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FOREWORD

The four independent reviewers estimated each paper and recommended 81 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 23rd International Scientific Conference "Research for Rural Development 2017" held at the Latvia University of Agriculture, in Jelgava, on 17 to 19 May 2017.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation more than 155 researchers with very different backgrounds. There were 124 presentations from different universities of Poland, Kazakhstan, France, Czech Republic, Lithuania, Estonia, India, Russia and Latvia.
Thank you for your participation! We are sure that you have learned from the presentations and discussions during the conference and you can use the outcomes in the future.

The cross disciplinary proceedings of the Annual 23rd International Scientific Conference "Research for Rural Development 2017" (two volumes since 2010) are intended for academics, students and professionals. The subjects covered by those issues are crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, environmental engineering, landscape architecture, information and communication technologies. The papers are grouped according to the sessions in which they have been presented.

Finally, I wish to thank Organizing and Scientific Committee and the sponsors for their great support to the conference and proceedings.

On behalf of the Organizing Committee
of Annual 23rd International Scientific Conference
"Research for Rural Development 2017"

Ausma Markevica
Latvia University of Agriculture

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FORESTRY AND WOOD PROCESSING

DESCRIBING NON-INDUSTRIAL PRIVATE FOREST OWNERSHIP IN ESTONIA: A QUALITATIVE APPROACH

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Abstract
Private forestry and forest owners in Estonia have become more profound research subjects in the last decade. One of the most common research topics has been the identification of private forest owners' (PFO) values and objectives. However, much of the existing studies rely on quantitative data which usually describes more active owners and owners whose forest property is larger than the average. Thus, the aim of the present study is to identify the management objectives and motives of non-industrial private forest owners using a qualitative approach – something that has been rarely used in forest owners' research in Estonia. Eight forest owner interviews form the dataset, which is analysed in order to identify PFO forestry objectives and forest use. The results of our qualitative content analysis show, in principle, similar generalized forest owner types of motivations. However, the qualitative approach opens up these generalized types even more. Income motive goes beyond just continuous financial returns and means in many cases a sense of security. Forest ownership is closely linked with "home" interpretations. We conclude that qualitative methods in small-scale forestry research provide new insights to forest ownership and its meanings to private forest owners.
Key words: private forest, interview, small-scale forestry, forest management, NIPF owner, motives.

Introduction

Changes in forest ownership is a normal phenomenon. However, many Central and Eastern European (CEE) countries have experienced changes rapidly and first hand due to the collapse of the Soviet Union. In Estonia, private forest ownership was re-established with the land and ownership reform initiated in 1993 (Meikar & Etverk, 2000). The process has led to a restructured forest ownership where, according to the National Forest Inventory (NFI) data the state owns 44% and private owners 48% of the total (2.2 million hectares i.e. 50% of land cover) forest land (Keskkonnaagentuur, 2016). It is expected that the share of private forest increases even more as about 177 000 hectares (8%) of forest is still subject to privatization (Keskkonnaagentuur, 2016). With this change, also the number of forest owners has increased. In 1994, it was estimated that there are 10,200 private farms that own forest (Leemet & Karoles, 1995), in 2011, the number of private forest holdings reached 52,000 (Toivonen *et al.*, 2005). However, in most cases the estimates on PFO structure have mostly been approximate expert opinions. Only quite recently, in 2011, the Ministry of Environment (2011) initiated a study on private ownership structure and forest use. The results indicate that most of the forest owners are private individuals. Such forest owners are often referred to as non-industrial private forest owners (NIPF), mostly in the US (e.g. Bliss & Martin, 1989; Zhang, Zhang, & Schelhas, 2005). Recent trends in Estonia show that the number of NIPF owners is increasing while their share of forest ownership is decreasing (table 1). This indicates that the degree of ownership fragmentation is significant and poses questions why these processes are taking place and how this influences the future management of these forests.

Table 1

General trends of private forest ownership

Indicator	2015[1]	2010[2]
Number of private individual forest owners (from total, %)	107,170 (95%)	93,271 (96%)
Number of legal (companies, trusts etc.) forest owners (from total, %)	5752 (5%)	4001 (4%)
Forest land in private individual forest ownership, ha (from total private forest, %)	688,246 (65%)	747,827 (74%)
Forest land in legal forest ownership, ha (from total private forest, %)	377,747 (35%)	262,960 (26%)
Average forest area for private individual forest owners, ha	6.4	8.0
Average forest area for legal forest owners (companies), ha	65.7	65.7

[1] Ministry of Environment 2015
[2] Ministry of Environment 2011

Private forestry and forest owners in Estonia have become more profound research subjects in the last decade. More recent research in Estonia (e.g. Toivonen *et al*., 2005; Sepp, 2008; Põllumäe *et al*., 2014a; Põllumäe, Korjus, & Paluots, 2014b; Põllumäe, Lilleleht, & Korjus, 2016) about private forest owners includes questionnaire survey data obtained from owners of larger than the average forest holdings. The data collection has involved samples taken from forest owners' organisation (FOO) networks or support measure databases. There are several reasons for that. One has been the need to have valid survey results (Toivonen *et al*., 2005) and others link with administrative reasons (e.g. Põllumäe, Korjus, & Paluots, 2014b). For example using existing FOO enables to gain the data with lower costs and with better response rates. For these reasons, it is difficult to extend many of the previous research findings to the overall forest owner population. Moreover, there is even a larger gap in understanding the management or ownership rationale of small-scale PFO as to date there is very little knowledge about the Estonian NIPF owners. Therefore, the aim of this study is to identify the management objectives and motives of selected NIPF owners using qualitative methods (as suggested by Põllumäe 2015). The choice of the method was driven mainly by the interest to have more detailed information that could go beyond the descriptive characteristics of quantitative data. Fischer *et al*. (2013) states that qualitative methods reflect better the attitudinal or psychic perceptions of the subjects.

Theoretical background and literature

Describing forest owners has been often limited to using quantitative solutions (e.g. cluster analysis). Põllumäe (2015) has outlined that PFO classifications are useful for designing more long-term objectives for forest policy due to the high level of generalization of these owner types. However, such broad descriptions might not be appropriate to implement everyday policy tools because the success of the implementation depends on how the design of the tools reflect the target group (Pregernig, 2011). Qualitative methods could be one way to overcome this barrier. Bliss and Martin (1989) used qualitative methods already in the end of 1980es to investigate NIPF management motives in the US. Among other things, they found more explained reasons for NIPF owners to produce timber. Former quantitative and neoclassical approaches had viewed private forest owners has rationale profit maximizers. In mid-1990es, Lönnstedt (1997) used qualitative methods to analyse the goals of ownership and found in case of Sweden that NIPF owners have long-term perspectives towards their ownership. A more recent qualitative forest owner study from Lithuania concludes that the regulation of forest policy does not fit well with the existing forest ownership characteristics (Stanislovaitis *et al.*, 2015). In Estonia, Grubbström (2011) used both qualitative and quantitative methods and investigated the emotional obstacles to land sale in Northwest Estonia. She concluded that there are often strong values attached to the land and that these values are mostly non-monetary in that particular region. Overall, value- and objective-based forest ownership typologies are the rather common subjects in forest policy research (Weber, 2012). Also in Estonia, Sepp (2008) used cluster analysis to segment the owners. He found that four main types – timber producers (23%), multiple-users (versatile producers. 10%), bystanders (27%) and indifferent or less active owners (40%). In 2014, Põllumäe, Korjus, & Paluots (2014) went a bit further by using principal component analysis to calculate the motives of forest owners. They did not group or segment the owners in specific groups. Instead, they concluded that different motives are present at the same time and situational aspects (policies or life events) initiate or prevent the realization of different motives (conservation motive, non-wood motive, income motive, 'home' motive, self-consumption motive). However, both (Sepp, 2008; Põllumäe, Korjus, & Paluots, 2014) studies rely on data, which reflects more the attitudes of larger-than-average forest owners. In addition, both studies generalize in a way, which might not reveal the actual diversity in different motives or objectives. Similar pros and cons between qualitative and quantitative approaches in PFO research have also been found for example by Bengston, Asah, & Butler (2011), Fischer *et al*. (2013) and Stanislovaitis *et al.* (2015).

Materials and Methods

In order to obtain the data and reach the respondents, different strategies of purposeful sampling (with emphasis on similarity) were used (Patton, 2014). Firstly, the interviewees were reached through personal connections or through some key informants (i.e. chair persons of regional forest owners associations). It is therefore that the sample is purposeful in order to provide full information about the targeted subjects as the interviewees were chosen mainly according to their property size (forest area not more than 20 hectares). Our aim was to have a small-scale forest owner's perspective (criterion strategy). However, another aspect that was considered was that the owner would reflect 'an average' NIPF owner. Therefore, forest owners with active forestry background or connections were not chosen for the interview. The data was gathered using a semi-structured interview guide. Eight NIPF owners between November 2014 and August 2015 were interviewed. The interviews lasted between 20 to 70 minutes. All the interviews

Table 2

General information about the interviewed forest owners and their forests

Number of interview	Gender	Age (years)	Number of parcels	Forest area (ha)	Owner since (year)	Distance from forest (from residence, km)
FO1	Male	22	1	8	2014	40
FO2	Female	28	8	20	2008	55
FO3	Male	32	1	18	2013	35
FO4	Male	62	2	3	2013	33
FO5	Female	46	1	14	2008	237
FO6	Male	39	1	18	2008	25
FO7	Male	55	4	5	1993	2
FO8	Male	50	3	6	1993	0.2

were recorded with the interviewees' permission. The recordings were fully transcribed for the analysis. A general overview about the interviewees and their characteristics is provided in Table 2.

A motive is a reason that makes (or might make) a person choose to act in a certain way and the reasons might reflect e.g. persons' needs or objectives. The latter are more general and long-term thus depending on the situation and institutional arrangement a particular behavioural choice might not reflect the person's initial objective. The objectives and motives were therefore analysed using two main themes. Firstly, all the transcriptions were analysed several times and all the parts where objectives were mentioned the text was indexed as 'OO' (ownership objectives). Secondly, the past or present forest use was identified and indexed with the abbreviation 'FO' (forest use). During this first step, the principles of open coding were applied. The second step of analysis included a segmentation of the identified parts of the text into categories of ownership objectives adopted from Põllumäe, Korjus, & Paluots (2014b). This was done in order to simplify and systematize the data for further analysing. Within these segments, the different aspects of these broader categories of ownership motives were then explored. The interviewees were also asked to describe themselves as forest owners and talk about their forest. In addition, the ways of becoming a forest owner were explored. Specific issues like ownership duration, past and present forestry activities, future plans, objectives, actual forest work conduced, frequency of forest visits and duration of stays were asked. Eventually, the interviewees were asked what forest ownership means to them.

Results and Discussion

Ownership objectives

Financial considerations or income motives were found in all eight interviews. One very distinct form of this motive was the indication that the forest provides some economic security either for the current or future generations. In addition, it is as a pension fund or a safe capital investment in case of emergent needs. This income motive is further looking and considers future prospects.

> "[the forest] *gives confidence, it is a guarantee in case something happens. If, for example, there is a shortage of money or if there are some health issues*." FO1.
> "*Forest ownership means that you are like a king. It´s like independence – if you have land then you are "the man", you are independent. It is like a great trump ace in your pocket, just in case, whatever happens.*" FO2

None of the eight forest owners indicated that there would be some direct (or sustained) economic expectations from forest management. Nevertheless, there were cases of forest harvesting and income generation. In many of such situations, the income was at least partially re-invested in forest management. In two specific cases, the forestry income was or is important to develop the property or own business further. Clear cuts have mostly been done by professionals and organized by forest owners' associations (FO2, FO3, FO5). In case of smaller-scale work (sanitary cuttings, thinnings) either local workforce (FO1, FO4) was used or the forest owners themselves (FO7, FO8) had done the work. In most cases (FO2, FO3, FO5, FO7, FO8) the forest owners had valid forest management plans and the plan seemed to be an important part of their forestry behaviour.

> "*The numbers* [income from harvesting] *were so small, but I had to do it, because I* [when buying the property] *booked my money into this, but I still need funds to make and maintain some roads.* /…./ *The market conditions were*

> *not good, among others. But the plan was in place and it* [the harvest] *was done. I am not the "let's-wait and see" kind of person*". FO 3
> "*The main business is still the livestock. If I have to buy more agricultural land, then I would sell some forest.*" FO7

In addition, it seemed that owners with stronger emotional bonds to the land were more eager to invest back to the forest (e.g. reforest, afforest, young stand development etc.) or they saw their activities as not just forest management, but as 'fixing' the forest (e.g. FO1). Other income motivated plans or activities were mostly related to the particular forest. However, they differed fundamentally from the previous cases where the income was re-invested. In the latter case, the forest owners saw it as an investment to other land uses, whereas the other owners saw it as an investment to forestland development.

> "*The purpose of the harvesting was that this money could be used to take care of the rest of "underwood"* [referring to the need to make thinnings]." FO5
> "*It makes sense to re-invest back to the forest. Most idiotic would be getting the money and spend it on just things. Most reasonable would be the* [investing into] *maintenance of young growth forests or standing forest.*" FO2

Consumption for own needs was quite an integral part of the investigated forest owners' objectives. In six cases out of eight such notions of objectives were identified. In all these cases, some need for firewood was mentioned. In most cases, the forest owners and their families themselves gathered the firewood. Moreover, if some additional help was used it was usually the local neighbouring persons. In addition to firewood, Christmas trees (FO2) and saw logs (FO2, FO7, FO8) were also mentioned. In such cases, the saw logs were also used for own purposes and in some cases (FO7) further processed to have boards.

> "*I could bring a Christmas tree from my own forest. And I could take firewood, because why should I pay for it when I´ve got my own forest. I don´t have to have a permit to make firewood from my own forest, do I?*" FO2
> "[when asked how much time the owner spends in the forest] *To fill up the shed with firewood and to makes sure the edges of the fields are clean from brushes.*" FO7

Different notions where the forest property was linked to be a 'home' were found in seven interviews out of eight. It was expressed directly either if there was a summer house nearby (FO1, FO3, and FO4) or the forest owner lived on the property (FO7). There were also future plans expressed (FO2, FO6) where forest owners indicated the wish to live on the property. In one case (FO5) the physical linkage with the property has weakened as the forest owner lives almost 240 kilometres away in the city; however, the emotional connection is still present.

> "/..../ *And since I do not have any connections anymore in South-Estonia and I still have to go to a salaried work.* /..../ *We indeed still have the foundation there in the property but no house. I could go with a tent, but…no*." FO5

Non-wood products and services were of less significant to the owners. In four cases out of eight different non-wood motives were present. In one case, such motive was not even linked to the forest itself but the property in general (FO3) as the forest owner indicated the wish to have some small agricultural beds. In addition, the same owner mentioned a plan to have a hiking trail on the property. Most of the non-wood uses were identified with owners who lived close to the property or who frequently visited the place. Most commonly, not only mushroom and berry picking was mentioned (FO6, FO7), but also hunting (FO7).

> "[When asked about how often the owner visits the forest] *Five times a week. If I include hunting, then even more I guess. If I´m hunting, then in autumn 4-5 times a week plus weekends.* /..../ *I´m not an active picker, but when I´m there I definitely pick the chanterelle* [*Cantharellus cibarius*], *some 3-4 times at least. For self-use. In autumn, some lingonberries and cranberries. But not on a large scale, it is still a hobby.*" FO7

In addition, nature conservation was not identified directly as a motive or objective. However, in at least two cases some notions were identified where forest owners reflected the importance of their forest on a more landscape level. For example, FO3 wanting to establish a home on the property, indicated that the "*forest is like a fur coat around the house*". Also, it "*keeps privacy and creates environment and cosiness*". Such deeper motives for ownership were mostly present in cases where the forest owners also related to the 'home' motive presented earlier.

> "*In fact, the forest around the house is more like a part of the surrounding greenery. I do not think of it as a source of income, but as a part of the landscape*. /…/" (FO4)

Many of the interviews reflected directly to previous or past ownership of the forest. Only in a few particular cases the owner had either bought the property nevertheless he characterized himself as a city person (FO3) or he and his family directly lived on the property (FO7, FO8). However, in the FO3 case, the objective of the ownership was also establishing home in the long term with the forest being a part of the landscape on the one hand, and on the other hand providing some financial resources to develop the property as a whole.

> "/..../ *There is a strong emotional bond. I could not imagine selling it off.* /…./ *It is not like an apartment in Tallinn what you can just buy and sell. It is different.* /…./" (FO3)

In most of the interviewees the forest had been inherited and quite an important part of the ownership seems to be linked to the roots of the ownership (FO4, FO5), the influences of past generations (FO1, FO2) or to a sense of responsibility (FO1, FO2, FO5). Some forest owners knew very well the historical background of the forest property even dating back to early 20th century (FO4). Others had more close connections with the previous owning generation (FO1, FO2).

> "/…./ *Their* [parents] *intention was that land has value and it will ensure welfare.* /…./" FO2

For example, FO5 lives approximately 240 kilometres away from the forest, but still stresses the importance of regeneration of the harvested stands and still manages in her own way the forestland despite of the weak physical connection.

> "*It's an issue of identity, that there would be some continuity.* /…./ *We won´t get anything anymore, but our children perhaps if there is something. When times are bad, you can take something. Or like a land, like a piece of Estonia, I have the land.*" FO5

However, in many cases where such sense of responsibility can be identified it can also be seen that it is individual and it is not somehow imposed to future generations (FO2, FO4, FO8). There are no expectations from the future generations to hold on to the property. In this case, also exceptions existed (MO1, MO2).

Põllumäe, Korjus, & Paluots (2014b) identified empirically five general motives for forest ownership and management in Estonia – a conservation motive, non-wood motive, income motive, 'home' motive, and self-consumption motive. Following the same categories, it was found in the interviews, that indeed, quite similar categories are present. The biggest distinction with Põllumäe, Korjus, & Paluots (2014b) was that there were no identifications of special forest conservation objectives in the interviews. Instead, some landscape-related aspects were identified. The level of specificity of other motives was much more abundant. The qualitative approach enabled to distinguish different aspects of single objectives. We found strong linkages between the family ties and personal identity. Grubbström (2011) who concludes that these emotional linkages are often historical has drawn similar findings. She stresses three main reasons – the loss of land during the Soviet annexation in 1940; the representation of the land as family roots and the presence of memories of the land and area in general. Very similar conclusion could be also drawn from this analysis, as there were forest owners with very detailed knowledge about the past of the property (e.g. FO4); there were important family roots (e.g. FO1, FO2, FO5) or some family- or place-related memories (FO1, FO4). Jörgensen & Stjernström (2008) show that stronger emotional linkages are present in case of inherited forest, and that the emotional values often outweigh the economic ones. However, our study also suggests the opposite with the FO3 have bought the forest and presents strong emotional values with no intentions to sell. But in case of FO8 who inherited the property, the willingness to sell it off if it would be needed, is clearly present. Bengston, Asah, & Butler (2010) when analysing an open-ended question for ownership reasons, also found a variety of family related aspects like legacy and heritage. Also Lind-Riehl *et al.* (2015) have found very strong links between NIPF decision-making and family influences. Such similar diversity and deeper meanings in the present study were also identified under the 'income' theme. The forestland is thus seen as a solid investment or back-up in case of sudden emergencies. In addition, it is a source for further property development. Lönnstedt (1997) found that most NIPF owners he studied also indicated the wish to preserve and develop the forest area further. Bengston, Asah, & Butler (2010) presented also similar results. So actually, the "income" motivation encompasses a huge variety of different considerations and preferences, which influence the actual behaviour of the owner.

The core values and objectives establish the foundation for PFO individual decision-making, but also the situational and institutional aspects influence the final decision (Karppinen, 1998) or actual behaviour. Such a relationship was also present in almost all of the studied interviewees. Much of the income-related motivations were influenced by other external factors such as market conditions (FO2),

stand condition (e.g. beetle, wind damage in FO4) or planned or unexpected financial needs (FO3, FO8). Bliss and Martin (1989) concluded also that different external influence merely adjusts the timing and scale of forest management activities. This approach is different from what Stanislovaitis *et al.* (2015) indicated. Stanislovaitis *et al.* (2015) stated that the forest characteristics (size, condition) influence the objectives. However, relying on Bliss and Martin (1989) and the current study, we would oppose Stanislovaitis *et al.* (2015), and propose that the forest property characteristics only influence the realization (both in terms of quality and quantity) of the aim. In principle, the value-based general objective itself (e.g. income) is universal. It is therefore that different policy tools (i.e. an institutional aspect) influence the behaviour of NIPF owners, but they still do it under certain situational conditions. Each external situational aspect (e.g. the NIPF owners' forest is damaged by wind) also has different influences to the forest owners since their physical or emotional linkages with the property differ. In addition, there are varieties of situational aspects that are present and interact at the same time as well. Forest policy can deal with this in many ways – have more costly policies and tools, which might provide better results from an individual forest owners perspective or have a more generalized approach, which is cheaper, provides more generalized results and might not accost to the individual forest owner.

As the approach of this study has been qualitative and the objectives and motives of NIPF owners have been under investigation, there are no generalizations or statistical analysis in this paper. It is therefore impossible to weigh or measure otherwise the proportions of one or another motive. Instead, we focused on having a rich data, which could enable us to go deeper than just one generalized objective or motive. From a methodological point of view, it is important that both quantitative segmentation and qualitative approaches have their place in forest ownership research. Moreover, the results of this study indicate that using both approaches in a mixed-method way could be even more appropriate and enlightening, as from a broad classification (based on generalized values) the researcher would be able to specify each of the group deeper (i.e. looking at the institutional and situational aspects) offering a continuous look at the development of objectives and thus decision-making. Fischer *et al*. (2013) who used such mixed-methods approach have also made such a conclusion. Also, many aspects of forest ownership seem to link with the place itself and how the forest property is understood and perceived. Therefore, we suggest that the different aspects of place attachment could be one possible future option in small-scale forestry research. This would provide us with a deeper understanding how the place itself influences the person-place relationship and whether and how different objectives of forest ownership are influenced by the forest property.

Conclusions

Based on the previous sections of the paper, we draw the following conclusions and general remarks for future research directions:

1. Among our respondents, we have identified even broader ownership objectives than just conservation, non-wood, income, 'home' and self-consumption motives and explained how such objectives actually come to life and influence the decision-making of the forest owners and thus the landscape itself.
2. The presented diversity of forest ownership illustrates quite well that in order to preserve a multiple-use forestry and diverse landscapes it would be reasonable to encourage diverse NIPF ownership. On a general level such as a country, diverse ownership could cover all the environmental, social and economic components of sustainability.
3. Qualitative methods in small-scale forestry research provide new insights to forest ownership and its meanings. This approach enabled us to open up rich and colourful meanings of some generalized ownership classifications that are often used to characterize and generalize forest ownership and owners.

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OCCURRENCE OF *LOPHODERMIUM* SPP. IN YOUNG SCOTS PINE STANDS IN LATVIA

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Abstract
Scots pine (*Pinus sylvestris* L.) is one of the most widespread coniferous species in boreal zones and it has a considerable economic importance in the Baltic countries. The impact of fungi on Scots pine has been known also historically, but it is predicted that it will increase in future due to climate changes that will have a positive effect on incidence and vitality of various species of fungi. The aim of the study was to characterize the occurrence of *Lophodermium* spp. in young stands of Scots pine in Latvia. Needle samples were collected from young pine stands (aged 1 – 14 years) located in all regions of Latvia. DNA extraction from needles was done using modified CTAB protocol, presence of *Lophodermium* in total extracted DNA was detected using a PCR method. Meteorological data – air temperature and precipitation – were obtained from all 34 observation stations of the Latvian Environment, Geology and Meteorology Centre. The presence of *Lophodermium* spp. was detected in all surveyed stands, and occurrence differences between the western and eastern regions were significant ($p = 0.004$). The occurrence of *Lophodermium* spp. in the eastern and western regions was affected by the meteorological conditions in autumn and winter of the previous years, as well as differences in the given years' air temperature and precipitation. In 2016, the eastern region of Latvia had a higher May-August precipitation and, to a lesser extent, temperature, which correlated with a higher occurrence of *Lophodermium* spp.
Key words: needle cast, disease distribution, meteorological conditions.

Introduction

Pine forests have considerable economic importance in the Baltic countries, as demonstrated both by the contribution of the forestry sector to the Gross Domestic Product (GDP) and the proportion of wood and wood products in total export value. Therefore, it is important to understand threats that might negatively affect vitality and growth of Scots pine trees in Latvia. Many threats are associated with rapidly progressing climate changes (Edenhofer *et al*., 2014). The relationship between meteorological factors and increment (both height and radial) of Scots pine has been well studied and the obtained results mainly demonstrate a slightly positive influence of the predicted changes on tree growth (Jansons *et al*., 2013a, 2013b, 2015a, 2016a; Rieksts-Riekstins *et al*., 2014). One of the threats to young Scots pine trees in particular is a needlecast disease caused by fungus *Lophodermium seditiosum*. *Lophodermium* spp. are highly diverse endophytic fungi with woody hosts, representing a range of symbiotic interactions with their host plants, from commensals and mutualists to latent pathogens. They have a one-year development cycle (Ortiz-García *et al*., 2003). *Lophodermium seditiosum* is one of the most important pathogens of Scots pine (*Pinus sylvestris* L.) needles in nurseries and young stands (Drenkhan, 2011). If the infection of this fungus in the forest nursery is not resolved, it can spread to young pine stands (Kļaviņa *et al*., 2012). Therefore, research has been done to develop effective fungicides to combat the disease (Millar, 1975; Ormrod, 1976). Weather conditions favorable for this disease are increasingly observed during recent decades – humid and warm autumns (as well as the end of summer) and mild winters (Martinsson, 1979; Diwani & Millar, 1990; Stenström & Arvidsson, 2001), making this period of time suitable for the studies of occurrence and impact of this disease. Hanso & Drenkhan (2007) indicate that Estonian climatic conditions (cold winters) are not an obstacle to the spread of the disease, which are favorable in late summer and autumn. Host-pathogen co-evolution as well as tree breeding had ensured development of resistance of local Scots pine individuals (genotypes) against the disease (Reich *et al*., 2003; Booy *et al*., 2000).

In recent years, other diseases significantly affecting needles have also been identified in Latvia: *Diplodia pinea* (Desmo.) J. Kickx and *Dothistroma septosporum* (Dorogin) M. Morelet (Adamson *et al*., 2015). The occurrence of new pathogens as well as increased influence of the already present ones is expected in future. Change of dominant tree species is a feasible option only in limited areas (Jansons *et al*., 2016b) due to specific soil requirements and economic importance of Scots pine. Tree breeding (and use of selected material in forest regeneration) can have an important role in reduction of the *L. seditiosum* and other needlecast diseases, since it is well developed and financially viable activity in Latvia (Jansons *et al*., 2015b) with proven effects on quantitative traits of Scots pine (Jansons, 2008; Jansons *et al*., 2006). Therefore, the existing breeding and propagation opportunities such as experimental

trials, infrastructure, as well as seed orchards, can be used to improve the resistance of needlecast simultaneously with other traits. Earlier studies had indicated the potential role of genetics in resistance to the needlecast (Liesebach & Stephan, 1996), but this effect may vary between the populations within a species. The aim of the study was to characterize the occurrence of *Lophodermium* spp. in young Scots pine stands in Latvia, and to investigate the correlation with meteorological factors.

Materials and Methods

Twelve young stands of Scots pine (further in the text 'objects') (Table 1) were selected – 5 stands in Western (W) Latvia, 5 stands in Eastern (E) Latvia and two stands in the central (C) part of Latvia. A report describing the presence of *Lophodermium* spp. in Scots pine stands in Latvia (Moročko-Bičevska *et al*., 2010), was utilized to select the sampling locations. In each stand the needle samples from 24 trees (aged 1 – 14 years) were collected. The total surveyed area of young stands was 20.7 ha (Fig. 1). The analyzed stands were located up to 1 km from the young stands that were surveyed in 2009 and had similar tree dimensions and growth conditions (mineral soil, surrounded by other Scots pine stands). Location of young stands was determined by GARMIN eTrex 20 navigator.

In each young stand along the longest diagonal, 6 – 15 needles of the previous year damaged by *Lophodermium* spp. were collected from 24 trees and inserted in sealable plastic bags. Needle samples were collected in August. Accurate monthly average values of air temperature (°C), precipitation (mm) and relative air humidity (%) for the selected study objects in years – 2008, 2009, 2015 and 2016 were obtained by spline interpolation. With spline interpolation method the image raster cells whose dimensions were 100 x 100 m in nature were created, reflecting accurate weather data information about the above-mentioned areas. Meteorological data – air temperature and precipitation in 2008, 2009, 2015 and 2016 – were obtained from all 34 observation stations of the Latvian Environment, Geology and Meteorology Centre.

In total, 288 samples were collected in 2016 from all regions of Latvia. Both objects from the central part of Latvia were excluded from further analysis to sufficiently differentiate the western and eastern regions. Consequently, results from only 240 needle samples were used for further mathematical analysis. DNA extraction from all samples was done using modified CTAB (Doyle & Doyle, 1987) protocol. Presence of *Lophodermium* in total extracted DNA was detected using a PCR method as described previously (Stenström & Ihrmark, 2005).

Descriptive statistics was used to characterize the data; chi-square criterion was used to analyze differences between the occurrence of *Lophodermium* spp. infection in the western and eastern regions and differences in infection frequency between years in these regions (Arhipova & Balina, 2003).

Table 1

Location and inventory parameters of studied objects

Object No.	Regions	Object location		Stand area, ha	Stand age, years	Stand average H, m	Stand average DBH, cm	Stand density, trees ha^{-1}
		Latitude	Longitude					
1	Western	56°40'30.13"N	22°16'42.30"E	3.6	6	1.9	3.5*	3100
2	Western	57° 2'15.66"N	23°10'20.08"E	1.6	7	3.2	5.2*	3200
3	Western	57°27'44.09"N	22°40'47.95"E	1.0	10	4.8	5.8*	2800
4	Western	57°20'59.24"N	22° 6'14.71"E	0.7	12	9.2	9.5*	2500
5	Western	57° 3'41.59"N	21°48'44.67"E	0.7	4	1.5	2.7*	3350
6	Eastern	56°36'8.05"N	25°35'5.24"E	0.7	6	2.2	3.7*	2700
7	Eastern	56°48'41.10"N	26° 7'46.21"E	2.7	4	0.9	1.8**	3300
8	Eastern	56°34'43.14"N	26°40'28.90"E	0.7	3	1.1	2.1**	3250
9	Eastern	56°27'11.62"N	27°17'20.52"E	0.5	10	6.5	6.2*	1900
10	Eastern	56° 5'28.54"N	26°21'42.05"E	2.8	5	2.1	2.9*	3400
11	Central	56°43'12.75"N	23°41'9.75"E	1.3	11	8.9	9.3*	2200
12	Central	56°55'55.09"N	24°23'23.74"E	4.4	3	0.9	1.9**	2900

*DBH – mean diameter at breast height; ** mean diameter at root collar; H – mean height.

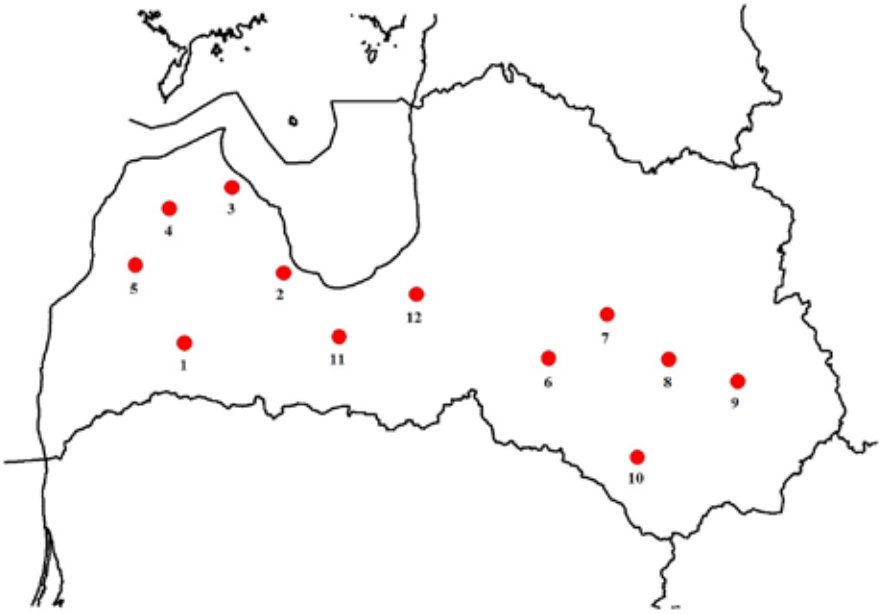

Figure 1. The location of research objects.

Results and Discussion

Correlations were not found between the young stand height, diameter and density indicators and their impact on *Lophodermium* spp. Therefore, data were further analyzed as a single set of data, evaluating them between years. The study shows that *Lophodermium* spp. were present in needles collected from all analyzed objects. This correlates with results from a previous report, where *Lophodermium* spp. were present in similar localities (Moročko-Bičevska *et al*., 2010).

The proportion of individuals where *Lophodermium spp.* were detected using a PCR method ranged from 50.0% (Stand 4) up to 100% (Stands 7, 8,

Table 2

Detection of *Lophodermium* spp. and meteorological data at each object

Object No.	Region	*Lophodermium* spp. detection, % in 2016	Average autumn precipitation, mm		Average winter temperatures, °C		Average temperature from May to August, °C		Average precipitation from May to August, mm	
			2008	2015	2008	2015	2009	2016	2009	2016
1	Western	83.3	206.0	235.0	-2.0	-0.7	14.6	15.8	282.2	317.7
2	Western	87.5	166.8	199.3	-1.2	-0.2	15.0	16.3	307.2	357.3
3	Western	87.5	207.2	190.4	-1.1	-0.1	14.2	15.4	386.0	360.0
4	Western	50.0	260.0	228.6	-1.4	-0.3	14.3	15.2	328.7	315.9
5	Western	95.8	300.8	253.5	-1.5	-0.3	14.2	15.9	282.2	279.1
Average western		**80.8**	**228.1**	**221.4**	**-1.4**	**-0.3**	**14.5**	**15.7**	**317.3**	**326.0**
6	Eastern	79.2	206.9	233.8	-2.9	-2	14.7	16.4	315.7	425.4
7	Eastern	100	199.0	174.9	-3.3	-2.5	14.4	16.1	308.4	341.5
8	Eastern	100	176.3	241.4	-3.1	-2.4	14.8	16.4	294.2	320.3
9	Eastern	87.5	175.7	285.0	-3.5	-2.4	14.4	16.2	334.0	291.0
10	Eastern	100	141.7	180.4	-2.8	-2.1	15.0	16.5	315.8	366.9
Average eastern		**93.3**	**179.9**	**223.1**	**-3.1**	**-2.3**	**14.7**	**16.3**	**313.6**	**349.0**
Total average		**87.1**	**204.0**	**222.2**	**-2.3**	**-1.3**	**14.6**	**16.0**	**315.5**	**337.5**

10). The overall proportion of Scots pine individuals where *Lophodermium* spp. were detected was 87.1% (Table 2).

Objects were divided into two groups (eastern and western region) to find the regional differences between detection of *Lophodermium* spp. *Lophodermium* spp. were more frequent in the eastern region ($p = 0.004$) (Fig. 4). Differences in occurrence of *Lophodermium* spp. in young stands of Scots pine in eastern and western regions could be explained by differences in meteorological (e.g. temperature and precipitation) conditions in the previous year (autumn and winter temperature) and during the survey year during *Lophodermium* spp. development from May to August.

A previous study (Moročko-Bičevska *et al*., 2010) had surveyed the occurrence of *Lophodermium* spp. in Latvian pine stands using a fungal culturing method. Samples for this study were collected in 2009, and the current samples were collected in similar locations, in order to enable the comparison of meteorological data. In 2016, using PCR detection, *Lophodermium* spp. were found in 87.1% of analyzed needle samples. The occurrence data were obtained using different approaches (cultivation on media in 2009 and PCR detection in 2016), however, the occurrence of *Lophodermium* spp. was lower in 2009. A significant difference in occurrence between the eastern and western regions was found in 2016. Based on the fact that autumn and winter meteorological weather conditions of the previous year affect the development of *Lophodermium* spp. in the next year (Drenkhan, 2011), it was concluded that in 2015 winter was warmer (-1.3 ± 0.8 °C) than in 2008 (-2.3 ± 0.3 °C), and the amount of precipitation in autumn of 2015 was higher (222.2 ± 2.7 mm) compared to the amount of precipitation in year 2008 (204.0 ± 5.9 mm). The autumn precipitation in 2015 was similar between the western and eastern regions (221.4 mm and 223.1 mm), and the average winter temperatures were lower in the eastern region (Table 2). However, the average precipitation in May-August 2016 was higher in the eastern region, and the average temperature in May-August 2016 was also slightly higher in the eastern region. These differences in May-August precipitation and temperature were not as pronounced in 2009 (when no significant differences in occurrence of *Lophodermium* spp. were found between the eastern and western regions).

Air temperature and precipitation during the time of *Lophodermium* spp. development (from May to August) in 2009 was 14.6 ± 0.2 °C and the amount of precipitation was less than 315.5 ± 6.2 mm, but temperature in 2016 was 16.0 ± 0.3 °C and precipitation was 337.5 ± 6.5 mm. The previously mentioned differences between the analyzed years were statistically significant ($p < 0.001$). When analyzing air temperature and precipitation differences between eastern and western regions in 2009 and 2016, the differences during the period from May to August are small, but could affect the development of *Lophodermium* spp. Air temperature in eastern region in 2009 was 14.7 ± 0.5 °C, the amount of precipitation was 313.6 ± 9.0 mm, but in western region air temperature was 14.5 ± 0.6 °C, the amount of precipitation was 317.3 ± 10.5 mm. In 2016, the air temperature in eastern region was 16.3 ± 0.3 °C and amount of precipitation was 349.0 ± 13.8 mm, but in western region it was 15.7 ± 0.4 °C and 326.0 ± 4.5 mm, respectively. In several other studies – temperature and precipitation were mentioned as some of the main factors for the occurrence, development and spread of needlecast (Drenkhan, 2011; Rajkovic, Markovic, & Rakonjac, 2013). Researchers in their studies have found that the fungus is capable of sporulation and its spores germinate in any time during the year

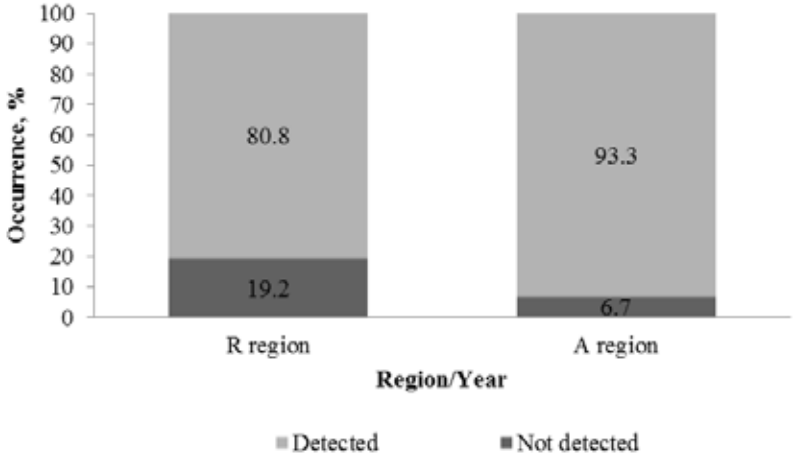

Figure 4. Occurrence of *Lophodermium* spp. in eastern and western regions.

when air temperature is above +5 °C and there is enough moisture (Thyr & Shaw, 1964). The optimum temperature for the development of ascospores (including *Lophodermium* spp.) is from +14 °C to +22 °C, minimum temperature is from -2 to +1 °C, with a maximum from +25 °C to +35 °C (Karadžić & Milijašević, 2008; Peterson, 1967; Gadgil, 1974). In the study, *Lophodermium* spp. in majority of cases have been observed directly on the previous year's needles, and in some cases also on the current year's needles. This shows that the degree of *Lophodermium* spp. infection significantly affects the current year's height increase of a tree and tree growth potential, and it is also noted by other scientists (Staley & Nicholls, 1989; Kanaskie, 1990). Needlecast primarily damages the current year (autumn infected) needles, which have a greater role in ensuring the growth of tree overground parts than older needles (Drenkhan, Kurkela, & Hanso, 2006). Martinsson (1979) found a significant negative correlation ($r = -0.80$, $\alpha = 0.01$) between the current year's relative increase of tree length and needle loss determined by needlecast infection degree. A similar correlation was found by other researchers (Baumanis, 1975; Squillace, La Bastide, & Van Vredenburch, 1975).

The two surveys of the occurrence of *Lophodermium* spp. in young Scots pine stands (Moročko-Bičevska *et al*., 2010, and this report) in Latvia utilized different methods for fungal detection (culturing vs PCR). In both cases, although only needles showing needlecast symptoms were collected for analysis, *Lophodermium* spp. were not detected in all samples. It is not clear, if this is due to technical limitations of the utilized detection techniques leading to false negatives, or additional, undetected pathogens could be inducing similar symptoms. In addition, the differences in occurrence frequency between the two reports may be due to differences between the detection techniques utilized. Therefore, additional studies are needed to obtain more accurate data about correlations between *Lophodermium* spp. occurrence and meteorological weather conditions in eastern and western regions, as well as throughout the entire territory of Latvia.

Conclusions

1. The presence of *Lophodermium* spp. was detected in all surveyed stands, and occurrence differences between the western and eastern regions were significant ($p = 0.004$).
2. The occurrence of *Lophodermium* spp. in the eastern and western region was affected by the previous year's autumn and winter meteorological weather conditions and differences in the given years' air temperature and precipitation.
3. In 2016, the eastern region of Latvia had a higher May-August precipitation and, to a lesser extent, temperature, which correlated with a higher occurrence of *Lophodermium* spp.

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MEAN BASIC DENSITY AND ITS AXIAL VARIATION IN SCOTS PINE, NORWAY SPRUCE AND BIRCH STEMS

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Abstract
The objective of this study is to evaluate the performance of models developed by Repola (2006) to estimate the vertical changes of the basic density of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* [L.] Karst), and birch spp. (mainly *Betula pendula* Roth) stemwood in the forests of Latvia and to develop a model for the estimation of average knot-free stem density with bark (SD) using diameter at breast height as an independent variable. The study material comprised a total of 81 spruce, 102 pine, and 105 birch stems representing a wide range of tree growing conditions in Latvia and covering all age classes.
The knot-free stemwood density (SWD) of pine and birch demonstrate strong vertical dependence along the stem, while for spruce the variation pattern was less pronounced and seemed to be nearly constant along the stem. The SWD estimated by Repola's models was 4.3% lower for spruce, but 3.4% higher for pine and 2.2% for birch comparing to average values obtained in this study.
Sigmoidal regression equations constructed in our study explained 67%, 27% and 54% of variations for predicting SD for pine, spruce and birch, respectively. Birch stemwood has a highest basic SWD – 470 kg m^{-3}, followed by pine – 397 kg m^{-3} and spruce – 385 kg m^{-3}. According to our results, the birch bark turned to be denser than the birch stemwood, being vice versa for the studied coniferous species.
Key words: stemwood density, bark density, density variations, pinus silvestris, picea abies, betula pendula.

Introduction

Basic density is one of the key wood properties for evaluation of the mechanical wood qualities for commercial use, determining the suitability of wood for different end use purposes. Wood density has numerous variation sources and is well correlated to many other physical properties of wood, like strength, stiffness and performance in use (Saranpää, 2003). It is a key variable for the estimation of tree mass (Henry *et al*., 2010) Low density wood is more suitable for pulp and paper products than for structural timber, where high density and strength is preferred (Saranpää, 2003).

Worldwide, there has been an increasing focus on the carbon footprint of buildings and recognition that design professionals are uniquely positioned to reduce greenhouse gases in the atmosphere by creating high performance structures using wood (Cabeza *et al*., 2013; Gustavsson, Pingoud, & Sathre, 2006). The density of wood tells how much carbon the tree has allocated into the wood (Chave, 2005); denser wood products stored more carbon. Carbon constitutes approximately 50% of the dry mass of wood and when wood is used to produce products, the carbon is stored for life in that product.

As it is presented in Figure 1, one of the most important factors affecting wood density is the moisture content. There are varieties of wood density definitions in reference to moisture content. Foresters usually measure the air-dried or green weight of the wood. In addition, there are different understandings of air-dry weight – the water content remaining in the wood may be 12% or 15% (Chave, 2005).

Information about correct wood basic density is needed to determine the stem biomass. In many studies, oven-dry stem biomass (water content is 0%) has been determined by multiplying the stem volume by the average stem wood basic density e.g. (Repola, 2008; Repola, 2009; Skovsgaard, Bald, & Nord-Larsen, 2011). Wood density interpretation and comparison at divergent meanings causes considerable misunderstandings in the literature. When reporting wood density, the moisture content at which the weight and volume of wood were measured must be indicated in the manuscript, as well the drying temperature when wood density is measured, since the influence of different drying temperatures on a dry weight determination has been reported (Petersson & Stahl, 2006). Below 100 °C the wood sample may not be dried completely, and above 105 °C it may char (Ilic *et al*., 2000).

Different methods have been applied to determine the average wood density of the stem. In biomass studies, the average stem density has been most commonly derived from sample disks taken at different heights along the stem by destructive sampling method (Repola, 2006; Repola, 2008; Repola, 2009). An alternative method is to use the increment borer extracting a small dowel-like piece from a tree (Chave, 2005; Liepins & Rieksts-Riekstins, 2013). The within-tree variation of wood density can be divided into radial variation from the pith to the cambium and axial variation along the stem (Jyske, Mäkinen, & Saranpää, 2008), and these variation patterns need to be considered when estimating the weighted density from wood samples.

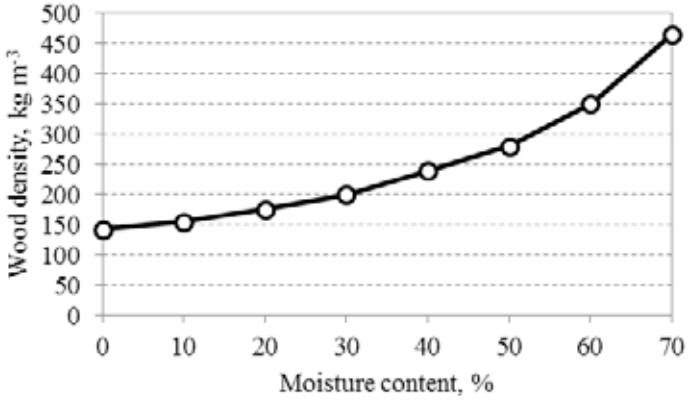

Figure 1. Relationship between wood density and absolute moisture content in whole tree Sitka spruce wood (Kofman & Kent, 2007).

In the Nordic and Baltic countries, Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* [L.] Karst), and silver birch (*Betula pendula* Roth.) are tree species with high industrial and ecological importance. Variation of basic stem density from the stem base to the tree top of these species has been studied in Finland (Repola, 2006) deriving models to describe vertical dependence of density along the stem and average stem density. Repola (2006) found that birch and pine wood had strong vertical dependence of density, encountering a downward trend from base to top along the stem while spruce had a moderate vertical variation.

Despite the importance of the aforementioned species, so far the average basic density values obtained in Russia and European countries have been exploited in Latvia, and there is no information about the most suitable one for local conditions (Millers & Magaznieks, 2012). Earlier studies of wood density variations within silver birch stems (Liepins & Rieksts-Riekstins, 2013) showed that Repola's model had a good fit for empirical data collected in birch plantations on former farmlands in Latvia, reporting the average wood density values that are similar to those achieved in other studies in the Baltic sea region.

The aim of this study is to evaluate the performance of models developed by Repola (2006) to estimate vertical changes of the basic density of Scots pine, Norway spruce, and birch stems in forests of Latvia and to develop a model for estimation of knot-free stem density with bark (SD) using diameter at breast height (D) as an independent variable.

Materials and Methods

The study material comprised a total of 81 spruce, 102 pine, and 105 birch stems representing a wide range of tree growing conditions in Latvia and covering all age classes (Table 1). In each of the selected forest stands three sample trees representing the range of social status of the dominant trees were felled down. Empirical material is also described in previous studies (Liepiņš & Liepiņš, 2015). The tree stems were cross-cut into 1 m or 2 m sections towards the top depending on the stem length (1 m sections for stems with a length below 20 m, 2 m sections for stems with length over 20 m). To obtain the average density of the stem and to describe vertical changes of density, sample discs were collected at the beginning of each stem section as well at the height of 1.3 m and at the midpoint of the first section. The vertical location of samples along the stem was expressed as the relative height ranged from 0 (butt) to 1 (top). The sample disks were stored in plastic bags and transported to the laboratory for measurements of dry weight and basic density.

In the present study, basic density is technically defined as the ratio of the oven-dry mass of a wood sample divided by the mass of water displaced by its green volume. The specimens for the basic density measurements were prepared from the sample discs using modified scheme reported in Herajarvi (2004) studies. The wood and bark specimens were measured separately and density was determined using Precisa XB 220A scales equipped with a Precisa density determination set (Part no: 350-8556). For the calculation of basic (water content is 0%) wood and bark density, all specimens were dried at 105 °C until a constant weight was achieved. Before the density measurements were conducted, all specimens were saturated with water by immersion for 24 hours (Ilic *et al*., 2000).

The weighted average formula was used to calculate the mean basic density of each sample disc from the specimens and their corresponding areas. The volume of each stem section (between two sample discs) was estimated using Smalian's approach often applied in similar studies e.g. (Smith *et al*., 2014). The

Table 1

Sample tree characteristics by tree species

	Pinus sylvestris			*Picea abies*			*Betula* spp.		
	D, cm	L, m	T, year	D, cm	L, m	T, year	D, cm	L, m	T, year
Mean	19.0	17.3	54	17.5	16.6	41	14.7	18.1	35
Std	9.4	9.2	39.1	9.0	8.9	26.9	7.5	8.1	23.6
Min	1.5	1.9	6	2.3	2.8	9	2.7	4.8	8
Max	45.2	34.5	141	36.3	30.8	97	37.1	32.3	92

D – diameter at breast height, L – stem length, T – tree age (forest inventory data).

average density of each stem section was calculated as mean from both sample discs at the ends of section, except top sections where only base sample disc was used. The weighted average formula was used to calculate the mean density of each stem from the average density of sections and their corresponding volumes.

The linear mixed models proposed by Repola (2006) were used to calculate vertical dependence of the knot-free stemwood density (SWD) of Scots pine, Norway spruce, and birch stems on the basis of measured tree variables (D, tree height, tree age, relative height of position in tree) in this study.

Sigmoidal regression equations were constructed to estimate SD using tree D as the only variable. The general form of the equation is as follows:

$$Y = \beta_1 / \left(1 + \beta_2 e^{-\beta_3 D}\right) \quad (1)$$

Where: Y is the average stem density (kg m^{-3}), D is breast height diameter (cm), β_1, β_2 and β_3 are the coefficients.

Regression analyses and descriptive statistics were carried out using the CurveExpert Professional 2.2 software.

Results and Discussion

The results are indicating the distinctive patterns of SWD along the stem for the studied tree species (Figure 2). Pine and birch demonstrated strong vertical dependence, reducing the SWD with an increase in stem relative height. For spruce the axial variation of SWD was less pronounced and seemed to be nearly constant from the stump height to the top of tree which is consistent with the results reported in previous studies (Hakkila, 1998; Repola, 2006; Jyske *et al*., 2008). Our findings and discovered SWD variation trends for pine, spruce and birch stems are in line with Repola's (2006) findings who also reported the distinct variations in wood density with an increasing tree relative height.

In our study, the SWD estimated for our data set with Repola's models was systematically lower for spruce (average 4.3%) but not for other species (Figure 2). Pine and birch SWD, estimated with Repola's models, resulted in an overestimation (average 3.4% and 2.2%, respectively) mostly in the upper part of stems. Different D distribution of the sample trees can be one of the reasons why models resulted in a small overestimation for pine and birch. Repola's data set did not include the stems from young stands, and the minimum D of the sample trees was 6 cm. In our study, an equal number of trees of

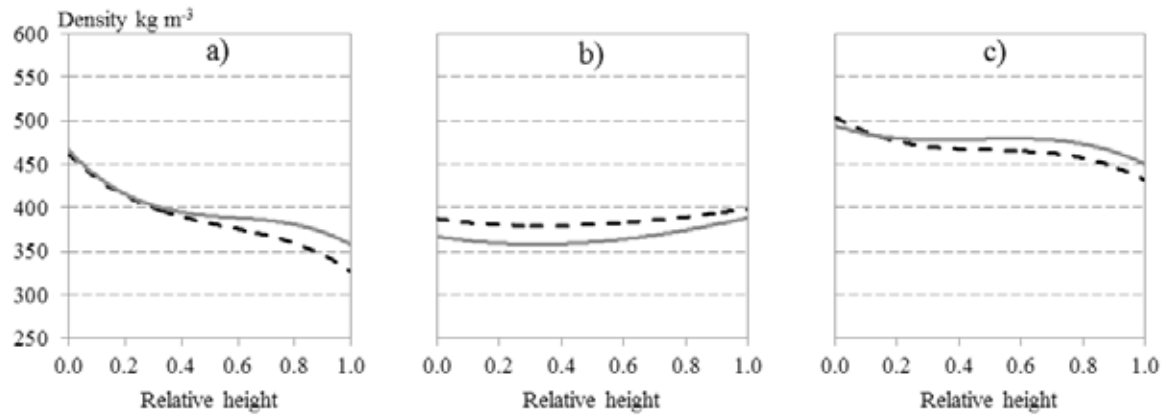

Figure 2. Predictions of SWD in the vertical direction along pine (a), spruce (b) and birch (c) stems according to Repola's (2006) models (grey line) and our measurements (black, dashed line).

determined age groups (young, middle aged, mature) were selected and minimum D of the trees was 1.5 – 2.7 cm depending on the species (Table 1). Generally, SWD is higher for mature trees than for young trees, but these variations are also depending on the interactions of the species and the environment (Ilic *et al*., 2000; Saranpää, 2003; Jyske *et al*., 2008; Millers & Magaznieks, 2012).

The average SWD for spruce reported by Repola (2006) is 385.3 kg m^{-3}, being very close to the value achieved in our study – 378.0 kg m^{-3}. The reasons why the use of Repola's model produced markedly lower SWD for spruce (359.5 kg m^{-3}) are unclear, especially taking into account that for pine and birch the aforementioned model predicted very similar values with those reported in his study. If plotted against our results (Figure 2), the predicted SWD values according to Repola's model along pine and birch stems are showing very good fit for pine and birch but not for spruce.

In both studies, the density estimates were based on knot-free sample disks, therefore a 1% correction is recommended in order to obtain realistic values (Repola, 2006). Tree branches are always denser than wood, for instance, branch density for spruce decreased along the crown level and was about 15 – 20% higher than wood density (Dibdiakova & Vadla, 2012).

It has to be noted that there are numerous studies performed on SWD and its variations worldwide, however, the mean stem density (SD) has been studied much rarer. In terms of SWD, Rikala (2003) found that 3% and 50% of the variations could be explained for basic density as a function of relative height for spruce and pine, respectively. For spruce the largest (49 – 80%) variation in SWD was found within the annual rings (Jyske *et al*., 2008). The predictive models were developed for average SWD using density data from one or more cross-sectional discs (Singh, 1984; Repola, 2006). Applying of models may be a more accurate method than the use of average basic density values to estimate mass of stem or wood sample due to the large variations in wood density. However, time consuming measurements needed for estimation of some key variables is the reason why many of the published functions are not practiced. Mostly the functions are focused only on the estimation of stemwood density; however, the bark should be included in the models if the calculation of whole stem biomass is the aim, as it is in the case of carbon accounting. Prediction equations based on commonly measured tree variables, such as D, tree height and age, would be the most useful and convenient ones to obtain the estimates of mean stem density. Among those, D is the easiest variable to measure; therefore it was chosen in this study.

In our investigation, the sigmoid growth model widely used in investigations of growth of forest trees and to describe various biological processes (Birch, 1999; Pödör, Manninger, & Jereb, 2014) was applied. The modified form of the sigmodal model (equation 1) gives three basic curve parameters that can be used in further investigations on mean SD in pine, spruce and birch stems. The relationship between mean SD and D and the general form of basic density curves is presented in Figure 3. A wide prediction band in Figure 3 indicates considerable fluctuations of basic density that is influenced by other factors. The summary of fit statistics (Table 2) displays that 67%, 27% and 54% of the variations could be explained with basic density as a function of D for pine, spruce and birch, respectively. The confidence bands for all species demonstrate that standard error of the regression is greater at small and large tree D, likely because of the smaller number of measurements at given dimensions (Figure 3).

There is a strong evidence of SD variation depending on tree dimensions and, consequently, on the tree age being more obvious for the pine. SD of young pine was 281 kg m^{-3} that is 34.2% less than for matured trees (427 kg m^{-3}). There is the same regularity for spruce and birch, however, the difference in SD for young and matured trees is markedly less pronounced. The range for mean values for spruce was 335 kg m^{-3} for young trees and 398 kg m^{-3} for matured trees (difference 15.8%) while the same values for birch 416 – 524 kg m^{-3} (difference 20.6%).

The difference between mean SD and SWD values (Table 3) shows that among tree species, the oven-dry stem bark is less dense than wood for

Table 2

Summary of fit statistics and parameter estimates

	Parameter values			R^2	SE kg m^{-3}
	β_1	β_2	β_3		
Pinus sylvestris	441.423	0.691	0.089	0.67	28.5
Picea abies	418.551	0.291	0.048	0.27	30.6
Betula spp.	528.197	0.354	0.104	0.54	26.4

R^2 – coefficient of determination, SE – standard error of the regression.

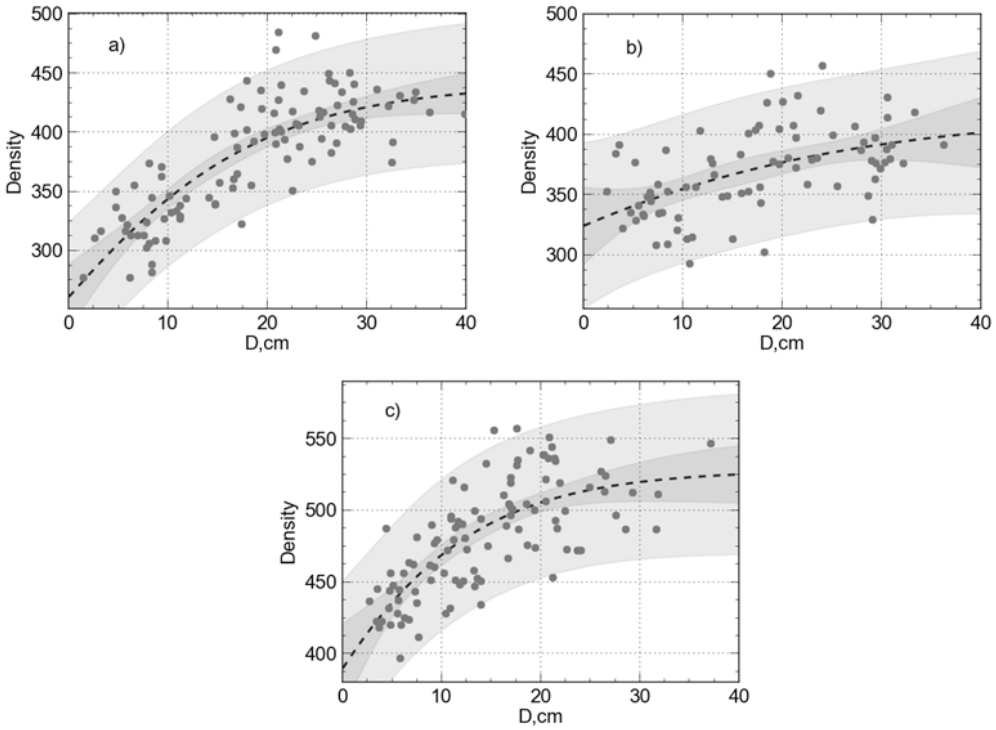

Figure 3. Relationship between average stem basic density (kg m^{-3}) and diameter for pine (a), spruce (b) and birch (c) stems according to the derived model. For all regressions, the confidence bands (dark grey) and prediction bands (light grey) at 95% confidence level are displayed.

coniferous species but for birch bark is more dense than wood.

The Intergovernmental Panel on Climate Change (Eggleston *et al*., 2006) guidelines suggest the use of mean wood density values of 420 kg m^{-3}, 400 kg m^{-3} and 510 kg m^{-3} for pine, spruce and birch, respectively, to estimate the wood biomass. Our results indicate that this approach for the studied tree species is leading to the overestimation of stem biomass for young trees because the suggested density values are correct for mature trees. Dibdiakova & Vadla (2012) found significant differences ($p < 0.05$) in bark density along the stem of Sitka spruce. In the aforementioned study, the bark density was reported higher than the wood density only on the base of trees. It has been reported in Latvia that bark density of Scots pine along the stem increases from 385 kg m^{-3} from the stump to 575 kg m^{-3} to the top (Millers & Magaznieks, 2012). Little information is available on variations of bark density for the studied tree species, highlighting the need for further investigations.

Conclusions

1. Pine and birch demonstrate strong vertical dependence in SWD, but for spruce the variation pattern is less pronounced and seems to be nearly constant.
2. The SWD, estimated with Repola's models, was 4.3% lower for spruce but 3.4% and 2.2% higher for pine and birch, respectively.
3. Tree D explains 67%, 27% and 54% of the variations in SWD for pine, spruce and birch, respectively.

Table 3

Pine, spruce and birch stemwood and bark basic densities (average values and standard deviation), kg m^{-3}

	SD	SWD	SB
Pinus sylvestris	380.5 ± 48.2	397.3 ± 49.0	260.1 ± 24.0
Picea abies	368.9 ± 36.7	378.0 ± 35.4	295.5 ± 27.5
Betula spp.	481.7 ± 46.5	470.3 ± 38.5	551.2 ± 36.7

SD – mean stem density, SWD – stemwood density, SB – stem bark density.

4. From studies, the tree species birch has the highest basic SWD – 470 kg m^{-3}, being lower for coniferous species – 397 kg m^{-3} for pine and 378 kg m^{-3} for spruce.
5. The mean stem with bark basic density for pine, spruce and birch are 380 kg m^{-3}, 368 kg m^{-3} and 481 kg m^{-3}, respectively.
6. According to our results, the birch bark turned to be denser than the birch stemwood, being vice versa for the studied coniferous species.

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CARBON BALANCE IN FOREST MINERAL SOILS IN LATVIA MODELLED WITH YASSO07 SOIL CARBON MODEL

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Abstract
Yasso07 soil carbon model was used to estimate soil carbon balance in dry forest site types (6 site types in total) in Latvia and the results were compared with data from Biosoil2012 soil surveys. Litter input, chemical quality and climatic data are required to run the model. Three different scenarios were used for climate data input – steady climate, climate change + 0.025 °C annually and climate change + 0.05 °C annually.
Forest mineral soil is a carbon sink for the whole modelled period - the years of 1990 – 2030. Under steady climate, the average carbon removal is 0.6 t CO_2 ha^{-1} yr^{-1}, under climate change (+ 0.025 °C) scenario 0.4 t CO_2 ha^{-1} yr^{-1}, but under climate change (+ 0.05 °C) scenario 0.3 t CO_2 ha^{-1} yr^{-1}. CO_2 removal at the beginning of the period (1990) was 0.35 – 0.38 t CO_2 ha^{-1} yr^{-1}. Carbon stock modelled with Yasso07 is lower than estimated in Biosoil2012 soil surveys. Differences between modelled and Biosoil2012 results vary from 2 t C ha^{-1} in the poorest and 41 t CO_2 ha^{-1} in the third poorest site type. Carbon stock modelled with Yasso07 increases from the poorest to the most fertile site type while Biosoil2012 shows an increase from the poorest to the third poorest, and a decrease from the third poorest to the most fertile site type. Underestimation and different trends between Yasso07 and measured carbon stock may be explained by inappropriate equations and models used to estimate non-woody biomass. It is necessary to improve accuracy of input data for non-woody biomass by elaborating national equations and models in order to include Yasso07 in the national GHG inventory.
Key words: carbon, mineral soil, forest, modelling.

Introduction

Soil is one of the largest terrestrial carbon pools (Schimel, 1995; Schlesinger & Andrews, 2000). One third to fifth of the total soil carbon pool is stored in organic soils (Gorham, 1991; Yu *et al*., 2010), the rest in mineral soils. It is estimated that approximately two thirds of terrestrial carbon pool in the long term are involved in active interaction between the atmosphere and soil (Post *et al*., 1982), and more carbon is stored in soil than is present in the atmosphere (Davidson & Janssens, 2006). Thus, alterations in this cycle may accelerate rates of global warming. Furthermore, the temperature increase may intensify fluxes of carbon from soil (Cox *et al*., 2000; Wieder, Bonan, & Allison, 2013).

A large proportion of carbon stored in mineral soils is in forest ecosystems (Pan *et al*., 2011), especially in high latitudes (Dixon *et al*., 1994). Although the importance of forest mineral soils to a global carbon cycle is high, it is not mandatory to report emissions from forest mineral soils in the National Greenhouse Gas Inventory. This is related to the complexity of those processes and lack of data (Buchholz *et al*., 2014).

The whole carbon cycling in forest ecosystem is not complete if soil carbon is not included. Some of the forestry practices may contribute to climate change mitigation through a higher energy wood production, for example, stump removal or removals of residues during harvesting (Lazdins & Mattila, 2012). At the same time, it may negatively impact soil carbon storage (Kataja-aho *et al*., 2012).

There are several factors which significantly affect the soil carbon balance. It is worth mentioning such abiotic factors as temperature (Schimel *et al*., 1994; Trumbore, Chadwick, & Amundson, 1996), moisture (Davidson, Belk, & Boone, 1998), soil texture (Richter *et al*., 1999; Krull, Baldock, & Skjemstad, 2003) and more complicated biotic factors as microbial activity (Wieder *et al*., 2013). Moreover, the interaction between those factors may significantly change intensity and direction of impact for each of those factors. Usually soil temperature rise causes increased CO_2 fluxes from soil (Schimel *et al*., 1994; Trumbore *et al*., 1996). But if the temperature rise interacts with drought, it may significantly reduce CO_2 emissions (Grünzweig *et al*., 2009; Dato *et al*., 2009; Joos *et al*., 2010). The complexity of impact of those factors limits the possibility to elaborate simple and reliable emission factors for mineral soil in greenhouse gas inventory. In order to solve the problem, soil carbon models can be used.

There are several models available to model soil carbon cycling in mineral soils, including Yasso07 soil carbon model. Yasso07 is a dynamic soil carbon model, created specifically for forest mineral soils (Liski *et al*., 2005). The impact of main climatic variables, such as temperature and precipitation, is considered in the model. One of the advantages of Yasso07 against other models is its simplicity. Input data can be easily measured or calculated from measured data.

Yasso07 model is used in this study to calculate the carbon balance in forest mineral soil at a national scale. The aim is to estimate the soil carbon balance in

dry forest site types in Latvian forests with Yasso07 soil carbon model and to evaluate the applicability to include results in the national greenhouse gas inventory.

Materials and Methods

Yasso07 is a dynamic soil carbon model. The model can be used for different mineral soils worldwide and it is not limited by climatic or geographic conditions (Liski *et al*., 2005; Tuomi *et al*., 2011). Litter input data, climate data and initial state of soil are necessary to run the model.

Litter input is formed by dead wood which is accounted directly in NFI and from living biomass. The average growing stock diameter data for each forest type of dry site type forests was used to calculate the total above and below ground living biomass. The data was collected from the National Forest Inventory (NFI). According to the Latvian forest classification system, there are 6 forest types under dry site type forests which represent dry mineral soils, ranked from the least to the most fertile: *cladinoso-callunosa (Cl), vacciniosa (Va), myrtillosa (My), hylocomiosa (Hy), oxalidosa (Ox), aegopodiosa (Ae)* (Zālītis & Jansons, 2013).

The growing stock is in m^3 ha^{-1} units. Biomass conversion factors created by LSFRI Silava were used to convert from volume to above- (stem, branches) and below-ground (stumps, roots) biomass units in tons (unpublished). Biomass in foliage and bark was calculated using biomass equations created by Repola (Repola, Ojansuu, & Kukkola, 2007; Repola, 2009). Fine root biomass was calculated depending on the tree species, from the results published by Finer *et al.* (2011). It was assumed that fine root biomass is dependent only on the tree species. The respective fine root biomass per hectare values is 3.38 t C ha^{-1} for pine and spruce and 3.20 t C ha^{-1} for deciduous trees.

It was assumed that litter production from living biomass is linear and proportional to the total biomass of each group of biomass. Coefficients of litter production rates are shown in Table 1. These litter production rates are created in Finland for boreal forests (Lehtonen *et al*., 2004; Starr *et al*., 2005; Liski *et al*., 2006).

Tree species are divided in three classes: pine, spruce and deciduous. Rates for deciduous trees are originally created for birch, but they are used for other deciduous species too.

Litter input from ground vegetation is 0.506 t C ha^{-1} yr^{-1} for *myrtillosa* and *hylocomiosa* site types. The source of this value is Finnish national greenhouse gas emission inventory, the litter production rate of ground vegetation for southern Finland. The ground vegetation litter production in *cladinoso-callunosa, vacciniosa, oxalidosa and aegopodiosa* site types is, respectively, 0.5, 0.75, 1.25 and 1.5 times of 0.506 t C ha^{-1} yr^{-1}.

Fallen and cut down trees are accounted directly in NFI and this is also a source of litter. All tree stems that have been fallen or cut down (including stumps of those trees) and left in the forest are accounted for as coarse woody litter (diameter > 10 cm). Other compartments are fine woody (< 10 cm) or non-woody biomass. Biomass of different groups of biomass of fallen or cut down trees was calculated using the same biomass equations as for living biomass.

Considerable litter input is produced from cut down and removed trees. Although stems have been removed from the forest, stumps and roots are left for decomposition. It is assumed that also 50% of branches are left in the forest, others are removed as biofuel.

Yasso07 requires input data for chemical quality of litter input. Chemical quality is divided into 4 classes according to the solubility of organic compounds in different solvents – acid soluble, water soluble, ethanol soluble and non-soluble (Table 2). There are 5 different groups of litter. The data about quality of different groups of litter are from Yasso07 user manual (Liski, Tuomi, & Rasinmaki, 2009).

Zero level was chosen for the initial state (no carbon in soil), because of land use history in Latvia. Vast areas of forest historically were agricultural lands which were afforested later. That is why the trend of carbon stock in soil should be increasing over the past century. The model was run for 200 years for calibration purposes. The input data of 1990s was used for calibration.

Table 1

Annual litter production rates proportionally to biomass of different groups of biomass

Litter production rates	Needles	Branches	Bark of stems	Bark of stumps	Roots >2mm	Fine roots
Pine	0.25	0.020	0.0052	0.0029	0.018	0.85
Spruce	0.10	0.013	0.0027	0.0000	0.013	0.85
Deciduous	0.79	0.014	0.0029	0.0001	0.014	0.85

Table 2

Annual litter production rates proportionally to biomass of different groups of litter

Groups of litter	Tree species	Acid soluble	Water soluble	Ethanol soluble	Non soluble
Stem	pine	0.67	0.03	0.01	0.29
	spruce	0.67	0.02	0.01	0.31
	deciduous	0.7	0.02	0.01	0.28
Branches	pine	0.46	0.02	0.08	0.43
	spruce	0.67	0.02	0.01	0.31
	deciduous	0.7	0.02	0.01	0.28
Fine roots	pine	0.58	0.13	0.06	0.23
	spruce	0.55	0.13	0.07	0.25
	deciduous	0.43	0.2	0.1	0.27
Foliage	pine	0.52	0.18	0.09	0.22
	spruce	0.48	0.13	0.07	0.32
	deciduous	0.43	0.2	0.1	0.27
Vegetation	all	0.27	0.47	0.23	0.03

Three different scenarios were used for climate input data. The first scenario is steady climate. Interannual climatic data were used for this scenario. The average temperature is + 5.9 °C, precipitation – 667 mm and monthly temperature variation – 7.8 °C. The second is climate change scenario. It was assumed that the annual raise of temperature is + 0.025 °C since 1990 in this scenario. The third scenario is climate change with + 0.05 °C annually. In both climate change scenarios interannual data is used until 1990.

Results of Yasso07 were compared with results from the forest soil inventory in 2012. Soil inventory throughout the country was done within Biosoil soil surveys in 2006 and 2012. Soil samples down to 80 cm soil profile were collected with soil volumetric sampler to estimate soil dry bulk density and carbon content.

Mean organic carbon stock in m^3 ha^{-1} was calculated from Biosoil data for 5 of 6 forest site types in dry mineral soils. No data was available for the most fertile site type – *Aegopodiosa*. In total, all 55 sample plots representing forests on dry mineral soil in Biosoil were used in calculation, respectively, 3, 5, 13, 21 and 13 sample plots for *cladinoso-callunosa, vacciniosa, myrtillosa, hylocomiosa* and*, oxalidosa.*

Results and Discussion

Under the steady climate scenario, the forest mineral soil is a stable carbon sink. The model shows carbon removals for the whole 1990 – 2030 period. High peaks and changes since 2009 are related to the availability of NFI data. This is the time when the first NFI cycle was completed in 2008 and the second cycle of NFI was started and more accurate information about tree biomass changes and dead wood production was available. The average NFI data values were extrapolated for the period before. The average CO_2 removals for steady climate scenario are ~0.6 t CO_2 ha^{-1} yr^{-1}. Removals vary from 0.2 t CO_2 ha^{-1} to 1.0 t CO_2 ha^{-1} yr^{-1} (Figure 1). Even if the climate change is considered, forest mineral soil still acts as a sink not source of CO_2. Under the most severe climate change scenario (+0.05°C annually), soil is a source of CO2 only for one year. All other time the forest mineral soil is CO_2 sink (0.1 – 0.7 t CO_2 ha^{-1} yr^{-1}).

De Wit *et al*. (2006) reports 0.29 t CO_2 ha^{-1} yr^{-1} for year 1990 in Norway, also modelled by Yasso07, which is slightly lower compared to our results 0.38 t CO_2 ha^{-1} yr^{-1}. Almost similar results to Wit *et al*. (2006) are also reported by Rantakari *et al*. (2012) for Finnish forest mineral soil 0.27 t CO_2 ha^{-1} yr^{-1}. Higher removals in our study can be explained by land use history. Our forests are relatively young. During the last century, the total area and growing stock in Latvian forests have doubled. The increase originates from afforestation of grasslands and croplands, which generally have a lower soil carbon stock.

The results are highly sensitive to carbon input data. Especially, the variation of total carbon input through woody biomass (Thürig *et al*., 2005). It is concluded by Thürig *et al*. (2005) that the removal of fallen trees after strong windthrows may turn soil from sink to source. Our study shows that there is strong correlation between changes in total carbon input in soil and changes in net carbon balance (r = 0.98). The peaks in CO_2 balance correlate with peaks in litter input. The smallest estimated litter input is in 2010 when the lowest CO_2 removals have been modelled, while the highest C input is in 2014, followed by the highest CO_2 removals.

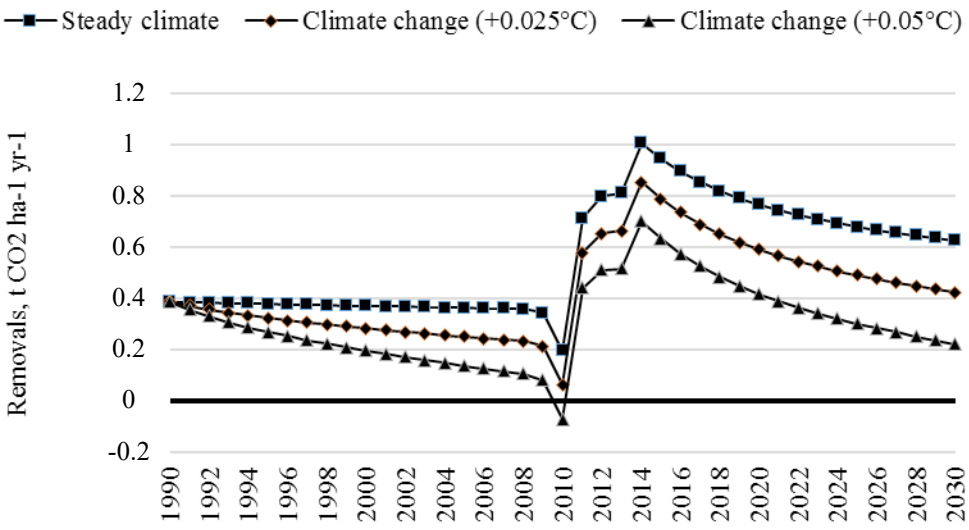

Figure 1. CO_2 net removals in forest mineral soils.

If the average forest productivity per area unit will keep increasing or stay at the current level, litter production rate also should increase or stay at the current level. And forest mineral soil will be carbon sink also for the next two decades. It is not projected that mineral soil in forest may turn to a carbon source even under climate change. Furthermore, it is predicted that climate change and following temperature increase and longer vegetation season may accelerate tree growth (Jansons *et al*., 2013, 2014, 2015).

Carbon stock modelled with Yasso07 (in 2012, steady climate scenario) was compared with data from Biosoil soil surveys to evaluate the modelled results (Figure 2). The trend is to underestimate carbon stock with Yasso07. In 5 of the 6 (no data for the most fertile site type in Biosoil) dry site forest types the carbon stock is smaller with Yasso07 compared to inventory results from Biosoil. There are only slight differences in the poorest *cladinoso-callunosa* site type and the second most fertile *oxalidosa* site type (6 t C ha^{-1} yr^{-1} and 2 t C ha^{-1} yr^{-1}). For those site types, with average fertility, differences are bigger, with the biggest for the third poorest site type *myrtillosa* (42 t C ha^{-1} yr^{-1}).

Results modelled with Yasso07 show an increasing trend in carbon stock from the poorest towards the most fertile site type, with the least carbon stock in the poorest *cladinoso-callunosa* site type and the biggest carbon stock in the most fertile site *aegopodiosa*. This trend is not present in Biosoil surveys. Biosoil shows an increasing trend from the poorest to the third poorest site type. Then the trend is getting opposite and carbon stock is decreasing from the third poorest to the most fertile site type.

Carbon stock modelled by Yasso07 strongly correlates with the total carbon input data. The highest average carbon input is in the *aegopodiosa* site type (most fertile) and is 76%, 56%, 41%, 25% and 20% higher than the other site types ranked from the poorest to most fertile (Table 3). Carbon input is the main factor influencing carbon stock in different forest types. Biosoil results highlight the impact of other factors, also influencing carbon storage in soil. Organic matter decomposition in fertile soils is usually faster than in poor soils. This is partly driven by vegetation differences between fertile and poor site types. Vegetation in fertile forests is dominated by deciduous trees and herbs in the ground vegetation, compared to coniferous trees, mosses and lichens in poor sites. Theoretically, the impact of type of vegetation on carbon cycling should be excluded by chemical composition data input in Yasso07. However, there is a lack of reliable input data for non-woody biomass, which is the main carbon input pool. There is almost no information about the ground vegetation biomass dynamic among forest types and vegetation types for Latvian forests. The share of total carbon input through ground vegetation is 8% – 12%. Furthermore, the biggest pool for carbon carbon input is fine roots (40% – 70% of total input), but this is the most uncertain pool at the same time. Additional errors may be caused by unsuitable non-woody tree litter production rates for our conditions (8% – 20% of total input). Litter production rates are calculated based on studies from boreal vegetation zone.

Overall, the share of non-woody biomass to the total carbon stock is 70%, of which the share of ground vegetation, fine roots and non-woody litter is, respectively, 15%, 9% and 46%. Considerable contribution to carbon cycling shows the necessity for more accurate input data for those pools. Currently, there are no suitable models or equations elaborated for local conditions to calculate carbon input to soil from non-woody biomass. All input data, except fine roots, for non-woody biomass have been calculated

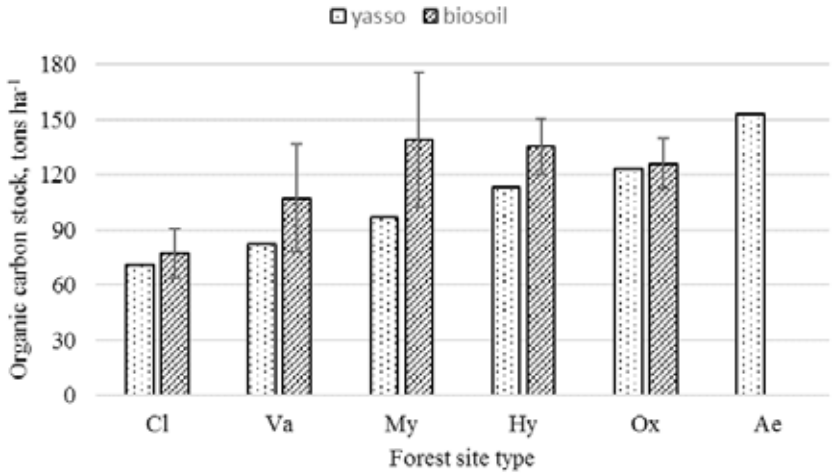

Figure 2. Organic carbon stock in different forest site types (Error bars shows 1 SE, Cl - *cladinoso-callunosa,* Va - *vacciniosa,* My - *myrtillosa,* Hy *hylocomiosa,* Ox - *oxalidosa,* Ae - *aegopodiosa*).

Table 3

Carbon input in soil by different groups of biomass and forest site types (all given values are in tons ha^{-1} yr^{-1})

-	Cl	Va	My	Hy	Ox	Ae
Coarse woody biomass (> 10 cm)	0.03	0.06	0.17	0.28	0.56	0.79
Woody biomass (< 10 cm)	0.23	0.38	0.46	0.73	0.57	0.80
Non-woody biomass	3.43	3.74	4.00	4.18	4.30	4.91
Total	3.68	4.18	4.62	5.20	5.44	6.50

using data from boreal vegetation zone. Fine root data reported by Finer *et al*., (2011) are compiled from literature for boreal, temperate and tropical forests. Still, the data are rough and with a high uncertainty as may be expected if the global datasets are used.

Conclusions

Soil at dry forest site types is a carbon sink. Soil will continue to sequester carbon at least for the next two decades even under changing climate. Results with Yasso07 show underestimation of carbon stock when comparing modelled results with measured ones in Biosoil2012 soil survey. There are also different trends between site types among fertility gradient. Yasso07 shows an increasing trend from the poorest to the most fertile site, while there is no such a trend in Biosoil2012. These inconsistencies may be explained by inappropriate non-woody biomass input data. In order to include Yasso07 in the Latvian national GHG inventory, national models or equations should be developed to estimate the litter production from non-woody biomass pool.

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THE IMPACT OF MANAGEMENT ON GROUND VEGETATION IN RIGA'S URBAN FORESTS

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Abstract
Urban forests provide ecological and social functions and significantly improve esthetical value of these ecosystems. These forests also function as recreational areas for urban residents. The aim of this study was to assess the influence of understory cutting of different intensity on vegetation and coincidence with *Myrtillosa* forest type in Mežaparks, Riga. In total nine study sites were established: three sites adjacent to roads where regular undergrowth management is practiced, three adjacent sites to edges of the pathways, where undergrowth management is practiced only occasionally and three adjacent sites to roads where there is no management activity at all. Combined 54 sample plots were created (each sized 10 × 10 m, area 100 m^2), where vegetation survey was done using Braun-Blanquet method. This study showed that with an increase in the intensity of undergrowth management, in general, the species richness and their coverage increases, or more specifically, the number of herbaceous and moss species increases, but the occurrence of shrub species decreases. In all study sites we observed the development of synantrophic species, especifically, the development of fructification which was indicated by the following species: *Sambucus nigra* L., *Sambucus racemosa* L., *Amelanchier spicata* Lam. If management intensity decreases, the proportion of competitor species decreases, but the proportion of CSR strategy type species stabilizes.
Key words: urban forests, vegetation, synantrophication, forest management.

Introduction

Urban forest ecosystems have evolved by interaction of human activities and natural processes. These ecosystems provide significant environmental (air purification and oxygen production, temperature and microclimate regulation, runoff reduction, soil protection, noise reduction, preservation of biodiversity) and social function (recreation, aesthetic values, cultural heritage sites, environmental education) (Emsis, 1980; Dwyer, McPherson, & Schroeder, 1992; Tyrväinen *et al*., 2005; Bell, 2008; Chen & Jim, 2008; Konijnendijk, 2008). Residents of urban forest prefer to use these forests for recreation closer to their place of residence (Konijnendijk & Randrup, 2004). This demonstrates the importance of social role of urban forests, and the conservation of biodiversity in these forests is closely related to the recreational and landscape features (Alvey, 2006).

Various natural or anthropogenic disturbances affect the development of vegetation in forest ecosystems (Priedītis, 1999; Drobyshev, 2004). However, urban forests are also affected by direct human activities, such as trampling, campfires, and waste disposal, and indirect – promoting the dispersal of adventive and invasive species, changes in the amount of soil nutrients. In addition, urban forest ecosystems are characterized by synantrophication, which promotes the pollution and eutrophication of soils, as well as the increase of anthropogenic pressure (Laiviņš, 1998; Priedītis, 1999). High pressure from recreational activities causes the degradation of the understory vegetation, for instance, better lighting conditions increase the ruderal and grassland plant introduction. This causes the patch structure formation where disturbed and undisturbed areas in urban forest landscape appear (Эмсис, 1989; Trušiņš, 1990; Bell, 2008). Therefore, the succession processes in the urban forest ecosystems are strongly influenced by anthropogenic loads and appropriate sustainable forest management planning is essential to preserve these areas in longer term.

Understory vegetation is a significant landscape-forming element, which increasesthe diversity in vertical structure and openness while dense understory limits recreation opportunities and hinders forest regeneration. It is known that increaseddensity of shrub layer promotes fructification (Priedītis, 1999). However, different studies have shown that for recreation activities people prefer to visit open forests or forested areas with low density in the undergrowth (Tyrväinen *et al*., 2005). Therefore it has to be taken into account and one of the most important management tasks is to provide the stability in terms of succession and improve the aesthetic values in these forests. The aim of this study was to assess different intensities undergrowth cutting effects on the understory plant diversity and conformation to *Myrtillosa* forest type in Mežaparks, Riga. The following study objectives were identified: 1) to characterize vegetation along the roads and trails in Mežaparks where understory cutting is done regularly, irregularly and left intact; 2) to compare and evaluate how different management intensity affects the understory vegetation.

Materials and Methods

The study was conducted in Mežaparks, located in Riga, the capital city of Latvia. Mežaparks is known since 1901 as a forest park. Its total area is 1182.1

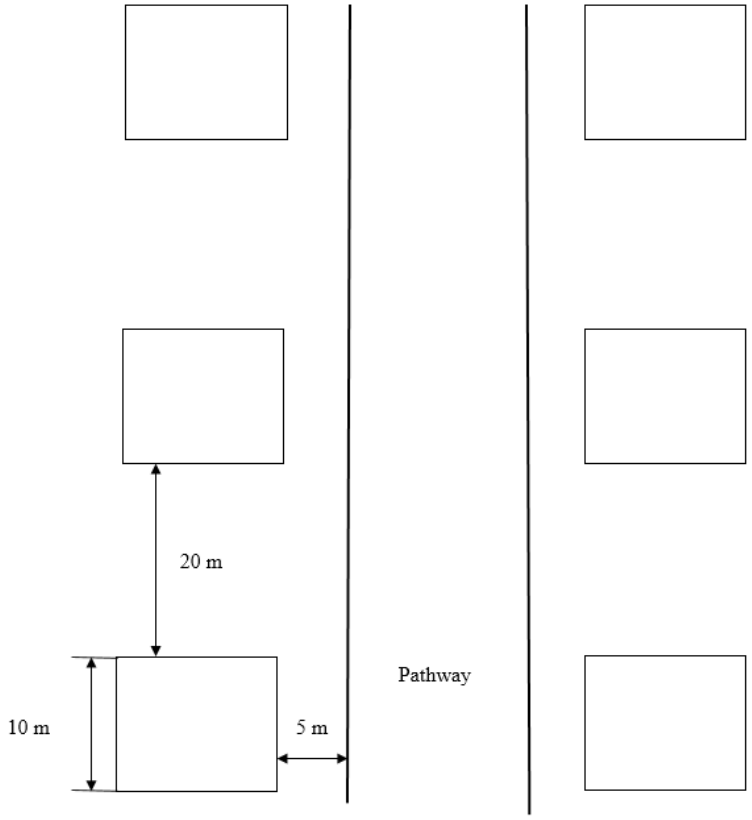

Figure 1. The schematic design of sample plot.

hectares large and it is dominated by Scots pine *Pinus sylvestris* L. forests with walking trails, recreational infrastructure, as well as residential houses on partly preserved sand dune terrain. The most important forest type is *Myrtillosa*, it occupies 55.9% of the total area. Despite the long-term anthropogenic impact, few forest stands correspond to the European Union protected habitat type *Western taiga forests* (Jakovičs, 2013). The territory is maintained by the company Rīgas meži Ltd. and the infrastructure improvements for recreation purposes are regularly done (understory cutting once a year in 20 m wide strips on both sides along the roads and trails from 2004), irregularly done (in sites along the roads and trails where surface is not covered by asphalt and understory cutting is practiced occasionally – in 2012 and 2014) and intact sites (management activities have not been done). A study was carried out in the vegetation season of 2015. In total we selected nine study sites which correspond to the intensity of the management: three sites with regular understory cutting, three with irregular understory cutting and three intact sites. In each study site, in both sides along roads and trails, three sample plots were established with size 10 m x 10 m and separating distance of 20 m (Fig. 1).

In each sample plot, the vegetation survey was done using Braun-Blanquet method. The total projective coverage of moss, herb (including vascular plants, dwarfshrubs, shrub and tree species up to the height of 0.5 m), shrub (shrub and tree species at the height from 0.5 to 7.0 m) and tree layers (tree species from the height of 7.0 m) as well as coverage of each separate species was evaluated in each zone as percentage (%). The occurrence for all plant species was calculated according to Raunkiaer constancy classes (I < 21% II 21 – 40%, III 41 – 60 %, IV 61 – 80% , V 81 – 100%) characterizing the plant species stability of objects (Markov, 1965; Muller-Dombois & Ellenberg, 1974). All species in the herb layer were characterized by plant strategy groups (C-S-R) (Grime, 1979) and according to the abiotic conditions (light, moisture, soil pH and soil nitrogen) were estimated using Ellenberg indicator scales (Ellenberg *et al*., 1992). For data statistical analysis the ANOVA test was used to estimate the significant variations between gradation classes. A confidence interval of 95% was used to define the statistical significance (Arhipova & Bāliņa, 1999).

Results and Discussion

Species richness. In the study sites where management activities were carried out regularly, 83 vascular plant and bryophyte species were found, in irregularly managed study sites – 86 species, but in

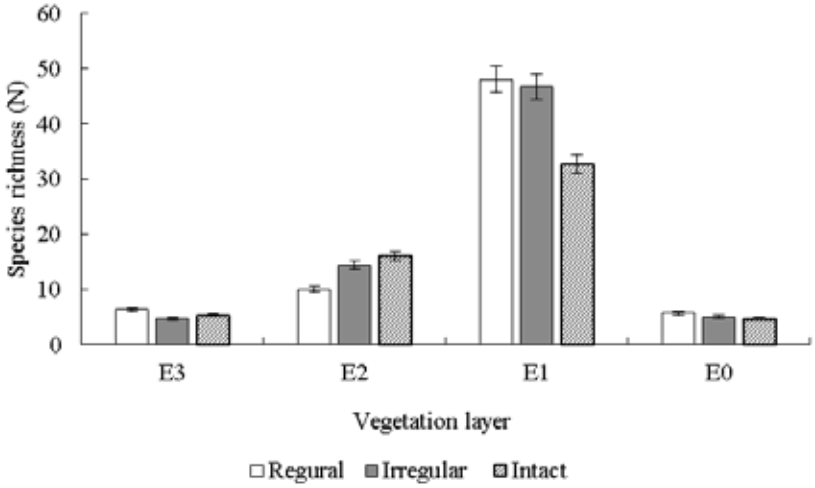

Figure 2. The comparison of species richness by vegation layers in sites with different management. Mean values ± SE are shown.

the intact study sites – 72 species. Statistical analysis shows that species richness has not been affected by the management activities. This study shows the tendency that species richness in all vegetation layers, except the shrub layer, increases when the management of understory has been done (Fig. 2). This explains the fact that management activities promote the species diversity in herb and moss layers. But when the number of shrub species increases, the species richness in the herb layer decreases.

Species occurrence. In Mežaparks the most common vascular plant species are *Pinus sylvestris*, *Acer platanoides* L., *Sorbus aucuparia* L., *Cotoneaster lucidus* Schltdl., *Fragaria vesca* L., *Veronica chamaedrys* L., *Luzula pilosa* (L.) Willd., *Vaccinium vitis-idaea* L., *Lerchenfeldia flexuosa* (L.) Schur, *Polygonatum odoratum* (Mill.) Druce and bryophytes – *Pleurozium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) Schimp. These species partly correspond to the vegetation characteristic of *Myrtillosa* forest type (Bušs, 1981; Liepa *et al*., 2014). In the studied sites where undergrowth management activities have been done regularly, the development of synantrophication and occurrence of invasive vascular plant species was observed, for instance, vascular plants – *Sambucus nigra* L., *Sambucus racemosa* L., *Symphoricarpos albus* L., *Amelanchier spicata* (Lam.) K. Koch., *Impatiens parviflora* DC., grasses – *Dactylis glomerata* L., *Agrostis tenuis* Sibth., also weeds and meadow species – *Stellaria media* (L.) Vill., *Galium verum* L., *Knautia arvensis* (L.) Coult. Also the species that correspond to forest types on rich soils were also found in these sites – *Mycelis muralis* (L.) Dum., *Geum rivale* L., *Geum urbanum* L., *Glechoma hederacea* (L.) (Liepa *et al*., 2014; Straupe, Indriksons, & Kazāka, 2014). Also the indicator species of trampling were found (for instance, *Plantago major* L.), too. It has been observed that after the management activities changes occur in the vegetation succession and soil conditions, increasing fertility, light and moisture availability (Laiviņš & Laiviņa, 1991). Due to the increase in the level of synantropisation in these habitats, the regeneration probability of *Pinus sylvestris* L. could decrease. In the study sites where irregular management of understory was found, the appearance of graminification was observed, for instance, the higher occurrence of *Dactylis glomerata* L., *Agrostis tenuis* Sibth. as well as a significant increase of typical forest species *Lerchenfeldia flexuosa* (L.) Schur (Laiviņš, 1998). This successional process could affect the natural regeneration of coniferous species. The dominance of broad-leaved species in the shrub layer (*Quercus robur* L., *Tilia cordata* Mill.) could replace the currently existing stands which are dominated by Scots pine (Straupe *et al*., 2012). In the intact study sites the most common species characterizes the stability in composition of vegetation system and slower succession process.

Projective coverage. The average projective coverage in the studied sites with regular forest management activities differs from 41% in the tree layer to 1% in the shrub layer, including 6% in the moss layer and 25% in the herb layer (Fig. 3). In the studied sites where irregular forest management activities have been practiced – 36% (tree layer), 6% (shrub layer), 14% (herb layer) and 6% in moss layer, but in the intact studied sites – 36% (tree layer), 16% (shrub layer), 5% (herb layer) and 6% (moss layer). When the management activity increases, the projective coverage of shrub layer decreases from 16% to 1%, but the herb layer increases from 5% to

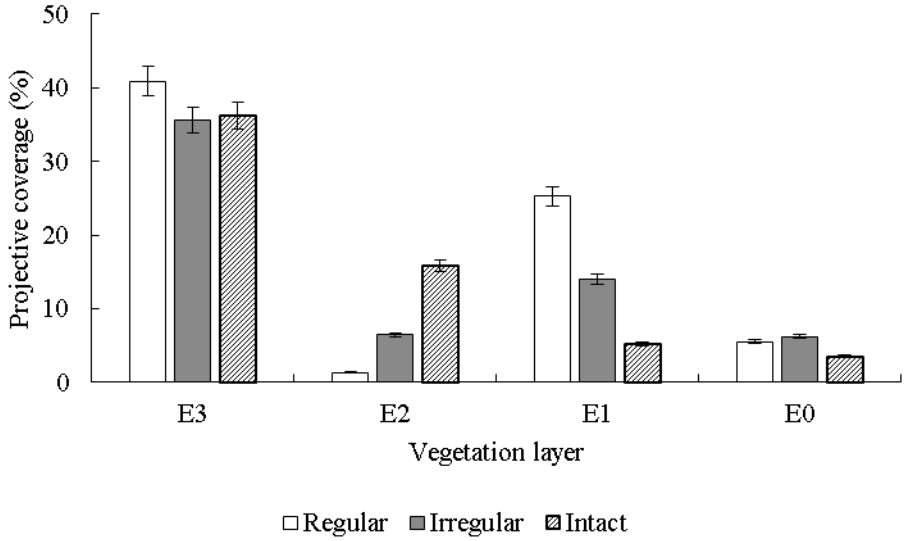

Figure 3. The comparison of projective coverage by vegation layers in sites with different management. Mean values ± SE are shown.

25%. The total vegetation projective coverage differs significantly when different forest management activity is practiced ($p < 0.05$).

The results show that the intensity of undergrowth management affects the shrub layer projective cover. After regular management activities, the projective coverage in the shrub layer decreases, but in the herb layer – increases. In the studied sites relatively high proportion in the tree layer consists of broad-leaved species, such as *Acer platanoides*, *Quercus robur* and *Tilia cordata* (Straupe *et al*., 2012). We also found several species which are indicators for rich soils and broad-leaved forests (*Acer platanoides, Quercus robur, Ulmus glabra* Huds*., Convallaria majalis* L. and moss species *Rhytidiadelphus triquetrus* (Hedw.) Warnst.). In these studied sites, the development of frutification is conducted by the indicator – *Amelanchier spicata.* In the intact studied sites, the mean cover of the shrub layer is three times higher than coverage in the herb layer. This fact is explained by shady conditions on understory from the shrub layer. In these studied sites, various tree and shrub species are dominant (for instance, *Populus tremula* L., *Acer platanoides* and S*orbus aucuparia*). The processes of frutification are observed by the dominance of species *Cotoneaster lucidus* and *Cerasus avium* (L.) Moench.

The herb layer by strategy types. It is observed that in the studied sites where understory cutting has been practiced regularly, 37% of all species are competitors (for instance, the most common species are *Acer platanoides*, *Frangula alnus* Mill., *Quercus robur*, *Sorbus aucuparia*, *Ribes spicatum* E. Robson and *Rubus idaeus* L.), but in the studied sites where irregular and intact management has been done 33% and 40% respectively. The CSR strategy type species are adaptive to different environmental conditions, for instance, meadow species *Galium album* Mill., *Rumex acetosa* L. and *Trifolium pratense* L. (41%, 43% and 48% respectively). The analysis of strategy types shows that with a decreasing the intensity of management the variablity of the strategy groups (for instance, CSR) increases and the proportion of competitor species decreases.

Ellenberg indicator values. The results show that soil pH increases when the intensity of understory management activities increases - these areas are characterized by the moderately acidic soils (pH) and are slightly rich in nitrates, but in the studied sites where irregular management activities were practiced – acidic to moderately acidic soils (pH) and poor to moderately alkine. The rich soil indicator species *Rhytidiadelphus triquetrus* (Hedw.) Warnst. have been surveyed (Liepa *et al*., 2014).

Conclusions

1. With the increase in intensity of understory management in Mežaparks, the species richness of herbs and mosses increases by 42%, but the occurrence of shrub species decreases by 45%.
2. The proportion of rare species increases in the stands where regular understory management is practiced and it decreases in sites with an absence of understory management, where the proportion of widespread species increases. This indicates the disruption of relative ecosystem's stability due to management which is considered to be anthropogenic disturbance, after the succession process of vegetation activates and the arrival of new species is observed especially in the herb layer.

3. In all studied sites, the development of synantrophication was found, especially where frutification was observed by the following species – *Sambucus nigra* L., *Sambucus racemosa* L., *Symphoricarpos albus* L., *Amelanchier spicata* (Lam.) K. Koch. In the studied sites where understory management was practiced also graminification and presence of ruderal process were also found (grasses – *Dactylis glomerata* L. and *Agrostis tenuis* Sibth., weeds – *Stellaria media* (L.) Vill. and *Impatiens parviflora* DC.).
4. In all studied sites the regeneration of most common species *Pinus sylvestris* L. is disturbed as the herb layer consists of excessive projective coverage where understory management was practiced. By contrast, the stands where management is not carried out, a large projective cover consists of a shrub layer, which dominates and creates shady conditions.
5. To continue the further management in these stands, it is essential to provide the presence of characteristic species. We suggest to continue the management activities in the understory, for instance, remove the schrub layer and allow the regrowth of the potential tree layer species.

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THE ASSESSMENT OF VEGETATION IN UNMANAGED NEMORAL FORESTS IN ZEMGALE

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Abstract
Zemgale geobotanical region historically was dominated by large broad-leaved forests, but last centuries the high anthropogenic pressure on these territories has reduced the area of these forests. In fragmented landscape small patches of natural broad-leaf forests are protected to preserve the forest structural features, which also function as habitats for rare and protected species. The aim of this study was to compare the natural broad-leaf forest habitats and structural elements and vegetation in woodland key habitats in production forests and protected areas (nature park 'Tērvete' and nature reserve 'Ukru gārša') in Zemgale. In total, 12 sample plots were established (the size of sample plot 0.1 ha) in Aegopodiosa forest type. In all sample plots forest structural features were measured and vegetation survey using Braun-Blanquet method was made. In this study, we found that average amount of dead wood varies between 78.7 m^3 ha^{-1} in woodland key habitats in production forests and 133.0 m^3 ha^{-1} special protected areas. Considerable amount of broad-leaf natural forests represented by nemoral species (50 – 58% of species richness), which corresponds to these forest type communities in the region.
Key words: Woodland key habitats, deciduous forest stands, forest vegetation, forest structural elements.

Introduction

Latvia is located in the hemiboreal zone – the contact zone of boreal coniferous forest and temperate broad-leaf forests (Krampis, 2011). Part of Latvian forests belong to the broad-leaved forest vegetation classes, where the dominant species is *Fraxinus excelsior*, *Quercus robur* and *Tilia cordata*, with other deciduous trees in admixture (Priedītis, 1999), sometimes also Scots pine (Ikauniece, 2013). The most important features for biodiversity are uneven age stand structure with large dimension trees, dead wood in various stages of decay, understorey dominated by decidous trees and shrubs, and the small proportion of Norway spruce (Priedītis, 1999). The gap dynamics is a common type of natural disturbance (Mežaka, 2009) where in open patches the development of first pioneer phase and broad-leaved tree species occurs (Ikauniece, 2013). Zemgale geobotanical region historically was dominated by large areas of broad-leaved forests, but in last centuries human activities affected the area and reduced the area of these forests (Zunde, 1999). Zemgale is one of the oldest and most deforested Latvian regions where forests are currently fragmented and occupy about 10-12% from region's area (Tabaka, 2001). The ancient broad-leaved forests where replaced by deciduous pioneer species, for instance, *Betula* spp., *Populus tremula*, *Alnus incana* and *A.glutinosa* (Suško, 1997; Ek *et al*., 2002). Consequently, the dominant tree species changed the structure of understorey (Laiviņš *et al.*, 2008; Laiviņš *et al.*, 2014). Woodland key habitats (WKH) are characterized by habitat specialist species, indicator species and stand structural features (Ek *et al.*, 2002; Lārmanis, Priedītis, & Rudzīte, 2002). These relatively small forest areas with high ecological value are considered to be cost-effective biodiversity conservation tool in fragmented production forests (Timonen, 2011). The high valuable broad-leaved forests is protected habitat type in EU level (code: 9020*) (Auniņš, 2013).

The aim of this study was to compare forest stand structural elements and vegetation in broad-leaved WKH in production forests and formally protected areas in Zemgale.

The hypothesis of this study is that structural elements and vegetation in broad-leaved WKH is significantly different in production forests and formally protected areas.

This aim requires following study objectives:

1. to analyze structural elements (living trees and dead wood – snags, stems and downed log pieces) of broad-leaved WKHs;
2. to assess the vegetation of broad-leaved WKH (tree, shrub, herbaceous and moss layers);
3. to compare broad-leaved WKH in production forests and formally protected areas.

Materials and Methods

Study area. The study was conducted in Southern part of Zemgale geobotanical region. The data were collected in 2012 – 2014. Altogether 12 study sites were chosen for analysis. The sites are in Aegopodiosa forest type – four broad-leaf WKH in production forests, four – the natural park 'Tērvete' and four – the nature reserve 'Ukru gārša' (Figure 1).

The properties of each site were measured on a sample plot size 20 × 50 m (size: 0.1 ha) with exposition S-N or SW-NE direction from stand edge to interior (Liepa & Straupe, 2012). Each plot was divided into five sublots (zones) with respective distance from edge to core: 0-10 m (1st), 10-20 m (2nd), 20-30 m (3rd), 30-40 m (4th) and 40-50 m (5th). The

Figure 1. Location of the studied area in Southern Latvia, Zemgale geobotanical region.

stand structural features were measured in all sample plots (20 × 50 m), but vegetation survey was done in the 1st, 3rd and 5th zone, which respresents the plant composition in all vegetation layers.

Stand structural elements. All live trees and standing dead wood was measured at breast height (DBH, 1.3 m) also downed dead logs and pieces (diameter ≥ 10 cm) were measured. For each element, the tree species were recorded. Live tree trunk volumes were computed using a species specific volume functions (Liepa, 1996). The volumes of individual snags, stems and logs were computed using formulas by Liepa (1996). The decay stages were characterized for all dead wood according to Hunter (Neville & Bastrup-Birk, 2006) where decay stage was determined using five classes: 1) dying recently before sampling, a knife penetrates less than 1 cm into bark; 2) fairly hard wood, a knife penetrates 1-3 cm into the wood; 3) soft wood, a knife penetrates over 3 cm into the wood; 4) wood soft throughout, a knife penetrates all the way; 5) wood almost decomposed and a hand penetrates throughout.

Vegetation survey. The Braun-Blanquet approach has been used to survey and describe plant communities: the total projective coverage of moss, herb, shrub and tree layers as well as coverage of each separate species was evaluated in each zone as percentage (%): tree layer (E3) (tree species from height 7.0 m), shrub layer (E2), (shrub and tree species at height from 0.5 to 7.0 m), herb layer (E1) (including vascular plants, dwarf shrub, schrub and tree species up to height 0.5 m) and moss layer (E0) (Liepa & Straupe, 2012). The nomenclature for vascular plants follows Garvrilova & Šulcs (1999) and that for mossess Āboliņa, Piterāns & Bambe (2015). For each separate species in herbaceous layer the constancy classes were described (Markov, 1965), which correspond to the index I (< 21%), II (21% – 40%), III (41% – 60%), IV (61% – 80%), V (81% – 100%) (Mueller-Dombois & Ellenberg, 1974). The plant ecological groups were described for abiotic conditions according to Ellenberg (Ellenberg *et al*., 1992), seed and spore dispersal types and life forms according to Raunkiaer (База данных Флора сосудистых растений Центральной России).

Data processing. In this study, descriptive methods were used for data of vegetation (mean, standard error (SE) with confidence interval 95%). A statistical distribution was assessed graphically. According to the results (symetrical distribution of data), parametrical methods were chosen. ANOVA test was used to estimate the significant variations between gradation classes and Tukey's HSD test. A risk level of 5% ($p < 0.05$) was used to define statistical significance (Arhipova & Bāliņa, 2006).

Results and Discussion

Stand structural elements. WKH structural elements are structures in forest, for example, living trees with various dimensions and dead wood, important for habitat specialist species (Ek *et al.*, 2002; Timonen *et al.*, 2010). We found significant differences ($p < 0.05$) in the volume of living trees: for broad-leaved WKHs in managed forests volume was on average 310.2 m^3 ha^{-1}, and the largest portion of it was *Populus tremula* L. (55%) – a pioneer species which colonises former broad-leaved stands with natural succession. In formally protected areas – the nature park 'Tērvete' and nature reserve 'Ukru gārša' – the volume of living trees was 321.4 m^3 ha^{-1} and 195.4 m^3 ha^{-1} respectively (made up by mostly *Fraxinus excelsior* L. – 48% and 58%)

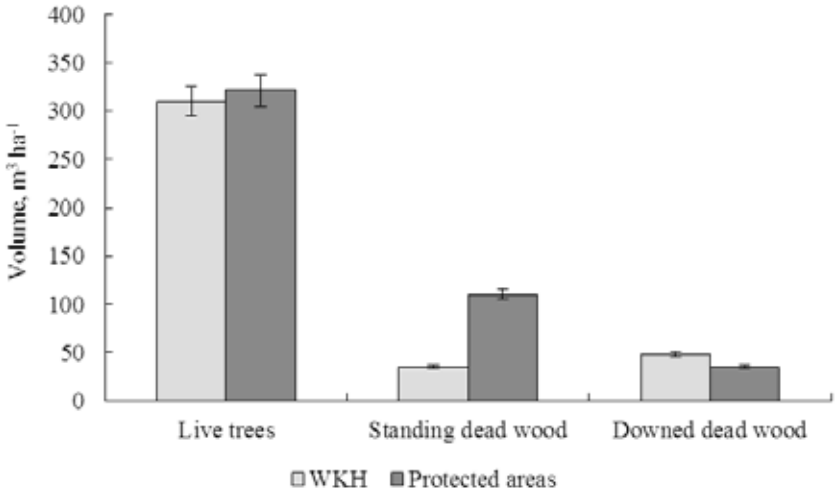

Figure 2. Diversity of forest stand structural elements. Values are means ± SE.

(Figure 2). In Eastern Europe and Latvia, stable are forest stands in which shade-tolerant tree species are dominant, for example, *Fraxinus excelsior* (Laiviņš, 2014). Average volume of living trees is significantly lower in the nature reserve 'Ukru gārša', which is explained by increased dieback of *Fraxinus excelsior* due to pathogenic fungus *Chalara fraxinea* (Kenigsvalde *et al.*, 2010; Pautasso *et al.*, 2013).

In sample plots, we found dead wood types - stumps, snags and pieces, and their volume in broad-leaved WKH differs significantly ($p < 0.05$) – in production forests dead wood volume reaches on average 78.7 m^3 ha^{-1}, but in formally protected areas volume is larger – on average 133 m^3 ha^{-1}. This is explained by natural dynamics in these forests – gap dynamics as well as with rapid dieback of *Fraxinus excelsior* trees. In production, forests dead wood volume is influenced also by the distance to populated places and the need for firewood by local inhabitants. Compared to the amount found in the Second cycle of Latvian National Forest inventory (23.5 m^3 ha^{-1}), dead wood volume in WKHs in production forests is almost three times larger, and in protected areas – almost six times larger (Meža nozares attīstības novērtējums, 1990 – 2013.). The average volume of stumps, snags and downed logs and pieces is significantly different ($p < 0.05$) between broad-leaved WKHs in production forests and protected areas. In the nature reserve 'Ukru gārša', the volume of stumps is larger (30.5 m^3 ha^{-1}), which is explained by recent dieback of ash trees. In the future stumps will form snags and pieces, thus securing the continuity of dead wood (Ek *et al.*, 2002). However, the largest volume of stumps (27.8 m^3 ha^{-1}) was found in WKHs in production forests. This is mostly due to the large proportion of *Populus tremula* in stands which have reached their biological age started to die or were damaged in windbreaks. Currently in Latvia standing dead wood volume is on average 9.1 m^3 ha^{-1} (Meža nozares attīstības novērtējums, 1990 – 2013.). In all study plots the largest portion of dead wood volume was made up by downed logs and pieces – on average 69% or 81.9 m^3 ha^{-1} (in average 14.4 m^3 ha^{-1}) (Meža nozares attīstības novērtējums, 1990 – 2013.). The volume of dead wood pieces in broad-leaved WKHs in production forests was 41.9 m^3 ha^{-1}, but in protected areas – 101.9 m^3 ha^{-1}. Dead wood pieces on forest floor decompose relatively faster, and they in the short term are inhabited by larger number of organisms – insects, mosses, lichens, fungi (Bobiec *et al.*, 2005). In broad-leaved WKHs in production forests the majority of dead wood volume is 18-26 cm in diameter which characterizes semi-natural forests (Bobiec *et al.*, 2005), but in protected forest it differs: in the nature reserve 'Ukru gārša' it is 20 – 32 cm but in nature park 'Tērvete' - 64 cm. In dry forest stands, dead wood is relevant for biodiversity with a diameter of at least 25 cm (Auniņš, 2013). Also, a study on moss species diversity managed forests shows that a larger diameter of dead wood correlates with a higher number of dead wood requiring species (Madžule, Brūmelis, & Tjarve, 2012). In broad-leaved WKHs in production forests strongly decayed dead wood (V stage of decay) was not found – this shows that the wood decay in these forests has started more recently (most common was dead wood at III stage of decay). In formally protected areas, dead wood was found at all five stages of decomposition that secures the continuity of dead wood and habitat for multiple organism groups (Blaser *et al.*, 2013). Dead wood volume is being used as an indicator of biodiversity qualities; however, diversity of dead wood and occurrence at different stages of decay is also important (Lassauce *et al.*, 2011). Furthermore, such forests are indicators of

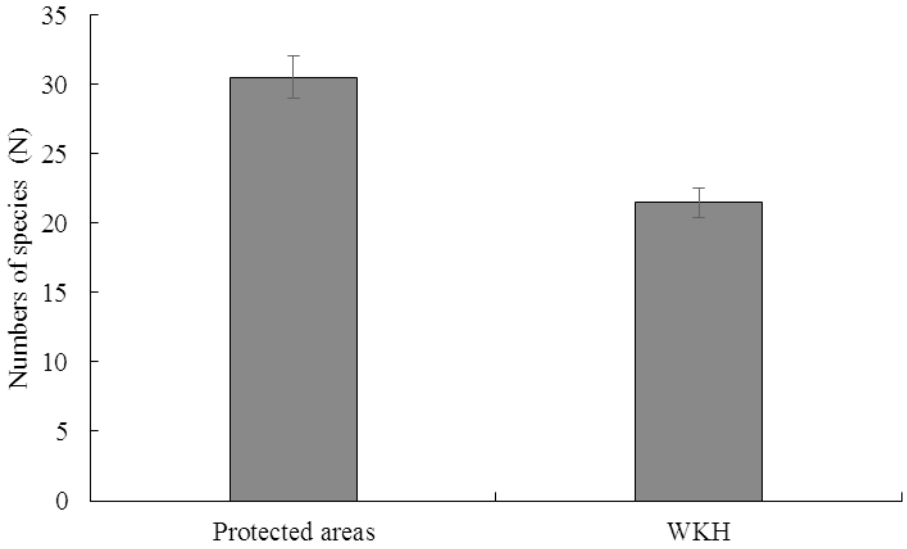

Figure 3. Numbers of species in protected areas and woodland key habitats (WKH). Values are means ± SE.

continuity (Bobiec *et al.*, 2005; Stokland *et al.*, 2012). Compared between WKHs in production forests and protected areas, a higher diversity of structural elements, environmental conditions and ecological niches is found in forests untouched by human activities (Brūmelis & Jankovska, 2013).

Vegetation survey. In broad-leaved WKHs in production forests in total 50 vascular plant and moss species were found (seven tree, nine shrub, 24 vascular and nine moss species) – on average 30 species per study site. Broad-leaved WKHs in the nature park 'Tērvete' contained 42 species (seven tree, six shrub, 24 vascular and five moss species), bet the nature reserve 'Ukru gārša' – 56 plant species (seven tree, seven shrub, 33 vascular and nine moss species) – on average 33 species (Figure 3). Numbers of species by vegetation layers and projective coverage in WKHs in production and formally protected areas was not significantly different.

In all study plots, nemoral biome species (50 – 58% of the total number of species) were most common. In broad-leaved WKHs in production forests, four European broad-leaved forest habitat specialist species were found, but in WKHs in protected areas – six habitat specialist species; thus, we conclude that studied forests correspond to European broad-leaved forests. Hemiboreal zone in Latvia is characterized by mosaic-type patterns determined mostly by soil richness (Laiviņš, 2014). In the study plots, *Fraxinus excelsior* L. (at tree, shrub and herbaceous layers), *Padus avium* Mill., *Corylus avellana* L., *Sorbus aucuparia* L. (shrub layer), *Rubus caesius* L., *Hepatica nobilis* Mill., *Oxalis acetosella* L. and *Paris quadrifolia* L. (herbaceous layer) were most common species. In broad-leaved WKHs in production forests the largest projective coverage was made up by a shrub layer (45%) (*Padus avium*, *Lonicera xylosetum* L.), but in protected areas – a herbaceous layer with *Aegopodiosa* forest type vascular species – *Aegopodium podagraria* L., *Anemone nemorosa* L., *Hepatica nobilis* (45% and 40% respectively). Species number and projective coverage in moss layer is small in all study plots characteristic to this forest type. Here, the most commonspecies was *Plagiomnium affine* T.J. Kop. – characteristic to broad-leaved forests on rich mineral soils (Liepa *et al.*, 2014). In all study sites, regeneration with *Fraxinus excelsior* was found, but in production forests it is hindered by a dense shrub layer. Plant communities of nemoral broad-leaved forests occur on rich soils, but if modifications of coniferous or broad-leaved plant communities are found, it demonstrates soil moisture and richness variability, anthropogenic actions or other environmental factors (Laiviņš, 2014). In all broad-leaved WKHs, perennial vascular plants or hemicryptophytes dominate (39%), ornitochorous are most common ones (39%), to a lesser degree – (20%) myrmecochores – mostly vernal plants which disperse with the help of ants as well as anemochorus plants (16%), (Bumbura *et al.*, 1967). Ecological variables for herbaceous layer in broad-leaved WKHs do not differ significantly ($p > 0.05$). In production, the forests' herbaceous layer is characterized by half-shade conditions moderately warm and moist neutral soils, rich in nitrogen; however, in protected areas half-shade to half-light conditions, moderately moist to moist neutral soils, rich in nitrogen are common.

Broad-leaved WKHs in production and formally protected forests differ, but production forests WKHs also contain the necessary diversity of structural elements and vegetation features.

Conclusions

1. In general, in broad-leaved WKHs in production forests and formally protected areas the volume of living trees is not significantly different ($p > 0.05$), dominated by respectively *Populus tremula* L. and *Fraxinus excelsior* L. The difference is significant in the nature reserve 'Ukru gārša' where rapid *Fraxinus excelsior* dieback was found.
2. Broad-leaved WKHs are characterized by all types of dead wood – standing dead wood and downed log pieces. Their volume differs significantly: in total, in WKHs in production forests, it is almost three times larger, but in protected areas – almost six times larger compared to the results of Second cycle of Latvian National Forest Inventory – 23.5 m^3 ha^{-1}.
3. In broad-leaved WKHs, the average volume of stumps is significantly different that is explained by a rapid dieback of *Fraxinus excelsior* in protected areas forests.
4. In all broad-leaved WKHs pieces are the dominant type of dead wood (on average 69% or 81.9 m^3 ha^{-1}).
5. Broad-leaved WKHs in production forests are dominated by dead wood with a diameter 18-26 cm in four decay classes, but in protected areas forests – 32 cm and 64 cm diameter in five decay classes which is a sign of forest continuity.
6. In broad-leaved WKHs in production forests, vegetation is formed by on average 24 species, but in formally protected areas by 33 species on average. Species richness by vegetation layers and projective coverage does not differ significantly. Since the species of nemoral biome dominates (50 – 58%), study plots correspond to European broad-leaved forests.
7. All broad-leaved WKHs were dominated by perennial vascular plants or hemicryptophytes (39%) and ornitochorous plants were common (39%).
8. Ecological variables for herbaceous layer in broad-leaved WKHs were not significantly different. In production forests, herbaceous layer is characterized by half-shade conditions moderately warm and moist neutral soils, rich in nitrogen; however, in protected areas half-shade to half-light conditions, moderately moist to moist neutral soils, rich in nitrogen are common.

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CHANGES IN FOREST FLOOR AND TREE VEGETATION IN THE LEVEL II MONITORING PLOT IN VALGUNDE PARISH

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Abstract
The objective of the research was to characterize the qualitative and quantitative changes in ground and tree vegetation within 10 years since the beginning of observations at Level II forest monitoring plot in Valgunde. Tree height, diameter, stem volume, radial increment, crown condition and cone yield were determined, as well as crown projective cover of each plant species was estimated. To study bioindication, the average Ellenberg's and Düll's indicator values for the monitoring plot were estimated. Species composition in the *Level* II *monitoring* plot in *Valgunde* is typical for *Myrtillosa* forest type – there is a distinct moss layer, in which *Hylocomium splendens* and *Pleurozium schreberi* predominate, and *Vaccinium myrtillus* is the most common in the herb layer. Shrub layer mainly consists of spruce seedlings, and the tree layer – of Scots pine. Changes in vegetation over 10 years are insignificant. It can be explained with no forest management activities occurring within the observation plot, such as logging or fertilization, and it may take a longer time to observe significant changes. Tree stand indicators correspond to the average in Latvia in *Myrtillosa* type forests, but the tree volume is higher in Valgunde monitoring plot.
Key words: *Myrtillosa,* Level II monitoring, vegetation, Ellenberg's indicator.

Introduction

Forests in Latvia cover 3.2 million hectares of land or 52% of the country's territory and standing wood volume is 631 million m^3 (Zemkopības Ministrija, 2017). The growth of trees and crown condition are key ecological parameters of forests, and thus highly important indicators of forest condition. Ground vegetation is another important component of forest ecosystems. A long-term study of vegetation changes helps to provide information on the status of forest organisms and environmental variables. Forests are greatly dependent on climate and numerous studies show that any change in abiotic factors can lead to considerable changes in biodiversity (Nordin *et al.*, 2005; Lindner *et al.*, 2010; Bell *et al.*, 2016). Growth and composition of vegetation are affected by many climatic factors – *precipitation*, *light*, *temperature*, *relative humidity* and wind. The possible negative effects on forests caused by climate change are spread of diseases and disappearance of native species, loss of needles or leaves, deforestation and soil degradation as a result of changes in pH, organic content and bacteria content. The overstorey trees have an impact on the ground vegetation through shading and regulation of the nutrient levels and moisture (Kuusipalo, 1985; Barbier *et al.*, 2008).

During the last century the average annual temperature in Latvia has risen by 0.8 – 1.4 °C, the amount of precipitation has increased, but periods of snow and ice cover are shorter. There is a slight increase in forest fire danger and net primary production, as well as considerable increase in frost events after budburst and spruce bark beetle attacks (Bergh *et al.*, 2010; Camia, Amatulli, & San-Miguel-Ayanz, 2008; EEA, 2005). The effect of winter-spring temperature on tree-ring width of European beech (*Fagus sylvatica*) and European larch (*Larix decidua)* has lost significance, suggesting a successful acclimation of stands growing northwards from their natural distribution in western Latvia (Jansons *et al*., 2015a). Studies of tree-ring width and mean area of early-wood vessel lumen of red oak (*Quercus rubra*) suggest that climate warming would increase vessel lumen area and result in a risk of embolism and xylem dysfunction (Matisons *et al.*, 2015). An increase in temperature burdens formation of tree-rings of hybrid poplars (*Populus balsamifera* × *P. laurifolia*) and hybrid aspens (*Populus tremuloides* × *P. tremula)* (Šēnhofa *et al.*, 2016). The influence of climatic factors on Scots pine height increment has changed. In western Latvia, the effect of temperature in preceding September and November has become significant, whereas in east the effect of temperature in October has become the main factor affecting height increment (Jansons *et al.*, 2015b). Due to climate change, the influence of precipitation amount on radial growth of Scots pine can increase, but the influence of mean air temperatures in winter will decrease, and Scots pine may become precipitation-amount limited in Latvia (Elferts & Jansons, 2012).

ICP forests (the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests), in cooperation with the European Commission, monitors the forest condition in Europe using two different monitoring intensity levels. The Level I monitoring is based on around 6000 observation plots on a systematic transnational grid of 16 x 16 km throughout Europe (Bārdule *et al.*, 2009). An overall estimation of tree health condition is done there. The Level II monitoring in Europe was started in 1994 with an aim of carrying out an investigation on the effect of atmospheric deposition

and other stress factors on forest ecosystem (Bārdule *et al*., 2010). The Level II intensive monitoring comprises around 500 plots in selected forest ecosystems across Europe. In-depth investigation of forest ecosystem is carried out in order to get a more complete picture of influence of air pollution and other stress factors on forest ecosystems. Currently, the program has set the objective to contribute to such important environmental issues as climate change and biodiversity as well. Its data is required for a range of institutions and conventions (MCPFE, CLRTAP etc.).

National forest monitoring in Latvia is a monitoring, analysis and forecasting system of forest resources and environmental status, where scientific methods are used. It includes monitoring of forest resources, Level I and Level II air pollution influence assessment, as well as scientific monitoring of forest pests and diseases. Information on the first level assessment of air pollution influence monitoring is obtained by carrying out annual observations in 115 observation plots on a systematic transnational grid, as well as in observation plots in grid of 16 x 16 km (Lībiete *et al.*, 2010).

The objective of the research is to characterize qualitative and quantitative changes in the forest ground and tree vegetation within 10 years since the beginning of observations in Valgunde plot. The general hypothesis is that changes in the forest ground vegetation are insignificant, whereas tree growth (increment) corresponds with the average indicators in similar growth conditions.

Materials and Methods

The Level II observation plot in Valgunde is rectangular (40 x 60 m) with a total area of 2400 m^2. The plot is divided into sections of 100 m in order to characterize the ground vegetation. In order to observe changes in species cover, vegetation was assessed in 2 ways: (1) within an area of 400 m^2 by dividing each rectangular plot (20 x 5 m) in four smaller squares 5 x 5 m (16 squares in total), considered as base units for vegetation assessment; (2) within an area of 26 m^2, placing six 1 x 1 m squares in each rectangular observation plot and taking them as basic units. Two additional squares are placed between rectangular plots. Vegetation is made up of 4 layers: trees, shrubs, herbs and mosses. Trees with height less than 0.8 m are counted as herbs. In this study, vegetation was inventoried over the period 2004 – 2012.

In surrounding areas, a 10 m wide unrestricted buffer zone has been established where samples are taken for several monitoring subprograms, such as precipitation, tree increment etc. Next to the tree stand observation plot, a 40 x 30 m large soil observation plot has been established. According to national soil classification, soil in terrain elevations of Valgunde plot is a typical podzolic sandy soil, but in declines – peaty podzolic gley soil. According to the FAO classification system, the soil type corresponds to Haplic Arenosols. It is coarse-textured, weakly developed soil.

Ellenberg's indicator values (EIVs) are commonly applied in Europe as bioindicators of primary environmental traits: light, temperature, continentality, moisture, soil pH and fertility (Szymura *et al.*, 2014). Ellenberg's indicator values range from 1 to 9 (Table 1). Ellenberg's indicator was initially developed for herb species in the United Kingdom, but it can be extended to other regions.

The value of a specific environmental factor at a specific site is estimated by using arithmetic means of the indicator values for this factor of all species (Ter Braak & Barendregt, 1986). EIVs are calculated by weighted average formula (Sürmen *et al.*, 2014):

$$\text{weighted average} = \frac{\sum_{i=1}^{n}(r_{ij} * x_1)}{\sum_{i=1}^{n} r_{ij}} \quad (1)$$

where r_{ij} is the response of species i in sample plot j, x_i is the indicator value newline of species i.

EIVs used in this study are approximated for Russian Federation (Czerepanov, 1995; Ignatov & Ignatova, 2003). Similarly as Ellenberg's indicator is used to calculate environmental factors of herbs, Düll's indicator is used for bryophytes. In this study, the mean EIVs and Düll's indicator values were calculated over years 2004, 2009 and 2012.

Table 1

Ellenberg's indicator values for main environmental factors (Nuamah, 2017)

Environmental Factor	Symbol	Indicator value
Light	L	1 = deep shade, 5 = semi-shade, 9 = full light
Temperature	T	1 = alpine-subnival, 5 = submontane-temperate, 9 = Mediterranean
Continentality	K	1 = euoceanic, 5 = intermediate, 9 = eucontinental
Moisture	F	1 = strong soil dryness, 5 = moist, 9 = wet, 10 = aquatic, 12 = underwater
Soil reaction (pH)	R	1 = extremely acidic, 5 = mildly acidic, 9 = alkaline

Tree growth measurements were carried out according to internationally accepted methodology (ICP Forests Manual on Estimation of Growth and Yield). Tree diameter was measured at 1.3 m height. Tree growth measurements (height, diameter and crown projection) were done for 60 selected trees. Tree height measuring sites were marked with stakes at a 20 m distance from the particular tree. Tree crown projections were estimated in four directions – N, E, S, W.

In order to measure tree radial increment, 15 manually readable tree increment bands were set up. Bands were placed randomly throughout the observation plot, including trees of Kraft's classes 1, 2 and 3. Measurements of tree increment bands were read systematically once in every 2 weeks.

To estimate tree canopy status 60 trees of Kraft's classes 1, 2 and 3 were randomly selected by dividing observation plot into 24 squares and by selection of 2 to 3 trees of each Kraft's class in every square. Defoliation in the first third or the whole canopy, dechromation, tree damages and cone yield of the observation plot trees were also measured and Kraft's class of the tree was determined. Defoliation is a loss of needles/leaves within the assessed canopy and it is the main indicator of tree health status. It is determined irrespective of the cause. Defoliation is evaluated in 5% classes. These classes are: 0 (no defoliation); 5 (defoliation > 0 – 5%), 10 (defoliation > 5 – 10%) etc. Cone yield is divided in 3 classes (1 – no yield, 2 – medium yield, 3 – good yield). Splitting of class 1 in subclasses (1.1 – cones are not observed, 1.2 – only a few cones have been observed) was started from 2014.

Results and Discussion

Vegetation in the observation plot is typical for mesotrophic pine forests – it has a distinct moss content and a herb layer. A few Norway spruce and oak seedlings (< 50 cm) are found in shrub and herb layer, but the tree layer mainly consists of Scots pine. The average projection of shrubs is the smallest, comparing with tree and herb projection: in 2004 it was 8.26%, in 2009 decreased to 4.06%, but in 2012 it slightly increased up to 4.5%. The average herb projection in 2004 was 55%, in 2009 it was 57%, but it 2012 – 65.63%. Tree crown projection has decreased in the following years. In 2004 it was 64.06%, in 2009 – 47% and in 2012 – 45%. Oaks were found only as young seedlings with small projection area during the entire observation period. The average moss projection slightly increased in years 2009 and 2012 and was 87% and 88%, respectively.

By further analysis, in total 21 plant species were identified in 2004. Five species from those found in the monitoring plot are also common in other parts of Europe: blueberry (*Vaccinium myrtillus* L.); bog bilberry (*Vaccinium uliginosum* L.); lingonberry (*Vaccinium vitis-idaea* L.); the small cow-wheat (*Melampyrum sylvaticum* L.); Scotch heather (*Calluna vulgaris* L.). Ten moss species were found, from which *Pleurozium schreberi* (39.4%) and *Hylocomium splendens* (42.4%) were the most common. Both moss species were also predominant in the following years and their projections slightly increased. Other identified moss species were *Dicranum polysetum* Sw., *Aulacomnium palustre* (Hedw.) Schwaegr., *Ptilium crista-castrensis* (Hedw.) De Not., *Brachythecium oedipodium* (Mitt.) Jaeg, *Hypnum cupressiform*e, *Pohlia nutans* (Hedw.) Lindb., *Polytrichum juniperinum* Hedw. *Dicranum scoparium* Hedw. was very rare and did not fit into the 100% scale with one decimal in 100 m^2 observation plots. *Pohlia nutans* (Hedw.) Lindb and *Polytrichum juniperinum* Hedw. were not observed in the following years, comparing with 2004. *Hypnum cupressiforme* was not observed in 2009, but was observed again in 2012.

10 species were found in herb layer, from which *Vaccinium myrtillus* (45.6%) predominated. Other species were *Calluna vulgaris* (L.) Hull., *Festuca ovina* L., *Goodyera repens* (L.) R. Br., *Melampyrum pratense* L., *Picea abies* (L.) H. Karst., *Quercus robur* L., *Scorzonera humilis* L., *Vaccinium myrtillus* L., *Vaccinium uliginosum* L. and *Vaccinium vitis – idaea* L. Tree species found in the herb layer were *Quercus robur* L. and *Picea abies* (L.) H. Karst. The most common herb species in the following years was the same as in 2004 – blueberry *Vaccinium myrtillus* L. *Festuca ovina* L. was not observed in the herb layer in the following years. In 2009, three new species were observed – *Dryopteris carthusiana* (Vill.) H.P. Fuchs (0.01%), *Luzula pilosa* (L.) Willd. (0.03%) and *Melampyrum sylvaticum* L. (5.91%). In 2012, two previously unobserved species were found – *Maianthemum bifolium* and *Plantago major*. Their projection was below 0.01%.

In 2004, there were only 3 species in the shrub layer – *Picea abies* (L.) H. Karst., *Quercus robur* L. and *Vaccinium uliginosum* L., and the projection was mostly formed of Norway spruce branches. In the following years, the shrub layer consisted only of Norway spruce.

Overall changes in vegetation are negligible. Presence and absence of some species with very small projections over time can be explained by methodological shortcomings rather than actual changes in species composition. No management activities, such as logging operations or fertilization, were carried out within the monitoring plot. Studies on short term responses to logging have shown that mosses and herbs are mostly affected negatively (Hannerz & Hånell, 1997; Roberts & Zhu, 2002; Fenton *et al.*,

Table 2

Mean EIVs for Valgunde observation plot over period 2004 – 2012

Parameter	Year		
	2004	2009	2012
Light (L)	5.0	5.0	5.0
Temperature (T)	3.2	4.1	3.9
Continentality (K)	5.0	5.0	3.9
Moisture (F)	4.0	4.6	4.4
Reaction (R)	2.0	2.0	2.0
Nitrogen content (N)	2.7	3.4	2.6

2003; Palviainen *et al.*, 2005; Caners *et al.*, 2013). The number and richness of forest floor vegetation species in European forests have changed over the last years due to wet and dry deposition of atmospheric nitrogen. Forest fertilization is another factor which increases soil nitrogen content. Such species as heather and lingonberry prefer nutrient-poor soils and are sensitive to nitrogen addition. Most of herb and moss species growing within the observation plot prefer soils poor in nitrogen. A drastic decrease in projection cover was not observed and it can be concluded that nitrogen deposition in Latvia is low. The increase in herb and moss projection cover could be a result of decrease in tree and shrub projection cover, thus allowing more sunlight to understorey vegetation. Herb species typical for *Myrtillosa* forest type are *Vaccinum myrtillosa*, *Calamagrostis arundinacea*, *Vaccinium vitis-idaea*, *Maianthemum bifolium*, *Luzula pilosa*, *Solidago virgaurea* L., *Pteridium aquilinium* (L.) Kuhn. and *Orthilia secunda* (L.). From mosses, the most common are *Hylocomium splendens*, *Pleurozium schreberi*, *Dicranum polysetum*, *Dicranum scoparium* and *Ptilium crista-castrensis*. *Vaccinum myrtillosa* and grasses are dominating in a typical *Myrtillosa* forest type. Nine of the above mentioned species were found in the monitoring plot. Grasses are not common in the observation plot.

In forestry, bioindication with the mean values of EIVs has been used to assess the site productivity (Bergès *et al.*, 2006) and to predict herb biomass production (Axmanová *et al.*, 2012), water relationships in soils (Häring *et al.*, 2013) and atmospheric deposition (Dobben *et al.*, 1999). Some studies suggest that bioindication can perform better than instrumental measurements and predicts plant species occurrence more accurately (Dupré & Diekmann, 1998; Jarvis *et al.*, 2016). Plants used for bioindication are supposed to illustrate relevant environmental factors for them over time, while measurements provide snapshots and depend on choices of the researcher – sampling, analytical methods and choice of measured factors.

According to Ellenberg's scale indicator values obtained from our study (Table 2), herb layer vegetation grows in semi shade, temperature is between alpine and temperate, continentality value is intermediate, soil is moist and acidic, but nitrogen content is low. According to our calculated EIVs, the temperature increased in 2009 and slightly decreased in 2012; continentality value decreased by 1 in 2012. Moisture value also has slightly changed over years – in 2009 it increased by 0.6 and slightly decreased in 2012. Nitrogen content also increased in 2009 and decreased in 2012.

Our calculated mean Düll's indicator values for light, temperature, continentality and reaction only slightly differ from the mean EIVs, while the mean value for moisture is higher by 3 units (Table 3). There is no change in the mean Düll's indicator values over

Table 3

Mean Düll's indicator values for Valgunde observation plot over period 2004 – 2012

Parameter	Year		
	2004	2009	2012
Light (L)	6.0	6.0	6.0
Temperature (T)	3.0	3.0	3.0
Continentality (K)	6.0	6.0	6.0
Moisture (F)	7.0	7.0	7.0
Reaction (F)	3.0	3.0	3.0

time. Düll's indicator values for nitrogen were not available.

Stem diameters were measured at breast height for 272 trees within the monitoring plot. The average height of trees was 24 m and average diameter was 21.5 cm. The average diameter of pines is 21.5 cm and the average diameter of spruces – 10.3 cm. The total basal area of a tree stand is 37.1 m^2 ha^{-1}. The volume of growing trees is 417.3 m^3 ha^{-1}. The average tree diameter in *Myrtillosa type* forests in Latvia is 30.9 cm and the average height of trees is 24.7 m, dominant species is Scots pine and the average age of trees is 85 years. The average total basal area is 33.56 m^2 ha^{-1}. The average tree count per ha is 936. Our results are similar to those overall in Latvia. The age of the stand is 90 years. Site index of the stand in Valgunde is I.

The average tree crown defoliation in 2004 was 9.8%. Undamaged and slightly damaged trees (0 – 10%) were 23% of evaluated trees. Dechromation was not observed. In years 2009 – 2013, the average defoliation did not change significantly. It was 15 – 20% on average, but did not exceed 30%. There was a slight decrease in defoliation in 2010. As in previous years, also in 2015 severely damaged trees with defoliation more than 60% were not observed. The tree health condition in the observation plot is good with a predominance of slightly damaged trees. More than half of trees (63.3%) belong to the defoliation class 5 – 15%. Comparing with year 2014, the proportion of trees with defoliation of 30% has increased from 3.3% to 11.7% in 2015. At the same time, the amount of undamaged or slightly damaged trees has increased from 43.3% in year 2014 to 50% in year 2015. The average condition of defoliation has not changed and the condition of tree crowns has not worsened over years. There is a slight decrease in average defoliation. Data from 2004 to 2008 may not be taken into account because the evaluation of the tree crown condition was done by other experts. It has significantly affected comparability of results. Foliage dechromation was not observed until 2008, when 5% dechromation was observed for one tree. The increase in dechromation was observed in 2014.

Cone yield has been estimated each year. Overall, during the past 11 years only minor changes in cone yield have been observed. In 2004, all 60 trees belonged to class 1. The average cone yield increased from 1 in 2004 to 1.5 on average in 2005. In 2006, there was a decrease to 1 again and in 2007 it decreased even more – 0.5 on average. In 2008, it increased again to 1.3, in 2009 – to 1.6 and in 2010 and 2011 remained approximately the same. In 2012, there was a slight decrease and in 2013 – an increase again. A slight decrease in 2015 was observed again. Trees with cone yield class 3 were observed in 2005 and afterwards from 2008. Cone yield is affected by flowering intensity and synchronicity as well as fertilization of strobiles. Those factors are affected both by heredity and environment (Sarvas, 1962; Dreimanis, 1973; Laura & Bērziņa, 1978; Zviedre, 1985). Flowering intensity is determined by meteorological conditions during bud differentiation in the previous vegetation period (Zviedre, 1985), whereas female strobiles are negatively affected by frost in late spring (Neimane *et al.*, 2014), as well as strong wind and rain or continuous draught. Accessibility of water and nutrients also is an influential factor (Karlsson & Örlander, 2002).

Radial increment was determined for 272 trees over the period of 11 years (2004 – 2015). The highest value of girth (perimeter) increment was 15.6 cm over 11 years and 1.42 each year. The average radial increment in 11 years was 1.11 cm and average annual increment was 0.1 cm. Tree radial increment was affected by meteorological conditions, tree age and genetic factors (Puriņa *et al.*, 2014). There was a reduction in tree radial increment over time. It is expected that tree annual radial increment will decrease even more in the next years due to increasing tree age and characteristics of the formation of the stem radial increment. Although radial increment of a tree is smaller, it does not mean it is growing slower – the increase in stem volume can be even bigger. It was found in the study that weather conditions can significantly affect measurement results due to swelling of bark after rainfalls.

Conclusions

1. There are no significant differences between tree stand indicators in Valgunde observation plot and in *Myrtillosa* type forests in Latvia on average, therefore the observation plot can be used to characterize processes in pine stands of respective forests.
2. The tree health condition in the observation plot is good with a predominance of slightly damaged trees. There is a slight decrease in average defoliation, but the overall condition of tree crowns has not worsened over years. Only minor changes in cone yield were observed.
3. Changes in the ground vegetation are insignificant and species composition is typical for *Myrtillosa* forest type.
4. According to Ellenberg's and Düll's indicators, vegetation grows in semi shade, temperature is between alpine and temperate, continentality value is intermediate, soil is moist and acidic, but nitrogen content is low.

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SOIL CARBON STOCK CHANGES IN TRANSITIONAL MIRE DRAINED FOR FORESTRY IN LATVIA: A CASE STUDY

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Abstract
The aim of the study is to evaluate the impact of drainage on soil carbon stock in a transitional mire drained for forestry. The study site is located in the central part of Latvia representing hemiboreal vegetation zone. Site was drained in 1960. It is located in a catchment area of the river Veseta. An undrained site at the same catchment area was chosen for control (ca. 2.5 km between sites). In both sites, the depth of peat is 4 – 4.5 m. Drained site is dominated by coniferous trees. Soil samples collected in 2014 were used to determine bulk density and carbon content, and to calculate soil carbon stock. Samples were collected down to 80 cm depth. Ground surface elevation was measured before and several times after the drainage to determine peat subsidence.
Carbon stock has increased by 0.3 tons ha^{-1} yr^{1} after drainage, although peat has subsided on average by 26 cm (13 – 48 cm). Subsidence was mainly caused by physical shrinkage of peat not by organic matter oxidation. Drainage was followed by compaction of aerated soil layer, which has caused most of the subsidence, especially during the first years after drainage. Soil bulk density has increased almost twice at soil surface layer 0 – 10 cm (from 75 kg m^3 to 141 kg m^3). Differences decrease at deeper sampling depths.
It is concluded that drainage is not always followed by reduction of carbon stock in soil. Increased above and below ground litter production rates may offset accelerated decomposition of organic matter after drainage.
Key words: drainage, carbon, organic soil, forestry.

Introduction

Organic soils store large carbon stock and are important carbon pools in the global carbon cycle (Gorham, 1991; Turunen *et al*., 2002; Yu *et al*., 2010). Management of organic soils may alter carbon cycling. The main threat for carbon storage is drainage, causing increased CO_2 emissions (Minkkinen *et al*., 2002; Hooijer *et al*., 2009). Drainage helps to remove the excess water and, consequently, the upper soil layer is enriched with oxygen. It accelerates the decomposition of organic matter (Lieffers, 1988; Bridgham *et al*., 1991). Soil surface aeration and increased amount of available nutrients for plants due to decomposition (Laiho & Laine, 1994; Indriksons, 2009) contribute to better plant growth.

In order to increase forest productivity, drainage is widely used in forestry, especially in the northern hemisphere (Zoltai & Martikainen, 1996). On one hand, the benefits of drainage on forest productivity are evident; it results in a better tree growth (Minkkinen *et al*., 1999; Zalitis & Indriksons, 2009). On the other hand, there may be negative consequences to soil carbon storage (Simola, Pitkänen, & Turunen, 2012; Pitkänen, 2013; Hommeltenberg *et al*., 2014). On the contrary, some authors have found that carbon stock in forest organic soils can remain stable or even continue to increase after drainage (Minkkinen & Laine, 1998b). One of the main drivers determining, whether forest organic soil will be carbon source or sink after drainage, is climatic variables. In the boreal vegetation zone drained organic soils in forest often continue to act as a sink (Minkkinen & Laine, 1998b; Flanagan & Syed, 2011; Lohila *et al*., 2011; Ojanen, Minkkinen, & Penttilä, 2013), at the same time soil carbon stock on boreal forest peatlands may also decrease (Arnold *et al*., 2005a; Lohila *et al*., 2007; Simola *et al*., 2012). Such differences are partly driven by soil fertility and tree stand type (Minkkinen & Laine, 1998b; Arnold *et al*., 2005b, 2005a; Minkkinen *et al*., 2007; Ojanen *et al*., 2010). Towards south, in temperate regions, drained organic soil is a net source of CO_2 emissions (Cannell, Dewar, & Pyatt, 1993; Hargreaves, Milne, & Cannell, 2003; Byrne & Farrell, 2005; Hommeltenberg *et al*., 2014). In some cases, emissions from soil can be large enough to turn the whole drained forest ecosystem into CO_2 emitter, especially in the long term (Hommeltenberg *et al*., 2014). On a broad scale, carbon loss from organic soil in temperate climate conditions is larger than in the boreal region (Armentano & Menges, 1986). No evidence of soil carbon or ecosystem net sink after drainage can be found for tropical climate (Hirano *et al*., 2008; Sundari *et al*., 2012; Jauhiainen *et al*., 2014). Drainage of tropical peatlands may result in extreme CO_2-C emissions (Comeau *et al*., 2013).

The impact of climate on carbon loss is clearly displayed in the Intergovernmental Panel of Climate Change (IPCC) guidelines for greenhouse gas (GHG) reporting (Hiraishi *et al*., 2014) in wetlands supplement. It is stated by default emission factors that net soil CO_2-C emissions in drained forests increase from boreal climate to tropical climate.

There is a number of publications concerning soil carbon storage in boreal, temperate and tropical climate conditions, but not for the hemiboreal zone. Only few studies deal with hemiboreal drained

peatlands (Minkkinen *et al*., 2007; Salm *et al*., 2012). It is obvious that the carbon cycle alterations after drainage is strongly affected by climate.

The aim of this study is to analyze the impact of drainage for forestry to soil carbon stock changes in a transitional mire which is located in hemiboreal vegetation zone in Latvia.

Materials and Methods

The study site is located in the central part of Latvia (N 56.7064; E 25.8544) in a catchment area of the river Veseta. This site was initially a transitional mire and it corresponds to hemiboreal vegetation zone. It was drained in 1960, and in 1963 a forest research station was established in this area. A closely located (~2.5 km from the drained site) undrained site in the same catchment was used as a control site. Peat depth on both sites is 4 – 4.5 m. Before the drainage, the site was dominated by pine trees with an average growing stock 50 m^3 ha^{-1}. Currently, the area is covered by pine and spruce forest (Zalitis, Jansons, & Indriksons, 2012) with an average growing stock 200 – 250 m^3 ha^{-1}. The age of tree stand in drained site is 40 – 110 years. Site type is *Myrtillosa turf. mel.*, according to the Latvian forest type classification system. This is ranked as third (out of four) most fertile forest type in the class of drained forests on organic soils.

In total, 20 sample plots (500 m^2) in the drained site and 10 sample plots in the control site were established near the ground surface measurement points and ground water wells installed in 1963. Sample plots are located at different distances from the drainage ditches. Soil volumetric samples and litter layer samples were collected in 3 replicates at each sample plot in 2014. Soil samples were collected at four depths: 0 – 10 cm, 10 – 20 cm, 20 – 40 cm and 40 – 80 cm. The volume of each sample was 100 cm^3. Litter samples were collected with 10x10x10 cm steel boxes. Diameter and height of trees were measured at each sample plot. Ground surface elevation was measured before (1960) and several times after (1966, 1970, 1972, 1975, 2014) the drainage with an optical level tool. Each time the same reference and measurement points were used.

Soil and litter samples were dried at 105 °C until constant mass. Carbon content was determined with LECO CR 12 analyzer at a temperature higher than 900 °C.

Soil samples were used to determine dry bulk density (kg m^{-3}) and carbon content (g C kg^{-1}) which was further used to calculate carbon stock (tons ha^{-1}) in the soil. Peat subsidence was considered when calculating carbon stock changes. Carbon stock at the control site is equal to soil carbon stock in 0 – 80 cm soil profile. Carbon stock at the drained site is equal to carbon stock in soil profile of 0 – (80-x), where x is subsidence in cm. Carbon stock changes were calculated as a difference of carbon stock between the profiles at the drained (0 – 80-x cm) and control (0 – 80 cm) site.

Stand volume was calculated as a sum of volume of individual trees (Liepa, 1996) to describe the effect of drainage on a tree stand productivity (1):

$$M = \sum \left(\psi * h_i^{\alpha} * d_i^{\beta * lgh_i + \varphi} \right) \quad (1)$$

Where ψ, α, β, φ – tree specific stem volume coefficient
h_i – height of i tree, m
d_i – diameter of i tree, cm.

Confidence interval for normal distribution at α = 0.05 was calculated to evaluate statistical difference of average carbon stock between the drained and control site. It was assumed that carbon stock data follows the normal distribution, although the data does not fulfill all the criteria of normal distribution. The same approach was used to calculate confidence interval for bulk density and carbon content.

Results and Discussion

The depth of peat has decreased by 13 – 41 cm (on average by 26 cm) 54 years after drainage. The annual subsidence rate is around 0.5 cm yr^{-1}. These results are comparable with 22 cm published by Minkkinen and Laine (1998b) and 14 – 43 cm published by Lukkala (1949), both studies were carried out in Finland. A lower rate of subsidence (8 cm on average) was observed by Rothwell (1996) in Canada's boreal peatlands. This author emphasizes the impact of ditch spacing to subsidence rate. Peat subsidence occurs faster if the ditch spacing is smaller. Subsidence is strongly variable along the climate and fertility gradient. Leifeld (2011) reports subsidence of 0.8 – 1.6 cm yr^{-1} on temperate fens. More extreme values have been reported from tropical regions, where subsidence rate can be as high as 2 – 7 cm yr^{-1} (Wösten, Ismail, & van Wijk, 1997; Schipper & McLeod, 2002; Hooijer *et al*., 2012).

Most of the subsidence took place during the first years after drainage – 11 cm after 6 years and 16 cm after 15 years (Figure 1). A similar trend was observed also by Lukkala (1949). After the drainage, the subsidence is rapid and it ceases later.

The main reason for subsidence during the first years after the drainage is the physical peat shrinkage after ground water level dropdown but not the peat oxidation, as it is sometimes stated. This hypothesis is confirmed by an increased soil bulk density and increased total carbon stock in the soil. Carbon storage 54 years after drainage has increased

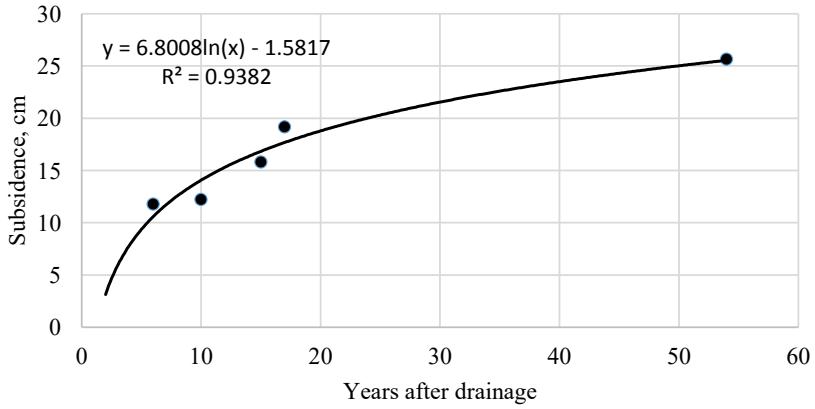

Figure 1. Peat subsidence after drainage.

by 0.3 tons ha^{-1} yr^{-1}. In total, carbon stock has increased by 15 tons ha^{-1} during 54 years. However, carbon stock changes are not statistically significant at 95% confidence level.

Soil carbon stock increase after drainage is reported also by other researchers for the boreal region in Finland (Minkkinen & Laine, 1998b; Ojanen *et al*., 2010; Lohila *et al*., 2011). Although drainage accelerates organic matter decomposition, it can be compensated by higher aboveground and belowground litter production rates followed by an increased tree biomass growth.

The growing stock at the control site ~50 m^3 ha^{-1}, but the growing stock at the drained site is at least four times higher (~220 m^3 ha^{-1}). It promotes higher litter production rates in the drained sites. Tree biomass growth and subsequent increase of litter production rate contribute to the increased carbon allocation into soil. Results show the formation of stable litter layer (~ 3 – 4 cm) on soil surface in the drained sites. At the control site, no litter layer was observed. Furthermore, a considerable quantity of carbon in the soil is allocated in the root biomass (Laiho & Finér, 1996; Bardulis, Jansons, & Liepa, 2012; Bardulis *et al*., 2015), especially through fine root production/ mortality. Fine roots may even contribute to ~ 70% from the total carbon cycle in forest ecosystem (Gower, Pongracic, & Landsberg, 1996; Bhuiyan *et al*., 2016).

The shrinkage of peat is followed by an increased soil bulk density (Figure 2). Bulk density in topsoil (0 – 10 cm) has increased almost twice from 75 kg m^{-3} to 141 kg m^{-3}. Differences of bulk density tend to decrease in deeper soil layers. However, differences are still significant at 20 – 40 cm depth. Furthermore, it seems that the impact of drainage may extend even deeper than the studied 0 – 80 cm soil profile. Although the difference of bulk density is not significant at the sampling depth 40 – 80 cm, still this difference is 23 kg m^{-3}. Similar results are reported

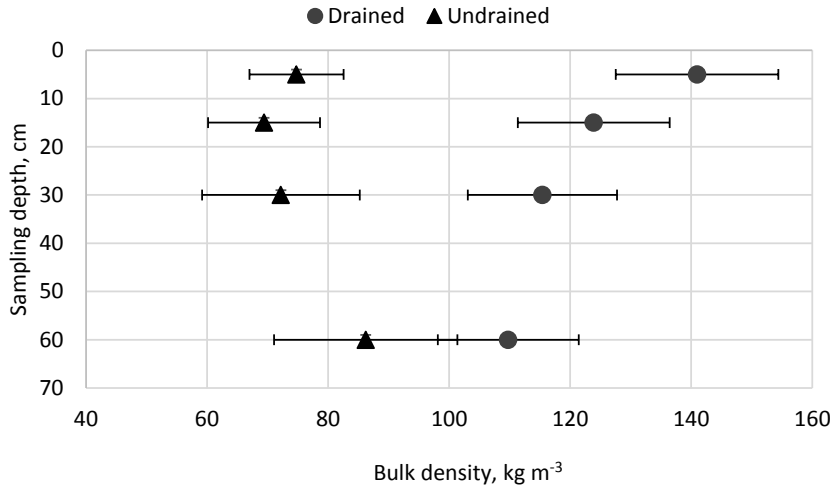

Figure 2. Soil bulk density in drained and undrained plots at different sampling depths. Results show mean ± CI for normal distribution at confidence level 95%.

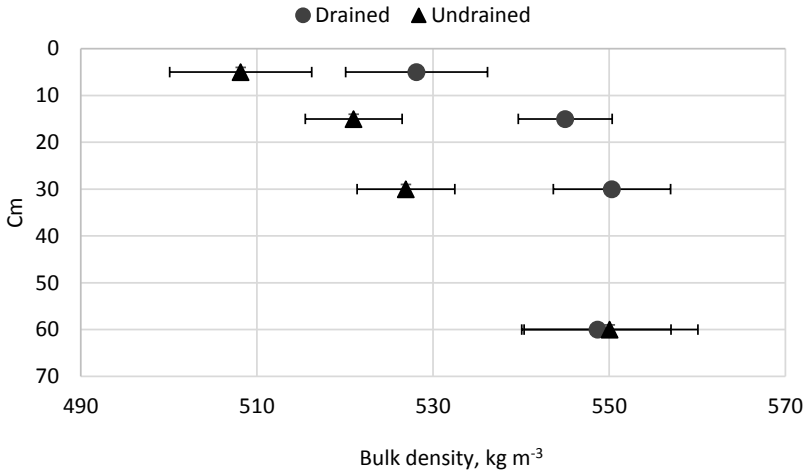

Figure 3. Carbon content in soil in drained and undrained plots at different sampling depths. Results show mean ± CI for normal distribution at confidence level 95%.

by Minkkinen and Laine (1998a), who found that soil compaction may occur also deeper than 80 cm.

It was expected that carbon content (g C kg^{-1}) in peat will decrease after drainage due to oxidation and mineralization of organic matter. The results show an opposite trend. The carbon content at drained sites is higher compared to the control site (Figure 3) and the difference is statistically significant in 0 – 40 cm soil layer. Carbon content at the drained site varies from 528 g C kg^{-1} in the 0 – 10 cm layer to 550 g C kg^{-1} in 20 – 40 cm layer. Carbon content in the control site varies from 508 g C kg^{-1} in 0 – 10 cm layer to 527 g C kg^{-1} in 20 – 40 cm layer. There are no differences at the deepest (40 – 80 cm) sampling depth.

These results may be explained by the vertical movement of easily dissolvable organic carbon compounds. Organic matter cycling on the soil surface is accelerated after drainage, and carbon from litter decomposition is penetrating from the soil surface into deeper soil layers (Charman, Aravena, & Warner, 1994; Domisch *et al*., 1998) and is subsequently stored there. Carbon content increase is reported also by Minkkinen (1999), who reports that the carbon content in peat has increased by 1.6%, while our results show even higher rates of increase - 4.1%.

It is necessary to do further research to get a better understanding about factors controlling the net balance of CO_2 in drained forest organic soils. Literature analysis shows a strong impact of climate and fertility gradient on the net CO_2 exchange. Still, there are lot of unanswered questions about controls which determine, whether soil after drainage will become source or will keep acting as a sink. More accurate information about the impact of soil temperature, moisture, drought, fertility, microbial activity etc. on CO_2 exchange would help us to model those processes and help to develop more accurate emission factors for national GHG inventory. Furthermore, it may contribute to thedevelopment of a more sustainable management of drained forests on organic soils.

Conclusions

In the hemiboreal vegetation zone, drainage of organic soils is not always causing carbon storage reduction. Carbon stock may even increase after the drainage. This is caused by the increase of above and belowground litter production rates. Subsidence followed by drainage is caused mostly by physical shrinkage of aerated soil surface not by peat oxidation.

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PRODUCTIVITY OF MECHANIZED WOOD ASH APPLICATION IN FOREST

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Abstract
Importance of the study is determined by increasing consumption of woody biomass as a renewable energy source. Whole-tree harvesting is commonly applied for bioenergy use, however intensified biomass removal from forests can cause significant nutrient loss in soil. Nutrients can be returned to forest ecosystem by fertilizing soil with wood ash, which is generated as a by-product of combustion in wood-*burning* power plants and heating plants. Studies on ash spreading technological processes and costs so far have been insufficient. Aim of the study is to compare the productivity and prime cost of ash spreading with modular spreading trailer and Amazone mineral fertilizer spreader. According to study results productivity of modular trailer is 0.57 ha h^{-1} and the spreading costs are 88 EUR ha^{-1}, but that of mineral fertilizer spreader Amazone – 0.61 ha h^{-1} and 41 EUR ha^{-1}, respectively. Despite lower productivity modular trailer is more suitable for spreading wood ash, because a larger amount can be delivered and ash is applied more evenly. Productivity of modular trailer can be increased by technical improvements, like equipping the tractor with a crane and a small excavator bucket. Comparing costs of ash spreading and deposition, additional 8.72 EUR ha^{-1} were required, when using modular trailer, whereas, when using mineral fertilizer spreader, 77.52 EUR ha^{-1} can be saved. The main reason for the difference is smaller dosage when using modular spreader and additional loading costs.
Key words: forest fertilization, wood ash, productivity.

Introduction

Intensification of forestry, for example, whole-tree harvesting method, which is getting more necessary because of increasing biofuel demand, can significantly increase nutrient loss in soil (Mälkönen, 1976). One of the unrated nutrient sources, which could be recovered back to forest ecosystems, is biomass ash that is left after biomass thermal processing. Biomass ash may consist of considerable amount of nutrients – Ca, P, K, Mg etc. (Misra, Ragland, & Baker, 1993; James *et al*., 2012; Vassilev, Baxter, & Vassileva, 2013; Okmanis, Lazdiņa, & Lazdiņš, 2015). In Latvia wood-based biomass is mostly used for heat and energy production. Studies show that wood-based biomass, comparing with other biomass forms, after combustion has relatively lower levels of indissoluble ash, which contains more compounds with low temperature melting point and causes slag, whereas the content of soluble - plant available nutrients is high (Hakkila, 1984; Nunes, Matias, & Catalão, 2016) thus providing better results on tree growth response comparing, for example, to peat ash (Hytonen, 2003). Numerous studies have been made on forest fertilization with biomass based on ash and contradictory results are revealed. Neutral or even slightly negative effect on tree growth was found on mineral uplands fertilized with wood ash, but increase of needle nutrient content was still observed (Jacobson, 2003; Moilanen *et al*., 2013). However in Nordic countries significantly larger tree increments on ash-fertilized drained peatlands are found, where mostly P and K deficiency is observed (Moilanen, Silfverberg, & Hokkanen, 2002; Sikström, Almqvist, & Jansson, 2010; Ernfors *et al*., 2010; Moilanen, Hytönen, & Leppälä, 2012; Hökkä, Repola, & Moilanen, 2012; Moilanen *et al*., 2013; Kikamägi, Ots, & Kuznetsova, 2013). Wood ash is considered even better than the commercial fertilizers because of slower nutrient release. Moilanen (2015) concludes that wood ash fertilization is economically feasible and already during commercial thinning annual income was three times higher than in control (Moilanen *et al*., 2015). Studies in Latvia also reveal that ash fertilization on drained organic soil induced additional volume increment from 2 to almost 5 m^3 ha^{-1} per year (Okmanis *et al*., 2016).

There is still insufficient research on ash spreading technological processes. Forest fertilization in Latvia is not common practice therefore fertilizer spreading productivity could not be implemented from agriculture because of various obstacles in forest. Aircraft is used over difficult terrain, where ground vehicles cannot operate effectively (Page & Gustafson, 1969), although ground based forwarder is two to three times cheaper (Väätäinen *et al*., 2011). Best results of fertilization could be gathered at conifer stands at age of 20 to 50 years, when productivity is already highest (Lībiete, Jansons, & Zālītis, 2009) and first commercial thinning is done – strip roads remaining for technological process. Purchase of specific fertilization machinery could be very costly. Solution of this problem could be adjustment of tractors used for other operations, for example, soil preparation. This could solve another problem of seasonal exploitation of machinery – while it is impossible to proceed with the soil preparation when soil is frozen, although possibility of element leaching should be assessed during thaw.

Although slightly negative impact of forest operation machinery on soil compaction and remaining trees has been already proven (Ampoorter *et al*., 2007; Horn *et al*., 2007; Petersons, Dreska, & Saveļjevs, 2010; Modig *et al*., 2012; Prindulis, Lazdiņš, & Kalēja, 2015), significant stand damages related to soil nutrition status have been observed in some cases of Norway spruce stands in Latvia (Lazdiņš, Miezīte, & Bārdule, 2011; Klavina *et al*., 2016; Okmanis *et al*., 2016). By now ash use in forest fertilization and forest fertilization has not been practiced in Latvia, however by certain activities and changes in regulations it could be as possible as in Nordic countries, therefore studies of ash mechanized application are necessary. The aim of this study is to analyse productivity of ground based mechanized wood biomass ash spreading using four-wheel agricultural tractor combined with modular ash spreader and regular fertilizer spreader.

Materials and Methods

First experiment of ash spreading was held at the end of November 2014 with forest-adjusted four-wheel farm tractor Valtra 6350, which has 105 hp engine output and was equipped with four-wheel modular ash spreader. The second experiment was made with a more powerful (170 hp) four-wheel farm tractor Valtra T191 equipped with Amazone three point hitch fertilizer spreader during October 2016. In both cases operators had no experience in forest fertilization. According to Table 1, using full bearing potential of modular ash spreader, total mass of equipment would be 16 660 kg (considering that ash density on average is 1000 kg m^{-3}), which is close to loaded medium forwarder that is often used in commercial thinning in Latvia. However, because of rut formation on trails, on average only 2 tonnes of wood ash were loaded in trailer. The full weight of Valtra T191 equipment was only 8 250 kg. First case machinery was also about 5 m longer and ground clearance was 160 mm lower, which could limit agility and affect productivity (Table 1).

In both cases treatment was carried out in stands, where first commercial thinning was done in recent years and every 20 m had remained the skid trails (strip roads) with laid forestry residues. Forest stands in first case were distributed in Joint Stock Company 'Latvia's State Forests' Viesite forest district. Experiment of wood ash spreading was made in four (two *Hylocomiosa* and two *Oxalidosa turf. mel.*) Norway spruce (*Picea abies* (L.) Karst.) stands from age of 43 to 48 years (Table 2). About half of each stand was treated and in total 20 t of wood ash were spread on 10 ha (treatment dose 2 tonnes ha^{-1}) forest land. In second case stands represent only drained soils, which are located in Jelgava, Jaunkalsnava and Smiltene and are managed by Forest research station agency. In second experiment also birch (*Betula pendula* Roth) stands were included. About half of total area (5 ha) was fertilized and 15 tonnes (dose 3 tonnes ha^{-1}) of wood ash were spread with Amazon fertilizer spreader.

Weather during the first experiment was cloudy, but with insignificant precipitation, temperature varied from 0 to -7 °C. During the second experiment sky was overcast, but no precipitation was observed and air temperature varied from +9 to +14 °C.

Wood ash was taken from wood pellet plant 'NewFuels' and from wood pellet plant 'Latgran' (both plants are operating furnaces with moving grates, both have 20 MW capacity and burn only wood based biomass). Before transportation ash was poured with water and loaded into big-bags of 500 kg each. Properties of utilized ash are shown in Table 3, origin of samples is not provided in the table. In first case (Sample 1), when dose was 2 tonnes ha^{-1}, only 10.2 kg of P on 1 ha forest area were brought, while

Table 1

Specifications of ash spreading machinery

Parameter	Valtra 6350 equipment	Valtra T191 equipment
Tractor mass, kg	4660	5950 + 1000 (hitch)
– length, mm	4534 + 1000 (hoist)	5150 + 1000 (hitch)
– width, mm	2280	2330
– height, mm	2730	3400
– ground clearance, mm	500	590
Spreader mass, kg	4000	300
– length, mm	6200	1420
– width, mm	2600	2440
– ground clearance, mm	440	600
– volume, m^3	8	1

Table 2

Parameters of treated stands

Stand	Forest type	Area, ha	Species/ age	Basal area, m^2 ha^{-1}	Stock, m^3 ha^{-1}
Valtra 6350					
1-1	*Hylocomiosa.*	6.3	Spruce/48	23	235
1-2	*Oxalidosa turf. mel.*	5.7	Spruce/48	21	214
1-3	*Oxalidosa turf. mel.*	2.1	Spruce/43	21	214
1-4	*Hylocomiosa.*	4.4	Spruce/48	18	171
Valtra T191					
2-1	*Myrtillosa mel.*	4.8	Spruce/45	26	314
2-2	*Oxalidosa turf. mel.*	0.9	Spruce/46	23	243
2-3	*Myrtillosa mel.*	2.1	Birch/29	19	182
2-4	*Myrtillosa turf. mel.*	2.1	Birch/28	14	121

Table 3

Wood biomass ash properties

Parameter	Sample 1	Sample 2
$pHCaCl_2$	11.9	12.6
$C_{carb.}$, g kg^{-1}	15.5	13.7
$C_{org.}$, g kg^{-1}	19.7	9.1
$S_{tot.}$, mg kg^{-1}	16.6	37.7
$N_{tot.}$, g kg^{-1}	0.2	0.1
P, g kg^{-1}	5.1	11.6
HNO_3 extractable elements		
K, g kg^{-1}	16.3	35.6
Ca, g kg^{-1}	101.7	167.4
Mg, g kg^{-1}	16.7	32.1
Mn, g kg^{-1}	1.3	3.0
Fe, g kg^{-1}	4,1	6.5
Cd, mg kg^{-1}	8,8	3.5
Pb, mg kg^{-1}	77.4	104.7
Cr, mg kg^{-1}	348.1	122.9
Ni, mg kg^{-1}	24.5	27.5
Cu, mg kg^{-1}	62.6	172.4

in second case (Sample 2; 3 tonnes ha^{-1} of wood ash) dosage was 34.5 kg ha^{-1}. Amount of nutrients brought into forest in both cases differs significantly, referring to suggestion to determine ash chemical composition before using them as fertilizer.

Time for all work elements (wood ash loading, arriving from loading place to stand, wood ash spreading, driving out and also stump cutting) in total productive time consumption for each object was registered. Alternative income of ash utilization as fertilizer was calculated according to formula 1:

$$I = DW - \frac{S}{P}, \quad (1)$$

where

I – income of wood ash use in forest fertilization, EUR ha^{-1};

D – costs of waste deposition, EUR t^{-1};

W – wood ash dose, t ha^{-1};

S – implement service cost of farm tractor, EUR h^{-1};

P - productivity of mechanized fertilization, ha h^{-1}.

Wood ash landfilling costs are 39.50 EUR t^{-1} and 1 hour implement service of machinery costs are 25.00 EUR (average farm tractor rental cost including operator by the experiment time).

Results and Discussion

In first case 17.54 hours were spent for 10 ha fertilization and in second case 8.20 hours for 5 ha fertilization. So the average productivity of modular spreading trailer in ash spreading was 0.57 ha h^{-1}, but that of Amazone mineral fertilizer spreader was 0.61 ha h^{-1}. The result is two times lower than in Sweden and almost three times lower than in Finland, when forwarder with a centrifugal spreader was used (Emilsson, 2006; Vaatainen *et al*., 2007). When working with ash spreader, another tractor with front-end loader was used for ash loading, therefore 2 technical units were included in costs. Under conditions of production, the second tractor had to be replaced with crane, which was mounted onto 3-point-hitch. Installation of crane would reduce ash spreading costs. When working with mineral fertilizer spreader Amazone, ash was loaded with a lifting device strengthened for the spreader, which has a limited functionality and it is reflected in working time consumption. One of solutions is strengthening the lifting device and equipping with electric winch to pull in ash bags. Mineral fertilizer spreaders are adjusted for spreading pre-packed material, which impose additional restrictions.

When comparing both technical solutions for ash spreading, a range of factors should be considered – distance from ash loading site to the stand, stand configuration, soil bearing capacity etc. Amazone mineral fertilizer spreader is intended for spreading granulated and free-flowing material, therefore during trials feeder had to be cleaned regularly and ash had to be mixed in order not to adhere to sides and sieve. Modular spreader is suitable for spreading unscreened, non-granulated, heterogeneous wood ash. Both devices have a problem with material freezing at sides when spreading wetted ash in cold weather, therefore recommendations prepared in this research include a suggestion to change metal plates for plywood. None of devices can spread dry ash with a significant proportion of light fraction. Ash processing with self-hardening, pelletizing or granulation technology is an obligatory requirement for ash application in forest.

Differences in work productivity are caused by technical gauges and ability to manoeuvre as well. When spreading ash with modular trailer, most time (48%) is consumed by driving into the stand (Figure 1) as gauges of tractor and trailer are unsuitable for driving in a roundabout way in thinning technological corridors. Operator had to drive into the stand in reverse motion (with a trailer in front), in order to spread ash by pulling the trailer. Tractor used for this trial was too light to drive a loaded trailer (6-8 tonnes, depending on load weight and moisture content in ash), therefore even small inclines could cause towing. In practice, when using a tractor with suitable size (unloaded weight at least 7 tonnes), work productivity would significantly increase. An alternative solution is mounting the current ash spreading device to the frame of a forwarder (LSFRI Silava has started working on adapting ash spreader for Vimek 610 and Vimek 610 BioCombi forwarders) or equipping rear tandem with hydraulic drive. As a result of the research, ash spreader has already been reconstructed, lifting higher the spreading mechanism and shortening frame length.

When spreading ash with mineral fertilizer spreader, only 13% of total working time was consumed by driving into the stand (Figure 2), because device's ability to manoeuvre was not limited and towing risk was relatively low. At the same time in first case 42% of all time was spent on reaching the stand. Opposite situation was observed with loading – much less time was spent in first case, when additional tractor with wheel loader was used. Clearance of modular spreaders is lower than that of

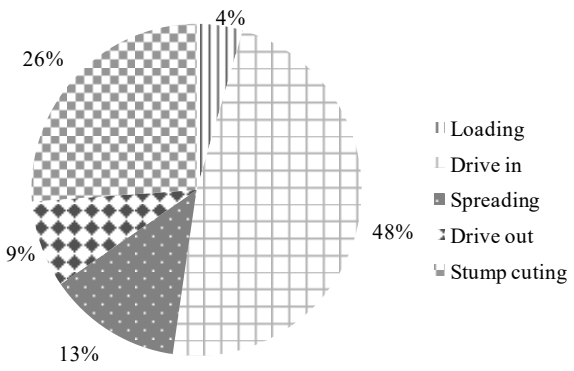

Figure 1. Working time distribution of wood ash spreading with modular trailer.

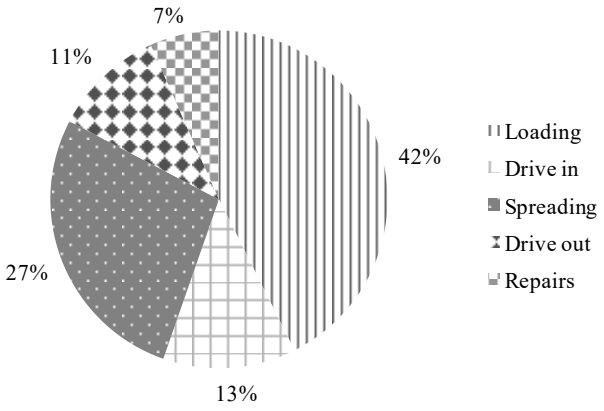

Figure 2. Working time distribution of ash spreading with mineral fertilizer spreader Amazone.

Amazone spreader, therefore, when working with ash spreading trailer, 26% of the total time was consumed by cutting higher stumps in order to protect spreading mechanism and hydraulic engine from possible damages. Observations on time consumption suggest that better technical solution should be developed by combining qualities of both machineries.

Productivity would significantly increase if spreading mechanism was lifted at one level with the trailer frame and installation of additional protection, because stump cutting is no longer required. Movement time would be shortened as well, because trailer is shorter by half a meter.

Amazone mineral fertilizer spreader is suitable for spreading granulated ash, which has to be pre-packed in bags with a maximum weight of 500 kg. This method has advantages if ash is timely delivered to the stand and spreader is not tied to ash delivery. Granulated ash, which is pre-packed in bags, can be stored in application site without a risk of getting soaked by the rain. Small volumes of bags enable precision of ash doses for each stand and tractor driving time is shortened significantly when delivering ash to each stand. These advantages can be used when working with ash spreader trailer. Ash bag size is limited by the power of installed crane.

Ash spreading costs, excluding load size and time consumed by loading, unloading and driving, are determined by technological corridor location in the stand – total driving distance in the stand and average driving speed. Productivity and costs of wood ash spreading can be easily modelled.

Ash spreading costs in trial with modular trailer were 43.86 EUR ha^{-1}, but with mineral fertilizer spreader – 40.98 EUR ha^{-1}, excluding service of frontal lifting device. Approximate ash deposit costs are 39.5 EUR t^{-1}. Since in the second scenario (Valtra 6350+Amazone) 3 tonnes of wood ash per hectare were spread, alternative income of ash spreading in forest, instead of deposition, is 77.52 EUR ha^{-1}. When using modular ash spreader frontal loader service was included – total costs 87.72 EUR ha^{-1} and only 2 t ha^{-1} of ash were spread, as a result additional 8.7 EUR ha^{-1} should be paid for ash spreading. Wood ash spreading costs with modular trailer can be reduced by installing crane on the tractor. Reduction of costs can be achieved by increasing ash dosage by amount, which ensures optimal addition of nutrients. In trials with modular trailer, in order to apply sufficient amount of potassium and phosphorus to the soil, ash dosage must be increased by up to 5 t ha^{-1}. In this case, reduction of costs would be greater, of course ash spreading productivity would also change by changing dosage.

Unprocessed ash was used in trials, therefore in practice ash hardening, crushing and quality control will cause additional costs. However increased tree growth is expected and with sufficient information about additional increments IRR (internal rate of return) could be calculated.

Conclusions

1. When working with modular trailer, ash spreading productivity is 0.57 ha h^{-1}, but with mineral fertilizer spreader – 0.61 ha h^{-1}.
2. When using modular spreading trailer and additional tractor with frontal loader for ash loading, ash spreading costs are 87.72 EUR ha^{-1}, but when using Amazone spreader with hydraulic lifting device – 40.98 EUR ha^{-1}.
3. Comparing ash spreading costs with expenditure for its deposition, in the first trial, when ash was spread in the forest using modular trailer, additional 8.72 EUR ha^{-1} were required, but in the second trial, when using mineral fertilizer spreader, 77.52 EUR ha^{-1} could be saved.

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BIOLOGICAL PROTECTION OF CONIFERS AGAINST *HETEROBASIDION* INFECTION – INTERACTION BETWEEN ROOT-ROT FUNGUS AND *PHLEBIOPSIS GIGANTEA*

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Abstract
The biological control agent Rotstop, composed of spores of *Phlebiopsis gigantea*, is used for treatment of conifer stumps to reduce the spread of *Heterobasidion* root rot in commercial forests. Two experiments were conducted to compare the antagonistic ability of the Rotstop isolate and nine Latvian isolates of *P. gigantea* against root rot fungus *Heterobasidion*, in wood of Scots pine and Norway spruce. Billets of conifer trees were first sprayed with a spore suspension of *P. gigantea* and then with *Heterobasidion* at different concentrations. The presence of fungi in billets was evaluated by morphological characteristics of mycelium. The Latvian isolates of *P. gigantea* showed similar or even higher values of efficacy against *Heterobasidion* (average efficacy 84% in spruce and 89% in pine) than the commercially manufactured Finnish preparation Rotstop (61% in spruce and 90% in pine). Latvian isolates of *P. gigantea* have a potential to be used for preparation of biological control agents in the future.
Key words: biological control, Norway spruce, Scots pine, *Heterobasidion*, *Phlebiopsis gigantean.*

Introduction

Annual economic losses caused by *Heterobasidion* root rot in the European Union comprise ca. 500 million euros (Korhonen & Holdenrieder, 2005). In southern Finland annual financial losses in coniferous stands have been estimated to be around 35 million euros (Bendz-Hellgren *et al*., 1998). In Latvia, Gaitnieks *et al.* (2008) reported that losses due to root rot in final felling of spruce stands were 800-4790 euros per hectare, depending on timber yield and infection frequency in the stand. As stands of spruce comprise 584 thousand ha (18.27%) of the total forest area of Latvia (Jansons, 2014), losses caused by root rot in spruce stands are considerable. Calculations made in Latvian State Forest Research Institute 'Silava' show that total losses caused by root rot in spruce forests of Latvia are at least 7 million euros annually (unpublished data). *Heterobasidion* root rot causes considerable economic losses also in Scots pine stands, especially in young stands. In Lithuania, *Heterobasidion* root rot was present in 53% of surveyed young stands of pine (Василяускас, 1989).

Spores of *Heterobasidion* infect fresh conifer stumps and wounds of growing conifers (primary infection). Mycelium of the fungus spreads along roots and moves from tree to tree via root contacts (secondary spread). Primary infection initiates development of new disease centres in stands where infection was not present before. Fresh conifer stumps cut during logging are the main source of *Heterobasidion* infection, but if growth conditions are favourable for spores, wounded roots in the soil can be infected as well (Redfern & Stenlid, 1998; Stenlid & Redfern, 1998). Sporulation of *Heterobasidion* in Northern countries and Latvia reaches its maximum in summer and autumn (Yde-Andersen, 1962; Kallio, 1970; Brandtberg, Johansson, & Seeger, 1996; Brūna, Gaitnieks, & Vasaitis, 2015). Fresh stumps cut at this time can be protected against *Heterobasidion* infection by treating them with biological (or chemical) control agents (Holdenrieder & Greig, 1998; Thor, 2005). The biological control agent Rotstop® ('Rotstop F', Verdera Oy, Finland), composed of spores of the non-pathogenic wood-decay fungus *Phlebiopsis gigantea* (Fr.) Jülich, is used for stump protection also in Latvia, where its efficacy is similar to efficacy in other countries (Kenigsvalde *et al*., 2011; Kenigsvalde *et al*., 2016). However, research in Sweden has shown that sometimes native isolates of *P. gigantea* have higher efficacy than Rotstop, which contains a Finnish isolate of *P. gigantea* (Berglund *et al.*, 2005). Moreover, large-scale use of a single genotype of fungus for a long time can negatively affect local populations of fungi in forest ecosystems (Vasiliauskas *et al*., 2004). Therefore, native isolates of *P. gigantea* can potentially be used for stump treatment in the future if the isolates provide similar or better protection compared to Rotstop.

The aim of our work was to compare the efficacy of Rotstop and nine Latvian *P. gigantea* isolates against different spore concentrations of *Heterobasidion* in stem pieces of spruce and pine.

Materials and Methods

Field experiments

Two experiments were carried out: Experiment 1 on 6th of July 2009, and Experiment 2 on 27th of October 2009. In each experiment two Norway spruce trees (*Picea abies* (L.) H. Karst.) and two Scots pine trees (*Pinus sylvestris* L.) were felled and cut into one meter long stem pieces. They were then transported to the site where experiments were carried

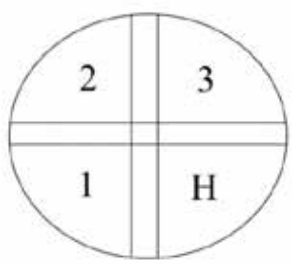

Figure 1. The cut surface of each billet was divided into four sectors and sprayed with two spore suspensions: a) *P. gigantea* suspension – asexual spores from one *P. gigantea* culture, b) *Heterobasidion* suspension – mixture of asexual spores from one *H. annosum* and one *H. parviporum* culture. Sector treatments: (H) *Heterobasidion* only, (1) *P. gigantea* and *Heterobasidion*, (2) *P. gigantea* and *Heterobasidion* two times; (3) *P. gigantea* and *Heterobasidion* three times.

out. Immediately before treatment, the stem pieces (diameter 13 – 16 cm) were cut into ca. 30 cm long segments (billets), and the upper surface was divided into four sectors (Figure 1).

Four Latvian *P. gigantea* isolates were tested in field Experiment 1 and five isolates in greenhouse Experiment 2. The sectors 1–3 were first sprayed with an oidial suspension of a *P. gigantea* isolate. One hour later the whole cut surface was sprayed with a suspension of *Heterobasidion* conidiospores. After another hour, sectors 2 and 3 were again sprayed with *Heterobasidion* (same suspension as before), and after another hour, sector 3 was sprayed with *Heterobasidion* for the third time. During the treatment other sectors were covered with a paper sheet. An empty space of 2 cm width was left between sectors to avoid cross contamination. In both experiments, four replicate billets of spruce and four billets of pine were treated with each isolate of *P. gigantea*. The billets were placed on folded garden fabric to avoid contamination from soil, and incubated 3 – 4 weeks outdoors (Experiment 1) or in a greenhouse (Experiment 2). Experiment 2 was conducted in a greenhouse, as weather conditions in October were not suitable for incubation outdoors. The billets were watered regularly to provide suitable moisture content in the billet for development of the fungi. Mean daily air temperature during incubation outdoors was 17 °C (Experiment 1) and temperature during incubation in the greenhouse was 18 °C (Experiment 2) with

Table 1

Some properties of the Latvian *P. gigantea* isolates cultivated on malt extract agar medium in Petri dishes at room temperature

Isolate of *P. gigantea*	Host	Growth rate on agar medium, mm day^{-1}	Growth rate over *Heterobasidion* colony on agar medium, mm day^{-1}		Number of spores produced in Petri dish, millions
			H. annosum	*H. parviporum*	
G1[1]	*P. abies* or *P. sylvestris*	7.1	0.9	1.4	47.3
K4[1]	*P. abies*	8.6	0.9	1.2	21.5
J4[1]	*P. sylvestris*	8.0	0.9	1.4	12.8
T207E[1]	*P. abies*	6.5	0.7	0.7	182.8
K107P[2]	*P. sylvestris*	5.9	0.6	0.7	22.2
Kn107E[2]	*P. abies*	6.5	0.7	0.8	18.5
Le107E[2]	*P. abies*	6.1	0.6	0.8	23.7
Le507P[2]	*P. sylvestris*	6.7	0.7	0.8	22.2
J207P[2]	*P. sylvestris*	5.1	0.5	0.7	24.7
Mean		6.7	0.7	0.9	41.7

[1]isolates used in Experiment 1
[2]isolates used in Experiment 2

relative humidity 30%. After incubation, four to six 2 – 3 cm thick sample discs were cut from the billets and transported to the laboratory.

Analyses of the discs

In the laboratory, the discs were debarked, washed with a stiff brush under running tap water, and incubated 5 – 7 days in loosely closed plastic bags at room temperature. Both sides of the disc were examined. A grid consisting of 0.42 cm^2 squares was fixed on each disc with pins. Each square was screened with a dissection microscope for the presence of *Heterobasidion* conidiophores, and the area, colonised by the fungus, was marked on the disc. Area colonised by *P. gigantea* was recognised on the basis of its typical orange brown colour in wood. The surface area occupied by *Heterobasidion* and by *P. gigantea* was redrawn on transparent paper and measured using a planimeter (PLANIX 10S 'Marble', Tamaya, Japan).

Fungal isolates

Four Latvian isolates of *P. gigantea* (G1, K4, J4, T207E) were used in Experiment 1 and five isolates (J207P, K107P, Kn107E, Le507P, Le107E) in Experiment 2. Rotstop was included in both experiments. The Latvian isolates had been previously tested in the laboratory for their growth rate on malt extract agar, production of asexual spores (oidia), and antagonistic ability against *H. annosum* and *H. parviporum* (Table 1), according to methods used by Sun *et al.* (2009a).

Treatment suspensions

Each *P. gigantea* isolate was cultured in several Petri dishes on malt extract agar medium for 3 weeks at ca. 20 °C. Spore suspensions were prepared by washing the asexual spores several times from one Petri dish with tap water, agitating the colony gently with a Drigalski spatula. The spore suspension obtained from the Petri dish was filled to one liter and 0.5 mL were transferred and spread evenly on a Petri dish containing malt extract agar medium. After 24 hours, germinated spores of *P. gigantea* were counted under a microscope (magnification 100x) within 30 sight fields per dish. The number of spores per original Petri dish was calculated, taking into account the area of the sight field and the area of the Petri dish. Treatment suspensions were prepared 2 – 4 hours before the treatment of billets from a replicate Petri dish culture, and the spore concentration in suspension was adjusted to ca. 5000 spores mL^{-1}. Suspension of *Heterobasidion* conidiospores was prepared as a mixture from one heterokaryotic *H. parviporum* (Rb175) and one *H. annosum* (358Rv) isolate originating from Sweden. *Heterobasidion* spore concentration in suspension was ca. 500 spores mL^{-1}.

Calculations and statistics

Efficacy of *P. gigantea* treatment was calculated by comparing the area occupied by *Heterobasidion* on treated sectors of the disc (sapwood and heartwood included) to the area of *Heterobasidion* on the control (H) sector. The efficacy was calculated from depths 3 and 9 cm from billet surface, and the results were presented as means of the four billet replicates. The following formula was used to calculate the efficacy of different treatments:

$$E(\%)=100-(100*n_t/n_u), \quad (1)$$

where n_t and n_u represent percentages of area occupied by *Heterobasidion* in treated and control sectors.

Control efficacy of *P. gigantea* and area occupied by both fungi were compared by the non-parametric Mann-Whitney test in RStudio (RStudio Team, 2015). Proportions were arcsine transformed before analyses.

Results and Discussion

Isolates of *P. gigantea* used in experiments were selected on the basis of previous tests in which the growth rate, spore production, and competitive ability against *H. annosum* and *H. parviporum* was measured in laboratory. Investigations by Sun *et al.* (2009a, 2009b) showed that efficacy of a *P. gigantea* isolate against *Heterobasidion* correlates mostly with its growth rate in wood. The growth rate of Latvian *P. gigantea* isolates on agar medium at room temperature varied from 5.1 to 8.6 mm day^{-1}. Within the material investigated by Sun *et al.* (2009a), consisting of 64 *P. gigantea* isolates, the corresponding variation was from 3.8 to 10.8 mm day^{-1}. Abundant sporulation of a *P. gigantea* isolate is a useful property for easy manufacture of the preparation for practical stump treatment; the spore production of the nine Latvian isolates varied from 12.8 to 182.8 million spores per Petri dish. In the material investigated by Sun *et al.* (2009a) the range was from 2.0 to 271.6 million spores per Petri dish.

Considerable differences were noted in the colonisation of spruce wood by different *P. gigantea* isolates in Experiment 1 – area occupied by *P. gigantea* isolates varied from 5.0 to 58.8% at the level (depth of) 3 cm below billet surface, and from 0 to 50.8% at the level of 9 cm. Comparable variation has been observed also in other investigations (Sun *et al*., 2009b; Berglund *et al*., 2005). As noted by other researchers (Webber & Thorpe, 2003), *P. gigantea* colonises more effectively pine wood than spruce wood. In Experiment 1, the colonisation of pine wood by different *P. gigantea* isolates varied from 79.1 to

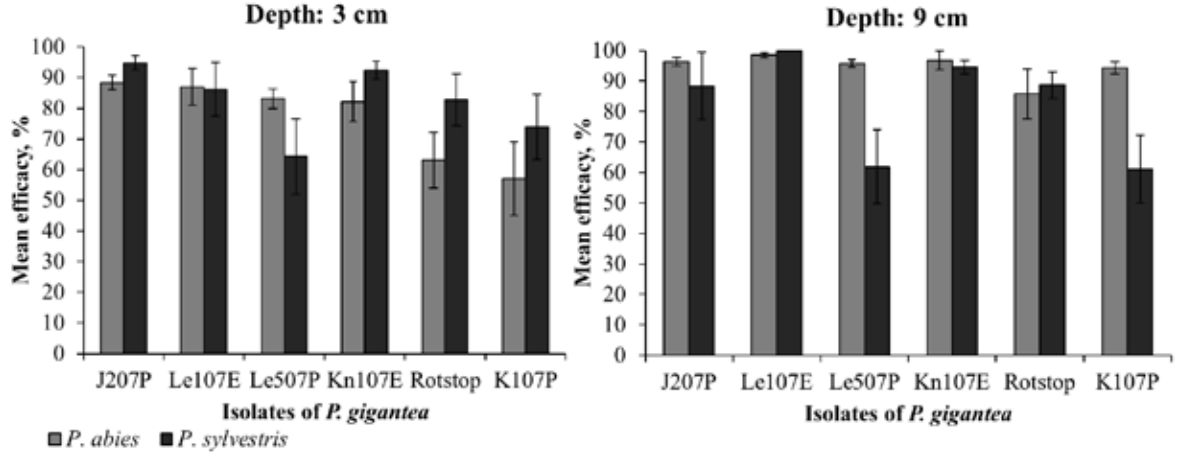

Figure 2. Mean efficacy of *P. gigantea* isolates in depth of 3 and 9 cm in billets (Experiment 2).

89.9% at the depth of 3 cm, and from 69.4 to 97.7% at the depth of 9 cm.

In Experiment 1, started at the beginning of July and incubated in the field, mean control efficacy of Latvian isolates of *P. gigantea* in spruce wood at the depth of 3 and 9 cm from billet surface was 77% and 79%, respectively. Corresponding values for the Rotstop isolate were 79% and 19%. Berglund & Rönnberg (2004) noted that the efficacy values of biological control agents were higher in the surface layers of a spruce stump, probably because *Heterobasidion* grows faster than *P. gigantea* in spruce wood (Holdenrieder & Greig, 1998). It is possible that the Rotstop isolate did not reach the depth of 9 cm during the incubation time (Sun *et al.*, 2009a) – mean area occupied by Rotstop isolate at the depth of 9 cm was 1%, whereas the occupation of the four Latvian isolates varied from 6 to 35%. In pine billets of Experiment 1, the efficacy of all analysed *P. gigantea* isolates (including Rotstop) was 99 – 100%, and the mean area occupied by *P. gigantea* in depths of 3 and 9 cm was 87% and 89%.

In Experiment 2, started at the end of October and incubated in a greenhouse, Rotstop and *P. gigantea* isolate K107P had the lowest values of control efficacy and occupied area in depth of 3 cm in spruce billets (Figure 2 and Table 2).

In wood of spruce in depth of 9 cm, there was no correlation between area occupied by *P. gigantea* and efficacy values although occupation of Rotstop and *P. gigantea* isolate K107P were rather small compared with occupation of other isolates (Table 2).

P. gigantea isolates K107P and Le507P had lowest efficacy values in wood of pine in depth of 9 cm: 61.1% and 61.8%, respectively, despite the fact that the latter isolate had occupied a big part of wood (68.7 – 82.3%) (Table 3).

Calculations of control efficacy of *P. gigantea* against *Heterobasidion* can be based on two kinds of data: the number of stumps infected by *Heterobasidion*,

Table 2

Area occupied by *P. gigantea* on sample discs cut from spruce billets (Experiment 2)

Number of treatments with *Heterobasidion* spore suspension (500 spores mL^{-1})	Area occupied by isolates of *P. gigantea*, % from total sector area					
	J207P	Le107E	Le507P	Kn107E	Rotstop	K107P
analysed depth: 3 cm						
1	14.2 ± 7.5	29.7 ± 15.9	31.0 ± 12.6	40.4 ± 15.2	8.4 ± 1.4	0.0
2	29.5 ± 4.2	20.5 ± 4.3	37.8 ± 14.9	49.6 ± 7.6	17.9 ± 8.6	0.5 ± 0.5
3	33.0 ± 8.7	22.8 ± 7.9	37.7 ± 13.3	48.6 ± 12.4	25.4 ± 6.4	0.3 ± 0.3
analysed depth: 9 cm						
1	17.9 ± 12.2	21.5 ± 9.2	28.3 ± 14.6	44.8 ± 13.5	7.3 ± 2.4	0.0
2	22.7 ± 9.7	21.7 ± 12.0	36.5 ± 10.0	46.4 ± 12.8	5.1 ± 3.8	8.7 ± 8.7
3	9.3 ± 5.5	21.3 ± 12.6	28.0 ± 11.3	33.3 ± 6.0	3.7 ± 2.1	2.3 ± 1.5

Table 3

Area occupied by *P. gigantea* on sample discs cut from pine billets (Experiment 2)

Number of treatments with *Heterobasidion* spore suspension (500 spores mL^{-1})	Area occupied by isolates of *P. gigantea*, % from total sector area					
	J207P	Le107E	Le507P	Kn107E	Rotstop	K107P
analysed depth: 3 cm						
1	85.6 ± 3.6	80.7 ± 4.4	84.4 ± 1.5	86.3 ± 1.6	84.8 ± 2.3	63.7 ± 17.1
2	88.7 ± 3.8	87.9 ± 2.9	88.9 ± 1.1	94.0 ± 0.6	85.3 ± 2.9	67.2 ± 9.7
3	91.8 ± 2.9	91.4 ± 3.7	88.6 ± 1.4	90.0 ± 2.1	86.3 ± 0.7	91.0 ± 1.7
analysed depth: 9 cm						
1	55.4 ± 17.1	80.2 ± 2.8	82.3 ± 1.6	71.1 ± 4.6	53.8 ± 17.4	41.4 ± 15.5
2	54.0 ± 13.3	78.4 ± 5.1	80.1 ± 3.6	65.8 ± 21.1	51.2 ± 17.6	45.7 ± 13.7
3	57.3 ± 11.7	81.6 ± 7.6	68.7 ± 9.2	60.2 ± 15.3	47.7 ± 16.7	46.4 ± 14.3

or wood area colonised by it in stumps (Kenigsvalde *et al.*, 2016). The results obtained with these two methods can differ more than two times (Thor & Stenlid, 2005). We consider that occupied area is a better indicator of control efficacy of a *P. gigantea* isolate, because infection frequency of stumps can be affected by stump size and amount of spores in the stand (Redfern & Stenlid, 1998).

Efficacy of *P. gigantea* isolates is affected by properties of wood and isolates (Berglund *et al*., 2005). In some earlier investigations Rotstop was shown to have higher values of efficacy in spruce (Korhonen *et al*., 1994; Korhonen, 2003) than in the experiments presented above. It is possible that Latvian isolates of *P. gigantea* are more adapted to local conditions. Also in Sweden, Rotstop showed lower efficacy values compared to native isolates of *P. gigantea* (Berglund *et al.*, 2005). However, in Experiments 1 and 2 in depths of 3 and 9 cm, mean efficacy values of Rotstop (mean from all analysed sectors) in spruce (61.5%) and pine (90.5%) were rather similar to values obtained in previous investigations conducted in Latvia: 89% in spruce and 95% in pine - efficacy values calculated based on wood occupied by *Heterobasidion* (Kenigsvalde *et al*., 2016). Mean efficacy of Latvian isolates of *P. gigantea* in Experiments 1 and 2 in depths of 3 and 9 cm (mean from all analysed sectors) was 83.6% in spruce and 88.7% in pine.

In our experiments, the efficacy of *P. gigantea* isolates was not affected by increasing number of *Heterobasidion* spores applied to billet surface. Meredith (1960) and Rishbeth (1963) found that if the spores of *Heterobasidion* and *P. gigantea* are mixed together in one suspension, then even a low concentration of *P. gigantea* spores can significantly reduce the development of *Heterobasidion*. Moisture content of wood is very significant for the growth of *Heterobasidion* and *P. gigantea* in wood (Redfern, 1993; Sun *et al.*, 2009a). In our experiments, one sector on the billet surface was treated twice and one three times with *Heterobasidion* spore suspension. Together with higher number of spores, more water was applied to these sectors. Increased moisture content of wood may have inhibited the development of *Heterobasidion* in these sectors. In the greenhouse Experiment 2, surprisingly, *P. gigantea* isolates showed lower values of colonisation and efficacy in pine than in spruce, especially in depth of 9 cm (Table 3). A possible explanation is the absence of wind in greenhouse; it slows down or even prevents the drying of billet surface. This may have, for example, favoured the growth of mould in wood, and affected the interaction between *Heterobasidion* and *P. gigantea* more in pine than in spruce billets.

Conclusions

1. Efficacy of biological control agent Rotstop against *Heterobasidion* spp. was 61% in spruce and 90% in pine.
2. Latvian isolates of *P. gigantea* showed similar (89% in pine) or even higher values (84% in spruce) of efficacy against *Heterobasidion* spp. comparing to the Rotstop isolate.
3. Latvian isolates of *P. gigantea* Le107E and Kn107E can potentially be used for preparation of a biological control agent in the future.

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COMPARISON OF RESPONSE REACTION OF *PINUS SYLVESTRIS* L. AFTER FIRE IN *HYLOCOMIOSA* AND *VACCINIOSA TURF. MEL.*

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Abstract
Forest fires in Latvia occur every year. Research about vegetation and forest regeneration after forest fire is being carried out in Latvia, and sanitary conditions are being evaluated too; however, there are no large studies about the forest fire impact on radial growth dynamics of trees. The aim of the study was to analyse and compare the response reaction of a forest stand after fire in two forest site types – *Hylocomiosa* and *Vacciniosa turf. mel*. The object of the research was fire affected middle–aged managed Scots pine (*Pinus sylvestris* L.) stands in the aforementioned forest site types. The study compares radial growth dynamics of trees, tree mortality and forest stand sanitary conditions after fire in *Hylocomiosa* and *Vacciniosa turf. mel*. The observed fire impact on radial growth dynamics of trees was negative in both forest site types. Negative fire impact on sanitary conditions was observed, too.
Key words: forest fire, *Pinus sylvestris* L., forest stand sanitary conditions, growing stock after forest fire.

Introduction

Forests cover 3.07 million ha of Latvia's territory (State Forest Service, 2017 a). Forest industry plays an important role in the national economy – 20% of total country's export in 2015 was forest industry export and value added share of the forest industry in Latvia's gross domestic product in 2015 was almost 5.2% (JSC 'Latvian State Forests', 2015). One of the most common tree species is Scots pine – 34% ha of all forests are occupied by this species (State Forest Service, 2016 a). According to the State Forest Service (2016 b, 2017 b) data, fire in forest occurs every year, the annual average area of last ten years is around 333 ha (there is no data from year 2016). According to predictions about the climate change, the situation could be even worse. It is foreseen that the average air temperature will increase during the fire danger period, and frequency of extreme hot periods, too. Moreover, it is expected that temperature will not increase evenly during the summer months, but increase most during the extremely hot periods. One more prediction about favourable conditions for forest fires – it is expected that precipitation will increase only by 0 – 2% during summer, or even be reduced by 10 – 15% (Jansons, 2012).

Fire can have both positive and negative impact. Ground vegetation biodiversity after forest fires usually increases (Marozas, Racinskas, & Bartkevicius, 2007; Marozas *et al*., 2011), which might be explained with an extra nutrients, released after burning of humus layer, where most of nutrients were inaccessible for the plants before fire (Certini, 2005). Fire can have a positive effect on Scots pine radial growth dynamics on dry mineral soils, but the impact of fire on tree radial growth dynamics in peat soil is negative (Freimane *et al*., 2013), which might be explained with forest fire type. Subsurface fire damages tree roots more than surface fire (Freimane *et al*., 2013, Freimane, 2015). In Lithuania five years after low intensity surface fire on sandy soil growing middle–aged Scots pine, fire impact to tree–rings growth is not observed (Marozas *et al*., 2011). In Northern Italy it is observed that after severe fire tree–rings in the first year are very narrow (Beghin *et al*., 2011). Research carried out in Norway shows that during the first five years after forest fire the annual growth decreases, then it increases 1 – 20 years after forest fire, but after 20 years it decreases again (Blanck, Rolstad, & Storaunet, 2013). Certini (2005) pointed out that the availability of nutrients after forest fire often increases, but it is not in long–term.

Forest fire impact on tree stand is also expressed as tree mortality. Tree mortality depend on several factors – age of tree, diameter of tree, species of tree, forest fire type, intensity and duration. Older Scots pine trees are more resistant to fire than younger; in the young Scots pine stands were observed higher mortality after low intensity surface fire (Sidoroff *et al.*, 2007; Piha, 2011). It is found out that tree diameter is one more factor which affect tree resistance against fire (Fernandes *et al*., 2008; Donis *et al*., 2010; Piha, 2011, Freimane *et al*., 2013). Tree species are next factor which determine tree viability after fire. Comparing fire resistance of three most common tree species in Latvia – Scots pine, Norway spruce (*Picea abies* (L.) Karst.) and silver birch (*Betula pendula* Roth) – most resistant is Scots pine (Donis *et al*., 2010). It could be explained with its thick crust bark. Forest fire type, intensity and duration also determine tree viability after forest fire (Freimane, 2015).

Fire influence the forest stand sanitary conditions negatively, mostly insect damages are observed (Donis *et al*., 2010, Freimane *et al*., 2013, Freimane, 2015), but there is observed quite a lot cracked bark to trees growing on dried peat soil (Freimane *et al*., 2013). Cracked bark opens up unprotected wood which is bounded by wood lumps and formed in the result of mechanical abrasion of bark, as well as in the

result of fire (Вакин, Полубояринов, & Соловьев, 1980).

The aim of research was to analyse and compare response reaction of the forest stand after fire in two forest site types – *Hylocomiosa* and *Vacciniosa turf. mel*.

Materials and Methods

Research was carried out in Latvia, Zemgale district. Empirical data was collected at the end of vegetation period of 2012 and 2014 in managed middle–aged Scots pine stands – three growing in the forest site type *Hylocomiosa* (data collected in 2014, coordinates of the forest stand No. 1 – latitude 56.60991, longitude 23.81109, forest stand No. 2 – latitude 56.60599, longitude 23.66905, forest stand No. 3 – latitude 56.940588, longitude 23.925927) and one stand in forest site type *Vacciniosa turf. mel*. (data collected in 2012, coordinates of forest stand – latitude 56.63839, longitude 23.88408). In forest site type *Hylocomiosa* three circular sample plots in every forest stand were placed in the part unaffected by fire (control plots) and two sample plots were placed in the part of forest stand affected by fire, in forest site type *Vacciniosa turf. mel*. – five sample plots in the part of stand affected by fire, five - in unaffected by fire. Size of each sample plot was 500 m^2.

For this study, the impact on tree radial growth dynamics was evaluated six years after the forest fire. Forest fire type in *Hylocomiosa* – low (height of scorching up to 0.5 m) to medium (height of scorching from 0.5 to 1.5 m) intensity surface fire, but in *Vacciniosa turf. mel*. – severe (height of scorching more than 1.5 m) intensity surface fire and shallow (depth up to 0.25 m) subsurface fire (Roga, 1979; Bušs & Vanags, 1987).

Kraft class for every tree was determined in every sample plot (Miezīte *et al*., 2013). Tree diameter at 1.3 m height above root collar was measured in the forest stands both affected and unaffected by fire. Peat layer measuring (with measuring rod) was done in *Vacciniosa turf.mel*.

To describe the forest fire impact on forest stand radial growth dynamics, 20 dominant stand fire affected trees and 60 fire unaffected trees were bored with Presler increment borer (I, II and III Kraft class trees) towards the sample plot center at 1.3 m height from tree root collar, for bored trees the tree height was measured (Liepa *et al*., 2013). The collected wood samples were dried at room temperature and then sanded. Tree–ring widths were measured with Lin TAB microscope system. Forest fire impact on radial growth and fire impact effect was analysed using method for evaluation of the impact of environmental factors. The method is based on comparison of tree–ring widths in environmental factor affected and unaffected forest stand (Liepa & Zaļkalns, 2014).

For the evaluation of the tree stand sanitary conditions after fire, the maximum height of scorching was measured. Trees with insect damage, exposed roots, cracked barks were listed.

The collected data were processed mathematically and analyzed using Microsoft Office. Statistical significance was tested by ANOVA and t-Test: Paired Two Sample for Means.

Results and Discussion

After analysis of measured tree–ring widths, it was found that first six years after forest fire tree–ring widths are narrower than before fire occurred in both forest site types *Hylocomiosa* and *Vacciniosa turf. mel*. It is a reason for negative effect of forest fire impact (Fig. 1). In the first year after forest fire in forest site type *Hylocomiosa* the growing stock losses are 0.7 m^3 ha^{-1}, over the next four years | it is approximately 1.8 m^3 ha^{-1} per year, and then in the sixth year – 9 m^3 ha^{-1}. The average loss of growing stock per year is 1.5 m^3 ha^{-1}. A longer period is needed after fire for better evaluation. It might be that in the first year after fire trees have stress, but later situation becomes better and positive impact of forest fire might be observed. For more objective explanation of fire impact on tree–ring widths and

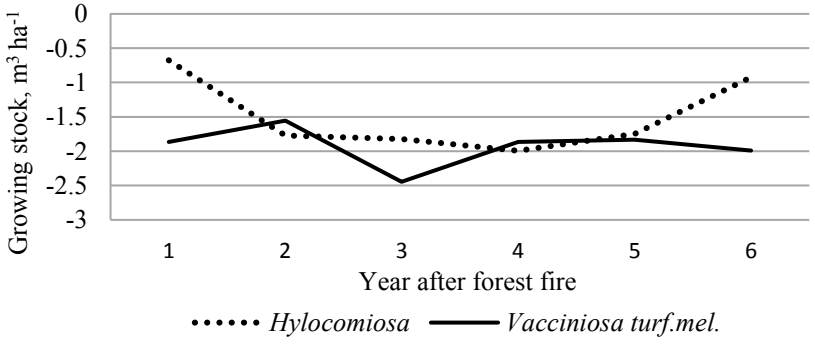

Figure 1. Effect of forest fire impact on growing stock.

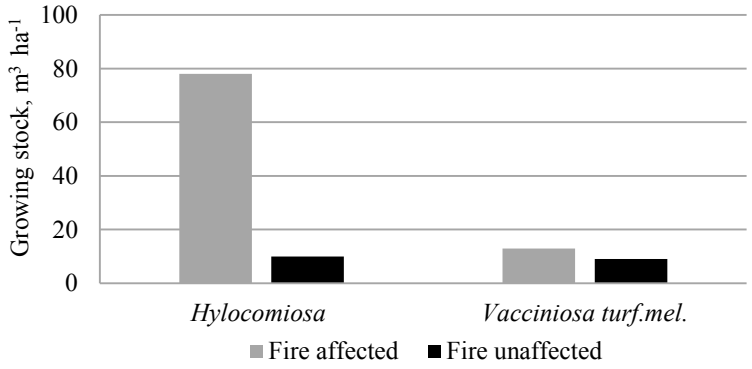

Figure 2. Tree mortality (m^3 ha^{-1}) in fire affected and unaffected forest area in forest site type *Hylocomiosa* and *Vacciniosa turf. mel*.

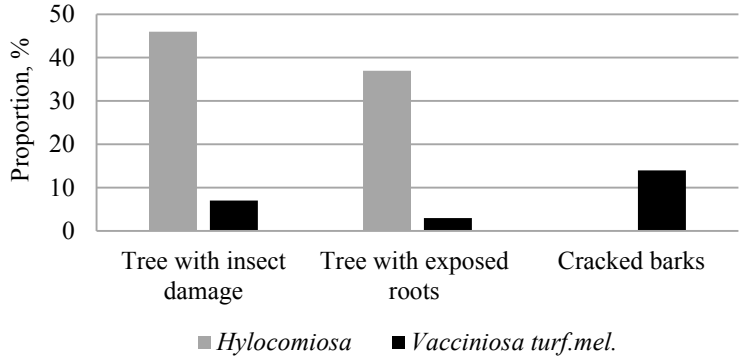

Figure 3. Type of damage and proportion in fire affected stands in forest site type *Hylocomiosa* and *Vacciniosa turf. mel*.

growing stock of middle–aged Scots pine growing in forest site type *Hylocomiosa* and *Vacciniosa turf. mel*. a larger study is needed. Research carried out by Marozas *et al.* (2011) shows that low intensity forest fire does not impact tree–ring growth within five years after fire.

In forest site type *Vacciniosa turf. mel*. the average loss of growing stock was higher – 2 m^3 ha^{-1} per year. And the situation improvement was not observed in the sixth year, which could mean that fire impact on tree–ring widths and growing stock in *Vacciniosa turf. mel*. is more negative than in *Hylocomiosa*.

Tree mortality was observed in both forest site types *Hylocomiosa* and *Vacciniosa turf. mel*. (Fig. 2). Tree mortality after fire is also mentioned in literature (Sidoroff *et al*., 2007; Piha, 2011; Eriksson *et al*., 2013). In forest site type *Hylocomiosa* the tree mortality in fire affected part was higher than in *Vacciniosa turf. mel*., respectively 78 m^3 ha^{-1} and 13 m^3 ha^{-1} on average, but in fire unaffected part the tree mortality was more or less the same – 10 m^3 ha^{-1} (in *Hylocomiosa)* and 9 m^3 ha^{-1} (in *Vacciniosa turf. mel*.). In both forest site types there was no significant difference between tree mortality in fire affected and unaffected parts (in *Hylocomiosa* $p > 0.05$, in *Vacciniosa turf. mel*. $p > 0.05$), but for more objective evaluation a larger data set is needed.

The impact of fire on the forest stand sanitary conditions was negative. Figure 3 shows three different damages and their proportion of all measured trees in the fire affected stands. Trees with insect damages (46% of all fire affected trees in *Hylocomiosa*, 7% in *Vacciniosa turf. mel*.) and exposed roots (37% in *Hylocomiosa* and 3% in *Vacciniosa turf. mel*.) were observed in both forest site types, but cracked bark (14% of all fire affected trees) only in *Vacciniosa turf. mel*. It could be explained with forest site type, fire intensity (severe surface fire, average scorching height 2.2 m) and fire type, too (surface fire plus subsurface fire). The reason of a quite high difference between proportion of trees with insect damages in *Hylocomiosa* and *Vacciniosa turf. mel*. could be possible because of insect occurrence in certain tree stands in *Hylocomiosa* before forest fire.

Conclusions

Research results show that forest fire does not impact tree radial growth dynamic positively in forest site types *Hylocomiosa* and *Vacciniosa turf. mel*.

There are no significant differences between fire affected and unaffected tree mortality in forest site types *Hylocomiosa* ($p > 0.05$) and *Vacciniosa turf. mel*. ($p > 0.05$).

Fire had a negative impact on forest stand sanitary conditions in both forest site types. Trees with insects and exposed roots were observed both in *Hylocomiosa* and *Vacciniosa turf. mel*., but craced bark only in *Vacciniosa turf. mel*.

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FINANCIAL ASSESSMENT OF *FAGUS SYLVATICA* STANDS IN LATVIA

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Abstract
European beech (*Fagus sylvatica* L.) timber value is high in southern Europe due to a great demand from Asia. Since the timber market is global, over a long time we can expect gradual increase in demand also in other countries, including Latvia, where climatic conditions becomes increasingly more suitable for this species due to ongoing climatic changes. In order to develop recommendation for the use of beech in our country, the aim of the study was to assess the potential financial gain from *Fagus sylvatica* stands in Latvia. Assortment structure, defined by top-diameter and length, as well as their prices was set based on the literature survey. Results showed higher income from older (115 years) than form younger (58 years) stands: 9108 EUR ha^{-1}, and 7939 EUR ha^{-1}, respectively. However, the opposite was true for the net present value (r = 3%): 1423 EUR ha^{-1} and 304 EUR ha^{-1}, respectively.
Key words: European beech, noble broadleaves, NPV, introduced tree species.

Introduction

European beech (*Fagus sylvatica* L.) is one of the most widespread broadleaved tree species in central and western part of Europe. In the middle of the 19th century, European beech was introduced in Latvia. Here beech trees grow in isolated areas outside of their natural distribution range (Puriņa *et al*., 2013). Climate envelope models suggest a significant shift of species distribution areas already by the end of this century (Hickler *et al*., 2012) and the conditions also in our country will become more favourable for European beech. However, due to slow natural migration and overwhelming human influence on forest ecosystem, beech might become more widespread in Latvia only in case if strategic decision of its wider use will be implemented. Such decision requires a sound basis, including analysis of potential financial viability as well as risks.

A number of studies so far have demonstrated a positive influence of already ongoing climatic changes on growth of trees in Latvia (Jansons *et al*., 2013a, 2013b; 2015a, 2016a; Rieksts-Riekstins *et al*., 2014). Climate-growth relationships for beech seem to be similar to native tree species, e.g. Scots pine (Jansons *et al*., 2016b), suggesting improvements also in growth of this tree species.

Beech wood market had been unstable in Europe; however, during last decade increasing tendencies had been observed due to export of its logs and saw-timber to Asia (Pöhler *et al*., 2005). The wood is highly appreciated for use in furniture, floorings as well as for plywood, veneering and pulp (von Wühlisch, 2011). Timber quality and value of beech trees depend on the tree traits and stand characteristics, site conditions and forest management (Poljanec *et al*., 2010). One of the most important traits determining assortment structure is branchiness (Haapanen *et al*., 1997; Puriņa *et al*., 2014). Also colour of beech wood important factors determining the price of the log (Pöhler *et al*., 2005). The read heartwood in the living trees is

Table 1

Parameters of dominant beech trees in experimental sites

Experimental site	Area, ha	Age, years	Number of trees, ha^{-1}	Relative number of trees*, %	Basal area, m^2 ha^{-1}	Stand volume, m^3 ha^{-1}	Average $DBH_{1.3m}$, cm	Average tree height, m
21-27	0.10	58	1270	100	54.5	637	23.0	23.2
14-3	0.10	58	650	100	32.7	374	24.8	23.1
23-25	0.2	101	424	87	31.3	408	30.7	27.0
23-25	0.2	115	395	100	34.3	456	32.2	27.2
26-7	0.45	105	222	86	36.7	520	45.9	30.5
26-7	0.45	110	193	84	38.2	555	50.2	31.5
21-1	0.30	115	280	100	35.7	474	40.3	27.9
21-25	0.33	101	364	100	42.8	686	38.7	33.8
21-25	0.33	115	352	100	47.9	786	41.7	34.8

*Number of dominant beech trees related to number of all dominant trees in the stand.

the cause of an important loss in timber quality thus also monetary value (Albert *el al*., 2003; Pöhler *et al*., 2005). Appearance of false heartwood also notably affects the potential use of beech wood (Johansson, 2005). Hillis (1987) indicated that the proportion of the false heartwood significantly increases with the tree age. Optimal harvesting age for beech is 80 – 120 years (von Wühlisch, 2008) and value increases significantly with the dimensions of the tree (Poljanec & Kadunc, 2013).

The aim of the study was to assess the potential financial gain from *Fagus sylvatica* stands in Latvia.

Materials and Methods

The study was done in European beech stands, located in the western part of Latvia, near to Skede (57°14' N; 22°39' E). Data from six repeatedly measured permanent sample plots (0.10 – 0.45 ha) in *Oxalidosa* forest type with beech as dominant tree species (Tab. 1) were used. Measurements of individual tree height and $DBH_{1.3m}$ at different age were used to calculate assortment classes. Assortment outcome forecast was made using Donis (2009) improved Ozoliņš (2002) designed assortment algorithm.

Trees were divided into five assortment classes by their diameter according to Poljanec and Kadunc (2013), as described in Table 2. Analysis of economic value of experimental beech stands was based on beech wood prices used in Slovakia where beech is one of the tree species with great economically importance (Sisák, 2013). Experimental stands were joined into three groups according to their age: 1) 58, 2) 101, and 3) 115 years (including one stand, hat was measured also at the age of 110 years).

Results and Discussion

Three age groups of European beech (*Fagus sylvatica* L.) were analyzed in the study, to determine financial value of timber base on potential assortment structure (Tab. 2). For comparison National Forest Inventory (NFI) data on the most widespread broadleaved tree species in Latvia – birch (*Betula pendula* and *Betula pubescens*) were used. Its dimensions in stand on fertile mineral soils with

Table 2

Assortment classes by diameter at the top end and current monetary value

Assortment[a]	Length, m	Beech assortments[b]		Broadleaved tree assortments[c]	
		diameter at the top end, cm	EUR m^{-3}	diameter at the top end, cm	EUR m^{-3}
Sawlogs I	3	≥ 44	150	≥ 26	70
Sawlogs II	3	38-43	100	21-25	65
Sawlogs III	3	30-37	64	18-20	60
Industrial wood	3	16-29	50	14-17	40
Firewood	3	6-15	41	6-13	20

[a] sawlogs I, II, III – large-sized, middle-sized and small-sized sawlogs, respectively;
[b] according to Poljanec and Kadunc (2013);
[c] according to data from Central Statistical Bureau (CSB) of Latvia (http://www.csb.gov.lv/).

Table 3

Assortment structure and income of beech

Stand age, years	Average income, EUR ha^{-1}	Net present value, EUR ha^{-1}	Proportion of assortments*, %				
			Sawlogs I	Sawlogs II	Sawlogs III	Industrial wood	Firewood
Estimation according to beech wood in Slovakia (Sisák, 2013)							
58	7939	1423	0	0	0	56	32
101	8513	430	0	6	25	48	10
115	9109	304	0	9	35	39	6
Estimation according to broadleaved tree wood in Latvia (http://www.csb.gov.lv/)							
58	5602	1009	0	0	96	2	1
101	6592	333	44	28	8	5	4
115	7109	237	54	22	4	5	3

*Assortments are described in Table 2.

normal moisture regime (as in beech stands) at the age 58 years (roughly 10 years before the final harvest) were: mean breast height diameter 25.3 cm, yield 248.8 m^3 ha^{-1}, mean height 25.6 m, basal area 21 m^2 ha^{-1}. In beech stand at the same age mean breast height diameter was slightly (not significantly) smaller – 23.9 cm, standing volume, due to high density – almost double that in birch stands, reaching 505 m^3 ha^{-1}, mean height slightly lower, reaching 23 m, basal area 43.6 m^2 ha^{-1}. In 101 years old beech stand mean diameter was 38.4 cm, but at the age 115 years 41.1 cm, also standing volume in beech stand was slightly increasing with age and reached 538 and 568 m^3 ha^{-1}, respectively, indicating a notable reduction of number of trees. Average tree height in older beech stands reached 30.4 m; consequently, basal area was slightly lower than in younger stand, reaching 37 m^2 ha^{-1} at the age of 101 years and 39 m^2 ha^{-1} at the age of 115 years.

Even in the oldest stand included in analysis sawlog I dimensions (top-diameter for a log 44 cm) had not been reached (Tab. 3). Also proportion of sawlog II assortments, presented in oldest stands (101 and 115 years) was small: 6% to 9%, respectively. Proportion of saw-logs III assortment in the oldest stands ranged from 25% to 48%, consequently three times lower portion of firewood was obtained than in younger (58 years) stands.

Calculated income from beech stands changed with its age: at the age of 58 years income from sales of timber assortments was 7939 EUR ha^{-1}, at the age of 101 years (only 33m^3 more) – 8513 EUR ha^{-1}, but at the age of 115 years – 9108 EUR ha^{-1}. It is worth reminding, that incomes from potential commercial thinnings are not included in the analysis. Reverse trend was observed for the net present value changes with stand age – it was highest for youngest stands. It can at least partly be explained by absence of assortments of highest value in oldest stands.

Such assortment was present, if the dimensions used for the broadleaved tree wood assortment in Latvia currently were applied. In this case at the age of 58 years 96% of the timber was sawlogs III (diameter at the top end 18-20 cm). Most of the trees reached the broadleaved saw-log dimensions at the age of 101 and 115 years: 44 to 54 % of first and second class sawlogs, respectively.

Comparison of the timber value in birch and beech stand, considering the dimensions and volume as well as price differences, reveal, that financial value of beech stands at the age of 58 years is notably higher than that of birch stands. However, for neither of the tree species in our calculations the optimal management had been applied. Recent study in Latvia (not published) found, that in birch stand with low initial spacing financial value as high as 9900 EUR ha^{-1} can be reached already at the age of 40 years. Valkonen & Valsta (2001) created birch economical models by cost and returns in mixed stands with spruce (no discounted value), and average net return varied from 1400 to 1900 EUR ha^{-1}.

Considering the assortment structure Tullus *et al.* (2012) modeled, that at the age of 60 years on abandoned agricultural land in Estonia most of birch wood would be pulpwood (46 – 49%), but in older stands playwood and energy-wood. It indicates, that for birch at the age of 58 years, similarly to beech in oldest stands in our assessment, the dimensions are still too small (sub-optimal) for final harvest, if the largest income in this operation is the goal.

In our study according to broadleaved tree dimensions, most of the assortments in beech stand at the age 58 were saw-logs III, that would represent a notably higher value than a pulpwood, suggesting a financial reason for use of this tree species in Latvia.

Net present value with interest rate of 3% in birch stands in Estonia was 1064 – 1104 EUR ha^{-1} (Tullus *et al*., 2012; Niskanen; 1999). In Latvia, Dudelis (2013) assessed the financial value of birch stands at the age of 60, 80 and 100 years. At the discount rate 3 % the author achieved the values of 441, 532, 619 EUR ha^{-1}, respectively. The estimates are comparable to those for beech stands in our study. In both cases value of admixture of other tree species in stand or branch quality were not considered. It has to be noted, that also possibility of occurrence of red heartwood for beech was not considered. It can significantly affect the financial value of beech timber. Richter (2001) found, that in North Rhine-Westphalia value loss of beech timber due to red heartwood reached as much 5.1 million EUR. Tree breeding might be required to avoid or improve quality traits as it has been demonstrated for other tree species in Latvia (Jansons *et al*., 2006, 2015b; Jansons, 2008).

Large dimensions are required for beech to obtain the highest financial gain; the value is increasing until the diameter of the trees reaches approximately 60 cm. However, it is not reasonable to wait much longer, since the probability of formation of read heartwood for such large trees is high and the accumulated value can be lost (Poljanec & Kadunc, 2013). Tree species comparison studies (Knoke, 2002; Knoke & Seifert, 2008) reveal, that incomes from Norway spruce are stable, but in long term European beech may be profitable. Other study, carried out in Czech Republic (Aubrecht *et al*., 2016), had reported higher average timber price for Norway spruce (83 EUR m^{-3}) than for beech (59 EUR m^{-3}); however, such result might be influenced by different management regimes – higher initial stand density and thus larger amount of firewood obtained from the beech stands.

Conclusions

Mean height and breast height diameter of beech at the age of 58 years were similar to birch in fertile soils, but basal area and yield were notably higher. Monetary value of beech stand at the age of 101 year reached 8513 EUR ha^{-1}, but net present value (r = 3%) was 430 EUR ha^{-1}; if the beech wood would have to be sold as broadleaved saw timber its value at the same age would be notably lower: 6592 EUR ha^{-1}, but net present value 333 EUR ha^{-1}.

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INTRA-SEASONAL DEVELOPMENT OF RADIAL INCREMENT OF *PICEA ABIES* IN LATVIA

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Abstract
Norway spruce (*Picea abies* (L.) H.Karst.) is amongst the most important tree species for forestry in Latvia. It has been suggested that due to the foreseen climate change the productivity of Norway spruce in Latvia may decrease. Continuous observations of radial increment allow to identify periods with different growth intensity and to study the effect of environmental conditions on radial increment during them. The aim of this study was to analyse stem radial variation of Norway spruce in mixed-species stand in response to meteorological conditions over one growing season. Stem radial variation of one Norway spruce tree was monitored by band dendrometer throughout the growing season of 2013. Cumulative radial increment was divided into contraction, recovery and increment phases by the stem cycle approach. Four periods with distinct apportionment of these phases were identified – winter/spring dormancy, spring/summer growth, growth termination and autumn hydration fluctuations. Radial increment began in the second part of May and the most active increment was observed during period of spring/summer growth. This period lasted for 42 days with the mean amplitude of stem radial variations reaching 0.06 mm while the length of increment phase reached up to two days. Throughout the growing season increment was facilitated by an increase in the temperature. Meanwhile, the effect of precipitation was insignificant, presumably due to sufficient water availability and low interspecies competition for it, characteristic in mixed species stands due to differences in depth and distribution of root systems between the species.
Key words: Norway spruceband dendrometer, stem cycle approach, stem radial variation, dendroclimatology.

Introduction

Adaptation of forestry to the foreseen climate change scenarios has been considered as one the greatest challenges for modern day forestry (Lindner *et al.*, 2014). In this regard, radial growth of trees and its relationship with climatic factors have been vastly studied in recent decades (Sheppard, 2010; Speer, 2010). However, majority of these studies have focused on tree rings and anatomic elements of xylem formed over a longer period of time (Zweifel *et al.*, 2006; Matisons & Brumelis, 2012; Senhofa *et al*., 2016). Meanwhile, the effect of climate on radial growth with a higher temporal resolution is less commonly studied, presumably due to a more complex data gathering, processing and analysis (Bouriaud *et al.*, 2005; Zweifel *et al.*, 2006; McMahon & Parker, 2015). Nevertheless, findings in the studies of a high temporal resolution favour to understand ecophysiological processes affecting the tree radial growth (Deslauriers, Rossi, & Anfodillo, 2007; Michelot *et al.*, 2012; Van der Maaten, Van der Maaten-Theunissen, & Spiecker, 2012; De Swaef *et al.*, 2015).

Band dendrometers are amongst the most widely used and accessible instruments to obtain continuous measurements on stem radial variation (SRV) (Deslauriers, Rossi, & Anfodillo, 2007; McMahon & Parker, 2015) that can been used to study radial growth, water status and transport, nutrition, carbon relations, phenology of trees (De Swaef *et al.*, 2015). According to Daudet *et al.* (2005), SRV is a result of several simultaneously co-acting mechanisms – reversible contraction and expansion of dead conducting tissues due to fluctuations in internal tensions, hydration induced reversible shrinkage and swelling of living tissues, irreversible radial growth and thermal expansion and contraction. Thus, the extraction of radial growth from the time series of stem radial variation is a crucial step for further analysis (Deslauriers, Rossi, & Anfodillo, 2007; De Swaef *et al.*, 2015) and has been the main reason for criticism of dendrometers (Makinen, Nojd, & Saranpaa, 2003). Nevertheless, several methods such as 'stem cycle approach' and 'daily approach' have been elaborated to extract growth signal from the time series of SRV (Downes, Beadle, & Worledge, 1999; Deslauriers, Rossi, & Anfodillo, 2007; Deslauriers *et al.*, 2011).

While traditional forestry tends to focus on single species stands, ecological modelling has shown that stands with several species coexisting in complementary niches may be more productive due to a higher use of the available resources (Loreau & Hector, 2001; Morin *et al.*, 2011). Such mixed species stands are also believed to be less vulnerable to uncharacteristic and extreme weather events caused by climate change due to spatial and temporal differences of limiting factors of coexisting species (Shanin *et al.*, 2013).

Norway spruce is the third most widely used tree species in forestry in Latvia covering 18% of all forest area (State Forest Service of Latvia, 2017) and is commonly used in single species stands. The aim of the study was to assess the intra-annual SRV and the effect of meteorological parameters on SRV of Norway spruce growing in mixed species stand over one growing season.

Materials and Methods

Throughout the growing season of 2013, SRV was monitored for one 60 years old Norway spruce tree located in a mixed-stand with black alder (*Alnus glutinosa* (L.) Gaertn.), Scots pine (*Pinus sylvestris* L.) and common aspen (*Populus tremula* L.) on wet and nutrient rich peat soil in north-west Latvia (57°40' N lat., 22°19' E long.). The forest type corresponded to *Filipendulosa* (Bušs, 1976). In the study area, climatic conditions are affected by dominant oceanic air masses, providing this area with relatively mild weather throughout the whole year where 30-year mean air temperatures in July and February range from +15.2 to -3.6 °C, respectively (Draveniece, 2007). Meteorological parameters were monitored on-site by the meteorological station Wireless Vantage Pro2 (Davis Instruments). In order to calculate the vapour pressure deficit (VPD), saturation vapour pressure (e_s) was found:

$$e_s = 6.11 \times \exp^{\frac{LRv(1273-1T)}{}}, \quad (1)$$

where L is the latent heat of vaporization (2.5× 106 J kg^{-1}), Rv is the gas constant for water vapour (461 J K^{-1} kg^{-1}). Afterwards, calculation of VPD was done as:

$$VPD = e_s \frac{100 - RH}{100}, \quad (2)$$

where RH is relative humidity.

Monitoring of SRV started on the 113th day of the year (DOY 113) (23 April) and continued for 195 days (Fig. 1, (C)). Stem radial variation was measured hourly by automatic band dendrometer DRL26C (EMS Brno, Czech Republic) which was placed 130 cm above the ground. To reduce the impact of hygroscopic swelling and shrinkage, a partly loosened outermost layer of bark was removed before installing of dendrometer. Time series of cumulative SRV was divided into contraction (C), recovery (R) and increment (I) phases by the stem cycle approach (Downes, Beadle, & Worledge, 1999; Deslauriers *et al.*, 2003). This method defines: C as a period between the first maximum of stem radius and consecutive minimum; R as a period from minimum until the point where previous maximum has been reached or until the beginning of the next contraction phase; I as a period until the next maximum resulting in either positive or negative value, depending on whether or not the previous maximum has been exceeded (Deslauriers *et al*., 2003; Vieira *et al*., 2013). Cycle consisted of

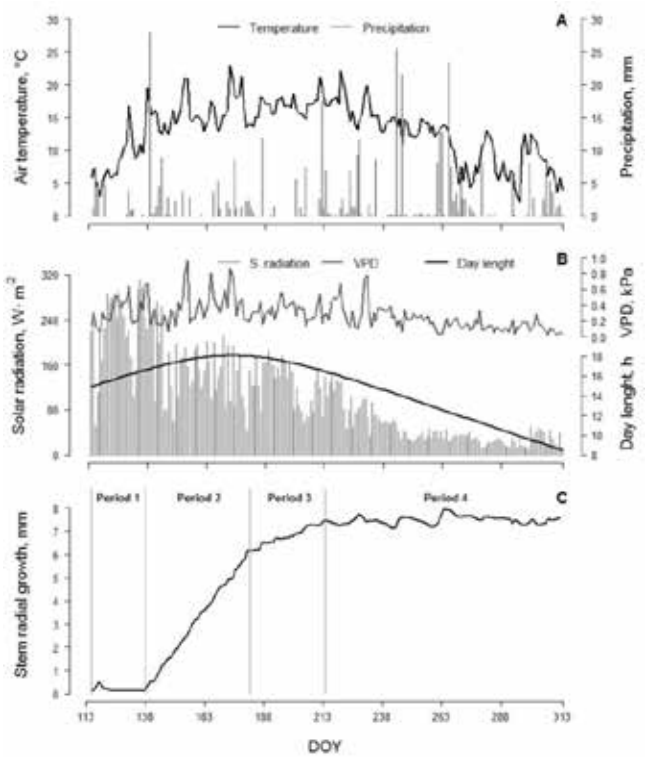

Figure 1. **(A)** Mean diurnal air temperature (black line) and precipitation sum (bars), **(B)** solar radiation (bars), VPD (grey line) and daytime length (black line), **(C)** stem radial variation per day of year (DOY). Vertical lines in **(C)** separate distinct periods of increment: Period 1 – winter/spring dormancy; Period 2 – spring/ summer growth; Period 3 – growth termination; Period 4 – autumn hydration fluctuations.

phase C followed by phase R and of phase I, in case the increment occurred before the next contraction. For each cycle duration and amplitude of variation was calculated. The amplitude of SRV and phase I were used as criteria to divide the series of cumulative SRV in four periods - winter/spring dormancy (P1), spring/summer growth (P2), growth termination (P3) and autumn hydration fluctuations (P4). These periods were determined as follows: P1 – as a period before the beginning of continuous positive increment; P2 – a period from the beginning of continuous positive increment until spring/summer maximum (the point after which increment continued notably slower); P3 from the end of P2 until SRV started to fluctuate greatly but with a relatively insignificant increment; P4 as a period of the greatest fluctuations in SRV but almost no increment. Besides the aforementioned criteria, in the stem cycle approach the exact division between periods depends also on expert opinion (Vieira *et al*., 2013), thus complicating a precise replication of the study. Nevertheless, the impact of expert opinion cannot significantly alter the results.

Pearson`s correlation analysis was performed to assess the effect of meteorological conditions on SRV at different phases and separately for each period. For the correlation analysis, hourly maximum and minimum air temperature, sum of precipitation, VPD and solar radiation were used. To avoid the misleading effect of missing data and different lengths of periods of SRV on results of statistical tests, correlation analysis was validated using bootstrap procedure with 10'000 iterations (Vieira *et al.*, 2013). All steps of data analysis were carried out using statistical package R 3.3.2. (R Core Team, 2013). Its package 'dendrometeR' was used (Van der Maaten *et al*., 2016) to apply the stem cycle approach.

Results and Discussion

Seasonal and diurnal SRV

SRV followed clear seasonal pattern showing notable differences in amplitude and duration of phases within each period (Fig. 1 C). During the P1, only slight fluctuations in SVR were present, except for the abrupt swelling and shrinkage observed at the very beginning of SVR monitoring. This extrinsic pattern might be caused by the sudden increase followed by decrease in temperature (Fig. 1 A), which may have led to the release of water from the living tissues as part of physiological mechanism to avoid intracellular freezing (Ameglio, Cochard, & Ewers, 2001; Charrier, Cochard, & Ameglio, 2013). A positive radial increment started at DOY 138, marking the start of P2. Within P2, cumulative curve of SRV followed a relatively sharp and steady increment with just minor (in terms of duration and amplitude) phases C and R (Fig. 1 A; Fig. 3). P2 lasted for 58 days and the mean amplitude of stem radial variation during it reached 0.06 mm while the length of phase I reached up to two days. Transition between P2 and P3 occurred in the second part of June, but was not as obvious as transition between the first two periods. During P3, the increment trend of cumulative curve of SRV started flattening due to decrease in duration and amplitude of phase I (Fig. 3 C & F) and increase of duration of phases R and C (Fig. 3 A, B, D & F). P4 started in the beginning of August, and during it, the greatest amplitude of all three phases and almost even apportionment of duration of these phases was observed, resulting in the most notable fluctuations in cumulative curve of SRV. The maximum stem diameter was observed at the end of September and can be attributed to rehydration of stem in response to replenishment of soil water content rather than radial growth (Deslauriers, Rossi, & Anfodillo, 2007).

Contrary to the results of studies on intra-annual SRV of trees growing in drought prone environment (Vieira *et al.*, 2013), in our study the increment continued to increase since the start of P2, while notable shrinkage of stem at late summer was not observed. An increase of temperature in late summer and its cumulative effect leads to decline in soil water content, while the transpiration demand increases with temperature and daytime length (Herzog, Hasler, & Thum, 1995). Thus, to minimize the risk of hydraulic failure the transpiration demand is reduced by stomatal closure, resulting in lower metabolic activity and, consequently, decline of radial growth (Zweifel *et al.*, 2006; King *et al.*, 2013). Apparently, in our study site water availability was sufficient throughout the whole growing season not imposing tree to enter quiescence. Low values of VPD and an increase in precipitation during P3 and P4 correspond to this assumption (Fig. 1 A & B).

Similarly as in the study of Vieira *et al.* (2013), diurnal fluctuations in SRV followed a rather similar trend during all four periods (Fig. 2). The highest value of stem diameter was observed from 4 till 9 AM, with the peak value earlier in the morning in P2 and P3 which were characterized with a higher daytime length. While, due to internal transport of water from storage to conductive tissues to meet the transpiration demand (Herzog, Hasler, & Thum, 1995; King *et al.*, 2013), stem diameter was the lowest during the middle of the day and started to replenish in the evening. Maximum mean diurnal amplitude of SRV (0.06 mm) was observed in P2.

Response of SRV to meteorological parameters

Response of amplitude and duration of SRV to meteorological parameters differed between phases and periods (Fig. 4). Contrary to the findings of Vieira *et al.* (2013), climatic signal in duration was

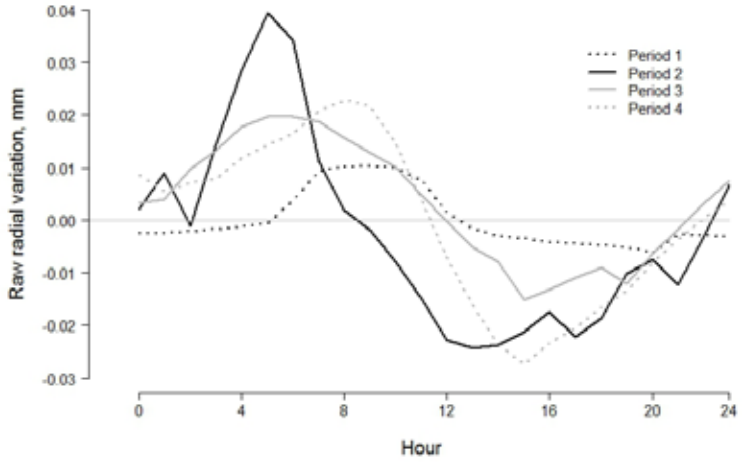

Figure 2. Diurnal cycle of stem radial variation in each period.

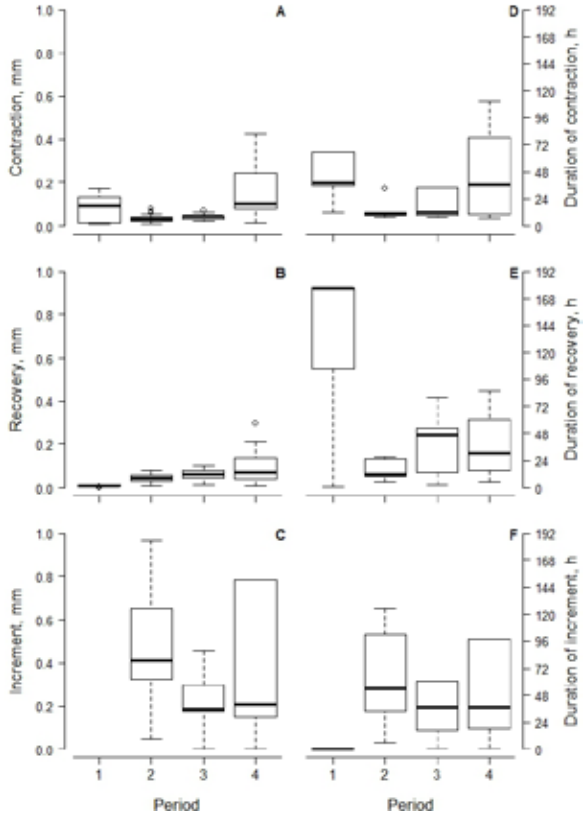

Figure 3. Amplitude (A, B, C) and duration (D, E, F) of stem radial variation per phase (contraction – A and D; recovery – B and E; increment – C and F). Bold line represents the median, box corresponds to lower and upper quartile, whiskers show minimum and maximum values (within 150% of interquartile range from median and outliers are shown by circles).

more clearly expressed than in amplitude of SRV phases.

During P1, a strong negative correlation was observed between the amplitude and duration of C and both maximum and minimum temperatures, while the temperature had a positive influence on duration of R. Such results might be explained by rehydration of stem before the beginning of growth that can be observed for trees in cold environment (Deslauriers *et al.*, 2003). In P1, this phenomena was clearly expressed during the abrupt swelling and shrinkage at the very beginning of SRV monitoring when the temperature fluctuated around the average minimum threshold (4 – 5 °C) for xylogenesis reported by Rossi

et al. (2008). However, after this abrupt swelling and shrinkage notable rehydration was not observed until the P2.

Duration of C showed a negative correlation with temperature also in P2, P3 and P4, while the amplitude of C had a negative correlation with temperature in P3 and P4. These results can be explained by above-mentioned diurnal cycle of internal water transport between the tissues to meet the transpiration demand during the warmest part of the day (Herzog, Hasler, & Thum, 1995; King *et al.*, 2013). The negative correlation between solar radiation and both amplitude and duration of C in P4 might be explained similarly, considering the strong relationship between solar radiation and temperature, especially in autumn when the daytime length is the shortest (Fig. 1 B).

Except for the amplitude in P3, the increment in terms of duration and amplitude was facilitated by both temperature parameters and solar radiation in all periods in which increment was observed (P2, P3 and P4), and this relationship was the strongest in P4. The positive effect of temperature on increment can be explained by the direct influence of temperature on assimilation and meristematic activity, e.g., cell division, differentiation and elongation, while solar radiation directly facilitates photosynthetic activity (Pallardy, 2008).

Moisture requirements of Norway spruce are relatively high, and a positive influence of increase in summer precipitation on radial growth of this species has been shown in numerous studies (Makinen *et al.*, 2002; Andreassen *et al.*, 2006; Koprowski & Zielski, 2006). However, in our study the influence of precipitation on amplitude and duration of I was negative in periods of the most active growth, e.g., P2 and P3. Furthermore, VPD positively influenced the amplitude and duration of I in both of these periods. Presumably, these results can be explained by sufficient soil moisture level in the sampling site throughout the season and possible negative effect of rise in moisture

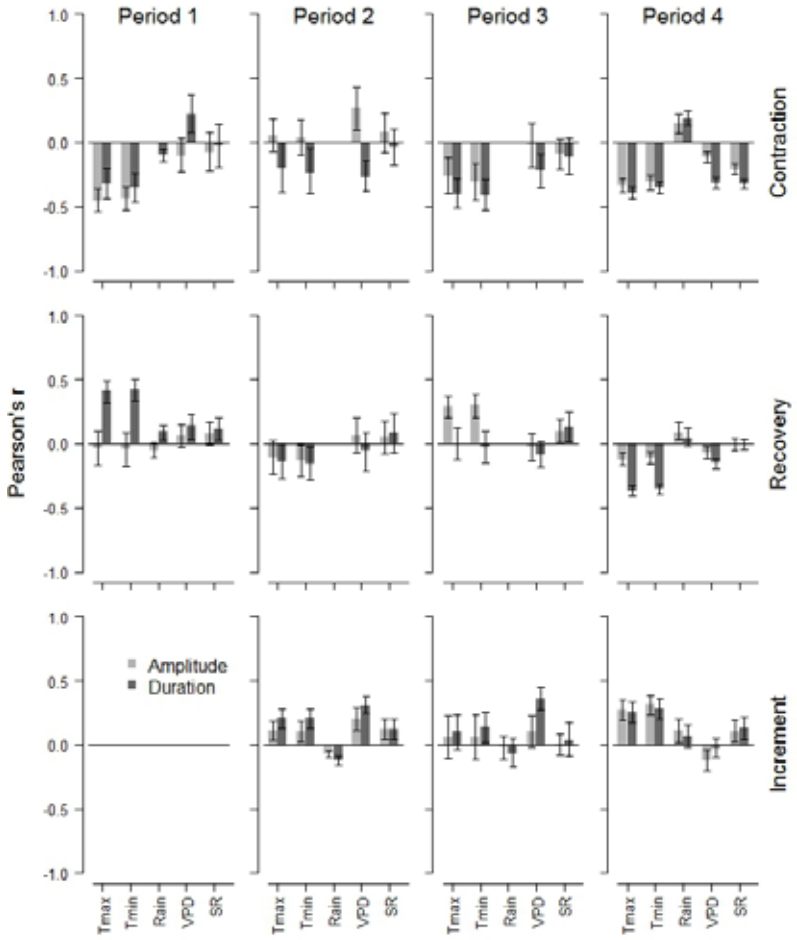

Figure 4. Bootstrapped correlations between both amplitude and duration of each phase of stem radial variation and meteorological parameters (Tmax, Tmin – maximum and minimum air temperature; Rain – sum of precipitation; VPD – vapour pressure deficit; SR – solar radiation). Correlations are significant ($p < 0.05$) if the confidence interval represented as vertical error bars does not include zero.

level caused by an increase in precipitation. In such conditions, water molecules may fill the capillary pores of soil, thus decreasing root respiration and water absorption that consequently lead to stomatal closure and reduced photosynthetic activity (Kozlowski, 1997; Parent *et al.*, 2008). VPD values during both of these periods, with a few exemptions, did not exceed 0.6 kPa (Fig. 1 B), meaning that the air was relatively saturated and did not cause notable transpiration (Oren *et al.*, 1999; Zweifel & Hasler, 2000). Furthermore, a lower competition between neighbouring trees for soil water due to differences in depth and distribution of root systems between species, may have facilitated the independence of increment from precipitation of the studied tree. Nevertheless, more comprehensive studies involving several coexisting species, the monitoring of sap flow and soil moisture level over several years are needed to properly address this question.

Climatic signal in R during P2 and P3 was less pronounced than in I and C. Sufficient soil moisture level throughout the monitoring of SRV, meaning that water resources did not limit the recovery, could be the reason for such results. While similar climatic signal in R and C during P4 might be related to interdependency between them, e.g., better rehydration leading to a higher possible contraction and *vice versa* (Vieira *et al*., 2013).

Conclusions

1. SRV monitoring revealed four distinct periods of fluctuations in and increment of stem radius of Norway spruce over one growing season. Presumably, a sufficient soil moisture level accompanied with a low competition for it between neighbouring trees of different species allowed for the studied tree to maintain the radial increment throughout most of the SRV monitoring.
2. The factors mentioned above might have decreased the dependency of the radial increment of the studied tree on precipitation during the growing season, which has been previously reported for Norway spruce.
3. The increment was facilitated by an increase in the temperature, indicating that in case of sufficient soil moisture the increment of Norway spruce could benefit from rising temperatures throughout the growing season. While artificial raising of soil moisture level might not be cost-efficient, our results indicate a potential for wider use of Norway spruce in mixed-species stands where due to contemporary niches of different species the negative affect of decrease in precipitation on productivity of this species might be lower.

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EVALUATION OF PRODUCTIVITY AND IMPACT ON SOIL OF TRACKED PROSILVA F2/2 FORWARDER IN FOREST THINNING

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Abstract
Tracked forwarders is one of the solutions for the forest soils with poor bearing capacity providing ability to use well-known technology for reasonable cost to improve accessibility of forest resources. Tracked forwarders are not common in forest nowadays and only a few models are produced serially. The ProSilva F2/2 is a new middle-class forwarder, whose frontal tracks are replaced with big tires, ensuring large contact surface, increased mobility of the machine and significantly reduced mass without loosing the load capacity (15 tonnes). The aim of the study was to evaluate productivity, soil impact and cost of the roundwood forwarding using the ProSilva F2/2. The study was conducted in Finland. Productivity (time per crane cycle, split into operations and loads), load size, depth of ruts and soil compaction were estimated during the study.
According to the study results, the average productivity is 16 m^3 per hour (driving distance to a landing site 200 m, driving speed 2.8 km h^{-1}, average load 9.7 m^3). The productivity can be significantly increased by more efficient utilization of the load capacity and by using gripper with the tilt function. The length of ruts on peat soil (56 m ha^{-1}) do not exceed the permitted threshold values in Latvia. The compaction of soil took place mainly in topsoil, no changes in soil density were observed in deeper soil layers. The prime cost of forwarding according to the preliminary estimates is 2.5 € m^{-3} if the annual production is at least 62000 m^3. Utilization rate is important to keep forwarding cost low.
Key words: tracked forwarder, productivity, forest thinning.

Introduction

According to the National Forest Inventory (NFI) data, the forest area in Latvia is 3.2 mill. ha, including 0.4 mill. ha of forests on drained organic soils and 0.3 mill. ha of forests on wet organic soils. Extreme forwarding conditions according to the national classification system can be an issue in 10 % of the stands and bad forwarding conditions – in 33% of the stands. Reduction of the mature forest area on dry and drained mineral soils will worsen the forwarding conditions in future and will raise a question about significant changes in harvesting methods and selection of appropriate machinery in forestry to access the forest resources in areas with extreme forwarding conditions.

According to the Joint Stock Company 'Latvia's state forests' (JSC) data, bad and extreme forwarding conditions (estimated as a share of extracted roundwood) reached 28% in 2016 and it had increased by 2% in comparison to 2015. Communication with the experts from JSC confirms that the share of the bad and extreme forwarding conditions will increase in future.

According to the NFI data about all felling sites where more than 10 m^3 ha^{-1} have been extracted during the last 5 years, extreme conditions can be an issue in 4% of stands and bad conditions – in 35% of stands. These numbers do not include potentially bad or extreme forwarding conditions between a stand and roadside. Similar results (4% of extreme conditions and 38% of bad conditions) were obtained if the clear-felling sites were evaluated separately. This result proves that the situation in private forests is considerably worse, especially in the share of bad forwarding conditions, than in state forests.

If extrapolated to the total forest area, the extreme forwarding conditions can be an issue in 10% of stands and bad forwarding conditions – in 33% of the forest stands. It means that in future forwarding conditions will worsen.

Damage to roots and stems, which is the primary result of bad or extreme forwarding conditions, can lead to fungal infection which can cause wood discoloration or even decay. The water regime and nutrition conditions in the forest soil can change as a result of soil settling (Ring *et al.*, 2015). The operation of forest machines is therefore avoided during the period of high soil compaction or rut formation risk, and the harvesting is usually postponed to the winter when soil is normally frozen in extreme conditions. It is estimated that the seasonal variation in timber procurement can cause considerable losses; in Finland alone these losses are approximately 100 M € annually (Pennanen & Mäkelä, 2003). Forest operations in poor bearing conditions increase time and fuel consumption and decrease the efficiency of harvesting operations (Sirén *et al.*, 2013).

Furthermore, deep ruts affect the general acceptability of the forest operations. The costs caused by extreme forwarding conditions could be decreased by additional information on soil conditions, especially soil bearing capacity. The load bearing capacity of soil is often described by its penetration resistance. Accordingly, forest operations could be planned to be performed during adequate bearing capacity or routed to avoid sections of poor

bearing capacity, thus minimizing the damage and maximizing the efficiency of harvesting. Another alternative is use of tracked forwarders with a considerably smaller impact on soil in comparison to conventional wheeled forwarders (Edlund, Bergsten, & Löfgren, 2016; Lupikis, Kaleja, & Lazdins, 2015; Cambi *et al.*, 2016).

The most popular forwarder model produced by ProSilva company up to now has been the fully tracked 15-4ST, which has been produced for more than 10 years. The 15-4ST forwarder is one of the largest and, at the same time, one of the most gentle forest machines, which can be used both for final felling and thinning on soils with low bearing capacity. In Latvia ProSilva 15-4 ST forwarder has been used both for thinning and final felling. It mostly transports wood from harvesting areas with long delivery roads, impassable for wheeled tractors, therefore forwarding costs of ProSilva are relatively high. The main advantages of ProSilva 15-4ST are stability on soils with low bearing capacity, which exceeds indicators of a standard wheeled forwarder several times, as well as the high load capacity (15 tonnes), which allows to reduce cumulative pressure on soil due to the reduction of number of passes in the same area and better distributed weight (Kaleja, Lazdins, & Prindulis, 2015).

Studies on productivity and costs of ProSilva 15-4 ST were conducted in 2014, where this tractor was compared with the mid-sized forwarder John Deere 810, equipped with chains on all axles or rear axles. In this study, the average time consumption for loading was 60 minutes, for unloading – 29 minutes, driving time – 17 minutes (the forwarding distance was 850 m), the average load – 8 tonnes (9.6 m^3). Evaluation of the prime cost of forwarding proved that ProSilva forwarder is an economically viable option in areas with extreme forwarding conditions, and it is crucial that the main strip-road is not damaged by wheeled forwarders before it is used by the ProSilva (Kalēja, Lazdins, & Zimelis, 2014b). The main drawback of ProSilva forwarder identified in the study was higher purchase costs. The main work method related issue was forwarding of assortments one by one, accordingly, the operators transported 1 or 2 assortments at once, thus considerably increasing the driving time and as a consequence – the impact on soil. Mixing of assortments in load is key to increasing productivity and reducing impact on soil using high capacity forwarders for thinning (Kalēja, Lazdins, & Zimelis, 2014a).

In 2015, repeated trials with ProSilva 15-4 ST forwarder were conducted in a collaboration with 'Serviss Betta' Ltd. The aim of the trials was to investigate the actual fuel consumption of the forwarder and load size. Studies revealed that fuel consumption of the forwarder ProSilva 15-4 ST does not exceed that of wheeled tractors with analogue load capacity (14 L per hour on average). Under extreme conditions, when the wheeled tractor created ruts after a few drives, the tracked one could extract 5 times more timber before rut formation. It was highlighted in this study that ProSilva 15 4ST with a standard frame is unable to load 2 rows of 3 m long logs. Thus, if the dominant length of logs is 3 m, the forwarder's capacity is always used insufficiently (Lazdiņš & Zimelis, 2015).

In 2015 ProSilva company developed a modified forwarder prototype, the front chains of which were replaced with a pair of big tyres (width 75 cm, height 150 cm). The purpose of chain replacement was to decrease machine weight, as well as to improve its manoeuvrability and efficiency in long driving distances. Increased pressure on soil due to replacement tracks is compensated by large tyre surface and hydraulic balancing system, which allows to equalize pressure on front and rear axles as well as pressure on both sides of the machine. In order to increase track usage time, thickness of tracks is increased. Applications of the new forwarder model have not been studied so far; whereas productivity and influence on soil of the tracked model was assessed in several studies in Finland and Latvia, positioning this forwarder as currently the most advanced solution for the forwarding on organic soils. The scope of the study is to evaluate if the productivity, impact on soil and cost of the roundwood forwarding using the semi-tracked ProSilva F2/2 forwarder are sufficient for commercial application in Latvia in extreme forwarding conditions.

Materials and Methods

The study was conducted in 3 forest stands in Finland (LKS92 coordinates of the stand centroids 582477.135,926606.244; 582634.384,926143.535 and 582093.954,926369.467). The total area of experimental sites was 5.4 ha, the average tree height – 13.3-14.0 m, growing stock – 108-129, dominant tree species – spruce and birch. The tracked ProSilva forwarder model F2/2 with large wheels on the front axle was tested in the study. Detailed specifications of the machine are not yet available. Indicative specifications are provided in Table 1. In comparison to a fully tracked model, this forwarder is lighter, faster and more manoeuvring.

Detailed time studies split the work time into 15 operations (Table 2). Load size was determined according to the operator's estimate. Additional information obtained was reason for breaks, applied service works etc. Time study did not include preparation for work, which took about 1 hour. Duration of shifts was 8 hours.

Table 1

ProSilva forwarder specifications

No.	Parameter	Values
1.	Dimensions	Length 9317 mm, width 2800 mm, clearance 702 mm, load capacity 15 tonnes, empty weight about 20 tonnes
2.	Hydraulic system	Sensors to avoid overloading, engine capacity 190 cm^3, hydrostatic transmission
3.	Engine	Iveco N67 ENT, capacity 175 kW, torque 1020 Nm at 1400 rpm
4.	Loading space	Lenght 4000 mm, loading area 4.5 m^2
5.	Crane	Kesla 800T
6.	Tracks	Rear – 75 cm wide tracks with rounded edges to improve adherence, front 75 cm wide tyres with chains

Table 2

Work operations in productivity studies

Type of operation	No	Operation	Explanation
Productive time	1.	Drive in	Drive into the stand without load
	2.	Reach logs	Reach logs with crane
	3.	Grip	Grip logs with fork
	4.	Load in	Load logs into loading space
	5.	Rearrange load	Rearrange logs in loading space, put back fallen logs
	6.	Drive during loading in	Driving in stand during loading
	7.	Prepare road	Putting slash into strip-roads
	8.	Drive out	Driving out from the stand with load
	9.	Reach during loading out	Reaching logs with crane during loading out
	10.	Grip during loading out	Gripping of logs during loading out in loading space
	11.	Load out	Putting logs into assortments pile
	12.	Rearrange assortments	Rearranging of landing yard (lining up of tops, putting fallen logs into pile etc.)
	13.	Drive in landing area	Driving in landing area during loading out
	14.	Other operations	Non-conventional operations (small repairs and service, checking driving conditions etc.)
Other work time	15.	Break	Time spent for non-work operations like eating, smoking etc., excluding longer breaks like serious repairs when engine is off

Harvesting was done with the tracked ProSilva S6 harvester. The number of produced assortments per stand – 2-3, mainly pulp wood, biofuel and small logs. Length of logs – 3-5 m. Harvesting residues were left dispersed in the stand except wet areas where they were put into strip roads. Time studies were done for 2 operators who both had experience in thinning, but limited experience with tracked machines carefully using load capacity of the machine. Experienced operators would work with larger loads.

Weather conditions during the study were good, the average temperature – 20-25 °C. Short rain falls (12 mm in total) were observed from 28 to 30 of June; heavy rain falls took place 2 weeks before the study (49 mm during 3 days).

The study was implemented in 2 sites with heavy forwarding conditions according to the contractor's decision: a stony site, where the rain did not affect forwarding, and peat soil (depth of peat more than 1 m), where topsoil after raining was wet and bearing capacity considerably reduced. The relative soil moisture (0 – 10 cm layer) in peatland was 47% during the studies.

Prime cost estimates are based on earlier studies (Lazdiņš, Liepiņš, & Zimelis, 2008; Lazdiņš & Zimelis, 2015). Operational costs of the ProSilva cannot be estimated because the machine was in a prototype stage during the trial, therefore the operational costs estimated earlier for a fully tracked version were used in the calculation (Kalēja & Lazdiņš, 2014; Kalēja

et al., 2014; Lazdiņš *et al.*, 2016). Fuel consumption was determined using the machine accounting system. Salaries were assumed according to the average salary range of the forest machine operators published by the State Tax Service. It was assumed in the calculation that the forwarder would work for 20000 hours.

Results and Discussion

The total amount extracted in the study was 145 m^3. The average load was 9.1 m^3, significantly bigger loads (9.7 m^3) in peatland. The productive time per load in peatland was 36 minutes and in the stony site it was twice more (75 minutes). The average driving speed was 2.8 km h^{-1}. The average productivity in peatland was 2.9 min. m^{-3}. Due to the relatively high proportion of time spent to pick and move assortments into load (46% of the total loading time in comparison to 32% using tractor equipped with the tilt function, Lazdiņš *et al.*, 2016), it was concluded that considerable increase of productivity can be reached by using tilt function in the crane so that logs can be moved in vertical position. Smaller dimension of logs in the stony site considerably decreased the productivity of loading. The main productivity figures are provided in Table 3.

Assuming that forwarder is taking only full loads (12 m^3 according to the conditions in experimental sites), productivity in peatland would increase by 4% and in stony site – by 12%. In real conditions the increase of productivity could be higher with full loads because of reduced time consumption for driving. Load capacity of the machine is about 15 m^3. In Latvia the most of assortments produced in thinning are pulpwood (3 m), firewood (2 to 3 m) and small logs (up to 4.2 m). Considering the structure of assortments, it is reasonable to increase the tractor frame length to 5 m to be able to load 2 parcels of short (up to 3 m) assortments.

The number of mechanically damaged remaining trees is considerably high (4.9% on average in both sites) and corresponds to the average data obtained in Latvia in early thinning (Kaleja, Lazdins, & Prindulis, 2015). The number of stem damages can be reduced by using the tilt function during forwarding, by more careful harvesting and by putting slash into strip-roads in the turning areas. Length of ruts in peatland in extreme forwarding conditions is 56 m ha^{-1}, in stony site – twice less.

The scope of measurement of the soil penetration resistance on peatland was to compare forwarding conditions with other trials implemented earlier in Latvia (Kalēja & Lazdiņš, 2014; Prindulis, Kalēja, & Lazdiņš, 2016). The soil penetration resistance in trials implemented in Latvia on soft soil with wheeled forwarder equipped with wide tracks was at topsoil level (11 – 20 cm) 1.1 to 1.3 MPa after forwarding of 5 loads (35 to 39 m^3), and the length of ruts did not exceed the limiting threshold values. The trials in Latvia were done in dry conditions, however it was considered that the conditions are not suitable for wheeled forwarder. In the study implemented in Finland, the soil penetration resistance on strip-roads on a peatland after forwarding of 4 to 5 loads was 0.26 MPa and did not increase significantly in deeper soil layers. It means that the soil penetration resistance in the study site on peatland is considerably smaller than in earlier studies in Latvia and the results are not actually comparable. But the relatively small amount of ruts still demonstrates that the ProSilva F2/2 is very efficient on peat soils. A significant impact on soil penetration resistance due to compaction was found in the stony area, however the compaction was found only in the upper 20 cm layer in contrast to the studies with wheeled forwarders, demonstrating considerable compaction in deeper soil layers – down to 80 cm (Prindulis, Kalēja, & Lazdiņš, 2016). It should be noted that measurement conditions were not favourable in the study in Finland (a lot of stones in soil), therefore the accuracy of measurement results in deeper soil layers may be affected by limited measurement data, and for accurate comparison the measurements should be repeated on stone free sites.

The new model of semi-tracked ProSilva forwarder is not yet in conventional use, therefore operational costs can only be assumed according to the results obtained with a fully tracked model, which are verified by operational costs of a tracked excavator in Latvia. Real operational costs will be available when the machines start to work in production. One of the most important cost positions is tracks, which can heavily affect the average hourly cost.

Forwarding costs at 200 m forwarding distance and 4320 productive work hours per year are 2.5 € m^{-3} (over bark), the total costs of harvesting, forwarding and road transport to 50 km distance are 14 € m^{-3}

Table 3

The main productivity figures

Study site	Loading in, m^3 per productive hour	Loading out, m^3 per productive hour	Productivity, m^3 per productive hour	Share of productive time	Loads per productive hour
Stony area	11	42	6	82%	0.8
Peatland	28	82	16	83%	1.7

Table 4

Logging costs in specified conditions in peatland

Parameter	Harvester	Forwarder	Roundwood truck
Annual cost, €			
Investments	€ 85 679	€ 56 723	€ 30 464
Personnel	€ 60 928	€ 40 516	€ 33 204
Operational costs	€ 122 129	€ 67 338	€ 72 900
Profit margin	€ 13 437	€ 8 229	€ 6 828
Total	€ 282 173	€ 172 805	€ 143 395
Productivity			
Productivity, m³ per productive hour	8.5	16.0	9.1
Annual production, m³	44364	69162	32475
Cost			
Roundwood under-bark, € m^{-3}	€ 7.07	€ 2.50	€ 4.42

(Table 4). Lifetime and replacement costs of tracks are taken from a heavy (16 tonnes) excavator. The ProSilva F2/2 forwarder can extract 69 thousand m^3 of roundwoovārds pārvietotsd annually. The productivity of forwarder is about 30% higher in comparison to harvester productivity in stands with similar dimensions of extracted trees according to earlier studies in Latvia (Kaleja & Lazdins, 2014; Skudra *et al.*, 2015); thus, in thinning the harvester and forwarder can work as a pair and ProSilva forwarder can simultaneously do additional tasks, such as forwarding of roundwood from intermediate storages over soils with weak bearing capacity. In the final felling the difference in productivity will decrease.

The sensitivity analysis of forwarding costs includes forwarding distance, load size, fuel consumption, fuel price, forwarding distance and number of productive hours per year. Forwarding distance has a considerable impact on the costs – if the forwarding distance increases to 1200 m, forwarding cost increases to 5.42 € m^{-3} (2.2 times). An increase of forwarding distance would have an even bigger impact in bad forwarding conditions when the forwarder should prepare the road and drive slowly. The situation when a tracked forwarder is used on a road with deep ruts left by a wheeled forwarder would have an even more harmful effect on forwarding cost. Size of load also has a significant impact on forwarding cost, especially when the forwarding distance is increasing. If the average load size increased to 11 m^3, forwarding cost would decrease to 2.27 € m^{-3}; if the load size decreased to 5 m^3, forwarding cost would increase to 4.54 € m^{-3} (by 45% in comparison to average results in the study). Fuel consumption and fuel price (considering the range of fuel prices during last 2 years) have a relatively small impact on the forwarding cost. The most significant impact on it has the number of productive hours per year. If the number of productive time decreases below 1300 hours, forwarding cost increases by 2.5

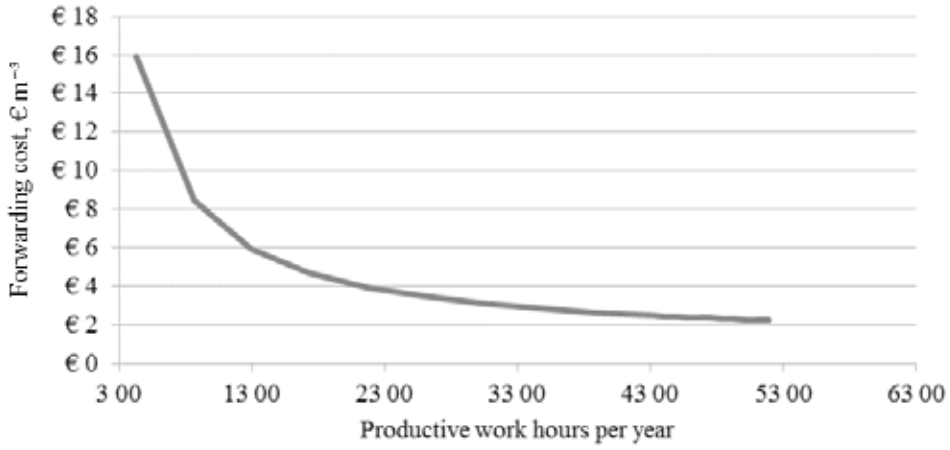

Figure 1. Sensitivity analysis of forwarding cost depending on utilization rate.

times, and rapidly grows if the number of productive hours continues to decrease (Figure 1). In practice, the increase of forwarding cost due to the reduction of productive time might not be so steep, because the calculation assumes that operators keep a constant salary regardless of the worked hours. However, if the reason for reduction of productive time is service, forwarding cost can increase even steeper.

There is limited experience with the ProSilva F2/2 forwarder to compare forwarding cost with the conventional wheeled machines, especially because the wearing of tracks, as well as schedule and costs of other service operations, are not yet known. The study proves that the machine can be used in extreme forwarding conditions where conventional wheeled machines cannot work at all, and productivity figures are as good as for a conventional wheeled machine in normal forwarding conditions. This means that the machine can be competitive if the operational costs are not too high and the machine is utilized to full extent. The optimal utilization rate is 4000 hours (or at least 2000) hours annually.

The estimates of prime costs of forwarding, obtained in the study, should be considered as preliminary information due to the fact that operational costs and durability of the machine were not evaluated in real life conditions during the study period. Therefore, the economic assessment, including prime costs, should be evaluated in separate studies, when more production data are available. The most critical issues are the wearing of tracks, average load size and time spent on maintenance and repairs.

Conclusions

1. The length of ruts even in extreme conditions normally harvested only when the soil is frozen do not exceed the threshold values for thinning in Latvia. In many cases, the reason for the formation of ruts was an inappropriate operation of the tracked harvester, which often drove off the strip road, thus damaging soil and stems of the remaining trees in the turning places, and putting slash into small piles in the centre of strip-road instead of putting them below tracks.
2. The annual productive time has the most significant impact on the forwarding cost, therefore planning of utilization of the machine is very important. The optimal number of working hours annually is 4000, minimum is about 2000 hours. Alternative uses, for example, ash spreading on peat soils, should be considered in order to increase the machine utilization rate.
3. According to the study results, the ProSilva F2/2 forwarder is suitable for extraction of biofuel and roundwood in extreme forwarding conditions on a peat soil, where wheeled forwarders cannot be used even with auxiliary tracks on all axles. To increase the load capacity, forwarder with a prolonged frame (5 m) should be used in Latvia instead of one with a standard frame.

Acknowledgements

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THE IMPACT OF FEED ROLLERS ON THE QUALITY OF TIMBER IN THE MANUFACTURING OF POSTS

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Abstract
The aim of the study was to determine the impact of the harvester cutting head feed roller type on the mechanical damages of processed roundwood timber in post manufacturing. The study presents the comparison of the gentle Moipu plate wheel rollers and conventional feed rollers having bigger spikes (hereinafter named - conventional feed rollers). The total amount of segments processed with the gentle Moipu plate wheel rollers was 2199, whereas the amount of segments processed using the conventional feed rollers was 5308. When processing roundwood with the gentle feed rollers, in the 1st flow 8.2% of timber was rejected as substandard due to defects. The corresponding figure in the 2nd flow was 5.2% of the material in which the damage in the form studs was caused by the feed rollers. When processing the timber with conventional feed rollers, the percentage of the damage – dents caused by these rollers in the 1st flow was 26.7%, in the 2nd flow – 43.7% and in the 3rd flow only 2.7%. Using the method of characteristics proportion comparison, significant differences have been observed between feed rollers ($t = 17.8 > t_{0.05;\infty} = 1.96$).
Key words: feed roller, timber damages, harvester.

Introduction

The damages to roundwood timber caused by feed rollers were already observed when the first harvesters were used in 1970. The modern harvesting machine - harvester - divides the stem into segments with a high precision and speed, but damages to wood caused by the feed rollers or delimbing knives are considered to be a side effect. The harvester head is fitted with a cutting mechanism and feed rollers, which can also carry out the the measuring operation. During the process of bucking and delimbing, the stem is pressed between the feed rollers when it is passed forward. As a result, damages in the form of dents occur in the bark and wood (Karaszewski *et al*., 2016; Nuutinen *et al*., 2010; Sowa, Gieralowiec, & Gaj-Gieralowiec, 2013; Uusitalo *et al*., 2000)**.**

Based on the data of 2006 obtained from the Finnish research institute, the annual allowable volume of felling by a harvester is 50.8 mil m^3 which is 98% of the entire volume of the processed wood. In Latvia, it is possible to compare the previous year's parameters and average harvesting volume of 84% (final felling, commercial thinning, cutting of damaged trees). Harvesters are most intensively used in the final fellings – 97%. Having such harvesting volumes it is important to choose the most suitable feed rollers, and to evaluate this parameter the following factors should be taken into consideration: work productivity, costs, efficiency and damages to roundwood.

The dents caused by feed rollers lead to blue stain in timber which is located in felling sites or the consumers' yards. Apart from the blue stain, the dents caused by the feed rollers also reduce the yield of the end product in the wood processing enterprises.

The studies on feed rollers and the possible solutions to the problems caused by them had been initiated since harvesters were introduced. Today the most popular on the market are the feed rollers made of metal. They have a higher throughput capacity, as a result of which the work productivity is higher compared to those feed rollers which are made of rubber (Nuutinen *et al*., 2010). In Scandinavia, the following types of feed rollers are widely studied: 'Ponsse taper-foot feed rollers', 'Mense', 'LogMax flexi-wheel', 'ponsse limited-pivot plates', 'John Deere V-type', 'Moipu' (Brunberg *et al*., 2007; Gerasimov, Seliverstov, & Syunev 2012; Nuutinen *et al*., 2010).

The aim of the study was to determine the impact of the harvester cutting head feed roller type on the mechanical damages of processed roundwood timber in post manufacturing.

Materials and Methods

Experimental harvesting tests were carried out in Vidusdaugava forestry using Ponsse Ergo fitted with the harvester cutting head H7. Using the gentle feed rollers, 19 felling sites with a total area of 18.69 ha were harvested (the average area of the site was 0.98). Using conventional feed rollers, 15 felling sites with a total area of 20.29 ha were harvested (the average area of the site was 1.35 ha); the distribution of species in the stands was similar. Logging was done from May 27, 2016 to June 30, 2017. Comparing harvester productivity figures to rainfall does not have a significant effect on technical productivity.

To carry out the research, spruce and pine roundwood timber was used, which had the entire sawn plane of the top part of the stem marked with paint. This type of marking is necessary to differentiate the timber processed with feed rollers of different construction types.

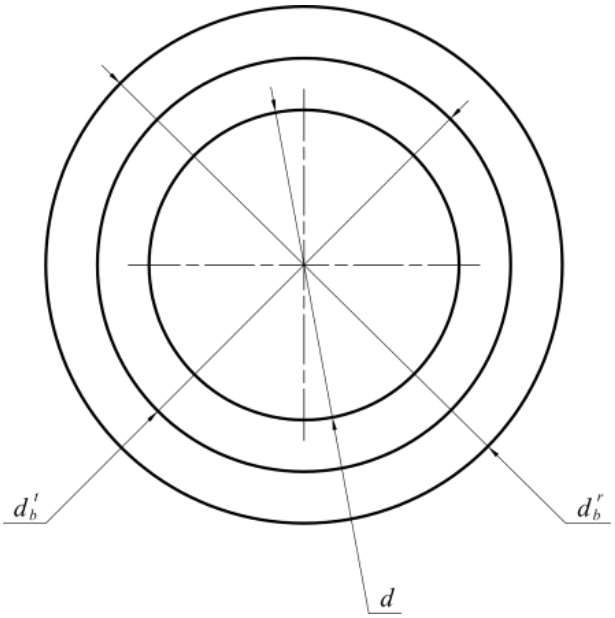

Figure 1. Roundwood timber milling scheme.
(d_b^t – roundwood timber top stem part diameter; – post diameter;
d_b^r – roundwood timber butt end diameter).

The total volume of roundwood timber processed with gentle and conventional feed rollers delivered to the enterprise for manufacturing posts was 138 m^3. In total, delivered and processed with the gentle feed rollers were 28 m^3 (2 199 segments) but with conventional feed rollers – 110 m^3 (5 308 segments), which were divided into the processing diameter classes.

In the enterprise, the roundwood timber (Figure 1) with the average diameter of the top of the stem of 70, 80, 95 and 120 mm is used for the manufacturing of posts, taking into account a 2 cm overmeasure in relation to the diameter of the end product. Consequently, all 4 diameter groups were checked within the framework of the study, but from the different lengths only those were selected in which a significant impact of the dents resulted in by the feed rollers on the quality of posts can be observed. Based on the information rendered by the enterprise, the most serious damages were observed on the posts manufactured from 3.3 m long roundwood timber. Thus, the roundwood timber of the appropriate diameter of the top part of the stem having the length of 3.4 m was prepared for the study, taking into account 0.1m length of overmeasure.

The amount of the roundwood timber processed within the framework of the study was equalled to the amount the enterprise processes during one shift. Based on the information given by the enterprise, the average feed productivity of the roundwood timber processing technological flow is from 40 to 50 m^3 roundwood timber in one shift. Consequently, within the framework of the study, the required total volume of all 4 roundwood diameter groups processed with one construction type harvesting machine's feed rollers was asumed to be 40 m^3 which corresponds to the useful load of a roundwood vehicle with trailer. The processed roundwood which within the framework of the research was delivered to the enterprise by trucks, before further processing was piled in the sorting and storage yard in separate non-sorted stacks where they were sorted according to the diameter of the top part of the stem.

The roundwood, sorted according to the diameter of the top part of the stem, was forwarded to two post manufacturing technological flows located in buildings 11 and 16 (Figure 2) where identical post manufacturing technology was used. In building 11 the roundwood with stem top part diameter of 70 and 80 mm was processed, and in building 16 (Figure 2) the roundwood with the stem top part diameter of 95 and 120 mm was processed. When starting the manufacturing of posts in building 11, the roundwood was conveyed to the separating and feeding equipment 3, where it was separated in a |single mode and conveyed to the longitudinal conveyor 4 which in turn conveyed it through the turning milling machine 5 to the longitudinal conveyor 6. From the longitudinal conveyor 6 the roundwood was conveyed to the transverse coveyor 7 and then further to the longitudinal conveyor 8, which fed it into the second turning and milling machine 9 (Figure 2), thus obtaining the required post diameter. The milled post templates were

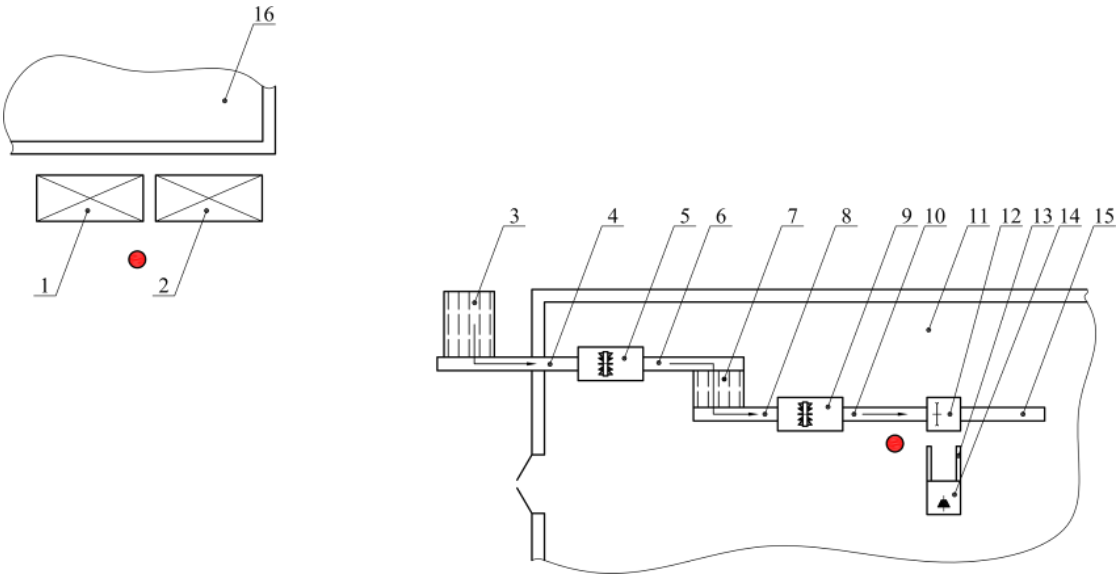

Figure 2. Technological scheme of the roundwood processing areas of an enterprise
(1,2 – rejected stacks of posts; 3 – roundwood timber separation and forwarding/conveying equipment; 4, 6, 8, 10, 15 – longitudinal conveyors; 5, 9 – turning milling machines; 7 – transverse transporter; 11 – building of the 1 st technological flow; 12 – cross cutting circular saw; 13 – the scaffolding bridge for loading bucked posts; 14 – cone shaping milling machine; 16. – building of the 2 nd technological flow; ● – evaluation positions of the impact of the dents made by the feed rollers of forest harvesting machines on the posts)

evaluated on the longitudinal conveyor 10 (Figure 2) to make sure that the respective template was suitable for further processing. If deep damages – dents - were found on the post templates, these posts were discarded from the technological flow and delivered further for storage in stacks 1 and 2 (Figure 2). The suitable post templates were bucked with a crosscuting circular saw 12 according to the nominal length of the posts and these bucked segments of the posts were forwarded to the scaffold bridge 13 from where the end cone was carried out with a milling machine 14 (Figure 2).

The impact of the dents made by the feed rollers of forest harvesting machines on the posts was evaluated prior to the bucking operation in the storage places where the discarded post stacks were stored (in the positions marked with a symbol ● (Figure 2). The evaluation was carried out by visual observation of all the template generatrix surfaces of posts determining whether the dents were observed on the generatrix surface. The result was expressed according to the number of post templates. Evaluating the dents caused by the feed rollers, the existing quality requirements of post delivery were not followed.

The amount of the damaged posts in proportion to the total amount of posts, which is possible to process from the delivered volume of roundwood, η% in the framework of each diameter group was calculated using the following formula:

$$\eta = \frac{10^2 \cdot \sum M_{bi}}{\sum M_i}, \quad (1)$$

where: $\sum M_{bi}$ – number of damaged posts of diameter group
$\sum Z_i$ – total possible number of posts of diameter group.

Economic aventages due to the use of gentle feed rollers were calculated by the enterpreneur on the basis of comparison of rejected logs, reduction of volume of logs and cost of repeated processing of poles. Production costs, as well as costs of the feedstock are considered as sensitive information by the enterpreneur and are not included in the article.

Results and Discussion

During the processing stage the recording was carried out in which the information was reflected on the amount of the processed and damaged products according to the types of the feed rollers (Table 1). Additionally, the recording of ‘other causes’ (other than feed rollers, such as bark and insect damages, etc.) concerning the damages to the product was carried out. In further calculations the roundwood damaged due to other causes was not separately distinguished. It was added to the total processed amount of product (Table)

Table 1

Total amount of roundwood used in production

Type of feed rollers	Flow	Damages caused by feed rollers, pieces	Products manufactured, pieces
Gentle feed rollers	1	181	2018
Gentle feed rollers	2	9	165
Gentle feed rollers	3	-	4
Conventional feed rollers	1	1416	3892
Conventional feed rollers	2	482	622
Conventional feed rollers	3	16	571
Conventional feed rollers	4	1	0

When processing the roundwood with gentle feed rollers in the 1st flow (the flow indicates the number of repetitions of how many times the material is repeatedly milled until it meets the quality requirements of the consumer) 8.2% was rejected and in the 2nd flow 5.2% of the material was rejected due to the dents - damages caused by the feed rollers. Processing the material produced with the conventional feed rollers resulted in 26.7% of logs rejected as defective in the 1st flow due to dents made by the feed rollers , 43.7% – in the 2nd flow and 2.7% – in 3rd flow. Consequently, 26.7% of the material had to be processed twice and 11.7% – three times and 0.3% was rejected completely at the end. A small number of rejected logs after the 3rd processing actually means that the most of the dents are shallower than thickness of stem layer removed during the processing.

Repeated reduction of diameter of poles during processing considerably reduces their value and results in additional production costs. In order to find out the economic advantages due to use of gentle feed rollers, the losses for the milled material have been calculated repeatedly. With the increase in the diameter of roundwood "posts" dimension within the range of 85-100 mm, the dents caused by harvester feed rollers increase significantly, irrespective of the type of the feed roller (Figure 3). The task of the feed rollers is to control and to move timber through delimbing knives, eliminating the possibility of their sliding out or getting serious dents (Leszczy, Przyrodniczy, & Ogrodniczych, 2014). Analysing the amount of the rejected products, it was found that conventional feed rollers cause more significant losses since their impact can still be observed after the third milling phase (Figure 4). Using the method of characteristics proportion comparison, significant differences were observed between the feed rollers ($t = 17.8 > t_{0.05;\infty} = 1.96$).

Analysing the total delivered volume after processing (Table 2) with the gentle feed rollers, the expected yield of the product is 17.24 m^3, whereas

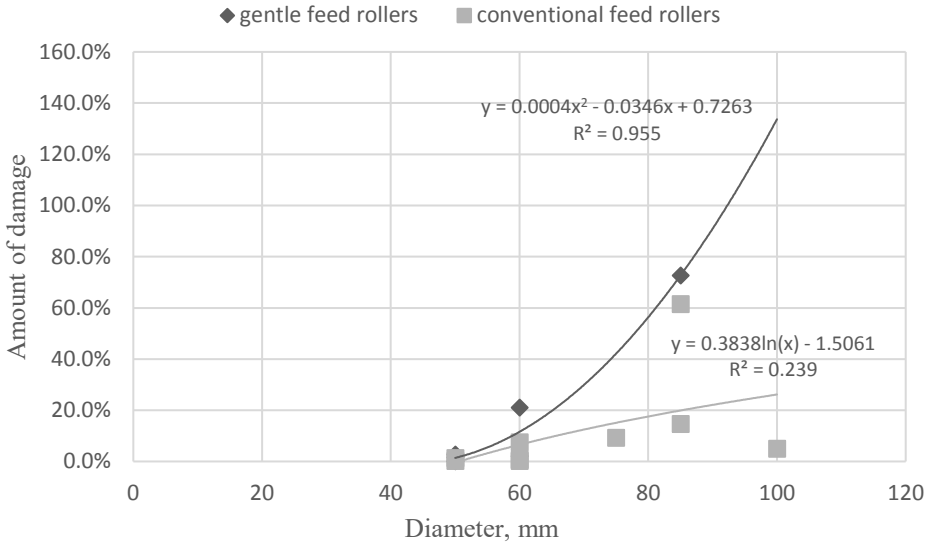

Figure 3. Changes of dents caused by feed rollers depending on diameter.

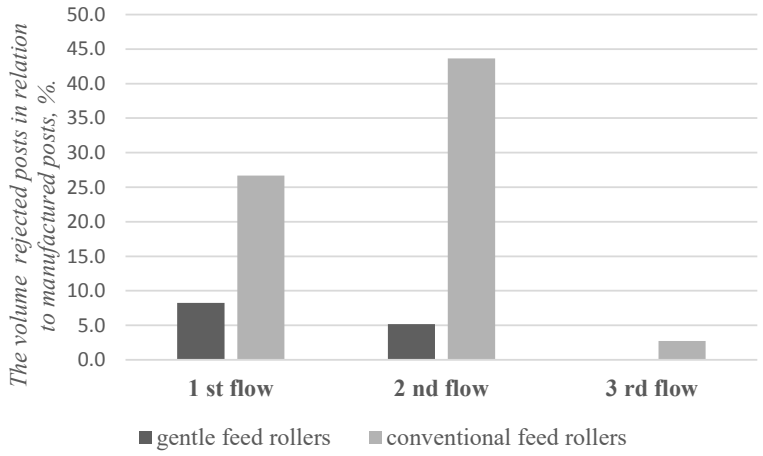

Figure 4. Breakdown of proportion of rejected materials by flows.

Table 2

Total volume of roundwood used in production

Type of rollers	Flow	Damages caused by feed rollers, m^3	Products manufactured, m^3
Gentle feed rollers	1	1.84 (± 0.86)	15.40
Gentle feed rollers	2	0.04	0.89
Gentle feed rollers	3	-	0.02
Conventional feed rollers	1	15.96 (± 6.44)	35.56
Conventional feed rollers	2	4.93 (± 1.51)	5.90
Conventional feed rollers	3	0.09	3.53

using the conventional feed rollers it is expected to be as high as 52.25 m^3.

When applying gentle feed rollers, the total lost volume of wood in the process of milling was 1.84 (± 0.86) m^3, but when conventional feed rollers were used, the corresponding figure was 15.96 (± 35.56) m^3. This amount was later used in the production of chips.

The loss was calculated based on the volume of roundwood timber of JSC 'Latvijas Valsts meži' delivered in 2015. Comparing the gentle feed rollers with the conventional ones, the loss in terms of money was 6.53 EUR per m^3.

Conclusions

1. As a result of the study, a significant impact of gentle feed rollers on the proportion of the rejected material in the manufacturing process of posts was stated – in the harvesting process with gentle feed rollers the amount of rejected posts after the first milling cycle was 8.2%, but for the material which was processed with conventional feed rollers, the amount of rejected posts was 26.7%
2. The most significant damages were to the posts within the diameter range of 85 – 110 mm.
3. Forest prepared amount of wood to the processing plant products are prepared by 59%, which points to the need to modernize the overall production process.

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COMPARISON OF PRODUCTIVITY OF VIMEK HARVESTER IN BIRCH PLANTATION AND YOUNG CONIFEROUS STANDS

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Abstract
The aim of the study was to compare the productivity and logging costs using the small class harvester Vimek 404 T5 in thinning of birch plantations and young coniferous stands. It was found in the study that due to a lower fuel consumption, maintenance costs and initial investment, the cost of a working hour of the harvester Vimek 404 T5 is significantly smaller than the cost of a working hour of the middle class harvester. The study confirmed that Vimek 404 T5 is a suitable harvester for thinning in a birch plantation; the study also confirmed the previous conclusions about the main advantages of Vimek harvester compared to a middle class harvester in the first thinning – low fuel consumption and costs of maintenance, high productivity and equipment compactness, which allow to reduce the strip road area. The average productivity for Vimek 404 T5 in birch plantation was 6.2 m^3 per productive hour, but in young conifer stand the productivity was 4.9 m^3 per productive hour. The average stem volume in the birch plantation was 0.06 m^3, but in conifer stand 0.05 m^3. Productivity for the same dimension trees in the birch plantation is not significantly different from the data obtained in the thinning of the coniferous stands.
Key words: productivity, Vimek 404 T5 harvester, thinning.

Introduction

Vimek 404 T5 is one of the smallest serially produced harvesters which is available in the market (Lazdiņš *et al*., 2016). This forest machinery is at least two times lighter than a middle class harvester (4.4 tons versus 10…12 tons). To work for JSC 'Latvia state forests' (LVM) as a service provider, the harvester measuring system must comply with StanForD-standard (Räsänen *et al*., 2010).

Only few studies on small forest machinery are implemented in the Nordic and Baltic region. A study in Sweden compared a conventional forest machinery with the smallest class forest machinery. The aim of the research was to determine economic gain in the first and second thinning, and also the impact on the remaining trees – how they react to wind and how the health condition changes in the stand. Leaving more trees on the strip roads in the first thinning when working with Vimek results in a bigger economic gain in the second thinning, which can be carried out with a middle class harvester (Jonsson, 2014). No differences were found in this study regarding forest health and wind damages in further years.

Using small machinery in the forest operations leads to a lesser number of damaged trees, which is approved by research carried out in Sweden. The study analyzed productivity, but it also addressed damages to the remaining trees and the possibilities of increasing the quality of the operation. The main advantages of small harvester are improved maneuverability and operator's location closer to the processed trees, which allows a better control of the felling and bucking while preserving the remaining trees.

In the studies of Latvian researchers in Sweden, it was found that forwarder Vimek 610 is more suitable in the early thinning than middle class forwarder. The forwarder used in the study was equipped with a tilt grapple which allows you to move logs in a vertical position, as it can be done with harvesters (Zimelis *et al*., 2016). The tilt crane allows loading of logs into forwarder following to the trajectory of the harvester crane.

In thinning the felling can be done in two ways, either with a chainsaw or harvester (Mederski, 2006). Working with Vimek 404 harvester secures a significantly better productivity than mechanized logging with chainsaw and, at the same time, without a significantly higher impact on the remaining stand (Zimelis *et al*., 2016).

An alternative for small class forest machinery is middle class forest machinery, equipped with a special grapple, which only allows production of biomass for energy. In theory, the advantages of these machines are higher productivity, but they require specialized strip road schemes and it results in a considerably smaller net income, because biofuel is still considerably less valuable in comparison to roundwood assortments (Bergström *et al*., 2010; Bergström *et al*., 2007).

The aim of the study was to compare the productivity and logging costs using small class harvester Vimek 404 T5 in the thinning of birch plantations.

Materials and Methods

Small class harvester Vimek 404 T5 was used in the study, which was carried out in LVM and JSC 'Latvijas Finieris' (LF) forests. Characteristics of the forest stands in LVM are provided in Table 1. LF stands were birch plantations located in the southwestern part of Latvia. Plantations were established on agricultural land where soil had been prepared before planting.

Table 1

Stand characteristics

Stand	Stand number	Average tree diameter, cm	Species	Forest type	Regeneration	Stand age, years	Area, ha
601-186-12	1	11.2	Pine	Mr	Artificial	37	3.4
602-28-19	2	14	Spruce	Mr	Artificial	26	1.9
602-32-8	3	8.9	Spruce	Dms	Artificial	39	1.3
711-358-5	4	8.8	Spruce	Vr	Artificial	26	3.5
601-186-16	5	16.2	Pine	Mr	Artificial	67	3.3
602-46-29	6	11	Spruce	Dm	Artificial	32	0.7
602-74-7	7	9.7	Birch	Vr	Natural	18	2.7

The average diameter of a tree – 11 cm; the number of trees – 1700 per ha.

During working process, the working time accounting was carried out with a specialized field computer Allegro II. The computer was equipped with a time study program SDI. Working time was split into 10 work elements and other operations (Table 2). Breaks and other activities that do not comply with the table set-time elements are described in notes. Time studies do not include preparatory work, which takes about 1 hour a day, but the working time records include moving part lubrication that is normally carried out during the shift. Time tracks were recorded in centiminutes (1min = 100 centiminutes).

The same operators of forest machinery were employed in both – young coniferous stands and birch plantations. Operators worked in 8 hours shifts.

In the birch plantations, two types of logs were produced – pulp wood and biofuel, which was determined by dominant tree dimensions in the plantation. Roundwood assortments in LVM stands were produced according to the company internal rules for roundwood production (JSC 'Latvia state forests', 2017). Biofuel in LVM stands was produced as firewood and partly delimbed small wood from tree tops. No residues were extracted for biofuel production, consequently, operators could leave residues distributed across the stand as far as they

Table 2

Working elements for time studies in a field work

Working time category	Working element numeration	Explanation
Information fields	1	Work cycle number
	2	Diameter of processed tree, d1.3, cm
	3	Number of processed trees per operation
	4	Felled half trunks
	5	Various notes, including brakes, travel, strip-road change etc.
Productive working time	6	Reaching for tree with crane
	7	Positioning of felling head
	8	Cutting of tree
	9	Delimbing and bucking
	10	Delimbing times (how many times trunk was dragged through delimbing knives)
	11	Log moving and stacking
	12	Undergrowth cutting
	13	Time spent on driving into a stand
	14	Time spent on leaving a stand
	15	Other non-standard operations, including machine maintenance
Unproductive time	16	Time spent on activities not related to harvesting

Table 3

Produced roundwwood volume (above bark) and characteristics of processed trees

Stand	Number of processed trees	Average diameter of processed tree, cm	Volume of processed trees, m^3	Average volume of processed tree, m^3
Plantation	1101	10 (± 1.33)	69	0.063
Naturally regenerated stand	2012	8 (± 3.25)	92	0.045
Artificially regenerated stand	11981	11 (± 4.47)	2600	0.085

were not making obstacles for piling of roundwood assortments.

In the birch plantations and young forest stands, the undergrowth trees with a diameter of less than 6 cm were left growing or felled down if they interfered with the harvester productivity, or a space for loading of logs had to be cleaned. Strip roads were organized asymmetrically and winding (bypassing the remaining target trees).

Air temperature during the study in the birch plantations ranged from 16 to 18 °C. Logging in the birch plantations took place from 12.09.2016 till 16.09.2016. Air temperature during the study in the young forest stands ranged from 6.5 to 24.9 °C. Logging in the young forest stands took place from 3.06.2016 till 15.08.2016.

Results and Discussion

Characteristics of the extracted trees and roundwood in the LVM and LF stands are given in Table 3. Stands are grouped by owners and regeneration type. In total, 46 stands were thinned in LF owned plantations. Detailed time studies were done in about 2 ha area.

Altogether, during the trials 16.8 ha were thinned by Vimek 404 T5 harvester. The proportion of felled trees divided by the diameter classes in each stand is provided in Fig. 1 and Fig. 2. Minimum diameter of the trees to be processed (delimbed and bucked) is set to 6 cm, based on the previous studies on impact of the tree diameter on the harvesting productivity (Lazdiņš *et al*., 2016). Smaller trees were felled mainly because they hindered productive work. Those small trees were felled and processed as biofuel or left intact. The dominant diameters in the group of extracted trees were from 8 to 14 cm.

The diameter distribution of extracted trees depending on the diameter of the average harvested tree in the birch plantation and coniferous forest stands is characterized by Weibull equation (parameters of the equation are provided in Table 4). This equation is used to model the prime cost of extraction depending on the average diameter of the extracted trees.

Productivity curve depending on the diameter of extracted trees can be characterized by polynomial equation (Error: Reference source not found). Comparing the results obtained in the birch plantation and forest stands, no statistically significant difference was found. In the birch plantations, the diameter of harvested trees did not exceed 20 cm, so, unlike to the young coniferous stands, no decrease of productivity was found while cutting trees with a diameter above 20 cm.

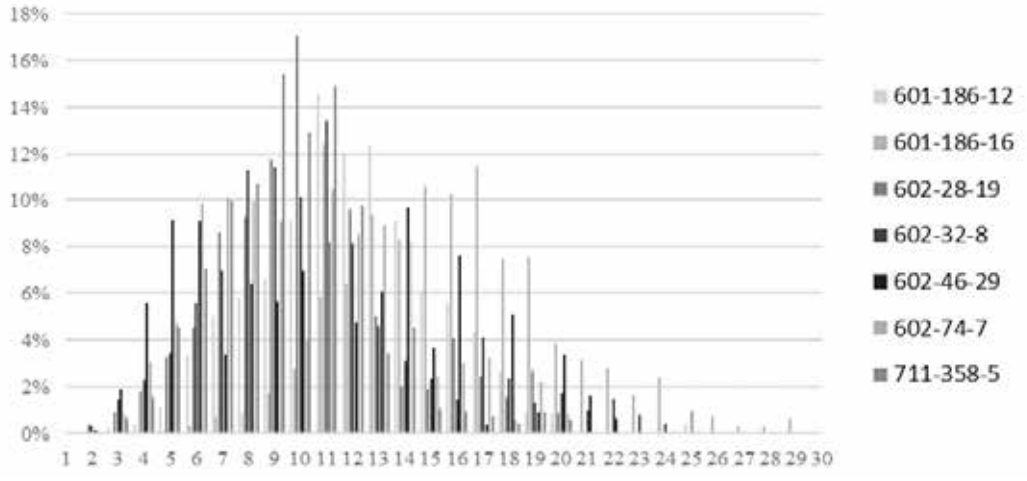

Figure 1. Diameter distribution of trees in thinned LVM stands.

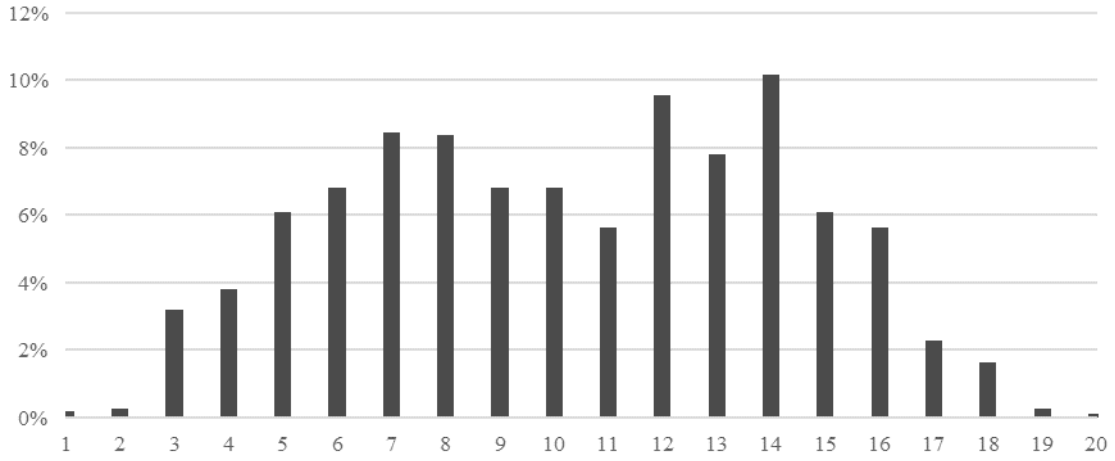

Figure 2. Diameter distribution of extracted trees in LF plantations.

Table 4

Parameters of Weibull

Equation parameters		Birch plantations	Conifer stands
Alfa	Inter	-1.93319	2.01004
	β	0.64682	0.14034
	β2	-0.02087	-0.00059
Beta	Inter	0.40971	0.19689
	D	1.08979	1.09419
Minimal diameter, cm		8	6
Maximal diameter, cm		20	18

While analyzing productivity data both in the young forest stands (Error: Reference source not found) and in the birch plantations (Fig. 5), it was observed that there is a significant increase in time consumption for a single tree processing, which is mainly determined by delimbing and bucking operations. On average, these processes take 51% of the productive time spent on a single tree processing. Delimbing and bucking of trunks in the diameter class till 5 cm consumes 36% from the whole tree processing time, in the diameter range from 6 to 9 cm it takes 45%, in diameter range from 10 to 13 cm it takes 48%, but if the tree diameter is above 14 cm delimbing and bucking time rapidly increases and reaches 64% of the total processing time. Time consumed to process a tree mainly depends on the feed roll speed and pulling force, which for small class harvester is not significantly changeable (Nilsoon, 1996).

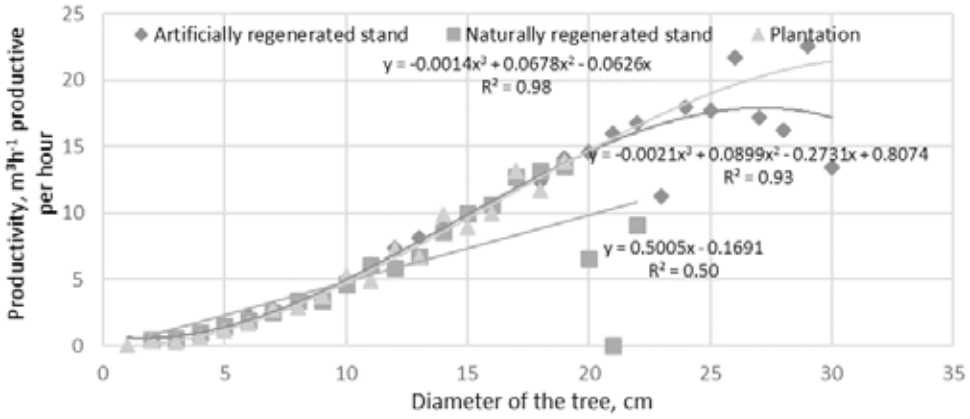

Figure 3. Harvesting productivity.

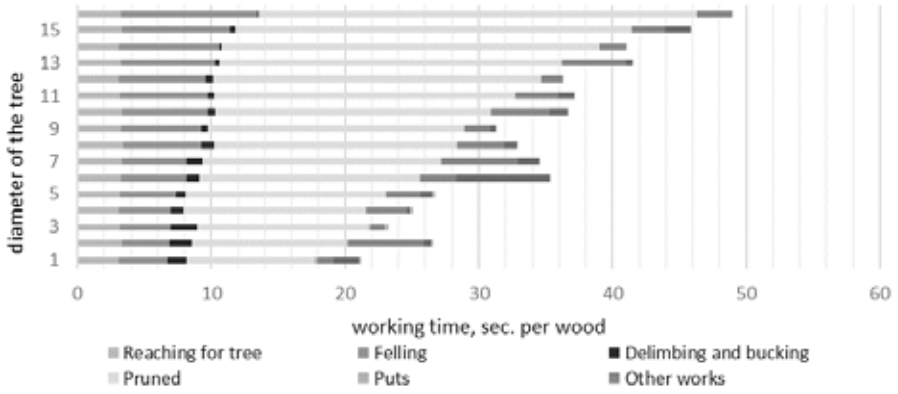

Figure 4. Distribution of work time consumed (sec. per processing of a single trunk) in different diameter classes in young coniferous stands.

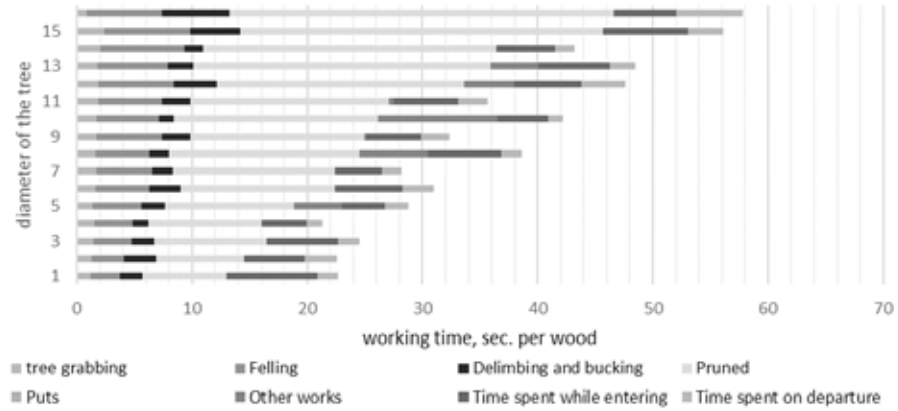

Figure 5. Distribution of work time consumed (sec. per processing of a single trunk) in different diameter classes in birch plantations.

Table 5

Average productivity and its distribution by diameter class

Stand type (regeneration type or plantation)	Diameter class, cm	A percentage of the total number of trees	Number of processed trees in productive working hour	Productive working time of total working time	Productivity, m³ in productive hour
Naturally regenerated	≥5	19%	151	93%	1.39
	6-9	54%	108	82%	3.15
	10-13	19%	81	61%	6.26
	14≤	8%	37	76%	5.90
	Average		94	78%	4.18
Plantation	≥5	14%	159	99%	0.88
	6-9	30%	132	99%	3.07
	10-13	30%	99	99%	6.66
	14≤	26%	65	99%	8.69
	Average		114	99%	4.82
Artificially regenerated	≥5	11%	114	77%	0.91
	6-9	32%	97	77%	3.10
	10-13	32%	83	77%	6.37
	14≤	25%	58	77%	10.65
	Average		88	77%	5.26

Summaries of productivity figures depending on the stand types and the diameter of extracted trees are provided in Table 5. In an artificially regenerated forest, the average productivity is 5.26 m^3 h^{-1} (min = 0.91, max = 10.56), in birch plantations 4.82 m^3 h^{-1} (min = 0.88, max = 8.69), in naturally regenerated stands – 4.18 m^3 h^{-1} (min = 1.39, max = 6.24). Increase in productivity can be observed in all types of the stands in the same diameter classes until the tree diameter reaches 15 cm. Productivity decrease can be observed in naturally regenerated stands, when cutting trees with a diameter above 14 cm.

The cost of a productive working hour of the Vimek harvester during the trials was 54 €, and average fuel consumption in trails was 4.5 L $hour^{-1}$. Logging costs, according to the average productivity figures in the artificially regenerated stands were 8.0 € m^{-3}, in naturally regenerated stands – 11.9 € m^3 and in birch plantations – 8.7 € m^{-3}.

Conclusions and Recommendations

1. The highest average productivity rate was achieved in the artificially regenerated stands – 5.26 m^3 h^{-1}. However, no statistically significant difference was found in the productivity of processing trees in the same diameter group. Therefore, the main factor affecting productivity and cost of harvesting is the diameter of extracted trees.
2. It is important to avoid processing of trees with a diameter below 6 cm to retain high productivity in thinning. Processing of small trees reduces the average productivity, however, further analysis is necessary to compare theoretical and practical possibilities to avoid processing of small trees.
3. The number of trees processed per working hour, which is a significant indicator of the harvester performance in thinning, ranged on average from 88 to 114. The highest number of trees processed per hour was in birch plantations – 114 trees. h^{-1} (min = 65, max = 159).

Acknowledgements

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QUALITY CHANGES DURING SUMMER – AUTUMN LONG TERM STORAGE OF BIRCH (*BETULA PENDULA*) ROUNDWOOD

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Abstract
The homogeneous and light wood of birch is suitable for different types of treatment processes and is highly valued for products where visual aspect is important, however, birch assortments are highly susceptible to damages caused by fungi during the storage. Such fungal damages cause discoloration of birch roundwood intended for further woodworking.
Sapwood discoloration development and its influencing factors for birch roundwood were analyzed in the summer - autumn period of the year 2016 (August, September and October). Control cut for assessment of sapwood discoloration development was made in four birch roundwood stacks in the districts of Auce and Daugavpils. In order to evaluate the influence of harvesting type, side surface damages, placement in pile, storage duration, diameter, distance from the end plane, daily average temperatures, precipitation amount and relative humidity on development of sapwood discoloration, 616 discs from birch log end planes were prepared.
After conducting multiple factor linear regression, it was concluded that a significant development of discoloration and its changes for birch roundwood are promoted by harvesting type, placement in pile, distance from the end plane, daily average temperatures and relative humidity ($R^2 = 0.450$). For birch logs that are prepared by harvester with the daily average air temperature + 6 °C, the first signs of discoloration appear after 17 – 18 days, but on all logs stacked in pile they appear after 68 – 69 days. Whereas, with the daily average temperature of + 18 °C, the first signs of discoloration can occur after only six days, but on all logs stacked in pile they appear after 23 days.
Key words: birch round assortments, storage, discoloration.

Introduction

Roundwood after logging is stored in the forest near the road from where it is later transported to the processing sites. Timber storage duration in such cases is uncertain both in the forest near the road and in the stowage area, so sometimes these materials remain vulnerable to various types of damage. Logs that are stored unprotected against different types of damage for a long time, remain susceptible to biological staining (Uzunovic *et al*., 2008).

During the summer, when storing birch logs, the main problem is sapwood fungal lesions that stain wood in brown colour, thus reducing the round timber quality. After logging, the sapwood colouring rises from the end surface inwards. The mechanical properties of both the conifer and birch wood are not affected, but the quality and economic value of lumber products significantly decrease. Colour changes may occur by oxidation when the air penetrates into the wood and reacts with the wood substance. One explanation is that the living parenchyma cells begin to produce substances that can react with oxygen (Corbo *et al*., 2001; Stahl *et al*., 2005).

Fungi and bacteria may also be involved in the sapwood staining processes. They have a major role in the staining processes of tree species sapwood (Shigo, 1965; Hallaksela & Niemisto, 1998). Mechanisms of sapwood coloring during the storage begin to work from the end plane and are not fully understood (Corbo *et al*., 2001; Stahl *et al*., 2005).

When storing birch and other tree species, staining of this kind occurs only in combination with slow drying of logs outdoors. In a similar way, during the storage other species - beech (*Fagus sylvatica*), maple (*Acer platanoides*), ash (*Fraxinus excelsior*), aspen (*Populus tremula*) – got stained (Nylinder *et al*., 2007).

Birch end surface sapwood discolouration may progress rapidly – by several hundred millimeters in just 2 weeks (Nylinder *et al*., 2001). Sapwood colour changes can be difficult to distinguish from the false heartwood, which is widespread birch defect appearing in the growth process.

The study that was made in Sweden (Jonsson, 2013) reveals birch and aspen sapwood discoloration trends, when storing logs in two different ways: standard – under natural conditions after felling, and when using irrigation. During the storage, the colouring increased for logs prepared in both winter and spring. Significantly more staining developed on birch logs compared to alder and aspen. There were no differences between logs prepared in winter and spring. Logs, which were sprinkled, showed less discoloration changes compared to the standard method of storage, especially after 12 weeks of storage.

The aim of this research was to determine the quality changes of birch (*Betula Pendula*) during summer–autumn long-term storage. When working with large volumes of roundwood, it requires constant wood forwarding and transport to the sawmills. This was the main reason for creating this study and getting an understanding on how to optimize the entire cycle from harvesting till the primary processing of the timber. In the future, this study may help to understand

the economic loss that a seller gets for every day that timber is stored for too long, thus failing to ensure the seller with maximum revenue from the log sales. Similarly, the results of the study can be used in practice by forest managers, harvester operators and timber logistics planners, who, knowing the factors that influence roundwood quality changes and their thresholds, can plan the technological process of logging and timber transport more accurately, avoiding (or minimizing) the quality reduction of roundwood.

Materials and Methods

Factors affecting birch round timber discoloration development were analysed in the study's third stage of the 2016 summer-autumn period (August, September and October). Control cuttings for determination of sapwood discoloration development were carried out in the open storages of birch round assortments in the Auce and Daugavpils regions.

In order to evaluate the preparation type, side surface damage type, placement in the storage, storage length, diameter, distance from the end plane, the daily average temperature, precipitation amount and relative humidity effects on discoloration development, 616 sample discs were prepared from the ends of birch round assortments.

Sample storage consists of 26 – 32 (on average 30) round assortments with 10 – 30 cm diameter and 1 meter length (see Fig.1). As it is known that sapwood discoloration and crooks are mainly developing from the end plane, 1 meter long assortments are enough to evaluate the quality changes from the round timber end planes.

The evaluation of round timber placement in the storages:

- 1 – 9 assortments are characterizing the quality changes at the bottom of the storage (round timber placement on the ground);
- 11 – 16 and 19 – 23 assortments are characterizing the quality changes in the middle of the storage;
- 10, 17, 18 and 24 – 30 assortments are characterizing the quality changes on the top of the storage.

In order to assess the variety of preparation ways, four timber storages were created simultaneously in each plot with different damages of side surface.

- Control – assortments without side surface damages.
- Damaged bark – assortments with the side surface of the bark bruised over the entire length.
- Harvester spike roller damages – assortments that have damages from harvester pike roller, but do not have bruised bark made by debarking knives.
- Harvester spike roller and bark damages – assortments with bruised bark and damaged side surface by harvester spike roller.

The inspection of birch round assortments was carried out at least once a week, but control cuttings started after 3 weeks of storage. In every control cutting, around 8-9 round assortments for determination of discoloration proportion were examined – 2-3 from the ground assortments, 2-3 from the middle and top assortments.

Discs of the assortment were sawn off in one survey, as shown in the scheme (Fig. 2). For the first disc on the outside, only sapwood was sawn 3-5 mm in depth, in order to assess the quality requirements of assortments at purchasing. Every subsequent disc was sawn in 50 mm.

After preparation of sample discs, it is necessary to mark the wood discoloration border. Further the numbering for taking photos was applied – the number shows the distance in cm from the end plane. To describe the proportion of discoloration, it is necessary to determine the percentage of wood discoloration. Therefore, sapwood and discoloration basal area of sample discs were calculated by using special image processing software 'ImageJ'. A lower average air temperature results in longer storage

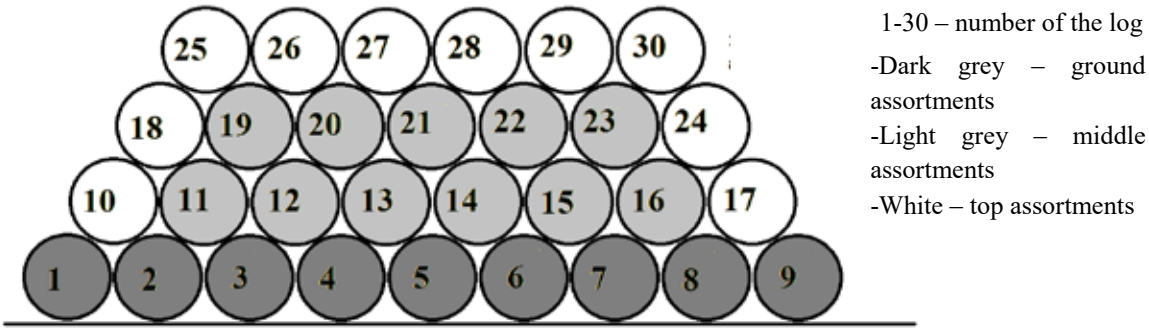

Figure 1. The scheme of assortment emplacement.

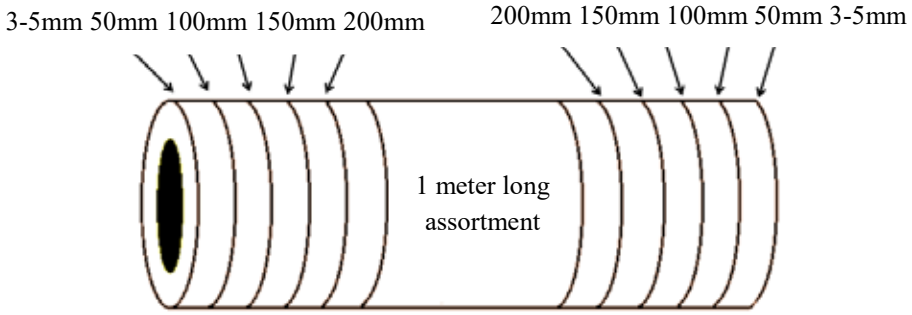

Figure 2. Roundwood cross-cuting section length.

periods, whereas a higher average air temperature results in shorter storage periods. In order to get the meteorological data, temperature and precipitation were obtained from www.meteo.lv by using the data of the nearest meteorological station. The sum of positive temperature during storage period was used.

In order to achieve weather discoloration area statistically significantly differ between birch round assortments depending on influencing factors two independent sample mean values are presented with ± 2 standard errors which are characterized by a 95% confidence interval. If two independent samples with 95% confidence intervals overlap with each other, then the difference between them will not be statistically significant.

Results and Discussion

In the start of the drying process, after the tree felling and cross-cutting, the moisture content of wood decreases in accordance with the current climate, wood properties and bark damages. Decreasing moisture content of wood results in an increased risk of discoloration. When the relative moisture of wood decreases below 50%, the risk of wood staining increases noticeably (Peek & Liese, 1974; Liese & Peek, 1984).

At the beginning, when surveying the birch round timber storages, the attention was paid to the point at which (the depth of 5 mm from the end plane) the first signs of sapwood discoloration would appear. For browning development, nutrients, oxygen, appropriate temperature and humidity are needed. In optimal conditions, discoloration can occur after just a few days. Therefore, in this analysis as a key influencing factor of discoloration development is the air temperature – the sum of daily average air temperatures during the storage period.

The development of the sapwood discoloration is shown in Table 1, depending on the average daily temperature and different side surface damages. In this case, by obtained results, all assortments can be divided into two birch round assortment groups:

- Control assortments and assortments with a damaged bark – prepared with the chainsaw;
- Assortments with the harvester spike roller damages and assortments with the harvester

Table 1

Intervals of sapwood discoloration depending on the daily average temperature

Daily average temperature, °C	First signs of discoloration	Discoloration on all assortments	First signs of discoloration	Discoloration on all assortments
	Storage time, days			
	Control		Harvester spike roller damages	
6	24	71	18	68
10	14	43	11	41
14	10	30	8	29
18	8	24	6	23
	Damaged bark		Harvester spike roller and bark damages	
6	23	69	17	69
10	14	42	10	41
14	10	30	7	30
18	8	23	6	23

spike roller and bark damages - prepared by the harvester.

The most attention was paid to two daily average temperatures + 6 and + 18 °C. At 5 to + 6 °C, sapwood discoloration had started, but at 18 to + 20 °C optimal conditions for sapwood discoloration fungi development were provided. When the birch round assortments are prepared with a chainsaw at the average daily temperature (+ 6 °C), the first signs of sapwood discoloration appear after 23 to 24 days of storage, but on all assortments sapwood discoloration appears after 69 to 71 days. If the average daily temperature reaches + 18 °C, the first signs of sapwood discoloration appear after 8 days, but on all assortments, sapwood discoloration appears after 23 to 24 days.

When the birch round assortments are prepared with the harvester at average daily temperature + 6 °C, the first signs of sapwood discoloration appear after 17 to 18 days of storage, but on all assortments sapwood discoloration appears after 68 to 69 days. If the average daily temperature reaches + 18 °C, the first signs of sapwood discoloration appear after 6 days, but on all assortments sapwood discoloration appears after 23 days.

Based on these results, it can be concluded that for birch round assortments, which are prepared with harvester at similar conditions, discoloration develops faster, compared to assortments prepared with a chainsaw.

Considering the above mentioned influencing factors, in further analysis it will be described how the placement in the storage influences staining process. It has been proven before that the placement in the storage and the preparation type also influence the water evaporation intensity; it also depends on wind and direct sunshine intensity.

For birch round wood assortments, which are prepared by the chainsaw and are stored for 3 weeks on the top portion of the storage, the proportion discoloration is around 48% from cross-sectional sapwood area. In further storage period, the same birch round assortments have no significant increase in the proportion of discoloration (47 to 50%). For the birch round wood assortments that are stored for 3 weeks in the middle section of the storage, the discoloration proportion takes up to 30% of the cross-sectional sapwood area. After 6 weeks the discoloration proportion more than doubles (77%) and after 9 weeks it is 84%. The most intensive development of sapwood discoloration for birch occurs for the assortments stored in the bottom section of the storage. Birch logs that are in direct contact with soil show 39% after 3 weeks and up to 98% of sapwood discoloration proportion after 6 weeks of storage.

For birch round wood assortments, which are prepared by harvester, discoloration tendencies are similar to assortments, which are prepared with the chainsaw. After 3 weeks of storage on the top portion of the storage, the proportion discoloration is around 55% from the cross-sectional sapwood area. After 6 weeks, for the same birch round assortments the proportion of discoloration is around 61%, but after 9 weeks around 78%. For the birch round wood assortments that are stored for 3 weeks in the middle section of the storage, the discoloration proportion

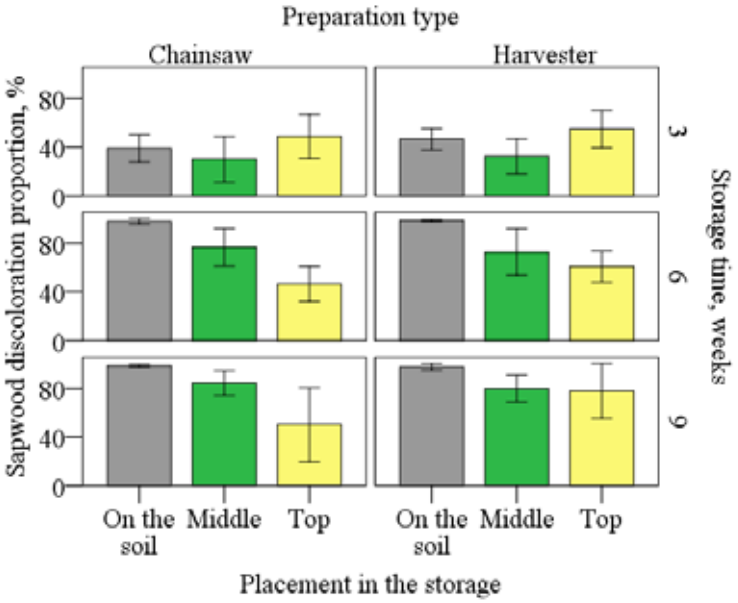

Figure 3. The development of birch sapwood discoloration depending on the preparation type, storage length and placement in the storage (with ± 2 standard errors).

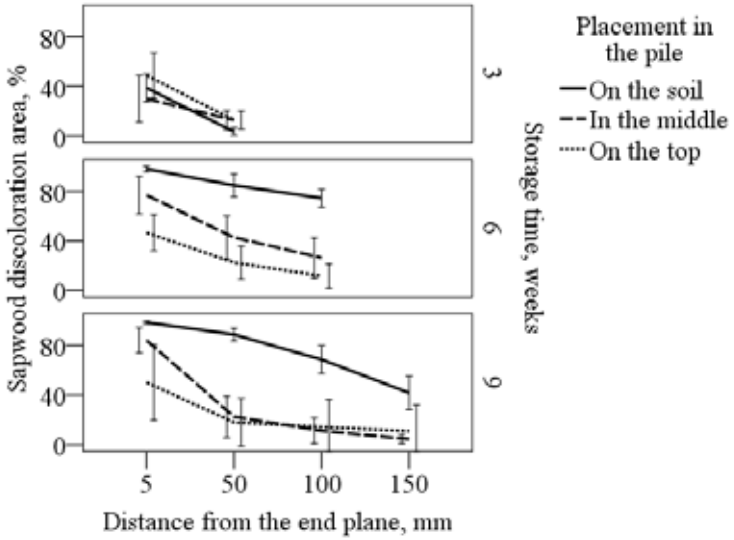

Figure 4. The development of birch sapwood discoloration depending on the preparation type, storage length and distance from the end plane – prepared with chainsaw (with ± 2 standard errors).

occupies around 32% of the cross-sectional sapwood area. After 6 weeks, the discoloration proportion more than doubles (73%) and after 9 weeks it is 80%. In this case also the most intensive development of sapwood discoloration for birch occurs for the assortments stored in the bottom section of the storage. Birch logs that are in direct contact with soil show 46% after 3 weeks and up to 98% of sapwood discoloration proportion after 6 and 9 weeks of storage.

By analysing the distance from the end plane, it can be observed that round assortments which are prepared with the harvester have a higher proportion of sapwood discoloration. It can be explained by the fact that the discoloration process in similar conditions for these birch round assortments begins faster compared to the assortments prepared with a chainsaw.

When the birch round assortments are prepared with a chainsaw, it can be observed that after 3 weeks of placement in the storage, it does not significantly influence sapwood discoloration development depending on the distance from the end plane – at 5mm distance discoloration proportion ranges from 30% to 49%, at 50mm it is from 3% to 13%.

After 6 and 9 weeks of placement in the storage, a significant influence can be observed on the sapwood discoloration development depending on the distance from the end plane. A higher proportion of sapwood discoloration is for assortments that are placed on the soil (see Fig. 4).

When the birch round assortments are prepared with the harvester, it can be observed that after 3 weeks of placement in the storage, sapwood discoloration development has not been significantly influenced, depending on the distance from the end plane – at 5mm distance discoloration proportion ranges from 32% to 55%, at 50mm distance from 8% to 21%.

After 6 and 9 weeks of placement in the storage, sapwood discoloration development is significantly influenced, depending on the distance from the end plane. A higher sapwood discoloration proportion is for assortments that are placed on the soil. After 6 and 9 weeks of storage, there are no significant differences depending on the distance from the end plane for assortments, which are placed in the middle or on the top of the storage (see Fig. 5).

In order to more fully describe the changes in sapwood discoloration proportions in the spring-summer period, the multiple linear regression analysis is used to analyse the wider effect of the influencing factors. The general task of regression analysis is to study the relationship between the dependent variable (sapwood discoloration proportion) and predictors (preparation type, side surface damage, placement in the storage, storage length, the diameter of round assortments, the distance from the end plane, the daily average temperature, amount of precipitation and relative air humidity) and to evaluate the correlation of predictors.

When regression analysis is applied, the birch round assortment placement in the storage is recoded in the digital form (1 – on the soil, 2 – in the middle, 3 – on the top). The type of preparation also is recoded in the digital form (1 – chainsaw, 2 – harvester).

Factors that significantly affect the sapwood discoloration development are: the preparation type, placement in the pile, distance from the end plane, the average daily temperature and relative air humidity (see Table 2).

Determination coefficient R^2 for linear regression is 0.450, indicating that 45% of the discoloration development cases of birch round assortments can be explained by these factors.

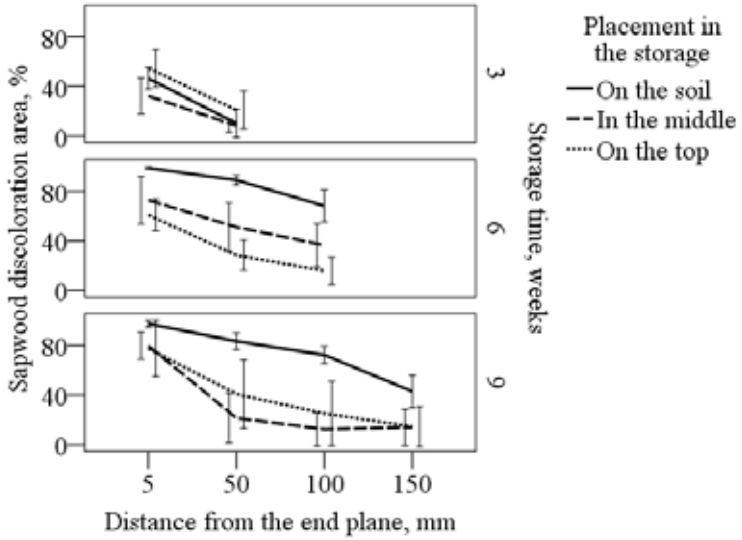

Figure 5. The development of birch sapwood discoloration depending on the preparation type, storage length and distance from the end plane – prepared with harvester (with ± 2 standard errors).

Table 2

The evaluation of significance of sapwood discoloration influencing factors

Indicator	Coefficient	Standard-error	t stat.	*p - value	Correlation coefficient (r)	Determination coefficient (R^2)
Constant	133.599	36.986	3.612	0.000	0.671	0.450
Preparation type	4.636	2.199	2.108	0.035		
Placement in the storage	-17.252	1.390	-12.408	0.000		
Distance from the end plane, mm	-0.417	0.025	-16.379	0.000		
Average daily temperature sum	0.193	0.021	9.232	0.000		
Relative air humidity, %	-1.884	0.568	-3.315	0.001		

If p values are smaller than 0.05, influence of the factor is significant.

In Sweden it was concluded that defects, such as cracks and rot, also contributed to the spread of discoloration on all studied tree species. For the birch top end assortments, on average, there is a little more stain than the for the butt end assortments. When comparing discoloration development, placing the roundwood top end on the sunny or shady side, there were no significant differences, only for birch logs prepared in winter and stored by the standard method on the sunny side, they had a deeper flame-like colouring than the ones stored in the shady side (Jonsson, 2013).

Conclusions

1. The main factors affecting the share of cross-sectional area of browning in the round assortments during storage are: the preparation type, placement in the storage, the distance from the end plane, the daily average air temperature and relative humidity of the air.
2. With the average daily air temperature reaching + 6 °C, the first signs of sapwood discoloration on birch roundwood prepared with a harvester would appear after 17 to 18 days of storage, and would spread to the whole stack of birch roundwood, prepared at the same time, after 68 to 69 days. However, with the average daily air temperature reaching + 18 °C, the first signs of sapwood discoloration may appear already after 6 days of storage and spread to the whole stack of simultaneously prepared birch roundwood after 23 days.
3. With the average daily air temperature reaching + 6 °C, the first signs of sapwood discoloration on birch roundwood prepared with a chainsaw would appear after 23 to 24 days of storage and would

spread to the whole stack of birch roundwood, prepared at the same time, after 69 to 71 days. However, with the average daily air temperature reaching + 18 °C, the first signs of browning may appear already after 8 days of storage and spread to the whole stack of simultaneously prepared birch roundwood after 24 days.

4. The most rapid development of browning is observed on the birch roundwood placed at the bottom of the stack; on the soil after 3 weeks of storage sapwood discoloration occupies 39 – 46% of roundwood end plane. After 6 to 9 weeks of storage, sapwood discoloration occupies 98% of round wood end plane.

Acknowledgments

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BLUE STAIN DEVELOPMENT OF SCOTS PINE (*PINUS SYLVESTRIS* L.) ROUNDWOOD AND ITS INFLUENCING FACTORS

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Abstract
The normal wood color changes due to the biochemical reactions as well as under the fungal exposure. Some of these fungi during all the exposure time cause only a discoloration (staining fungi), others change not only color, but also the structure of the wood in its further development (wood destructing fungi).
The development of blue stain in sapwood of pine roundwood and its influencing factors were studied in the spring-summer period of 2016 (April, May and June) in the districts of Nica, Jelgava and Daugavpils. 650 discs from pine log end planes were prepared in order to evaluate the influence of harvesting type, spreading type of fungi spores, placement in pile, storage duration, diameter, distance from the end plane, daily average temperatures, precipitation amount and relative humidity on blue stain of sapwood and rot development.
The most important factors influencing the blue stain development on pine roundwood during the storage are the harvesting type of roundwood, spreading type of fungal spores, placement in pile, storage duration, diameter, distance from the end plane, daily average temperature and amount of precipitation. By evaluating the daily average temperatures, it was concluded that blue stain of sapwood begins to develop when the average daily temperature reaches +5...+6 °C. But when the average daily temperatures reach +10 °C, depending on the harvesting type (chainsaw or harvester) and spreading type of the fungal spores (via air or bark beetle assistance) the first signs of blue stain may occur from 10 to 42 days of storage.
Key words: pine roundwood, blue stain, bark beetles.

Introduction

Blue stain is the most common cause of wood discoloration and it mainly affects conifer sapwood (Uzunovic *et al*., 2008). Blue stain is usually found on coniferous wood, like Scots pine (also Abies, Picea, Pseudotsuga, and other genus, respectively), more rarely blue stain can be found on deciduous trees (Fagus, Populus, Quercus, Fraxinus genus, etc.).

Blue stain causing fungi belong to the following genera: Alternaria, Cadophora, Diplodia, Discula, Graphium, Hormodendron, Hormonemia etc. These fungi are using the starch and sugars available in the sapwood. They cannot develop in heartwood, since there are no needed nutrients (Вакин *et al*., 1980).

Roundwood after logging is stored in the forest near the road from where it is later transported to the processing sites. Timber storage duration in such cases is uncertain both in the forest near the road and in the stowage area, so sometimes these materials remain vulnerable to various types of damage.

Logs that are stored unprotected against different types of damage for a long time, remain susceptible to biological staining, as these conditions are a favorable environment for microbial development. Such conditions allow different types of bacteria directly access the sapwood part, rather than the core, because sapwood is relatively rich in nutrients and contains no phenols and timber extractive matter, which hinders the growth of microorganisms. Although unstained timber can accommodate different types of organisms, it is important to determine the cause of each type of discoloration. In order to prevent future damages of the wood, it is necessary to distinguish between blue stain that is caused by bacteria or mold spots (Uzunovic *et al*., 2008).

Blue stain fungi are transmitted and transferred by insects that contribute to their release on the unprotected timber, where they act as pioneer species creating colonies. These fungi have little competition with other organisms, the impact of which is insignificant (Uzunovic *et al*., 2008).

Wood with an intact bark usually is not affected by blue stain fungi as fast as wood with bark defects, as blue stain has access to the outer part of sapwood through the damages of the bark.

The blue stain on logs usually appear in the form of wedge-shaped stripes in blue or gray color. In some cases, the blue stain has expanded all over the sapwood part. It is sometimes fully visible from the round timber terminal plane.

Blue stain fungus can spread even when there is only one infected log in the stack, because under favorable conditions blue stain fungus is able to spread at high speed, infecting most of the sawn timber, which is located in the stack. This spread can be slowed down or completely stopped, by drying wood in outdoor conditions so that they are not influenced by precipitation (Uzunovic *et al*., 2008).

Yang & Beauregard, (2001) conducted a study in the eastern Canada on seasonal effect on the development of the blue stain fungi in roundwood during the storage period. They concluded that the best time for the timber storage is winter. At this time of the year the development of blue stain fungi was

significantly reduced, but it rapidly accelerated after the winter period, starting from April. In the spring period, a significant development of the blue stain was observed after four-week long storage period. This project proved the theory of poorer development of the blue stain in the logs with undamaged bark, as peeled or rubbed logs suffered from blue stain much more. In cases when the timber implementation for various reasons has stopped and the blue stain begins to appear, logs must be immediately treated with certified chemicals that stop the fungus from spreading. When roundwood is drying, the spores of blue stain fungi do not develop if the timber moisture is not higher than 19%, except if the relative humidity remains very high for a long time (above 90%).

A big challenge for timber quality after storms is drying out. In addition to the timber dryness, drying also contributes to the spread of wood living fungi. Blue stain fungal penetration becomes a problem (spread through the air or with the help of insects) at the time when the tree is cut down. While storage rot is a relatively small problem in the first season, it becomes important in subsequent seasons (Tamminen, 1979).

Forest and timber industry is experiencing huge losses due to blue stain fungi. Blue stain lesions usually occur in the sapwood and represent only a "cosmetic problem" without serious changes of technical characteristics of the wood. Despite this, the timber with blue stain means that its market value will be lower. Friedl (2004) considered these losses in Austria after wood processing in mills and found that they reach more than 35 million euros per year. The acceptable discoloration level of blue stain fungi is dependent on the regulation of the specific roundwood entry place on sapwood discoloration depth.

The aim of this research was to determine the blue stain development of Scots pine (*Pinus sylvestris* L.) and its influencing factors. By having warmer spring and autumn, forest logging is possible all year round with a higher intensity, after that fast harvesting, forwarding and roundwood transportation should follow.

The study results can be used by forest managers, timber logging companies and timber logistics planners, who can plan logging operations and transportation of roundwood more precisely based on the knowledge about factors that influence roundwood quality changes during storage and their threshold values, in that way avoiding or minimizing the decrease of timber value reduction.

Materials and Methods

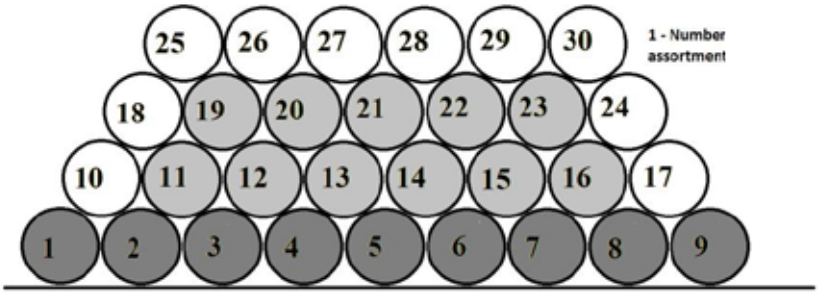
Factors affecting pine round timber blue stain development were analyzed in the spring - summer period (April, May and June) of 2016. Control cuttings for determination of blue stain development were carried out in four pine round assortment open storages in Nica, Jelgava and Daugavpils region. To keep the forest climate, all storages were placed in the forest under the tree crowns.

650 sample discs were prepared from pine round wood ends in order to evaluate the preparation type, side surface damage type, type of fungal spore spread, placement in the storage, storage length, diameter, distance from the end plane, the daily average temperature, precipitation amount and relative humidity effects on the blue stain development.

Sample storage consists of 26 – 32 (on average 30) round assortments with 10 – 30 cm in diameter and 1 meter in length (see Fig. 1). As it is known that sapwood discoloration and crooks mainly are developing from the end plane, 1 meter long assortments are enough to evaluate the quality changes from round timber end planes.

The evaluation of round timber placement in the storages:

- 1 – 9 assortments are characterizing the quality changes of the bottom of the storage (round timber placement on the ground);
- 11 – 16 and 19 – 23 assortments are characterizing the quality changes in the middle of the storage;
- 10, 17, 18 and 24 – 30 assortments are characterizing the quality changes on the top of the storage.



1-30 – number of the log

Dark grey – ground assortments
Light grey – middle assortments
White – top assortments

Figure 1. The scheme of assortment emplacement.

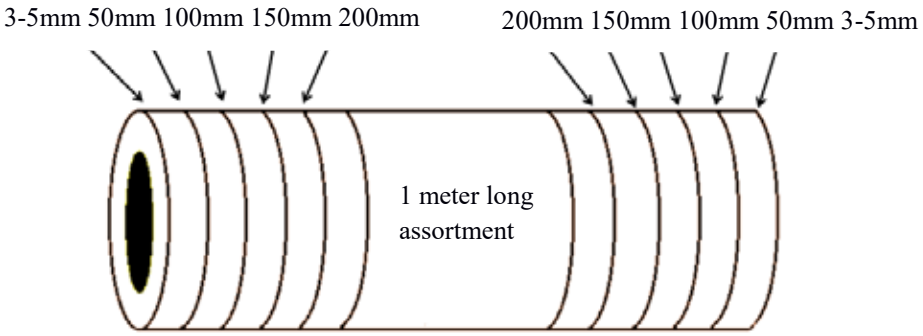


Figure 2. Roundwood cross-cutting section length.

In order to assess variety of preparation ways, four timber storages were created in the same time in each plot with different damages of side surface.

- Control - assortments without side surface damages.
- Damaged bark - assortments with the side surface of the bark bruised over the entire length.
- Harvester spike roller damages – assortments that have damages from harvester pike roller, but do not have bruised bark made by debarking knives.
- Harvester spike roller and bark damages – assortments with bruised bark and damaged side surface by harvester spike roller.

The inspection of pine round assortments was carried out at least once a week, control cuttings were started after 7 weeks of storage, but replications after every 2 – 3 weeks. In every control cutting around 8-12 round assortments for determination of blue stain were examined – 2-3 from the ground assortments, 3-4 from the middle and top assortments.

Discs of the assortment were sawn off in one survey, as shown in the scheme (Fig. 2). For the first disc on the outside only sapwood was sawn 3 – 5 mm in depth in order to assess the quality requirements of assortments at purchasing. Every subsequent disc was sawn in 50 mm.

After preparation of sample discs it is necessary to mark sapwood discoloration border. Further the numbering for taking photos is applied – the number shows the distance in cm from the end plane. To describe the proportion of blue stain, it is necessary to determine the percentage of sapwood discoloration by excluding heartwood. Therefore, sapwood and blue stain basal area of sample discs were calculated by using special image processing software 'ImageJ'. The total area of sapwood and blue stain was marked with different colors already in the forest. By establishing images with different distance from the end plane, we could assess damage intensity in the longitudinal direction. For further data analysis for every sample disc we made the measurement of heartwood basal area, sapwood basal area and area of discoloration. Lower average air temperature results in longer storage periods, whereas higher average air temperature results in shorter storage periods.

In order to get meteorological data, temperature and precipitation were obtained from www.meteo.lv by using data of the nearest meteorological station. The sum of positive temperature and sum of precipitation during storage period were used.

Two independent sample mean values ± 2 standard errors are characterized by a 95% confidence interval. If two independent samples with 95% confidence intervals overlap with each other, then the difference between them will not be statistically significant. To determine the significance of multiple factors, multiple factor linear regression was used. For this analysis IBM SPSS Statistics 20 was used.

Results and Discussion

To prevent the development of blue stain, it is necessary to maintain unfavorable conditions for the growth of fungi (keeping timber dry, temperatures above or below the ideal temperature for fungi development and protecting wood against insect attacks). Blue stain fungi are distributed with spores which are produced in very large quantities. Although the spores of other fungi are typically carried by the wind, the spores of blue stain fungi are sticky and are transmitted by insects. Ending up in nutritious environment these spores begin to grow rapidly. Blue stain spores can survive, but are not able to grow in wood with a humidity level of 20% or less, as well as in high or low temperatures. Temperatures above +65 °C are lethal for blue stain fungi (Simpson, 1991).

One of the possibilities for the presentation of results is to analyze and describe the sapwood discoloration development based on the length of storage. It is well known that climate is changing there are regular temperature fluctuations. We have found the way of analysis based on the sums of daily average temperature. In this way, the production companies will be able to plan logging, logistics and storage

Table 1

Development of the blue stain depending on the average daily temperature, storage length, preparation type and spreading type of fungi spores

Daily average air temperature, °C	First signs of blue stain	Blue stain observed on all round assortments
	Storage length, days	
Prepared with the chainsaw and the fungal spores spread by air		
6	70	101
10	42	60
14	30	43
18	23	34
Prepared with the harvester and the fungal spores spread by air		
6	50	94
10	30	56
14	21	40
18	17	31
Prepared with the chainsaw and the fungal spores spread by bark beetle		
6	17	69
10	10	41
14	7	29
18	6	23
Prepared with the harvester and the fungal spores spread by bark beetle		
6	23	75
10	14	45
14	10	32
18	8	25

of round timber based on simple climatic indicator value analysis, thus optimizing the maximum timber logging, delivery and storage time.

By evaluating the daily average temperatures, it was concluded that the sapwood discoloration begins to develop when the average daily air temperature rises to +5...+6 °C. Table 1 shows the development of blue stain depending on the average daily temperature, storage length, preparation type and spreading type of fungal spores. By analyzing a simple influencing factor such as air temperature, it can be concluded that by evaluating other influencing factors at the same average daily temperature, the first signs may appear within 6 to 23 days (daily average air temperature 18 °C). At lower average daily air temperature (10 °C), the first signs of blue stain can appear between 10 to 42 days.

If logging is done in spring (bark beetle flying time), from a reduction in quality point of view, the most dangerous is to prepare round assortments with the chainsaw by not removing bark, but in the summer time with harvester (average daily temperature – 18 °C) when debarked round assortments directly contribute to more intensive development of the blue stain. At this time already after 23-34 days of storage blue stain will be seen on all the round assortments.

There are many connections between bark beetles and conifer fungi, mainly including beetle species *Ips* and *Dendroctonus* and fungal species *Ceratocystis* and *Ophiostoma* (Webber & Gibbs, 1989). Different kinds of insects are inhabiting, breeding and feeding in the same wood together with blue stain fungi and other micro-organisms. Ophiostoma fungus rapidly attach to arthropods which promotes the spread of fungi (Blackwell, 1994). Blue stain fungal spores can be transmitted both through insect intestinal tract and the outer shells, which make a large part of insects the vectors of blue stain (Borden, 1995).

Figure 3 shows the sapwood discoloration area (%) depending on distance from the end plane, storage length and fungal spore spreading. This figure shows a very interesting trend for both types of fungal spore spread – 5 mm away from the end plane the proportion of sapwood discoloration is similar, while 10 and 15 cm from the end planes, the discoloration proportion is significantly higher when fungal spores are spread by bark beetle. This is explained by the

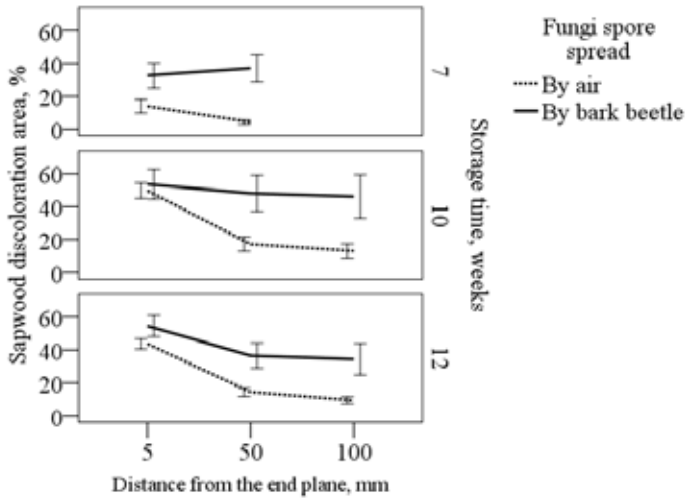


Figure 3. Sapwood discoloration area (%) depending on distance from the end plane, storage length and fungal spore spreading (with ± 2 standard error).

fact that insects make the aisles in round assortments' bark in all length by infecting the entire assortment and discoloration develops equally, regardless of the distance from the end plane. After seven weeks of storage, there is a trend to increase in the proportion of sapwood discoloration in the direction from the end plane.

Mechanized logging damages the side surface of the timber, uncovering and rubbing the tree bark, as well as creating up to a few centimeters deep gaps and penetrations with harvester roller spikes. And in this way the damaged timber gets exposed to the development of rot and discoloration (Uzunovic *et al*., 2004, Lee & Gibbs, 1996).

Figure 4 shows the sapwood discoloration area (%) depending on distance from the end plane, storage length and preparation type. Preparation type does not show significant influence on sapwood discoloration area development. Sapwood discoloration proportion tends to decrease from the end plane towards the middle plane.

By storing pine logs, depending on the storage length, the following discoloration development may occur:

- after 7 weeks of storage, at both 50mm and 5mm from the end plane the sapwood discoloration rate is around 20% and no statistically significant differences are found regarding the type of preparation;
- after 10 weeks of storage, at 5mm from the end plane the discoloration proportion is ranging from 45% to 55% depending on the preparation type, at 50 mm from the end planes, it decreases to an average of 30%, and at 100 mm from the end planes, it decreases slightly further to an average of 25%;

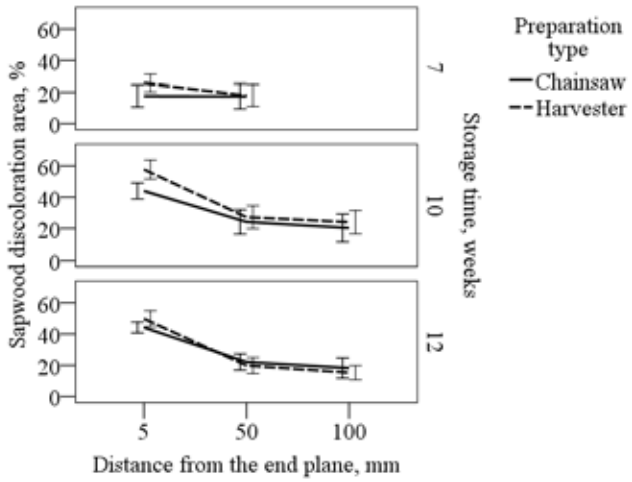


Figure 4. Sapwood discoloration area (%) depending on distance from the end plane, storage length and preparation type (with ± 2 standard error).

Table 2

The evaluation of significance of sapwood discoloration influencing factors

Indicator	Coefficient	Standard error	t stat.	*p - value	Correlation coefficient (r)	Determination coefficient (R^2)
Fungal spores spread by air						
Constant	-62.126	9.719	-6.392	0.000	0.721	0.521
Side surface damages	10.865	3.119	3.483	0.001		
Placement in the storage	1.838	0.832	2.210	0.028		
Diameter, cm	-0.286	0.117	-2.453	0.015		
Distance from the end plane, mm	-0.358	0.019	-19.234	0.000		
Average daily temperature sum	0.253	0.023	10.977	0.000		
Precipitation sum, mm	-0.847	0.084	-10.106	0.000		
Fungal spores spread by bark beetles						
Constant	29.282	15.243	1.921	0.056	0.615	0.378
Side surface damages	20.696	5.885	3.517	0.001		
Diamters, cm	-2.338	0.282	-8.288	0.000		
Distance from the end plane, mm	-0.125	0.038	-3.309	0.001		
Average daily temperature sum	0.165	0.042	3.899	0.000		
Precipitation sum, mm	-0.531	0.161	-3.303	0.001		

If p values are smaller than 0.05, influence of the factor is significant.

- after 12 weeks of storage, at 5mm from the end plane the discoloration proportion is around 40%, at 50 mm from the end planes it decreases to 22% and at 100 mm away from the end plane it decreases slightly further to an average of 20%.

In order to more fully describe the sapwood discoloration proportional changes in the spring-summer period, the multiple linear regression analysis is used to analyze the wider effect of influencing factors.

The general task of regression analysis is to study the relationship between the dependent variable (sapwood discoloration proportion) and predictors (side surface damage, placement in the storage, diameter of round assortments, distance from the end plane, the daily average temperature and amount of precipitation) and to evaluate the correlation of predictors. By analyzing the round assortment quality mitigating factors, a table 8.3.3 is made with values of factors influencing the development of sapwood discoloration, which will be used later for setting up the algorithm. For pine assortments two sets of factor values are created influencing the development of sapwood discoloration:

- Fungal spores spread by the air;
- Fungal spores spread by bark beetles.

By using values of these factors, it is possible to predict the trends if the round assortment quality changes. Factors that significantly affect the sapwood discoloration when fungal spores spread by air, are: a side surface damage, placement in the pile, round assortment diameter, distance from the end plane, the average daily temperature and precipitation sum. When fungal spores are spread by bark beetle, there is a significant impact on all of the above-mentioned factors except for placement in the pile. Both sets of factors show average correlation.

Determination coefficient R^2 for linear regression ranges from 0.371 to 0.521, indicating that from 37 to 52% of the discoloration development cases of pine round assortments can be explained by these factors (see Table 2).

Conclusions

1. The main factors affecting the share of cross-sectional area of sapwood discoloration in round assortments during storage are preparation type, fungal spore spread, placement in the storage, storage time, diameter, distance from the end plane, the daily average air temperature and precipitation amount.

2. With average daily air temperature reaching +18 °C, depending on the preparation type of pine round wood (chainsaw or harvester) and fungal spore spread (by air or bark beetle), the first signs of blue stain may appear after 6 to 23 days of storage. However, at average daily temperature of + 10 °C, depending on the preparation type and distribution way of fungal spores, the first signs of blue stain may appear after 10 to 42 days of storage.
3. Fungal spore spread by air causes sapwood discoloration reach 17% after 7 weeks of storage, but in the case of fungal spore spread by bark beetle – the average discoloration is significantly higher and reaches 37%.

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GIS BASED ANALYSIS OF FOREST SITE PREPARATION

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Abstract
The aim of this study is to improve the practice of mechanical forest site preparation (FSP) by identifying typical characteristics of FSP, including the direction of FSP machinery, manoeuvre count depending on configuration of parcel and forest type and width of manoeuvre track; to evaluate the productivity depending on different forest growing conditions; to create schemes of technological corridors for commercial thinning; to improve scheme of FSP according to the scheme of technical corridors for commercial thinning and to evaluate changes in the count of manoeuvres and total distance travelled.
In this study, we have developed methods to evaluate the quality of FSP. Methods used in this study include GIS analysis of vector data from FSP machinery tracking devices and LiDAR (*Light detecting and ranging*) data analysis for terrain information. Study shows that there is a significant difference in productivity when the machinery of FSP is driving in different angles to the longitudinal axis of parcel. Reduced productivity is justified by prioritizing topography of the forest floor. Slope is a decisive factor in the ground water movement and should be considered in FSP planning. Study shows that the developed method could be implemented in practice of forest management in 41% of sampled forest stands.
Key words: Forest site preparation, forest management, groundwater, GIS, DEM.

Introduction

Mechanical FSP is widely used to provide prompt and successful regeneration of harvested forest lands (Schmidtl & Macdonald, 1996). FSP may include changes of growth conditions in order to improve microsites for seeding. The manipulations may include such operations as changing soil moisture properties, increasing the amount of available solar radiation, changing soil temperature, increasing soil nutrient availability, reducing compaction of soil and competing vegetation control (Löf *et al*., 2012). FSP is important for tree establishment, early survival and growth (Hawkins, Steele, & Letchford, 2006).

FSP can lead to soil disturbance and can result in loss of carbon from soil (Jandl *et al*., 2007). Carbon loss may be significant in forest sites, which are located on slopes, especially in down-slope plowing conditions (Edeso, Merino, & Gonzalez, 1999). In sites without protection areas with trees and vegetation, the nutrients and organic matter may affect water quality in adjacent streams (Ahtiainen, 1992).

Heavy forest machinery may lead to disturbances of the groundwater flows. While soil compaction has little effect on ground water movement, rutting may lead to increased water table and dramatically restricted subsurface water flow (Aust *et al*., 1998). In order to characterise the groundwater movement in local areas, nests of piezometers and wells can be used, but on regional scale it may become expensive, impractical and intrusive (Whiteman *et al.*, 2012). On regional scale for modelling of groundwater movement and discharge, precise geological data (Levine & Salvucci, 1999) or thermal imagery could be used (Sass *et al*., 2014).

The aim of this study is to improve the practice of mechanical FSP in order to minimize the tree damage by commercial thinning. The objectives of this study are to identify common schemes of FSP; to calculate theoretical productivity of FSP at different angles to the longitudinal axis of parcel; to analyse FSP depending on slope and to develop improved planning scheme of FSP.

Materials and Methods

Joint Stock Company 'Latvia's State Forests' database of GPS data from FSP machinery is used in this study. 671 forest stands are used in analysis; the included forest types are: *Myrtillosa, Hylocomiosa, Oxalidosa, Mercurialosa mel.* and *Myrtillosa mel.* LiDAR data for topographical analysis of FSP is obtained from Latvian Geospatial Information Agency and the average point cloud density is 4 points m^{-2}.

FSP machinery direction analysis is based on trigonometrical principles, where productivity is calculated using different directions and longitudinal axis of parcels. Configuration of forest stands are from simple rectangles to complex shapes. Because of the complexity of polygons, we use simple geometrical values like length and width of the minimum bounding box of a parcel. We assume that for polygon to be outstretched, the division of width and length should be below 0.7 or 0.6. In this study we use both values (Figure 1).

Complexity of polygons is expressed as empty space in the minimum bounding box around the shape. We assume that forest stand is complex if empty space in the bounding box is more than 30% and 40%. In this study we use both values (Figure 2).

In order to calculate the direction of forest site preparing machinery, GPS data is generalized. The main direction of forest stand crossing is calculated by azimuth (α) and length (l) of each crossing as

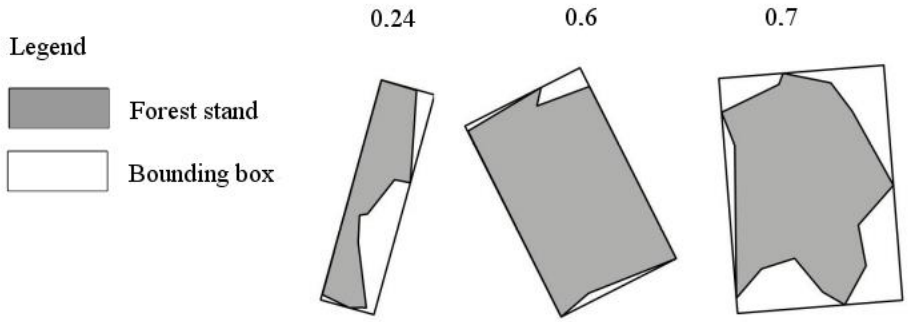

Figure 1. Example of outstretched forest stands.

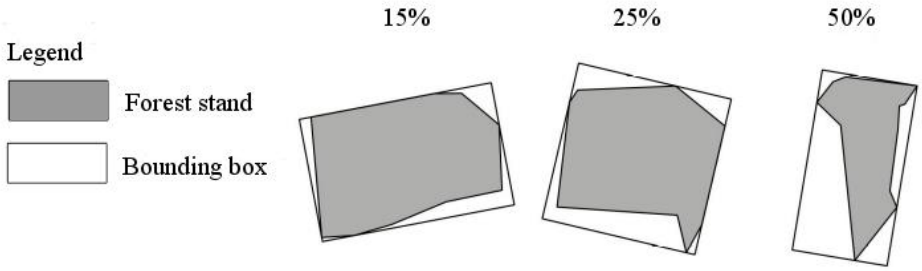

Figure 2. Example of complexity of forest stands.

weighted average with the maximum value of 180˚ (Formula 1).

$$a = \frac{a_1 * l_1 + a_2 * l_2 ... a_n * l_n}{\sum l} \quad (1);$$

In order to analyse longitudinal axis of forest stand parcel (α_n) relation with direction of FSP machinery (α), we use the following formula (Formula 2):

$$\Delta a = a - a_n \quad (2);$$

The value of relation may be in range from 0° to 90°. We analyse results in three categories with ranges 0°...25°, 0°...35° and 0°...40°. Boundary values of forest stand shape (ratio between shape width and length, complexity and ratio between longitudinal axis of forest stand parcel and direction of FSP machinery) are merged into the variable (40-40p-07; 40-40p-06; etc). The correlation between direction and longitudinal axis is stronger for the smallest values. In order to calculate the productivity of FSP, we use data about trajectory of FSP machinery, width of prepared zone and driving speed. The average width of the prepared zone is 7.5 m and the average driving speed is assumed to be 1 km h^{-1}.

To calculate the distance of FSP machinery turning manoeuvres in different forest types, GPS tracked trajectories are manually split into separate segments (Figure 3). The average turning distance is calculated for each forest type.

Figure 3. Turning manoeuvre of FSP machinery.

The terrain in all forest sites is classified in 4 aspect classes:

- North – south (0°...2.5°; 337.5°...0°; 157.5°...202.5°);
- Northeast – southwest (22.5°...67.5°; 202.5°...247. 5°);
- Southeast – northwest (112.5°...157.5°; 292.5°...337.5°);
- West – east (67.5°...112.5°; 247.5°...292.5°).

Classes are combined for opposite directions because longitudinal axis of forest site is calculated in 180° and optimal trajectory of FSP machinery is the same on opposite slopes. To avoid groundwater movement disturbance, it is advisable for harvesting machinery to move in parallel to a slope gradient. In order to avoid soil erosion, FSP machinery should be driven perpendicular to a slope gradient.

In most cases, in the studied area, the terrain has no single main aspect, and local depressions

are detected. Groundwater and surface water are concentrating in depressions without run-off and it is advisable for heavy forest machinery to avoid such places. In order to navigate through depressions, causing as little damage to soil as possible, complex groundwater movement calculations are needed. The amount of water in depressions depends on variables, such as soil type, texture of sediments, intensity of precipitation, transpiration, evapotranspiration etc. Accurate data for these parameters in the studied area are not available.

We assume that groundwater flow has the same direction as the aspect of the slope. In forest stands with different aspects and local depressions, empirical data is needed to evaluate characteristics of groundwater flow. Local depressions in the studied area are detected using LiDAR data, which are processed in QGIS software using *Fill sinks* algorithm.

Results and Discussion

Analysis of FSP and shape of a forest stand

Theoretical total distance of straight segments in different forest stand configurations is calculated using trigonometrical formulas and characteristics of simple figures. Calculations are made for 1 ha large polygons with different FSP machinery trajectory angles to the longitudinal axis and different coefficients of complexity and outstretchiness of a forest stand (Figure 4).

Results show that the total travelling distance is the shortest when FSP machinery is driven in the same direction as longitudinal axis of forest stand. The total travelled distance is growing till the coefficient of outstretchiness is about 0.7. After that there is no significant difference for driving parallel or perpendicular to the longitudinal axis. 76% of the studied forest sites are within coefficient values below 0.7.

The longest travelling distance of FSP machinery for whatever value of outstretchiness coefficient is at 45° angle to the longitudinal axis of the forest stand. The total distance decreases when the shape of the polygon is becoming more regular. Figure 4 explains why operators of FSP machinery tend to drive in parallel to the longitudinal axis of a forest stand.

There is a strong correlation between the total area of forest stand and total distance travelled (r = 0.97), which is logical. There is no correlation between the forest stand area and density of trajectories of FSP machinery.

A summary of forest stand complexity in the studied area is shown in Table 1. Complexity of a

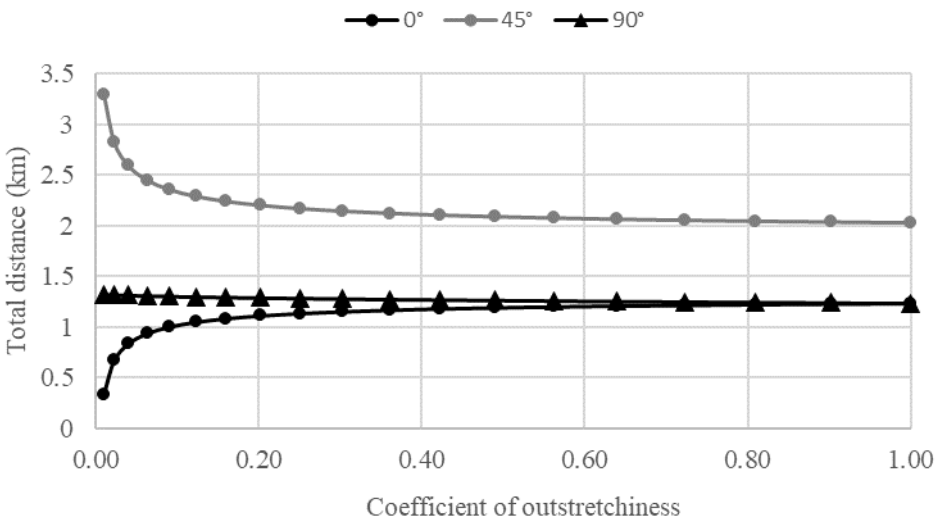

Figure 4. Total distance travelled in straight segments by different angles to longitudinal axis of forest stand.

Table 1

Complexity of forest stands in the studied area

Outstretchiness	Complexity less than 30%	Complexity less than 40%
> 0.6	287 ha	466 ha
	27%	44%
> 0.7	322 ha	546 ha
	30%	51%

Table 2

Characteristics of FSP machinery turning point

Forest type	Average turning distance (m)	Average turning count (count ha^{-1})	Total turning distance (km ha^{-1})	Total area (ha)	Forest stand count
Myrtillosa	32	20	0.63	15.71	12
Oxalidosa	29	16	0.47	70.09	33
Hylocomiosa	27	17	0.46	16.06	11
Myrtillosa mel.	27	19	0.52	22.37	15
Mercurialosa mel.	32	12	0.37	7.82	4
Average	28	17	0.48	132.05	75

forest stand affects the travelling distance of the forest site preparing machinery. More complex shapes tend to increase travelling distance and lower the productivity.

The average turning distance on the edges of forest stands is shown in Table 2. Turning distance characteristics depend mostly on the forest type. The average turning distance is 28 m and average total turning distance on 1 ha is 480 m. The biggest turning distance on average is in *Myrtillosa* forest type, because of a more complex pattern of soil scarification, most probably due to wet spots, which has to be bypassed. Lower ground bearing capacity means that the operator of FSP machinery should be more careful to avoid soil damage (rutting, compaction etc.).

Productivity of FSP is calculated theoretically and empirically using GPS data. Empirically calculated data shows that for 1 ha FSP 2 hours and 30 minutes are needed. Theoretical time for 1 ha preparation, if the operator is driving at 1 km h^{-1} is 2 hours and 38 minutes. Theoretical time for 1 ha of FSP in 45° angle is 2 hours and 46 minutes.

Analysis of FSP and terrain

For analysis of FSP in the context of terrain, we use forest stands where FSP machinery trajectories are close to the angle of the longitudinal axis of polygon, with regular shape and are relatively outstretched. In total, 105 forest stands are selected with a total area of 160.82 ha. LiDAR elevation data is available for all selected areas.

In order to evaluate the possibility of terrain data being used in heavy machinery movement planning in forest stands, aspect data is sorted in 4 classes in growing succession. We assume that a particular aspect class is dominant in a forest stand if it covers more than 30% of area. There is at least one dominant aspect class in 43.8% of forest stands and no areas with only one aspect class in the studied area (Table 3).

Terrain in the studied area is relatively flat. Slope in 81.5% of the studied area is within 0-3° (Table 4). Only in 15% of the studied area the slope is significant. Because of high vulnerability to the disturbances in groundwater movement in flat areas, the planning of heavy forest machinery movement across forest stands is increasingly valuable. Disturbances in the groundwater flow may lead to bogging process. Small standard deviation means that flat areas are with a low variety.

Local depressions are analysed to find out how large areas within forest stands are exposed to excessive water accumulation. Only in 18.1% of forest stands depressions take less than 10% of area (Table 5). In largest proportion of forest stands, depressions take between 10 and 50% of the total forest stand area. In 7.6% of studied forest stands depression area takes more than 50%.

Table 3

Average proportion of max and min aspect class

Forest stand area	Average maximal value	Average 2nd maximal value	Average 3rd maximal value	Average minimal value
Average	31.0%	25.6%	22.82%	20.5%
Minimal	25.5%	16.4%	9.0%	-
Maximal	55.1%	34.0%	25.9%	24.4%
Stdev	5.9%	2.4%	2.6%	3.8%

Table 5

Area of depressions within forest stands

Area of depressions %	Forest stands %
Less than 10	18.1
10...20	29.5
20...30	24.8
30...40	20
More than 50	7.6

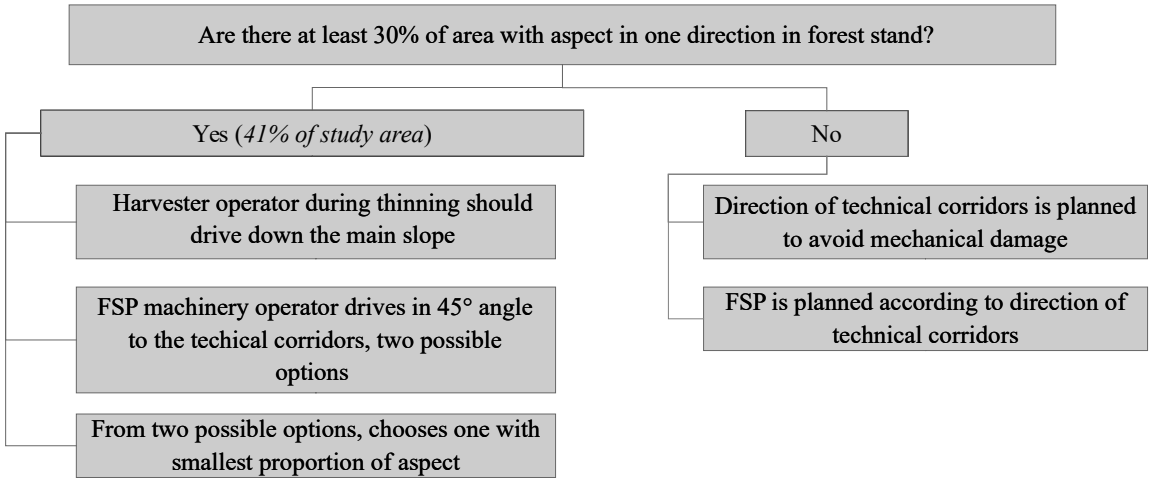

Figure 5. Decision support tree for FSP planning.

Drainage basins indicate complexity of terrain and groundwater movement in the area. 38% of forest stands are within one drainage basin, which means that groundwater drains in one direction. Forest stands with one drainage basin and with one dominant aspect in the study area are just 12.3%. In those forest stands, the planning of driving pattern of the heavy forest machinery theoretically is simple. 62% of forest stands in the study area are located within two or more drainage basins.

Results of this study show that data about forest stand configuration and terrain can be used in FSP planning. Summary of used analysis methods has resulted in recommendations for FSP planning (Figure 5). According to the proposed decision, support tree a stand is first evaluated for possibilities to optimize soil scarification so that groundwater flow is not affected and, if it is not possible, the scarification direction is subordinated to optimized strip-road pattern.

Conclusions

1. FSP usually occurs in the same direction as the longitudinal axis of a forest stand parcel without taking into account the terrain. The travelled distance of FSP and consumed time can be modelled in regularly shaped forest stands. Forest type does not influence the total travelled distance of FSP machinery.
2. Direction of FSP machinery to the longitudinal axis of a forest stand has significant influence on productivity and, by changing direction, the costs of FSP may increase. To accurately calculate the additional costs, empirical data about FSP machinery speed and fuel consumption is needed.
3. Results of this study show that in 41% of the studied forest stands it is possible to minimize the tree damage by implementing the developed methodology.

Acknowledgements

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RESEARCH OF SURFACE WATER QUALITY, TREATING IT IN RETENTION PONDS

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Abstract
Surface water runoff, formed in the company areas due to changing wastewater discharge and pollution fluctuations can be treated in retention ponds. Pollutants, released into the ponds, mineralize during complex natural biochemical processes in the aquatic environment. The paper presents the long-term observation data (2004 – 2016 m.) on the purification of surface wastewater, formed in the company's production territory, in retention ponds. The aim of this work is to identify surface wastewater purification efficiency in retention ponds. Wastewater samples were taken before and after biological treatment. The samples were investigated in the Chemical Analysis Laboratory of the Water Research institute of Lithuania University of Agriculture. Biochemical oxygen demand (BOD_7) was determined with titrometric method, suspended solids (SS) – with gravimetric method, having filtered the substance through a mid-thickness filter. Concentrations of oil pollutants were determined with the help of a spectrophotometric device of infrared rays IKAN-1 in the Analytical Department of Agrochemical Study Center of Lithuanian Agricultural Institute.
Although the suspended solids, BOD_7, and oil hydrocarbon concentrations, released into the natural environment, were below the MAC when they are collected from the company's production areas, they are often contaminated; therefore, it is necessary to treat them. The purification efficiency of researched materials was satisfactory and reached 61, 64 and 91%.
Key words: surface wastewater, retention ponds, polluting materials.

Introduction

Surface wastewater management is still relevant, because surface wastewater transmits various contaminants from stationary pollution sources into the environment. As urban areas grow, the collected surface wastewater levels increase as well. Most urban surface wastewater is discharged untreated, significant amounts of wastewater reach surface water bodies from diffuse pollution sources. With the growing influx of vehicles, intensifying construction, conducting road maintenance, surface wastewater contamination increases (Göbel *et al*., 2006; Paul Meyer, 2008; Yang *et al*., 2010; Rentz *et al*., 2012). When assessing the period since 2004, it can be claimed that the key pollutants have dropped significantly: BDS_7 decreased by 55.5%, suspended solids – 30.7%, total nitrogen – 38.5%, total phosphorus – 61.5%, oil and its products – 51.4%. The crucial factor in the depletion of the amount of emitted pollutants was a more effective cleaning of sewage treatment systems (Mereškevičienė, 2015).

According to the data from the Environmental Protection Agency, during 2013 there was about 52.9 m^3 of surface (rain sewerage) wastewater discharged into surface water bodies. Surface wastewater from areas, where there are no sources of pollution to water environment with harmful substances - parks, pedestrian zones, lawns, playgrounds, etc. – is discharged into water bodies without treatment. Such runoff accounted for 87.5% of all surface water in 2013. The most commonly treated surface wastewater comes from industrial companies, waste treatment facilities, gas stations' areas. Treated wastewater constituted 12.5% of all surface wastewater. Of these, 89.4% of wastewater was treated to the required standards, 10.6% was discharged insufficiently treated (Mereškevičienė, 2015).

The worst water quality is close to the pollution sources. As it can be difficult to quickly reach the permissible limits in these areas, they have a longer transition period - the water near the sources of pollution will have to comply with the requirements of the directive by 2018. Meanwhile, by 2025 any direct discharge of pollutants into surface waters must be discontinued.

To protect the natural environment against pollution, the surface wastewater management regulation adopted on the 2nd April 2007 by the Lithuanian Minister of the Environment, established the environmental requirements for surface wastewater collection, treatment and discharge. This Regulation applies to all persons managing the areas on which surface wastewater forms or may form, persons who are preparing planning documents of such territories, construction projects, who are currently planning surface wastewater management systems, who are planning to discharge or are discharging surface wastewater runoff to the environment or to waste water treatment systems belonging to other persons, as well as institutions regulating and controlling surface wastewater management.

Surface wastewater management regulations contain concentrations of not only the most important pollutants, but also of other materials, harmful to the environment. According to it, the surface wastewater, collected from the company's production area, can be discharged into the natural environment only when the instantaneous and average

annual maximum allowable concentrations are as follows: suspended solids 50 and 30 mg l^{-1}, BOD_7 – 57.5 and 28.75 mg l^{-1} O_2, oil hydrocarbons – 7 and 5 mg l^{-1}, respectively (Surface..., 2007).

The observation of surface wastewater management in the meat processing plant is being carried out since 2004. The company's manufacturing and domestic wastewater is supplied to Kedainiai town water treatment plants, and the surface wastewater from the industrial area is collected by sewer networks and treated in a biological treatment ponds.

Technically sound vehicles are used for animal transportation, from which the pollution from oil products is possible only in case of an accident, and the production area is tidy: in winter snow is ploughed from the asphalt coating, in spring – sand is swept away.

The aim of the research is to identify surface wastewater treatment efficiency in biological treatment ponds. The study tasks of the research: to determine the concentrations of pollutants formed in the surface wastewater in the company's production area before treatment and to determine the concentrations of pollutants in the surface wastewater that is being released to the environment.

Materials and Methods

Company's production area is bordered by roads from the north and the west while from the east and south – by arable land. Buildings and communications' layout of the production area, occupying 10.6 hectares, is shown schematically in Figure 1.

Surface wastewater collected from the company's territory is flushed in closed collectors into the treatment plant. The treatment equipment is made up of oil sludge trap and a two-stage biological treatment pond. The efficiency volume of the oil dirt pollutants trap – 158 m^3, biological pond – 1500ml m^3. The latter has two stages – 1/3 of the pond (in the wastewater inflow area) is 1.8 m deep and is used for dust and sand deposition, 2/3 of the pond is only 0.7 m deep and is planted with aquatic plants (bulrush).

Water analysis was done by the Chemical Analysis Analytical Laboratory of the Water Resource

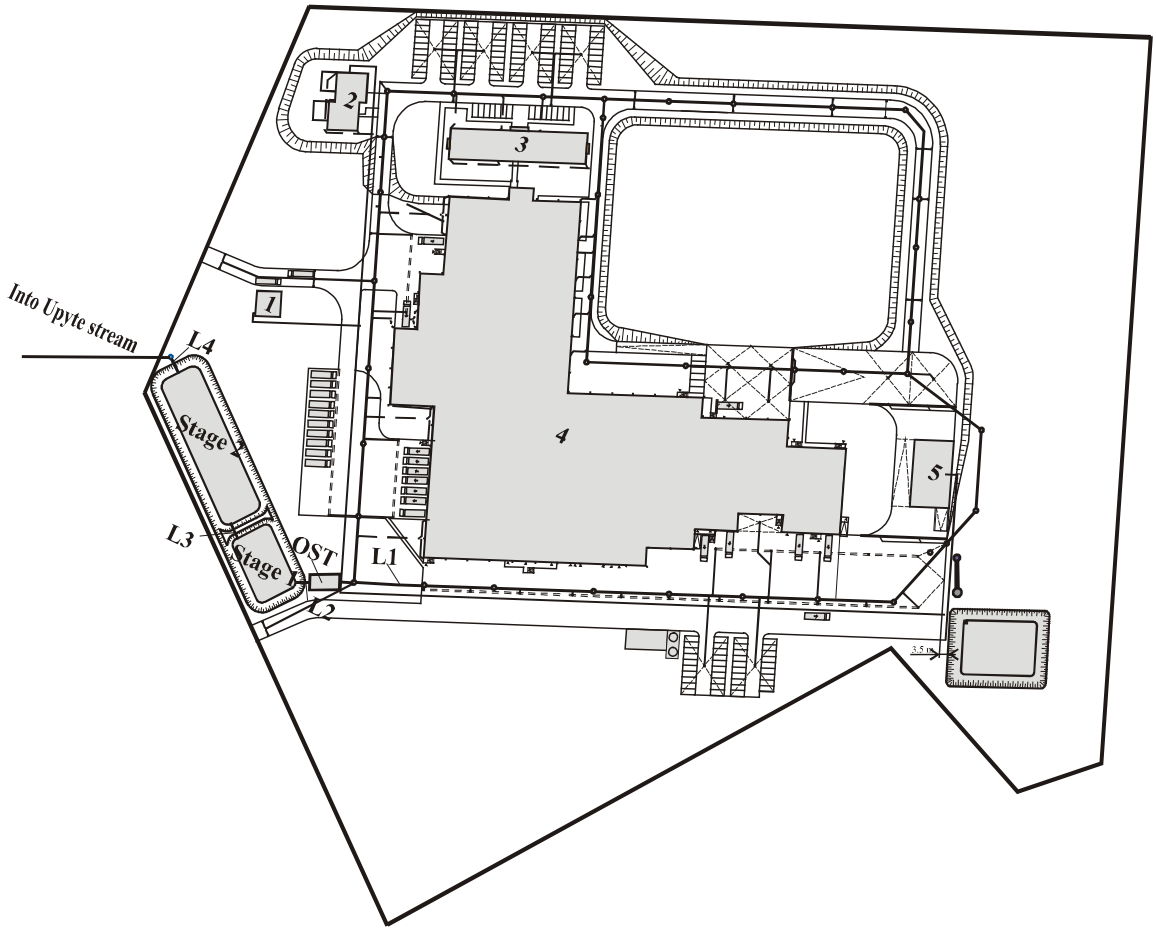

Figure 1. The scheme of company territory: 1 – watcher's building, 2 – flats of the enterprise, 3 – premises of the administration, 4 – buildings of production, 5 – washing premises of cattle's carriages, L1…L4 – surface water network, Stage 1…Stage 2 – biological pond; OST - oil sludge trap.

Engineering Institute of Aleksandras Stulginskis University. Suspended solids in the samples were identified by filtration through glass fibre filters, BOD_7 – the oxygen content difference was calculated after 7 days of incubation, oil products – identified by the infrared spectrophotometric method with IKAN-1. The analysis was carried out in the Agrochemical Research Laboratory of Lithuanian Research Centre for Agriculture and Forestry.

The efficiency of surface wastewater treatment is expressed as a percentage of the decrease in pollutant concentration in relation to the primary concentration. Surface wastewater treatment efficiency is calculated using the following formula:

$$E = \frac{C - C_0}{C} \cdot 100, \quad (1)$$

where E – treatment efficiency %;
C – pollutant concentration in surface wastewater, collected from company's production area mg l^{-1};
C_o – pollutant concentration in surface wastewater, released to the environment mg l^{-1}.

Statistical analysis of the data was performed using the computer program Excel.

Results and Discussion

The years with the highest precipitation were 2007, 2009, 2010, 2012 and 2016, when it reached 118, 128, 128, 128 and 127% of the climate normals, respectively. The lowest precipitation was in 2005, 2006 and 2015 – 83, 83 and 86% of the climate normals respectively. Precipitation level in 2004, 2008, 2011, 2013 and 2014 was broadly in line with the norm and accounted for 98, 101, 101, 95 and 99% climate normals respectively. During the entire research period, the average air temperature was higher than the climate normals, with the exception in 2005, 2010 and 2012 when it was lower than climate normals -0.3, -0.8 and -0.3 °C respectively. The warmest years were in 2008 and 2015, because the average daily air temperature was 1.5 °C higher than the climate normals (Fig. 2).

The key indicators of the surface water pollution are suspended solids, BOD_7 and oil products' concentrations. According to Li *et al.* (2014), suspended solids, forming sediments in surface water, have the greatest impact on water quality. Part of suspended solids settles as the wastewater flow rate reduces. During the research period, the higher concentration values of suspended solids in the wastewater runoff from the company's territory were most often established at the beginning of operation of the company, when there was a gravel road near the company. A large car traffic used to cause a cloud of dust, which would settle in the company's territory. After precipitation, the dust was being carried away with the storm wastewater to the collection system. In 2004 – 2007 the average concentrations of these substances from the company's production area were higher than the maximum allowable concentration and ranged from 38.3 to 60.8 mg l^{-1}, but the highest instantaneous concentrations ranged from 104 to 220 mg l^{-1} (Fig. 3).

Since 2008, after the asphalt road had been constructed, the situation due to suspended solids in rain wastewater improved significantly. Average concentration values of these substances have never exceeded the MAC until 2016, except some instantaneous concentrations in 2009, 2012, 2014 and 2016 that were 1.6, 1.3, 1.7 and 1.6 times higher than MAC, respectively. However, after the wastewater went through all the treatment steps, the quantity of suspended solids has decreased and, according to

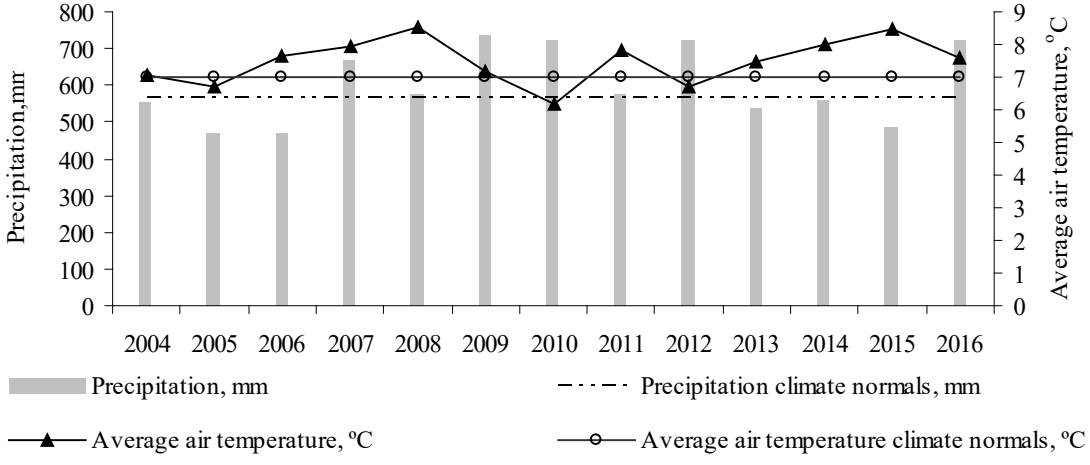

Figure 2. Meteorological conditions.

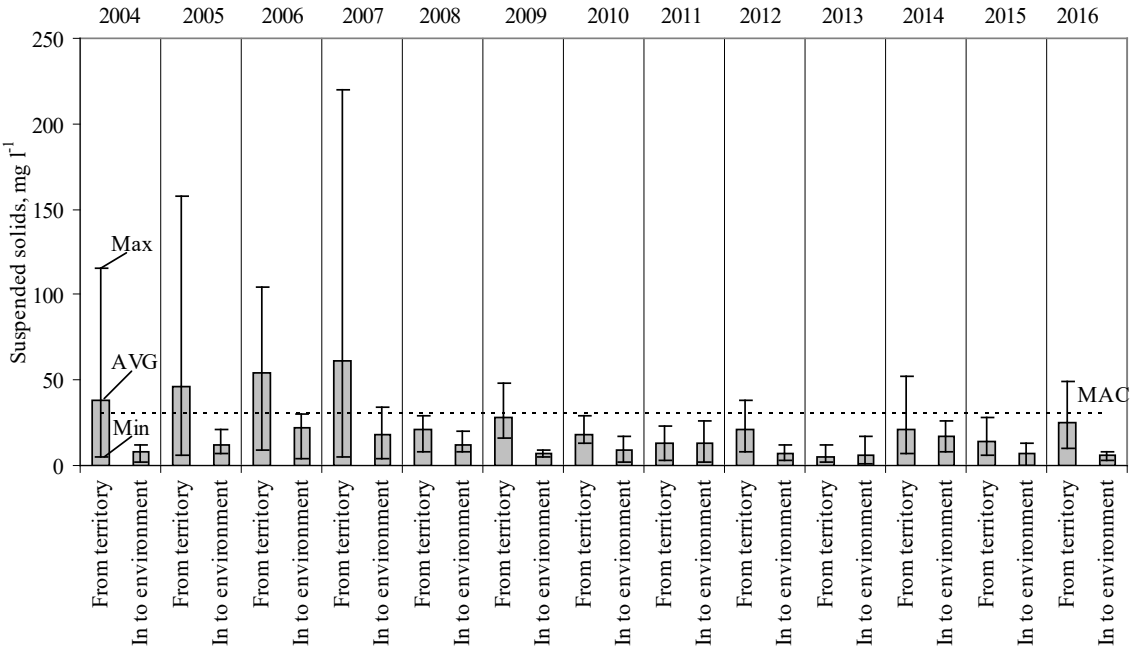

Figure 3. Fluctuations of suspended solids in surface water.

the aforementioned regulation, the average annual concentration of these substances that is released to the natural environment with the surface wastewater was 10.9 mg l^{-1} and did not exceed 30 mg l^{-1}.

Surface wastewater pollution by organic substances is indicated by the BOD_7 rate. During the research period, the values of this indicator in the surface wastewater runoff from the company's territory were higher than the MAC in 2006, 2007, 2011, 2012 2015 and 2016 – 36, 187, 55.9, 33.4, 185.6, 63.3 mg l^{-1}, respectively (Fig. 4).

Surface rain wastewater, after going through all treatment stages, was cleaned from organic pollution, because the BOD_7 concentrations in the wastewater that is released to the environment were determined to be low, as even the highest concentrations were 1.4 – 1.6 times lower than the maximum allowable instantaneous concentration of this indicator.

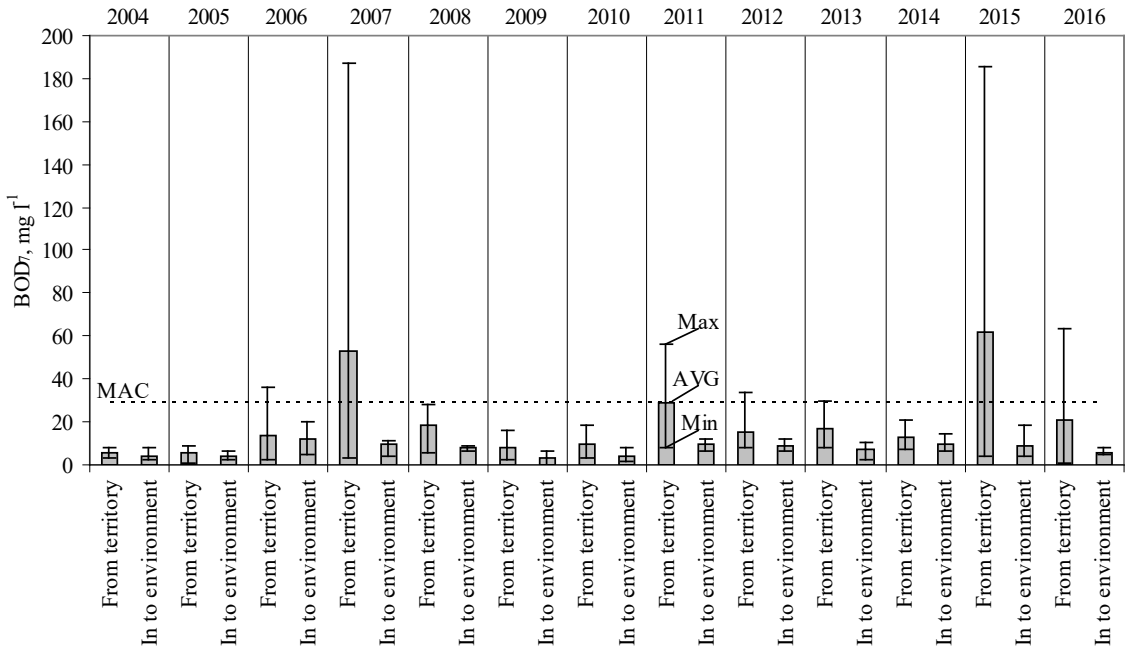

Figure 4. Fluctuations of BOD_7 in surface water.

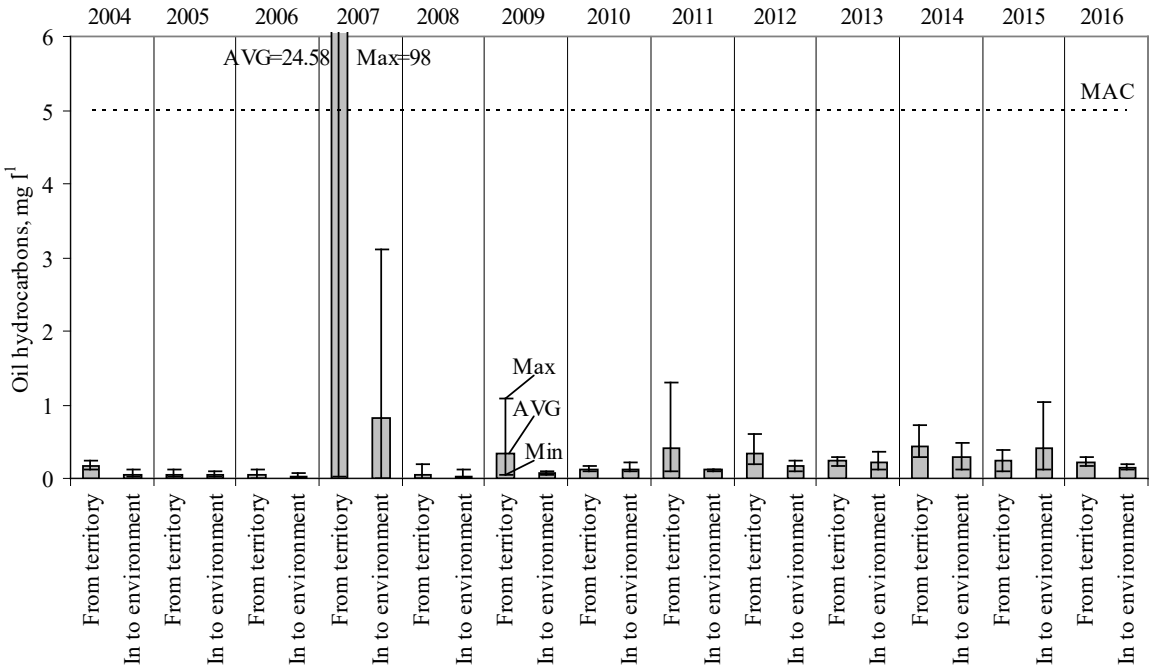

Figure 5. Fluctuations of oil hydrocarbons in surface water.

Table 1

Effectiveness indicators of surface wastewater treatment

Indices	Concentration, mg l^{-1}						The average treatment efficiency %
	Before treatment			In to environment			
	Min	Max	Average	Min	Max	Average	
SS	1.6	220	28.2	0.5	34	11.1	61
BOD_7	0.55	187	20	0	20	7.3	64
Oil hydrocarbons	0	98	2.1	0	3.1	0.2	91

The literature suggests that BOD_7 concentration in wastewater can be reduced by growing algae (Abdel-Raouf *et al*., 2012); however, the research shows that bulrush that was grown in the shallower area of the pond also reduced the values of this indicator.

Water bodies are polluted excessively by oil and its products. However, over a longer period of time this pollutant is decomposed by microorganisms (Das & Chandran, 2010). Higher oil hydrocarbon concentrations during the observation period were determined in the wastewater from the company's territory in 2007 and were as high as 98 mg l^{-1}, which is even 19.6 times higher than the MAC. However, this was an incidental case, as there were construction works being carried out on the company territory using faulty equipment. After the wastewater treatment, the average concentration of oil hydrocarbons decreased to 0.82 mg l^{-1}, and the highest value was 3.1 mg l^{-1}, which is respectively 6.1 and 1.6 times lower than MAC (Fig. 5).

In other years of research, oil hydrocarbon concentrations fluctuated in a small range and the maximum was observed in 2009, 2011 and 2015 – 4.6, 3.8 and 4.8 times lower than the MAC, respectively.

The concentrations of polluting substances in surface wastewater, after passing through biological ponds, were significantly lower than the concentrations referred in the surface wastewater management regulation. On average, during the observation years, the concentrations of suspended solids, BOD_7 and oil hydrocarbon, which have passed through the ponds, have decreased by 61, 64 and 91% and were below the MAC (Table 1).

Conclusions

1. It was found that the company's surface wastewater is mostly polluted by suspended solids, because during the research period, the average pollution in the water from the company's territory was determined to be 28.2 mg l^{-1}. However, after the treatment stages, the suspended solids decreased

by 61%, i.e. to 11.1 mg l^{-1}. This concentration conforms to the environmental requirements for the surface wastewater that is discharged into the environment.

2. BOD_7 concentrations in the water discharged into the natural environment, were very low and did not reach the maximum allowable instantaneous concentrations. The efficiency of wastewater treatment from BOD_7 pollution was 64%.
3. The amount of oil hydrocarbons in wastewater was also found to be low - the average concentration of 2.1 mg l^{-1}. The average concentrations of this product in the wastewater that is discharged into the natural environment, during the period were 0.2 mg l^{-1}, i.e. 25 times lower than the MAC.
4. The research has shown that in some cases rainwater runoff, collected from the company's production area is contaminated; therefore, it is important to treat it in pursuit of a better ecological status of streams' water.

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APPLICATION OF THE MODEL METQ FOR HYDROLOGICAL CALCULATIONS

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Abstract
In this study, a conceptual rainfall-runoff METQ model, version METQ2007BDOPT, to simulate daily runoff was applied. The model structure and parameters were fundamentally the same as in the METQ98 model with some additional improvement and semi-automatical calibration performance. The model has proved to be successful for both small (the Vienziemite Brook, 5.92 km^2) and large (the Daugava River, 81 000 km^2) drainage basins. The model METQ2007BDOPT was calibrated to the six different size river basins (the Pērse, the Malta, the Neriņa, the Imula, the Malmuta and the Iecava). These pilot river basins are characterised by one or two prevailing natural conditions such as hilly agricultural lands, agricultural lowlands, sandy lowlands, forested areas, swamps or lakes. The results of calibration showed good coincidence between the measured and simulated daily discharges. The Nash – Sutcliffe model efficiency coefficient *NSE* varies from 0.52 to 0.78 and Pearson correlation coefficient *r* from 0.65 to 0.88 for the six river basins with calibration and validation period from 1956 to 2015. In this study, we found some relationships between the model parameter values and physiographic sub-catchment characteristics.
Key words: hydrological modelling, calibration, conceptual model, river basin.

Introduction

Nowadays, hydrological measurement data has an important role of several hydrological challenges in water management. However, not always all data series of hydrological data can be measured or observed. One of the simplest and easiest ways to obtain hydrological data is application of hydrological models (Bergström, 1991; Seibert, 1999; Uhlenbrook *et al*., 1999; Merz & Blöschl, 2004; Beven, 2012).

During past decades in Latvia some versions of hydrological model METQ have been modified – METQ96 (Ziverts & Jauja, 1996), METQ98 (Ziverts & Jauja, 1999), METQ2005 (Ziverts & Apsite, 2005), METQ 2006 (Ziverts & Bakute, 2007) and METQUL2012 (Grinfelde, 2016). The model METQ is applicable to different dimensions of the river basins and lakes (Krams & Ziverts, 1993; Ziverts & Jauja, 1999; Apsite, Ziverts, & Bakute, 2008) althought the METQ model versions have been used for different hydrological tasks (Jansons *et al*., 2002; Bilaletdin *et al*., 2004; Zivers & Apsite, 2005; Apsite *et al*., 2005).

In this paper, the hydrological METQ2007BDOPT model is described and calibration results analysed.

The aim of this study was to calibrate parameters of the model METQ, version METQ2007BDOPT, of the six different river basins for simulation of daily discharge.

Tasks of the study are:

- to analyse and describe the study areas;
- to apply hydrological model METQ2007 BDOPT for study river basins;
- to analyse and assess calibration results of the METQ2007BDOPT model.

Materials and Methods

Hydrological model METQ2007BDOPT is a conceptual model which can be applied for simulation of daily discharge. Daily meteorological data were used as an input data for running the METQ2007BDOPT model.

Most of the parameters are physically based (Ziverts & Jauja, 1999) and the rest of parameters – A2, DZ, A3, PZ, CMELT, AMELT, DPERC, Beta, RCHR, RCHR2, RCHRZ, and RCHRZ2 were estimated by the semi-automatic calibration.

However, before to start to calibrate the model the climatic files were needed to prepare. Measures of precipitation and air temperature are used to create the climatic files. At least a 13-year period of daily discharge was applied for the calibration of the model parameters. To analyse the results of model calibration, the Nash – Sutcliffe model efficiency coefficient *NSE* (Nash & Sutcliffe, 1970), a Pearson correlation coefficient *r* and average values were used (Ziverts & Jauja, 1999).

In this study, six river basins by one or two predominant HRUs or natural conditions were chosen. The River Pērse basin was characterised by hilly agricultural lands and forests; the River Imula basin – agricultural hilly lands and lowlands; the River Neriņa basin – bog area and agricultural lowlands; the River Malmuta basin – bog area, the River Malta basin – lakes and the River Iecava (upper reaches) basin – sandy lowlands. The water balance and runoff of each HRU were simulated in three storages (Ziverts & Jauja, 1999). Surface runoff (Q_1), subsurface runoff (Q_2) and - base flow (Q_3) are components of total runoff (Fig. 1) from HRUs (Ziverts & Jauja, 1999).

In this study, the conceptual METQ2007BDOPT model was calibrated to such river basins: the upper reaches of the Neriņa (A = 73 km^2), the Imula (A = 232 km^2), the Iecava (A = 566 km^2), the Pērse (A = 249 km^2), the Malta (A = 797 km^2) and the Malmuta (A = 158 km^2).

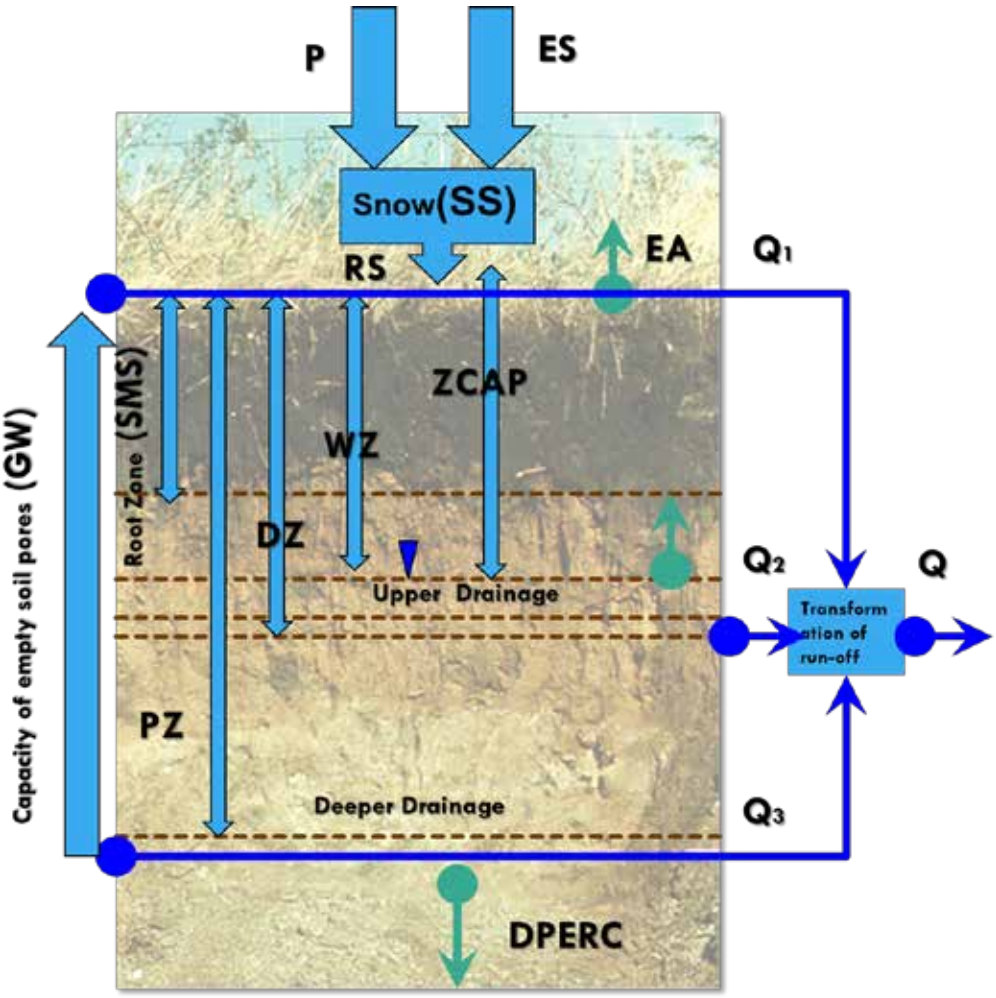

Figure 1. Structure of the hydrological model METQ2007BDOPT.
Source: author's flowchart, 2016.

Study Sites

In this study, the chosen six river basins are located in different places of Latvia and belong to the three largest river basins – the Daugava, the Lielupe and the Venta (Fig. 2). According to Pastor's (1987) regionalisation of Latvian small rivers, the River Pērse basin belongs to the rivers region of the Vidzeme Highland. The total drainage basin is 329 km^2, including upstream hydrological station Ūsiņi – 249 km^2. The average amount of precipitation is 800 mm per year. The area of the River Iecava drainage basin upstream hydrological station is 519 km^2, and it makes 1166 km^2 in total. The average amount of precipitation ranges from 650 to 750 mm per year. The River Iecava belongs to the hilly Upmale Plain and the Taurkalnes Plain. The Neriņa basin area is 118 km^2, and it belongs to the rivers of Piejuras lowland. The River Imula basin belongs to the Austrumkursas Upland, and the total basin area is 263 km^2. The average amount of precipitation varies from 650 to 700 mm per year. Compared to other river basins, the highest amount of precipitation receives the River Pērse because it is located in the Vidzemes Upland. This basin is also characterised by high percentage of forests – 47% cover of the entire basin. Irrespective of the Malta and the Malmuta river basins location in the same hydrological region, they are still different in predominant HRU. The River Malta is substantially affected by the lakes (approximately 35%), while the River Malmuta basin - by swamps area (approximately 40%). The River Iecava basin is quite different from other river basins regarding geomorphologic conditions. Sandy lowlands are dominating upstream of the River Iecava basin as well as forests. The River Neriņa basin is characterised by sandy and agricultural lowlands which occupy 46% of the total drainage basin. However, the River Imula basin is characterised by agricultural hilly lands and lowlands (62%).

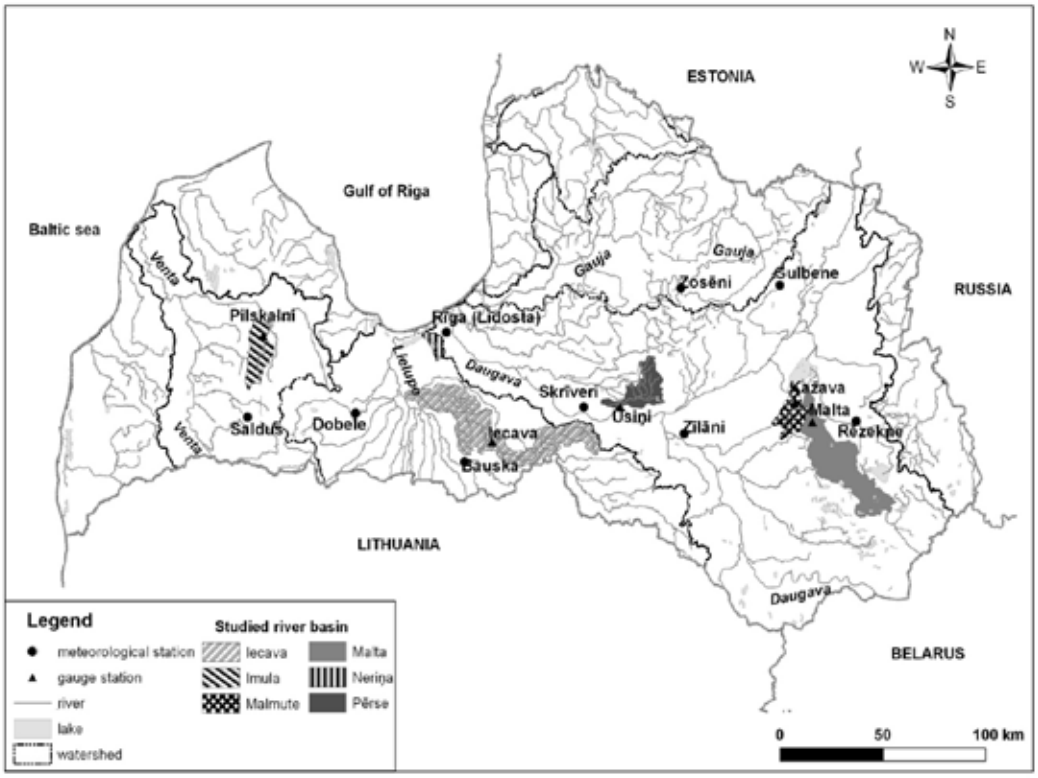

Figure 2. The location of study sites and observation stations (hydrological and meteorological).
Source: author's scheme, 2016.

Results and Discussions

In this research, the data series of at least thirteen years period of six hydrological and nine meteorological stations have been used for the calibration of a hydrological METQ2007BDOPT model for six different size river basins. We can conclude that for such catchment areas, the availability of observation points and the calibration periods is sufficient.

The results of the METQ2007BDOPT model calibration for the study river basins were showed sufficient or good coincidence between the observed and simulated daily discharges from 1956 to 2015: the Nash-Sutcliffe efficiency *NSE* varies from 0.78 to 0.52 and correlation coefficient *r* is from 0.88 to 0.77 (Table 1).

The best performance of the modelling results was obtained for the River Malta basin: *NSE* – 0.78 and *r* – 0.88 (Fig. 3). It is due to precipitation observations in the river basin. There is a meteorological station Viļāni, and its data could be used for the model calibration. The weaker results are obtained for the River Neriņa at Bulduri (Fig. 4).

The main source of difference between the simulated and observed daily discharge is the availability of meteorological stations in the river basins.

Table 1

The results of calibration of the model METQ2007BDOPT

Runoff gauge station	Results of calibration	
	NSE	r
Iecava – Dupši	0.66	0.82
Imula – Pilskalni	0.66	0.77
Malmuta – Kažava	0.52	0.65
Malta – Viļāni	0.78	0.88
Neriņa – Bulduri	0.55	0.78
Pērse – Ūsiņi	0.65	0.85

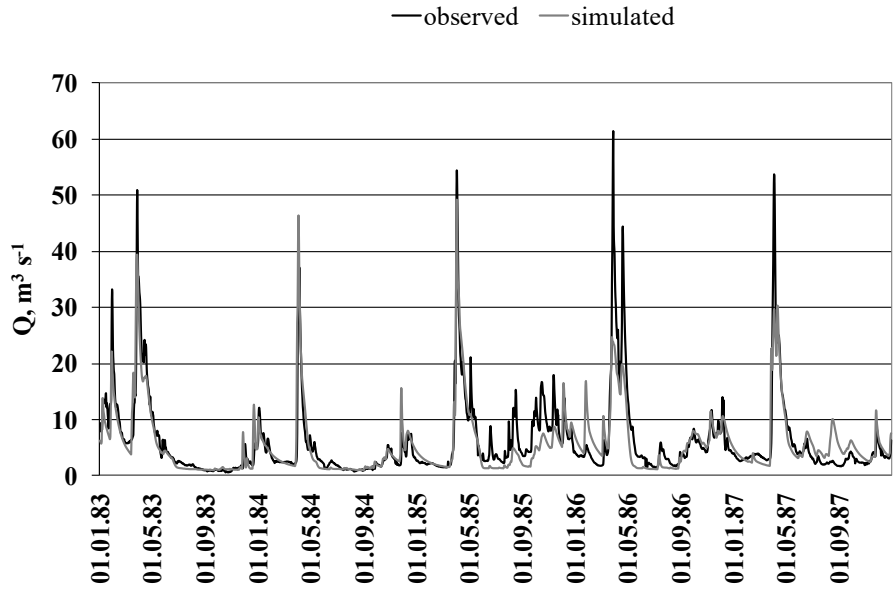

Figure 3. Simulated and observed discharge at Viļāni on the River Malta.

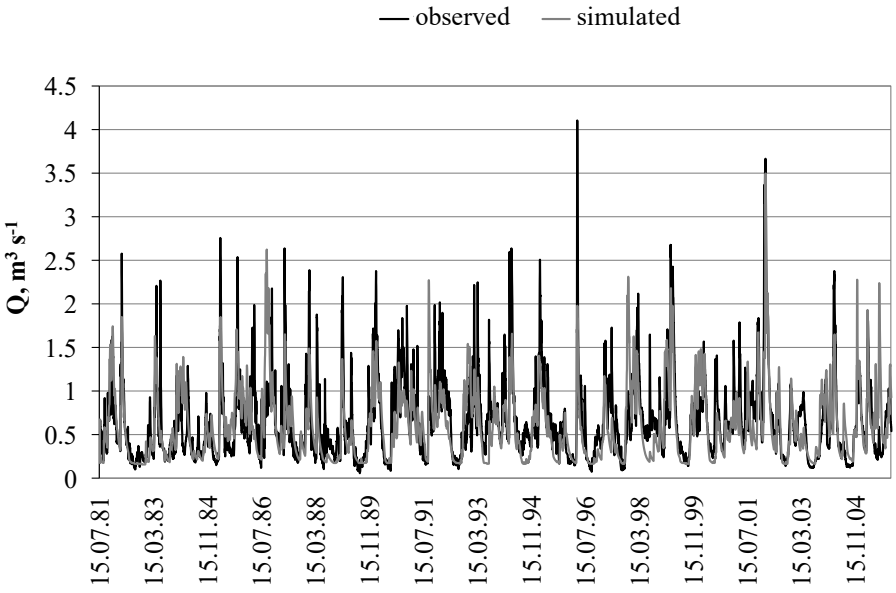

Figure 4. Simulated and observed discharge at Bulduri on the River Neriņa.

For instance, there are no meteorological stations in the River Iecava basin. Therefore, meteorological stations at Bauska, Skrīveri and Riga have been used. The weaker fit was also identified for the River Malmuta basin, and one of the reasons could be not sufficient meteorological observations to perform better model calibration. Since large areas of swamps in River basin play a significant role in the generation of the river runoff, meteorological observations of evaporation from wetlands are important for such basins. Another explanation is connected with a not well-marked riverbed.

The numerical values of model parameters for each basin reflect the physiogeographical conditions, including geomorphological, land use, soil, etc., of the studied drainage areas.

Estimation of a threshold value of water storage in the root zone is based on the previous studies of irrigations regime in Latvia (Ziverts & Jauja, 1999). In the river basins rich in swamps, i.e. the River Malmuta basin, values of WMAX is 20 mm. Soil conditions play a significant role in the runoff generation. According to the results, fillable porosity (ALFA) is one the main parameters which could reflect the geomorphologic conditions of rivers basins. The highest parameter

value of ALFA was defined for the River Iecava basin. It can be explained by dominating sandy lowlands. By the hydrophysical properties of the soil structure, the highest value of fillable porosity is for sands. The height of capillary rise (ZCAP) was identified for the heavy soils, i.e. the River Pērse basin, while these values are lower for light soils like sandy ones. Values of coefficient of snow melting (CMELT) in the river basin is higher in more open, not forested areas such as The River Neriņa basin.

Conclusions

1. The results of calibration of the model METQ2007BDOPT for the study, river basins are sufficient (NSE varies from 0.52 to 0.78).
2. The results of model calibration show that METQ2007BDOPT can be used for simulation of daily discharge of river basins with different HRUs – agriculture lands, forests, sandy lowlands, lakes and bog areas.
3. Using semi automatic calibration, model parameter values are found on start values of searching the parameters.
4. In this study, the numerical values of model parameters for each basin show relationship with the physiogeographical conditions, including geomorphological, land use, soil, etc., of the studied drainage areas.

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REVIVING PROSPECTS FOR LAKE RESTORATION - INVESTIGATING THE GEOCHEMISTRY OF LAKE ALŪKSNE SEDIMENTS

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Abstract
Lake sediments have a broad range of elementary and organic substance content. Bottom sediments collect decaying organic debris of aquatic plants and animals mixed by water drift with mineral constituents - deposited in the bottom of the lake in anoxic conditions. Lake Alūksne is situated in Northeast Latvia and is of glacial origin. Its water and bottom sediments chemical content depends on formation conditions – influenced also by anthropogenic activities. In order to determine environmental quality for restoration purposes and sediment recovery, geochemical research is needed. This paper aims to study bottom sediments – texture, ash part, organic compounds and metals to pinpoint necessary steps that are crucial for distinguishing environmental quality of the lake through geochemistry research. Bottom sediments contain sapropel with large admixture of organic compounds and microelements that may significantly influence biota and human health if concentration exceeds natural background levels. When bottom sediments are recovered, it may improve oxygene conditions in the lake, extracted sapropel can be used as a fertilizer, therapeutic agents, supplements for farm foods. Generally, sapropel is improving the structure of agricultural soils, increasing the cation exchange capacity and serving as binding material for complexes formation; thus diminishing ecotoxicological exposure threats of heavy metals, increasing yields and albumen and protein quantity in plants cultivation products. As these sediments can be used for soil fertilizing and crop production improvement in agriculture, mechanisms of ecotoxicological impact to various soil types and agricultural plant ecology should be researched.
Key words: ecotoxicology, contamination, organic fertilizers, resources, microelements.

Introduction

Lakes carry the history of natural and anthropogenic impact in previous times. Physical and chemical data that is gained from lake sediments or soils in coastal neighbourhood can tell a lot, e.g., identifying deposition and erosion processes as well as underlying lithologies (Anselmetti *et al*., 2004; Beres *et al*., 2006; Drew *et al*., 2010; Grunsky *et al*., 2010, 2013, 2014). Geochemical data includes plethora of exploration, visualisation and meta-analysis of gained information (Grunsky, 2010). Inorganic and organic substances play a huge role in determining the environmental quality and interactions in ecosystems of water bodies (Burlakovs, 2008; Grunsky *et al*., 2012, 2013).

Apart from direct pollution such as heavy metals and organic contaminants, aquatic ecosystems can receive the so called nutrients from human generated both point sources (industrial and urban sewage) and non-point (leaching of agricultural N, P and atmospheric deposition of N compounds from fossil fuel combustion) (Burlakovs *et al*., 2013b). A lot of nutrients are coming from synthetic N fertilizers used and produced for decades (Howarth, 2008; Tao *et al*., 2017). Eutrophication in many freshwater systems is under danger (Basu *et al*., 2010). Sediment is an integral part of aquatic system and N, P-loading is dominant eutrophication factor (Romo *et al*., 2007). Wetlands and lake areas provide us with important ecosystem services (Postel & Carpenter, 1997); nevertheless, there is a need to define the ecological quality whenever there is a need to revitalize or protect the natural habitats (Alvarez-Cobelas *et al*., 2001). One of influencing factors is intensive agriculture combined with industrial and common sense attitudes created surface and groundwater quality disturbances, these are the major threats for later absorbance of contaminants in bottom sediments and further development of poisoned food chains (Tockner & Stanford, 2002; Burlakovs *et al*., 2013b; Tao *et al*., 2017). Therefore, a lot of water covered areas such as lakes and wetlands are included in Biosphere reserves by UNESCO, as Ramsar sites and/or refuges for migratory birds and aquatic plants (Cirujano *et al*., 1996). However, every lake in the nature demands the best available attitude and concerns if significant threats to environmental quality appear there.

Sediment geochemistry in lakes are archives for studying long-term fluctuations of environmental conditions, climate, history of vegetation and human impact (Meyers, 2003). These insights are used in forecasting of ecosystem in future (Wetzel, 2001). In eutrophic lakes dominant in North Temperate Zone, algae and macrophytes dominate instead of mineralization processes in lakes (Cooke *et al*., 2005). Therefore, thick organic layers of gyttja form very often (Hansen, 1959). Gyttja or sapropel may be also prospective material for applications (Stankevica & Klavins, 2013; Stankevica & Vincevica-Gaile, 2017) and lake restoration combined with material recovery (Stankevica *et al*., 2013, 2014; Vincevica-Gaile *et al*., 2015), but analysis of sediment composition from pollutants point of view is of

great importance, helpful in reconstructions of industrial activities and raising understanding on aims that should be reached through ecosystem revitalization. Several lakes in Latvia have been intensively studied through scientific and applied research activities, some of them are tended and supported by municipalities that want to promote site clean-up for better local environmental quality (Purmalis *et al*., 2017; Burlakovs *et al*., 2016; Stankevica *et al*., 2015; Vircavs, 2006).

In turn of the 21th century up till now a lot of research had been done around. This paper describes the sediment quality in Lake Alūksne of glacial origin in Northeast Latvia. The aim of this paper is to pinpoint necessary steps that are crucial for distinguishing environmental quality of the lake sediments (probably polluted) through geochemistry research, both organic and inorganic substances. Following tasks were defined and fulfilled: 1) visual assessment and core sampling of sediments; 2) determination of basic parameters for sediments and textural properties; 3) measuring C, N, P elemental concentrations mainly responsible for eutrophication processes; 4) analyses of Pb, Zn, Cu, Cr, Ni, Cd and As to guideline exposure to toxic inorganics; 5) measurements of concentration for total hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) in sediments; 6) analysis of data and providing recommendations.

Materials and Methods

Lake Alūksne (Fig. 1) is relatively deep lake of Pleistocene glacial origin in Alūksne Highland developed in glacial environment. At present times, Lake Alūksne in its deepest part is 20.0 m deep, but the average depth is 6.7 m. It has four islands and is situated >180 m osl. Coasts of the lake are hilly, at several places they are urban and severely urbanized nearby the gulf where the investigation was concentrated on. Sediment samples of various types were derived from rich organic sediment layers (Figure 1).

Sediment sampling cores in lakes were performed in certain points selected according to the established network and coupled with sampling. Texture analysis, elemental and moisture content, loss of ignition (LOI) and pH were determined. Mostly organic material, mud and gyttja (sapropel) are covering mineral soil layer which is mostly sand, gravelly sand and rarely glacial till. The upper part consists of mud with low plasticity, deeper layered sapropel.

Coring of sediments was done using a Russian-type sampler equipped with a 1.0 m long (d = 5 cm) camera. Every sample was put into a non-transparent

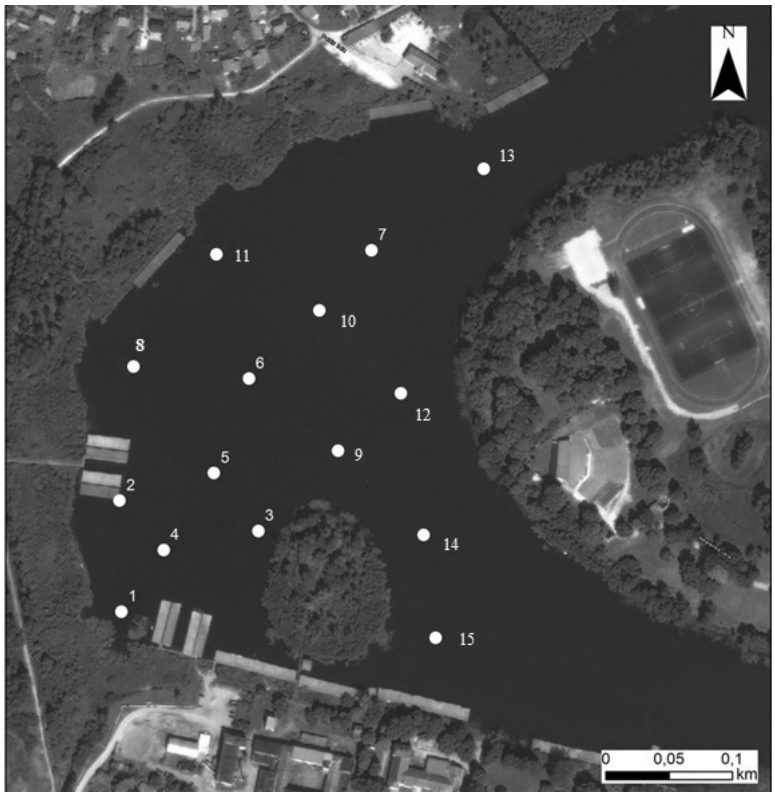

Figure 1. Map of location of sediment sampling points in Lake Alūksne, Southwestern part nearby urbanized areas with potential contamination.

air-tight plastic bucket with a lid and stored at constant temperature (+ 4 °C) to achieve *in situ* conditions during the storage. Sediment core was characterised by type of sediments.

Loss on ignition (LOI) method was applied for sediment analysis in order to estimate the moisture level and content of organic matter, carbonates and mineral matter in the sediments (Heiri *et al*., 2001). Moisture of sediments was determined after drying samples at 105 °C in drying oven. Content of organic and carbonate matter was analyzed by incinerating the samples sequentially at 550 °C for 4 h and at 900 °C for 2 h.

Quantitative content of metals in sediment was measured by atomic absorption spectrometry (AAS) (PerkinElmer AAnalyst 200) and total reflection X-ray fluorescence spectrometer (Röntec PicoTAX). Samples were mineralized in analytical-grade HNO_3 and H_2O_2 mixture. Total amount of C, N were determined with element analyzer (Eurovector). Total P was analyzed using ascorbic acid method (Hieltjes *et al*., 1980).

For the measurement of total hydrocarbons, fractions of hydrocarbons as well as PAH, samples were extracted in double distilled hexane and analyzed with gas-chromatography (Shimadzu GC-2010 Plus). The method used for extracting the PCBs from sediments was modified from the US EPA Method (USEPA, 1996). Sediment (30 g) (triplicates were made for each sample) was extracted for 8 h using the Soxhlet instrument with 200 ml (1:1) of *n*-hexane-CH_2Cl_2. Prepared extracts were analyzed with gas-chromatography (Shimadzu GC-2010 Plus).

Results and Discussion

Watershed and location in highland and city of studied lake have influenced sediment accumulation whose total depth in researched gulf reaches up to 13 m. The deepest part of lakes (> 2.5 m), its sediment consists of organic matter ~64%, carbonates ~2% and mineral sediments ~33%; 250 – 100 cm deep organic matter reaches ~70%, but shallower parts of the lake nearby coastline only 20 – 30%. Sediments with clearly identified anthropogenic origin contain ~10% of organic matter. Discovered variation of organic matter content in sediment causes material input from watershed of lake as well as from city. In some areas in lake sediments anthropogenic input from former wastewater source reaches thickness about 1 m. These findings provide answer to the question about factors responsible for rapid loss of water depth in the gulf of lake. In shallow water, intensive spreading of macrophytes increases, indicating beginning of eutrophication. Although N:P ratio of sediments is relatively high representing oligotrophic waters (Geider *et al*., 2002), but high C/N ratio (Table 1) indicates a prevailing allochthonous source of organic matter (Guilizzoni *et al*., 1996). Prospective overgrowing of researched gulf in the current development stage of lake is inevitable, thus highlighting importance of possible restoration.

In the researched gulf of lake in past decades manufactures of city have discharged their wastewaters. There is no exact data about total amount and quality of wastewaters, but there has been suspicion that sediment may be polluted, probably with oil products; therefore, total amount of oil products in sediments were analyzed on organics, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). The analysis of sediments shows relatively high concentrations of total amount of oil products exceeding not only in natural conditions background amount (Abdel-Shafy *et al*., 2016) (< 100 mg kg^{-1}), but also authorities posted regulations (> 400 mg kg^{-1}).

Total amount of oil products in sediments (Table 2) clearly represents human impact with source next to sampling point No.1. Contamination of oil products in the lake diffuses close by source due to lack of hydrological dynamics and properties of pollutant. Moreover, behavior of these products shows no or insignificant infiltration rate probably by binding with organic matter in sediments. Pollution has not migrated from the source further than 400 m and most of mass accumulates next by inlet. PAH's results show even smaller migration capacity due to their

Table 1

Element composition and their relations of sediment cover in Lake Alūksne (g kg^{-1})

No.	N	C	P	N/C	C:N:P
1	19.1	226.5	0.555	0.084	407:34:1
2	24.8	253.4	0.564	0.098	456:45:1
3	21.6	230.3	0.509	0.094	415:39:1
5	21.6	232.7	0.497	0.093	419:39:1
6	25.6	254.0	0.542	0.100	457:46:1
7	26.1	266.7	0.524	0.098	480:47:1

nature (Dąbrowska *et al*., 2008) and low solubility in water (Abdel-Shafy *et al*., 2016). Concentration as well as amount of pollutants steeply decreases starting from sampling point no.1 which strongly correlates with organic matter/ash content detected with LOI (Heiri *et al*., 2001) and pH, suggesting that organic pollutants form complexes with organic matter (humic substances) (Klavins *et al*., 2011; Burlakovs *et al*., 2013a) also approved by pH measurements. Average pH value of sediments is 6.0, but in sampling point no.5 in upper layers pH value reaches up to 6.81 and in no.1 up to 7.95 due to nature of PAH's, their concentration, neutralization of carboxylic groups of humic substances (Klavins *et al*., 2010) as well as possible toxicity (quiet low) to microorganisms (Abdel-Shafy *et al*., 2016). Although the greatest degradation rates at soil are at pH 7.5 (Pawar *et al*., 2015), at the same time during microbial degradation pH value can decrease up to 5.8 (Yenn, 2015) which strongly depend on species of microorganisms and abiotic factors.

To describe sediments polluted with oil better, the analysis of fractional content of hydrocarbons has been done for two samples (No. 1: 43 – 80 cm; No. 4: 10 – 30 cm) representing upper layer of sediments and layer with highest oil concentration. Fractions of hydrocarbons were divided to C_6-C_{10} (light fraction representing petrol, petrol ether); C_{10}-C_{24} (kerosene, diesel, aviation petrol); C_{24}-C_{40} (motor oil, paraffin waxes, mazut). Results show that more than 80% are heavy fractions of hydrocarbons in No.1. C_6-C_{10} – 0.3%; C_{10}-C_{24} – 19.1%; C_{24}-C_{40} – 80.6%, but in No.4, it is, 1.2%; 13.7%; 85.1% respectively.

Content of PAHs clearly shows their presence in lake sediments due to emitting polluted wastewaters form manufactures in Alūksne city. In the result, not only total amount is relatively high but also analysis of individual compounds of PAHs demonstrates significant level of sediment contamination (Table 3).

Distinguishing features of distribution patterns of PAH's and dominance of anthracene and other widely existing PAH's suggesting that source is pyrogenic and may be oil and burn residues (Wang *et al*., 2005; Abdel-Shafy *et al*., 2016). Similarly to oil products in this particular lake, also PAH's are surrounded with organic matter and therefore encapsulated. Reasons may be strong degradation product complexation with organic matter. Anthracene is the compound

Table 2

Total hydrocarbons, polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyls (PCB) in sediments of Lake Alūksne

Sampling No.	Depth, cm	Total hydrocarbons, mg kg^{-1}	Polycyclic aromatic hydrocarbon, µg kg^{-1}	Polychlorinated biphenyls, µg kg^{-1}	Sampling No.	Depth, cm	Total hydrocarbons, mg kg^{-1}
1	0-43	513	n.a.	n.a.	8	10-30	302
	43-80	906	3585	90		50-70	29
	110-140	149	5549	66	9	10-30	34
	170-190	169	n.a.	n.a.		50-70	35
	200-210	169	n.a.	n.a.	10	10-30	189
2	10-30	278	4554	n.a.		50-70	24
	60-80	208	n.a.	n.a.	11	10-30	27
3	10-30	302	4344	n.a.	12	10-30	81
	50-70	194	n.a.	n.a.	13	10-30	32
4	10-30	274	1753	63	14	10-30	73
	50-70	222	6634	n.a.	15	10-30	51
	80-100	229	n.a.	n.a.			
5	10-30	508	1015	4			
	50-70	153	1223	14			
	80-100	100	347	n.a.			
6	10-30	291	2732	n.a.			
	50-70	150	n.a.	n.a.			
7	10-30	343	1010	n.a.			
	50-70	87	255	n.a.			

Table3

Individual compounds of polycyclic aromatic hydrocarbon (PAH) in sediments of Lake Alūksne (µg kg^{-1})

Sampling No.	1		2	3	4		5			6	7	
Depth, cm	43-80	110-140	10-30	10-30	10-30	50-70	10-30	50-70	80-100	10-30	10-30	50-70
Naphthalene	77.2	54.4	9.3	33.9	63.0	90.7	31.3	14.1	9.6	8.3	10.3	9.4
Phenanthrene	465.6	836.9	40.6	70.9	129.1	763.6	97.3	19.8	7.1	30.6	13.0	7.7
Anthracene	465.6	170.3	8.4	21.2	129.1	150.8	25.9	4.6	1.6	6.1	3.0	1.5
Fluoranthene	465.2	377.2	79.0	119.7	287.9	331.7	165.7	28.7	7.7	38.6	20.9	7.0
Benz[a]anthrace	238.5	434.0	56.3	19.5	118.1	283.4	28.3	4.3	1.1	28.2	3.4	0.8
Chrysene	376.7	168.4	32.2	53.5	118.1	109.5	81.9	12.7	3.4	14.5	9.3	2.3
Benzo[k] fluoranthene	275.6	118.9	39.8	27.5	174.7	61.6	46.3	8.7	2.9	22.1	8.3	2.1
Benzo[a]pyrene	417.8	251.8	60.1	44.6	374.4	137.1	64.7	9.8	2.6	27.2	10.2	2.1
Indeno[1,2,3-*c,d*]pyrene	20.4	1.9	52.8	40.6	106.6	83.3	63.8	17.8	6.2	33.1	16.9	3.7
Benzo[g,h,i] perylen	<15	2.2	73.5	90.0	<15	2.6	129.4	30.8	10.8	42.4	34.6	6.9

Table 4

Content of heavy metals (mg kg^{-1}) in sediments of Lake Alūksne

Sampling No. and depth, cm		Cr	Ni	Cu	Zn	Cd	Pb	As
		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
1	0-15	11.3	9.5	16.3	66.4	0.08	4.1	<0.7
	40-50	10.1	8.5	17.3	85.9	0.29	7.1	1.1
	70-80	16.4	10.0	28.3	144.5	0.77	42.9	<0.7
	110-120	83.5	12.0	45.9	207.9	0.59	73.5	<0.7
	140-150	82.5	12.0	40.0	149.4	0.55	69.2	<0.7
	210-220	14.6	9.1	21.5	77.3	0.24	15.2	<0.7
	250-260	13.5	8.4	13.4	87.3	0.22	14.3	<0.7
	290-300	10.6	11.1	23.8	76.0	0.22	9.6	1.4
	380-390	15.3	9.3	36.1	80.4	0.20	21.2	2.3
5	10-20	25.2	12.9	40.1	280.9	1.22	60.6	<0.7
	30-40	23.0	12.7	48.5	249.3	1.10	69.2	<0.7
	50-60	20.5	11.5	24.4	118.8	0.59	38.9	<0.7
	80-90	10.0	7.7	12.5	77.0	0.23	11.6	<0.7
	110-120	12.4	8.6	18.0	68.7	0.19	10.7	<0.7
	150-160	15.2	8.2	10.0	75.5	0.21	14.2	<0.7
	180-190	12.8	8.3	12.1	84.1	0.17	5.7	<0.7
	200-210	16.1	10.1	12.5	73.1	0.22	9.4	<0.7
	230-240	8.7	9.3	19.8	84.3	0.32	10.7	<0.7
	270-280	17.0	10.1	11.0	69.5	0.17	5.8	<0.7

which very quickly underwent photodegradation yielding an thraquinone as the main degradation product (Dąbrowska *et al*., 2008), but major role to enhancing solubility of anthraquinone and their complexation plays humic substances (Klavins *et al*., 2008). These reactions and heavy fractions and properties of pollutants are main reasons why deeper sediment layers as well as far away located sediments

from inlet source do not show presence of enlarged oil and PAH's concentrations. Also, low solubility of PAH's in water (Abdel-Shafy *et al*., 2016) establishes their stability and low bioavailability and toxicity to food chains, for example, to fishes of Lake Alūksne (Rudovica *et al*., 2015).

Polychlorinated biphenyls were widely used as dielectric and coolant fluids in electrical apparatus and in heat transfer fluids affirming previously mentioned type of source – crude oil and burning residues. Because of their longevity and PCBs' environmental toxicity and high bioaccumulation rate, they have been classified as persistent organic pollutants (POPs) (Vorkamp, 2016). PCB production was banned for some time, but we still may detect these compounds in the environment till nowadays. Alongside increased concentration of heavy metals (Stankevica *et al*., 2015), also PCB's are distinctly correlated to human impact to environment. We have analyzed PCB's content in 5 samples and detected presence, hence amount which should take account before recovery activities – however PAH's may have elevated negative influence and risk to environment Individual compounds of PCB's have exceeded minimal requirements of government regulations up to 6 – 12 μg kg^{-1}, but they are not even close to concentration which allocates studied sediment as contaminated.

The content of metallic elements in sediments was detected for 60 samples (10 samples per coring) dispersed to the max depth 3.9 m. Despite the presence of organic pollutants, there was no identified contamination of heavy metals (Table 4). Metal concentrations did not exceed geochemical background and are very similar with concentrations in undisturbed natural lake sediments (Klavins *et al*., 1995; Klavins *et al*., 2001; Klavins *et al*., 2011). Although, when compared results, in sampling place no.1 they differ with a little bit higher metal (Pb, Cu, Zn, Sn, Cd, Cr) concentrations due to composition of pollutant. Metal ions may be strongly correlated with oil and PAH contamination (not detected in deeper located sediments) – those in lake sediments are Bi, Th, Y which can be concluded as pollution from former electro-technical factory, however concentrations are still slight and do not pose threats to environment and human health. Also, it was approved when tissues of fish were studied in Rudovica *et al*., 2015. Our research concluded that sediments shall be treated from hydrocarbons in urbanized gulf near Alūksne City, if sapropel is recovered, preliminary treatment is compulsory for further use as the resource.

Conclusions

The geochemical study in Lake Alūksne has shown that despite strong anthropogenic influence of emitted pollutants near Alūksne City, sediments are not strongly contaminated to put on the priority list for remediation. If sediments are extracted for lake remediation and possible resources recovery procedures, further research work must be performed in optimized mode in the gulf only. Crucial aspects in study success were careful preliminary studies of available geological and previously published data, careful interpretation of gained information on organic and inorganic contents in lake bottom sediments. Our research concluded that sediments shall be treated from hydrocarbons in urbanized gulf near Alūksne City, if sapropel is recovered, preliminary treatment is compulsory for further use as the resource.

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IMPACTS OF MUNICIPAL WASTEWATER TREATMENT PLANTS ON WATER QUALITY IN THE BERZE RIVER BASIN

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Abstract
Eutrophication caused by water pollution with nutrients (nitrogen (N) and phosphorus (P)) is one of the main environmental problems nowadays. Poor water quality might be caused by many natural and anthropogenic factors. The most common anthropogenic factors are water pollution caused by intensive agriculture (e. g. intensive fertilizer use, high density of livestock) and discharges from municipal waste water treatment plants (WWTP). In this study, nutrient load from WWTP to the river Berze basin in time period from the year 2005 to the year 2015 was described. In total, 23 WWTPs of the study area were analysed using descriptive statistics to calculate average values of nutrient amount and concentrations discharged to river Berze basin. From 2005 to 2015 average N and P load from WWTP to the river Berze basin have been reduced, but concentration of N and P in treated wastewater (WW) have increased. The largest WWTP of the study area – Krigeri (WWTP of city Dobele) is subject to the regulations of treated WW quality set by Republic of Latvia Cabinet Regulation No. 34. The WWTP Krigeri meets the regulations, but overall situation in the study area suggests that strict control of smaller WWTP should be made.
Key words: waste water treatment, point source pollution, nitrogen, phosphorus.

Introduction

Important environmental issue in the European Union is eutrophication of the Baltic Sea. The Baltic Marine Environment Protection Commission, also known as Helsinki Commission (HELCOM) as its aim has highlighted good environmental status of the Baltic Sea, this includes concentrations of nutrients in the water close to the natural levels of a region (HELCOM, 2007). In order to reach these goals water quality has to be improved.

Water quality at a river basin scale can be affected by many natural and anthropogenic factors (Vega *et al*., 1998). These factors can be furthermore categorized as point source or diffuse pollution. Example of typical diffuse pollution can be fertiliser use in agricultural lands or forestry. These inputs are usually continuous, with a little variability over time (Carpenter *et al*., 1998). Point sources are relatively easy to locate and include the direct inputs from municipal sewage systems and/or industrial discharges (Thyssen, 1999). Both diffuse and point sources cause significant water pollution with nutrients (N and P) (Vega *et al*., 1998; Pieterse, Bleuten, & Jørgensen, 2003; Withers *et al*., 2011).

Municipal wastewater (WW) is a very composite mixture. It consists of a high number of substances. Mainly these components are domestic WW (sewage from households, municipal establishments, and small businesses (<50 employees)), sewage from larger (>50 employees) industrial and commercial companies and hospitals (Pescod, 1992). In Latvia, the treated WW quality is controlled and set to fit concentrations of nutrients (Ministru kabineta noteikumi Nr. 34, 2002). For WWTP (waste water treatment plant) larger than 10 000 PE (population equivalent) concentration of total Nitrogen and Phosphorus in treated WW shouldn't exceed 15 mg l^{-1} for N and 2 mg l^{-1} for P. Smaller WWTP (PE<10 000) is not controlled, but is subject to HELCOM recommendations. Treated WW concentration of total nutrients for WWTP with PE 300-2000 should not exceed N - 35 mg l^{-1}; P – 2 mg l^{-1}; WWTP with PE <300 N - 25 mg l^{-1}; P – 5 mg l^{-1} (HELCOM, 2007).

The aim of this study was to determine municipal waste water treatment plant impact on water quality affected by nutrient load to the Berze River basin during the time period of 2005-2015.

This study has been done as a part of author's doctoral thesis. Data used in this study have been obtained and prepared for hydrological modelling with HYPE model (Lindström *et al*., 2010) as part of the MIRACLE project.

Materials and Methods

The Berze River basin was used as a study area (Figure 1). The Berze River is situated in the central part of Latvia and is the tributary of the Svete River. The length of the Berze is 109 km with an elevation difference of 108 m per 109 km. The Berze River has a contributing drainage area of 872 km^2. The river starts around 120 m above the sea level in drained meadows in the southern part of the Eastern-Courland highland. In the middle part of basin there is a hydro-power plant 'Annenieki' with a reservoir that can possibly influence nutrient retention.

According to CORINE land cover data, the study area consists of mostly agricultural areas (56.6%) and forest and semi-natural areas (384%), remaining area: wetlands (1.2%), water bodies (1.1%), artificial surfaces (discontinuous urban fabric, industrial or commercial units, dump sites, green urban areas, sport and leisure facilities) (28.0%).

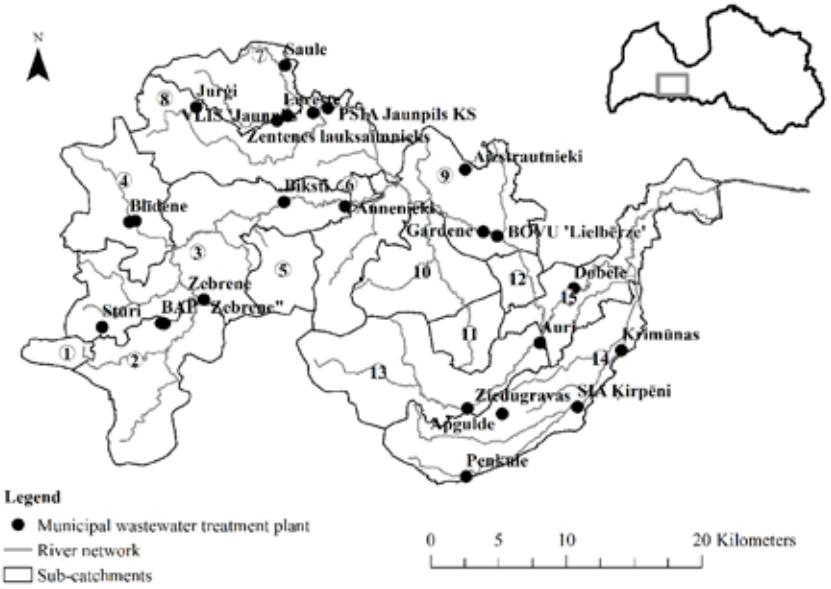

Figure 1. Location of the study area: WWTP in the Berze River basin.
(Map shows only still operating WWTP and the location of sub-basins).

Table 1

List of wastewater treatment plants in the Berze River basin

No.	WWTP title	Sub-basin ID	Population equivalent (year 2014)	Reconstruction/opened
1	'BAP Zebrene'	2	0*	Opened in 2011
2	'Zebrene'	3	20	2004
3	'Annenieki'	3	76	2015
4	'Stūri'	3	14	1988
5	'Blīdene'	4	450	1985
6	'Leveste'	7	119	2003
7	'Saule'	7	37	2004
8	'Biksti'	8	23	2010
9	'Jaunpils'	8	7000	2002
10	'Jurģi'	8	55	2003
11	'VLIS Jaunpils'	8	1	1968
12	'Lielbērze'	9	0*	1980
13	'Gardene'	9	427	2014
14	'Aizstrautnieki'	9	26	2010
15	'Krigeri'	12	12187	2001
16	'Zelta Druva'	12	-	Closed in 2007
17	'Auri'	13	24	1987
18	'Ziedugravas'	13	27	2010
19	'Penkule'	14	21	1989
20	'Krimunas'	14	44	2013
21	'Ķirpēni'	14	51	1982
22	'Strauti'	14	38	2010
23	'Šķibes'	15	-	Closed in 2007

* Non-municipal WWTP.

Water quality in the basin is affected by agricultural diffuse pollution and variable point sources. The largest point sources of nutrient (nitrogen and phosphorus) loading in the Berze River basin are wastewater treatment plants (WWTP). Overall 23 waste water treatment plants are located in the Berze River basin area. The largest of WWPT serves the city of Dobele (10 000 PE (population equivalent)). Other WWTPs service small communities with a PE <2000. WWPT of Dobele draws up to half of all waste water discharged form WWTP in the Berze River basin.

In this study, the data obtained from 23 WWTP is used (Table 1). Two of the WWTPs ('Zelta Druva' and 'Šķibes') was in operation until 2007. Two new WWTPs have been in operation ('BAP Zebrene') since 2011, due to negligible discharges these two WWTPs have been reported as one in this study. WWTP 'BAP Zebrene' serves for a landfill 'Zebrene' and is not municipal WWTP. The largest WWTP ('Krigeri NAI' – WWTP of city of Dobele) is located in the sub-basin ID 12.

Data on wastewater discharges and nutrient concentrations was obtained from the publicly available data source of '2 – Ūdens' maintained by the State Limited Liability Company 'Latvian Environment, Geology and Meteorology Centre.' In this study data reported from 2005 to 2015 was used. In most of the cases waste water sampling are done once a year and values of nutrients are calculated to tons per year. Microsoft Excel used to describe data from 23 WWTPs of river Berze basin. Descriptive statistics used to describe the data – average values of nutrient amount and concentrations and standard errors for the nutrient concentrations have been calculated using Microsoft Excel.

Results and Discussion

Nutrient load changes in time are shown in Table 2. Amount of nutrients loaded into river Berze basin varies, reasons of it should be examine considering error made by missing values (data missing in the reports provided by WWTP operators) in the data set. Also, the date of making water sample is not specified, but seasonality strongly affects waste water quality (Redeker, 2011; Vega *et al*., 1998). Another possible reason of differences in amount of nutrients loaded to river Berze basin might be reconstruction of WWTPs (date of WWTP reconstruction can be seen on Table 1).

Most of the Nitrogen from point sources to river Berze are loaded to sub-basin ID 12 (76.252 t). The lowest amount of nitrogen has been loaded from sub-basin ID 2 (0.055 t – sub-basin ID2 is not displayed on the fig. 2) and 15, but WWTPs in these sub-basins have been closed (sub-basin ID 15) or opened recently (sub-basin ID 2, also not municipal WWTP) causing total amounts being the lowest. The lowest amount of phosphorus from still existing municipal WWTP have been loaded to river Berze sub-basins ID 4 (4.165 t) and 7 (4.981 t) (Fig. 2).

Most of the phosphorus from point sources to river Berze are loaded to sub-basins ID 8 (10.131 t) and 12 (8.770 t). These sub-basins are mostly agricultural areas (Corine land cover data). Livestock research station is located on sub-basin ID 8 most likely causing large amounts of phosphorus leaking to river Berze basin. It is known that the livestock causes P accumulation in soils (Bouwman *et al*., 2013). These results suggest that large amounts of livestock affect waste water as well most likely sourcing from farm sewage systems.

Table 2

Total yearly nutrient (Nitrogen and Phosphorus) load from WWTPs to River Berze basin in time from 2005 to 2015

Year	Total Nitrogen (tons)	Total Phosphorus (tons)
2005	21.578	5.246
2006	20.924	3.841
2007	17.421	3.986
2008	16.786	3.133
2009	9.375	1.104
2010	16.008	0.968
2011	7.274	1.829
2012	10.434	1.864
2013	8.894	1.613
2014	12.584	2.193
2015	9.994	1.533

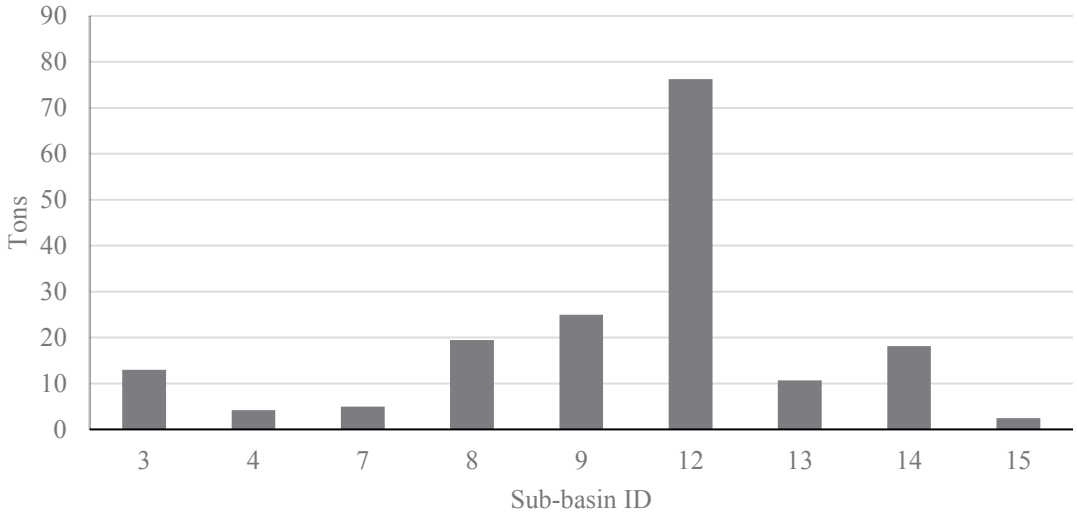

Figure 2. Total amount of Nitrogen loaded from WWTPs to river Berze basin from 2005 to 2015.

The lowest amount of phosphorus has been loaded from sub-basin ID 2 (0,000 tons – sub-basin ID2 is not displayed on the fig. 3) and 15 (0.326 t), but WWTPs in these sub-basins have been closed (sub-basin ID 15) or opened recently (sub-basin ID 2, also not municipal WWTP). The lowest amount of phosphorus from still existing municipal WWTP have been loaded to river Berze sub-basins ID 4 (0.850 t) and 7 (0.998 t) (Fig. 3).

As seen on Fig. 4 average nutrient (N and P) loads from single WWTP to River Berze basin have reduced in time, but not significant. Nitrogen spikes in year 2010 to average 1.21 tons of Nitrogen (15.458 tons of Nitrogen loaded to sub-basin ID 12). In year 2010 the largest WWTP (Krigeri – WWTP of city of Dobele) went under reconstruction (Table 1). Reconstruction might be reason of Nitrogen load peak, if the WWTP did not function properly at the time.

The maximum permitted concentration of N and P in treated WW are set by local legislation (Ministru kabineta noteikumi Nr.34, 2002) only applies to WWTP larger than 10 000 PE. For these WWTP maximum N concentration is 15 mg l^{-1} and maximum P concentration is 2 mg l^{-1}. Fig. 5 shows reduction of nutrient concentration in treated WW from WWTP ´Kriger' over time. The WWTP Krigeri meets N concentration regulations, but peaks in 2010 (15,90 mg l^{-1}) when the WWTP was under reconstruction. Starting with 2006 WWTP Krigeri meets regulation for Phosphorus concentration in treated WW.

Total average nutrient concentration in treated WW loaded to river Berze basin have not reduced

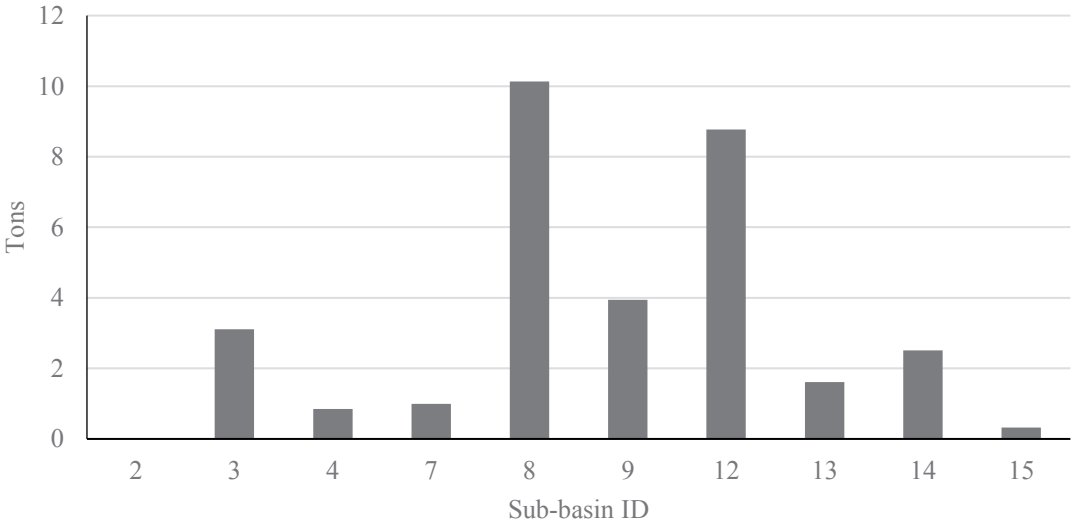

Figure 3 Total amount of Phosphorus loaded from WWTPs to river Berze basin from 2005 to 2015.

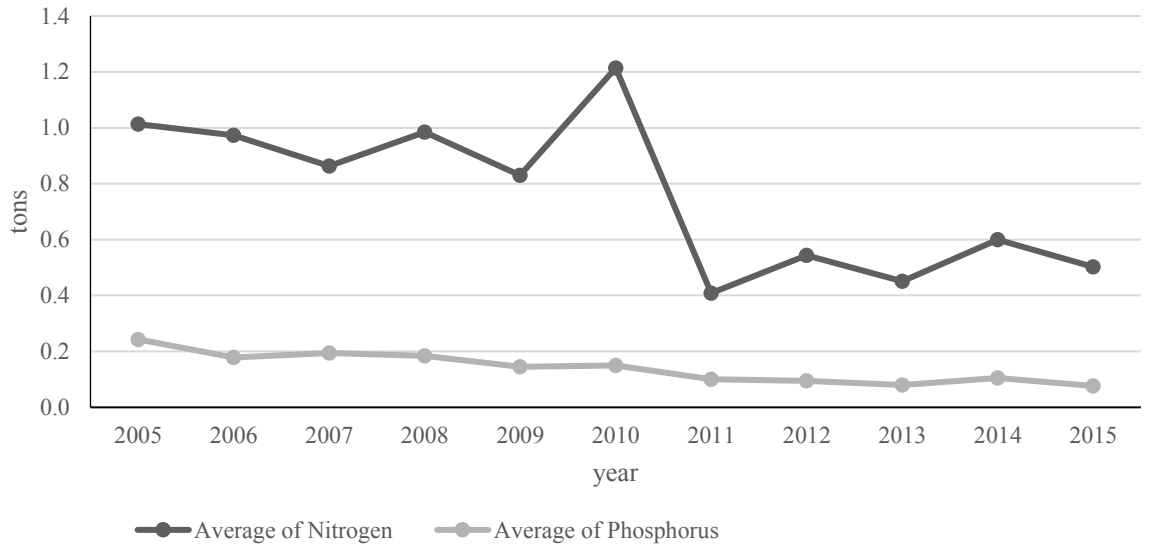

Figure 4. Average N and P load from WWTPs to the Berze River basin changes in time (2005 – 2015).

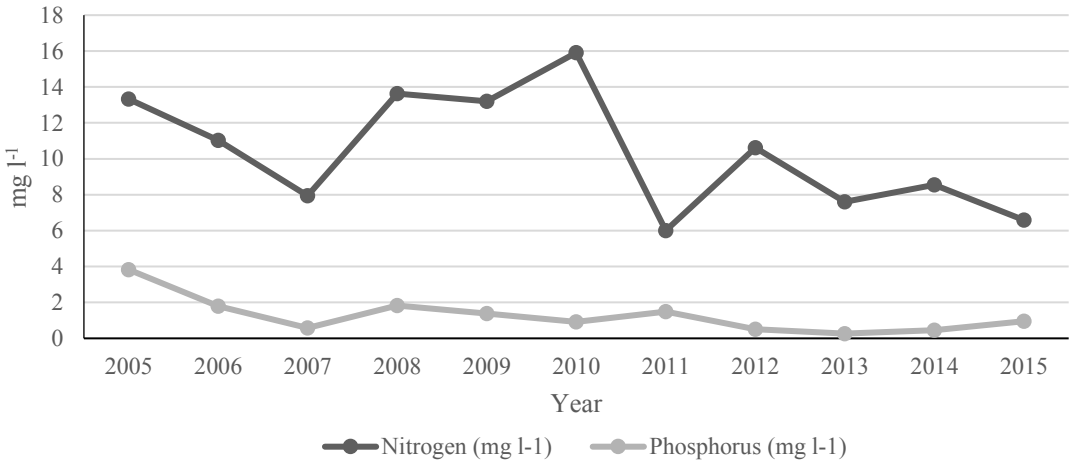

Figure 5. Concentration of nutrients (N and P) in treated WW from WWTP 'Krigeri' changes over time.

over time (Fig. 6). Average nitrogen concentration has met regulations (concentration of nitrogen 15 mg l^{-1}) only in years 2011 (14.56 ± 2.54 mg l^{-1}) and 2012 (14.04 ± 13.19 mg l^{-1}) and since then have increased to 32.57 ± 4.34 mg l^{-1} in year 2015. Average phosphorus concentrations have reduced from 7 ± 1.35 mg l^{-1} (in year 2005) to 3.69 ± 0.44 mg l^{-1} (in year 2015) and these concentrations are higher than set in the state regulations.

All the results indicate that to reduce N and P loads to river Berze basin from point sources small WWTP (PE < 10000) have to be targeted. The largest WWTP 'Krigeri' meets all the legislation set by state government and have reduced nutrient load to river Berze basin, but overall situation in the basin shows nutrient concentration increase suggesting other (PE > 10000) WWTP causing the problem. In Poland, poorly treated waste water has been recognized as one of the main eutrophication causes in the Baltic sea region (Kiedrzyńska *et al*., 2014). Results of this research stress the importance to target small WWTPs as the main pollutant with nutrients of the river basins in Latvia.

Conclusions

It was recognised in this study that the average amount of nutrients (N and P) loaded to river Berze basin from WWTP (waste water treatment plants) have reduced over time.

The largest amount of nutrients is loaded to sub-basin ID 12 where city of Dobele and its WWTP are located. In time from 2005 to 2015 in total 76.252 tons on nitrogen and 8.770 tons of phosphorus have been loaded to the river Berze basin sub-basin ID 12.

The WWTP Krigeri (the larges WWTP in the study area) meets N and P concentration in treated WW

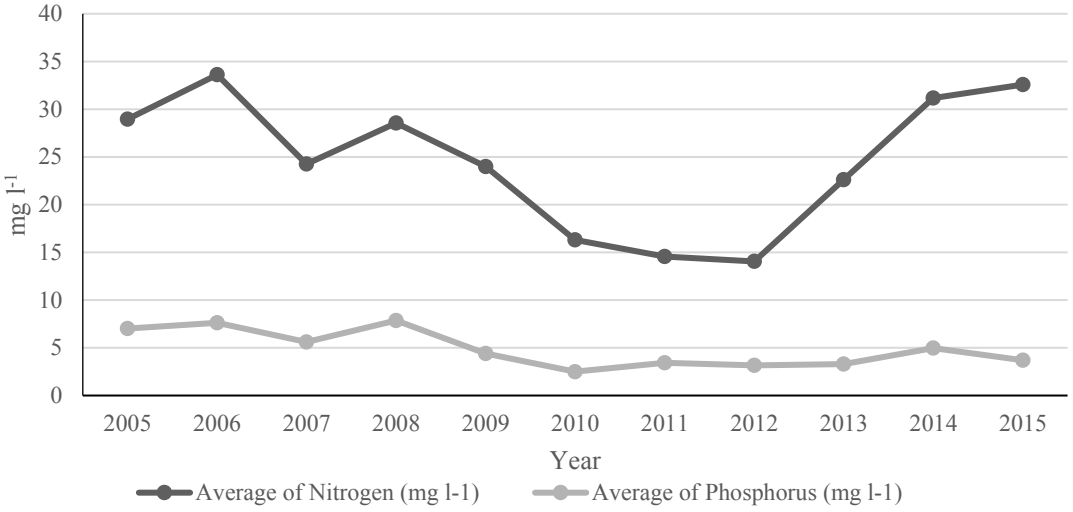

Figure 6. Average concentration of nutrients (N and P) in treated WW loaded from WWTPs to river Berze basin changes over time.

(waste water) regulations set by Republic of Latvia Cabinet Regulation No. 34. But the concentration of nutrients in treated WW loaded to river Berze basin haven't reduced in time pointing out that the problem might be the smaller WWTP (PE < 10000). Thus, to reduce nutrient load to river Berze basin small WWTP should be targeted.

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NUTRIENT REMOVAL BY SUBSURFACE FLOW CONSTRUCTED WETLAND IN THE FARM MEZACIRULI

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Abstract
A pilot-scale horizontal subsurface flow constructed wetland was installed at the farm Mezaciruli, Zalenieki county, Jelgava region, in the middle part of Latvia, in August 2014 to improve stormwater quality collected from the farmyard and demonstrate applicability of constructed wetland as a convenient treatment option for contaminated surface runoff. The examined water treatment system consists of a sedimentation pond as a pretreatment plant, a water pump, a water distribution well, and a horizontal subsurface flow constructed wetland with the surface area of 160 m^2. During the observation period of 27 months (2014 – 2016) water quality parameters such as total suspended solids (TSS), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), total nitrogen (TN), orthophosphate phosphorus (PO_4-P), and total phosphorus (TP) were monitored and nutrient removal efficiency of the system was examined. Water samples collected at the inlet and outlet were compared. Monitoring results obtained during this study show that on average concentrations of TN and TP were reduced by 22% and 80%, respectively. However, nitrate - nitrogen concentrations on average increased by 10%, as during the time period from June, 2015 to March, 2016 reduction in NO_3-N concentrations was not observed.
Key words: Constructed wetland, Subsurface flow, Nutrients, Stormwater.

Introduction

When water quality issues in agricultural areas are discussed, mainly the negative impacts of surface runoff and subsurface drainage from arable land, pasture, grassland or wastewater discharge from livestock farming are investigated. Research by Berzina & Sudars (2010) shows that nutrient concentrations influenced by point source pollution increases in high density livestock farming catchments in Latvia. Results from the long-term monitoring in Latvia show that nitrate concentrations are often higher than the nitrate limit value set by the Nitrate Directive for nutrient leaching from agricultural land (Jansons *et al*., 2011). The attention should be paid also on surface runoff from farmyards to assess the impacts of territories alongside farm buildings which usually can be described by a smaller catchment area comparing with the extension of agricultural lands. Farmlands may also be artificially drained or equipped with a stormwater handling system. Farmers frequently exploit their farmyards and nearest territories for temporary placing or storage of different agricultural machinery and byproducts. These areas can cause nutrients and suspended solids leakage to waters. In Latvia, which is a member state of the European Union, there is legislation related to the Nitrate Directive 91/676/EEC (1991) concerning storage and use of manure. Still there are not determined any regulations for water quality requirements of stormwater discharged from farmyard territories (LR MK, 2015). Therefore, in most cases stormwaters are directly discharged into streams as a surface runoff or through stormwater handling collector as a point source pollution causing undesirable losses of nutrients and other pollutants to water streams. That runoff may contain high concentrations of organic matter, plant and soil particles, residues from animal feed, litter, remains of mineral and organic fertilizers etc. Stormwater may also contain oil products from agricultural machinery, impurities from manure storage, dust and other dissolved or particulate substances flushed from roofs of agricultural building and other solid surfaces at a farmyard.

To reduce such negative impacts of agriculture on water resources, farmers and regulatory agencies require information on simple, cost-effective tools to control diffuse and point source pollution of watercourses (Tanner, Nguyen, & Sukias, 2005). In Latvia, it is suggested by regulations to include in-field and edge-of-field practices in artificially drained agricultural land to control surface and drainage runoff from agricultural fields in a more environmentally friendly and sustainable way. Interception and biofiltration of surface and subsurface runoff using sedimentation ponds, bottom dams, two-stage ditches, controlled drainage and constructed wetlands (LR MK, 2014) can supplement good agricultural practices to reduce rates of nutrient losses from agricultural lands (Tanner, Nguyen & Sukias, 2005) The same attention should be focused also on water collected at farmyards. Constructed wetlands may play an important role in reducing nutrient loads to receiving waters (Tuncosiper, 2009; Valkama *et al*., 2017). Constructed wetland is a well-known nutrient reduction measure as it is a low cost (Heistad *et al*., 2006) and low maintenance technology used for wastewater treatment around the world (Neralla *et al*., 2000; Vymazal, 2002), lately this measure is also recognized in Latvia. Choosing constructed wetland as a treatment plant for surface runoff, we should

comply with variation of discharge and contamination levels that generally depend on precipitation events and operations in a farmyard. Domestic septic systems are generally designed with a pre-treatment system (Heistad *et al*., 2006; Langergraber, 2007). At the farm Mezaciruli pre-treatment occurs in an open pond, which reduces a peak flow by receiving and compensating weather driven fluctuations of incoming water volume and allows the wetland to operate as evenly as possible. Pre-treatment and primary treatment is combined with elimination of solids (Vera *et al*., 2011), while subsequent treatment stages consist of a constructed wetland as a natural water treatment technology. The major removal mechanisms of nitrogen in constructed wetlands are nitrification and denitrification (Vymazal, 2002; Tuncosiper, 2009). Subsurface flow constructed wetlands require relatively shorter operation time and additional inputs in comparison to conventional treatment technologies (Vymazal, 2008). Moreover, treatment process can be operated and maintained by personnel without a specific knowledge or education (Garcia *et al*., 2005). The main purpose of this study is to inspect the treating efficiency of subsurface flow constructed wetland as a wastewater treatment plant under Latvia climate conditions in the pilot site. The objective of the article is to determine if the studied constructed wetland reduces nitrogen and phosphorus concentrations in rainwater.

Materials and Methods

Study site

A horizontal subsurface flow constructed wetland was installed at the farm Mezaciruli (56^0 34` 22 N, $23^0$ 29` 46 E), Zalenieki county, Jelgava region, Latvia. The study site is located in the nitrate vulnerable zone made in accordance with the criteria set out in the EU Nitrates Directive (EC, 1991), since intensity of agricultural production throughout the Zemgale region is high. The installation of the constructed wetland in Mezaciruli was started in August, 2014 with a purpose to improve the quality of stormwater collected from the hard surfaces in the farmyard territory. The examined water treatment system consists of a sedimentation pond as a pretreatment plant, a water pump, a water distribution well, and a horizontal subsurface flow constructed wetland with the surface area of 160 m^2. A filter material was filled in the subsurface part of the wetland at a depth of 0.9 m (Fig. 1).

There are 77% of hard surfaces covering 0.81 ha of the farmyard area, the rest are roofs of the farm buildings. Agricultural equipment and solid manure storage are covered with a polyethylene film and kept in the farmyard under the open sky. Greenhouses for growing vegetables and herbs, biogas plant, livestock buildings and office building are also located in the farmyard. Rainwater from the building roofs and paved area is collected in a stormwater capture wells or gullies and discharged through a close piping system to the sedimentation pond for accumulation and temporal storage, and further distributed to the wetland with the pump. Average concentration of suspended solids at the inlet was 83.36 mg L^{-1}. Color and turbidity of the incoming water visually differ from the water leaving the wetland at the outlet, which shows that the wetland successfully retains suspended solids.

In order to provide suitable conditions for effective wetland performance, which is particularly needed for rainwater treatment, the sedimentation pond before the constructed wetland was built. The dimensions of the sedimentation pond were estimated based on high precipitation events to ensure adequate storage capacity. The designed dimensions for the sedimentation pond are 135 m^2 in total area with an average water depth of 1 m. The pipe located at the outlet of sedimentation pond is equipped with a protecting sieve to prevent discharge of coarse material into the distribution well. The pump operates based on a predefined switch-on-tripping system preventing flooding from adjacent areas. Rainwater is discharged into the constructed wetland through a perforated infiltration pipe with the diameter 100 mm.

The surface area of the wetland is 1.2% of the catchment area. Common reed (*Phragmites australis*) is the most commonly used plant (Vymazal, 2002) in constructed wetlands because it is easy growing plant in moderate climate, therefore, it is chosen also in

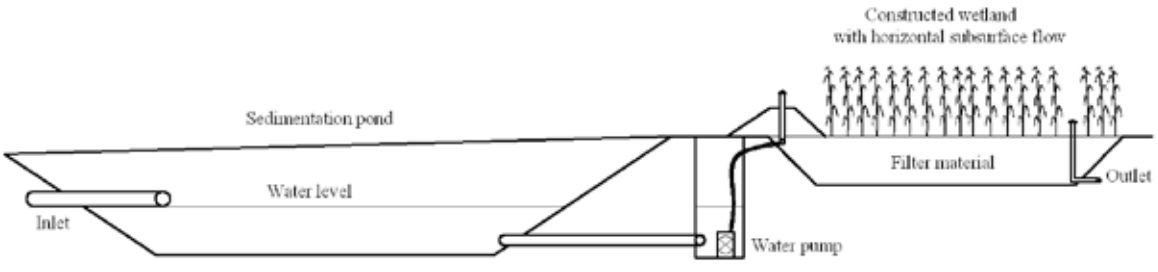

Figure. 1. Schematic drawing of the rainwater treatment system at the Mezaciruli farm.

Table 1

Analysis methods, Latvian Standards and equipment for the chemical analysis of water quality parameters

Water quality parameter	Latvian Standard	Analysis method	Equipment title
NO_3-N	FIA, ISO 13395:1996(E)	Flow analysis (CFA and FIA) and spectrometric detection	AutoAnalyzer Bran+Luebbe AA-3
NH_4-N	LVS ISO 7150-1:1984	Manual spectrometric method	
TN	LVS EN ISO 11905-1:1998	Method using oxidative digestion with peroxodisulfate	
PO_4-P	LVS EN 1189:2000	Ammonium molybdate spectrometric method	Spectrophotometer GENESYS 10S Vis
TP	LVS EN 1189:2000	Ammonium molybdate spectrometric method	

Mezaciruli. Most frequently used filtration media is gravel and crushed rock with size fractions of 4/8 and 8/16 mm (Vymazal, 2002), in Mezaciruli a filter layer consists of gravel and coarse sand with size fractions of 0.5 – 5 mm. The bottom part of the wetland is lined with a flexible waterproof membrane to prevent water leaching out of the system (Neralla *et al*., 2000).

Monitoring of water quality

To evaluate the efficiency of the rainwater treatment system, water samples were collected using a grab sampling technique at the inlet of the sedimentation pond and at the outlet of the wetland, once or twice per month depending on water discharge. If water flow at the inlet was not observed, water sample was not collected. The monitoring period for this study was from August, 2014 to October, 2016 or 27 calendar months. Water samples were analyzed for concentrations (mg L^{-1}) of total suspended solids (TSS), nitrate nitrogen (NO_3-N), ammonium nitrogen (NH_4-N), total nitrogen (TN), orthophosphate phosphorus (PO_4-P), and total phosphorus (TP) according to the Latvian Standards (Table 1) at the Hydrochemistry Laboratory of Latvian Institute of Aquatic Ecology. Methods used for the water quality parameter detection are described in Table 1.

To determine whether the nutrient concentrations change significantly after the treatment process in the wetland, data analysis offered by Statistical Package for the Social Sciences (SPSS) program was used.

Results and Discussion

The existing monitoring data show that the average concentrations of TN and TP at the inlet of sedimentation pond are 10.88 and 3.44 mg L^{-1}, respectively. Table 2 summarizes the average values and standard deviations calculated for the water quality parameters at the inflow and outflow of the rainwater treatment system. The results of standard deviations indicate that nutrient concentrations at the inflow and outflow vary greatly. Ammonium-nitrogen concentrations were generally high and quite similar at the inflow and outflow. Almost all of the monitored water quality parameters showed reduction. During the study period the concentrations of TN and TP was reduced on average by a 22%, and 80%, respectively. However, the concentrations of nitrate - nitrogen increased on average by 10%. Notably that during the

Table 2

Average, standard deviation and reduction values for the water quality parameters

Water quality parameters	Average concentrations at the inflow	Average concentrations at the outflow	Reduction, %
NH_4-N	1.78 ± 1.71	1.61 ± 1.17	**-9**
NO_3-N	2.96 ± 4.88	3.26 ± 4.73	10
TN	10.88 ± 8.29	8.49 ± 4.49	**-22**
PO_4-P	2.88 ± 4.88	0.54 ± 0.94	**-81**
TP	3.44 ± 5.67	0.67 ± 1.08	**-80**
TSS	83.36 ± 137.72	32.83 ± 22.14	**-61**

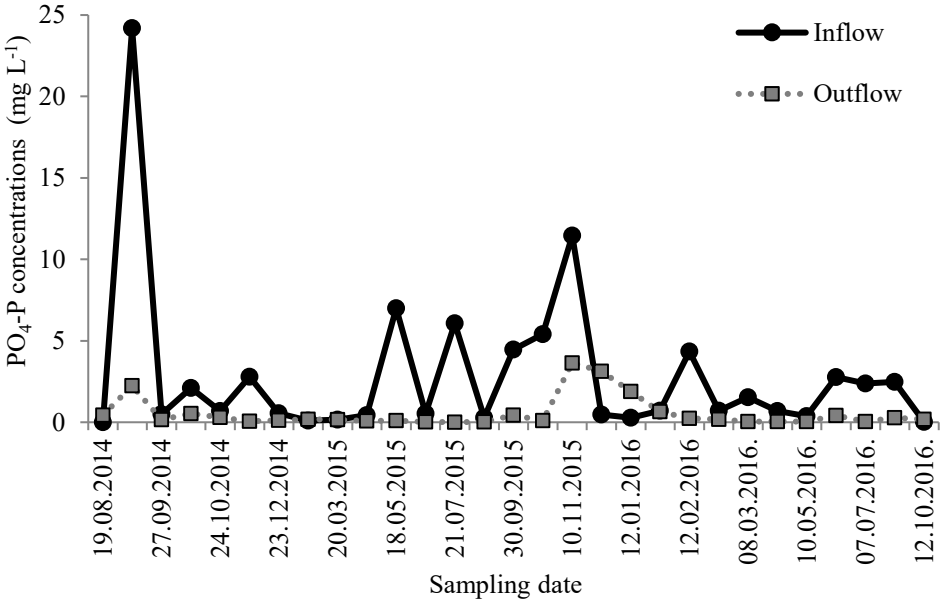

Figure. 2. PO_4-P concentrations (mg L^{-1}) during the study period.

time period from June, 2015 to March, 2016 reduction in NO_3-N concentrations was not observed.

To determine whether the nutrient concentrations change significantly after the treatment process in the wetland, ANOVA analysis was used. Null hypothesis was that wetland did not have a statistically significant impact on nutrient concentrations (Valkama *et al*., 2017). The results of ANOVA analysis showed that TP concentrations at the inflow were significantly different from TP concentrations at the outflow if the statistically significant differences were examined at a significance level of 0.05. Similarly PO_4-P concentrations at the inflow were significantly different from PO_4-P concentrations at the outflow at a significance level of 0.05. However, the difference was not significant for the concentrations of TN (α = 0.177), NO_3-N (α = 0.814), and NH_4-N (α = 0.668) when results from the inflow and outflow were compared.

Temporal changes in the concentrations of orthophosphate - phosphorus in the rainwater treatment system are presented in Fig. 2. The reduction efficiency depends greatly on the concentration of PO_4-P measured at the inlet. As PO_4-P concentration increases at the inlet, the reduction efficiency decreases. The study of Heistad *et al.* (2006) showed similar findings. Two episodes supporting this conclusion were observed in September 12, 2014 and November 10, 2015 when the highest concentrations of PO_4-P at the inlet were measured. In general, the system has performed well with respect to PO_4-P and TP removal throughout the study period.

The subsurface flow wetland did reduce the concentrations of TN in 52% of the sampling events (Fig. 3). The highest concentration of TN, along with the highest reduction rate was observed during the relatively dry period in October, 2015.

One of the factors strongly affecting the performance of constructed wetlands is vegetation (Vymazal, 2002). Garcia *et al*. (2005) have found that gravel of fine composition promotes greater growth of macrophytes, thus increasing ammonium retention through biomass uptake. Our results also showed that ammonium – nitrogen concentrations had a tendency to increase in vegetation period. The study of Kuschk *et al*. (2003) demonstrated the similar effect of seasonal temperature changes on the nitrogen elimination rates. Average air temperature during the warm season (April-September) was 13.7 °C, while during the cold season (October-March) air temperature decreased to 1.9 °C. Meteorological data obtained from Auce meteorological station.

Ammonium - nitrogen entering the wetland can be transformed into nitrate - nitrogen on the way to the outlet (Xinshana, Qina, & Denghuab, 2010). When different forms of nitrogen (Wojciechowska, Gajewska, & Ostojski, 2017) in water sampled at the inflow and outflow are analyzed, an organic form of nitrogen (TN - NH_4-N - NO_3-N = N_{org}) was 48% and 46% for the inflow and outflow, respectively. In contrast, the proportion of NH_4-N in TN was 21% and 22% at the inflow and outflow, respectively. Similar results were detected also for the proportion of NO_3-N in TN - 31% and 33% at the inflow and outflow, respectively. These results indicate that nitrogen uptake might be the primary nitrogen removal mechanism in this constructed wetland, but further research is needed to support this statement. Most likely phosphorus is absorbed by the filter material.

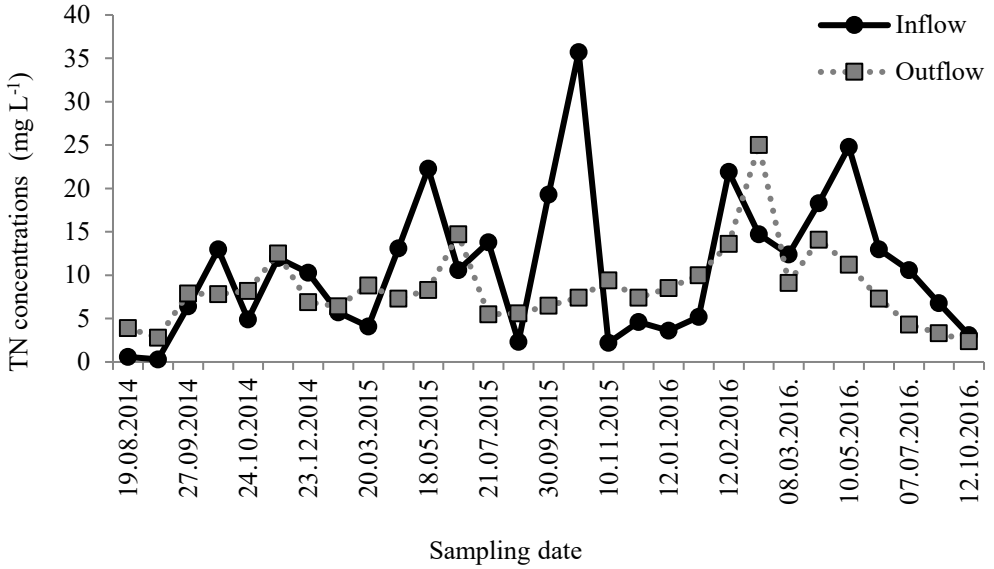

Figure. 3. TN concentrations (mg L^{-1}) during the study period.

Since the water pump is operated depending on the water level in the sedimentation pond, there may be cases where the water level maintains the pump on operating mode for the prolonged time period. A 19 m long infiltration tube inside of the wetland currently is able to distribute 0.72 m^3 of water. This amount of water can be infiltrated in 17 minutes, limited by the filtration coefficient of the filter material which is 60 m per day for coarse sand. Extended and intensive rainfall events or rapid snow melting can promote the situation when the surface of the wetland is covered with an open water layer which slowly infiltrates vertically into the filter. Presence of such conditions may negatively affect the efficiency of nutrient removal processes in the wetland.

Conclusions

This study showed that subsurface flow constructed wetland treating rainwater from agricultural farmyards has a potential to reduce nitrogen and phosphorus concentrations. Higher removal efficiency at the studied wetland was observed for phosphorus compounds as these compounds are mainly retained through physical processes such as filtration and absorption occurring in filter media. The reasons why nitrate – nitrogen concentrations increase after the constructed wetland remain an open question. Overall, additional and more detailed investigations are needed to understand processes behind nutrient removal in subsurface flow wetlands.

Acknowledgements

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AIR IONIZER AND INDOOR PLANTS INTERACTION IMPACT ON ION CONCENTRATION

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Abstract
The plants emit different types of volatile organic compounds (Bio VOC's) and can improve air quality: they effectively remove organic pollutions and reduce the number of microorganisms in the air by releasing phytoncides. The lack of negative ions in the air can cause deterioration of the health of humans breathing it. At the same time, an air saturated with negative ions can improve the state of health and provide a comfortable environment. In this article, the influence of the plants (*Cupressus macrocarpa*) on the number of ions is proved, based on a series of experiments performed with applying high-voltage pulses (air ionizer). This work is devoted to the elaboration of the mathematical relationship between the air ions concentration and the factors influencing it. For this purpose an experimental stand was made, consisting of two equal compartments: one contained the plants while another one was used as a control without plants. It was concluded that the plants, in general, are able to stabilize the ion concentration and to reduce its fluctuations. The plants help to increase the concentration of negative ions and to decrease the concentration of positive ones.
Key words: air ions, plants, microclimate, high-voltage pulses.

Introduction

Aerosol is a collective term for the myriad of particles present in the atmosphere. The size spectrum of these suspended particulates ranges from the smallest cluster ions to relatively large organic matter with radii of order 10^{-4} m (Pruppacher & Klett, 1998). Atmospheric ions are frequently classified into large ions ($r > 3$ nm), intermediate ions ($1 > r > 3$ nm), and small ions, which are typically 0.5 nm in radius. Large ions are often classified as charged aerosol particles and have a distribution of electrical charges, whereas intermediate and small ions have a unit charge (Hõrrak *et al*., 2000). Atmospheric particles with small diameters transform in different layers of the atmosphere. Atmospheric particles have an important role in cloud formation and global radiation processes. The most recent studies on the formation of atmospheric particles and their impact on the human organism are based on the importance of these processes (Kulmala *et al*., 2000).

Data on the presence of small charges in the layers of the atmosphere prompted a study of the causes and effects of air ionization. Several causes of formation of these charges need to be noted: free electrons, their deficit or excess, the presence of particles in the air to which atoms attach (Griffin *et al*., 1962).

The variable chemical composition of negative ions reduces the mean number of water molecules attached to the central cation. Consequently, the negative ions are slightly smaller than positive ones, and can move faster in the electric field. A schematic diagram of the atmospheric ion production mechanism is given in Figure 1 (Aplin, 2000).

The topic of many discussions was the impact of air ions on the human organism. The formation of negative air ions in the atmosphere has a beneficial effect. Whereas, migraines, nausea and ill health can be caused by the presence of positive ions. In the natural environment, air ions are found near waterfalls, with the presence of particular atmospheric currents, radioactivity fluctuations or cosmic rays (Clements-Croome, 2004).

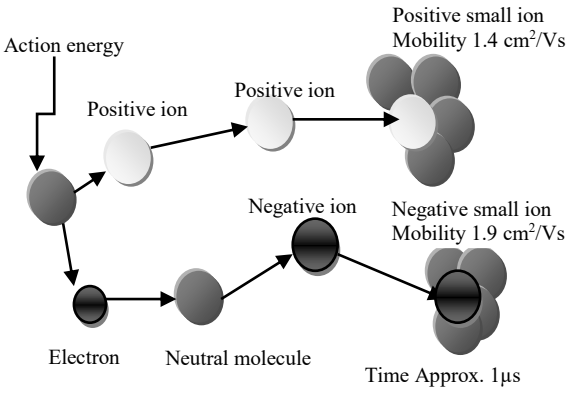

Figure 1. Schematic illustration of the production of atmospheric small ions from neutral molecules (Aplin, 2000).

The authors of many articles reviewed agree on the fact that most live organisms, including humans and plants, are affected by the balance and concentration of particles in the atmosphere (Smallwood, 1999). Any change in the proportions of air ions can affect biological organisms (Jovanic & Jovanic, 2001). Not only do air ions affect vegetation processes but plants themselves can generate ions, including negative ions under certain conditions (Nemeryuk, 1970). Therefore, not only temperature, pressure, humidity, daylight and the amount of minerals, but also the concentration of air ions in the atmosphere can be

considered an integral factor for the organization of the ecological microclimate of plants.

Negative and positive air ions are generated from different sources like lightning, the aerosols present in the atmosphere (Jagadesan *et al*., 2016). The presence of negative air ions (NAI) in the inhaled air is essential for normal functioning of human and animal organisms (Charry *et al*., 1987).

However, the NAI level is too small to be detected by conventional methods. In this paper, we propose that the generation of NAI by plants could be enhanced by electrization by high voltage pulses, thus providing for the high level of NAI. It has been proven that the production of air ions by plants and soil is observed under natural conditions. In particular, it occurs after a prolonged accumulation of air condensate near the plant, despite the fact that the formation of NAI is very slow (Tikhonov *et al*., 2004).

Some countries have already elaborated legal framework concerning the concentration of air ions in the work-rooms. On 16 June 2003, sanitary and epidemiological rules and regulations 'Hygienic Requirements for the Air-Ion Level of Industrial and Public Facilities SanPin 2.2.4 1294-03' (Санитарно-эпидемиологические правила и нормативы 'Гигиенические требования к аэроионному составу воздуха производственных и общественных помещений СанПин 2.2.4 1294-03') entered into force in the Russian Federation. According to these Requirements, optimal concentration of light negative ions amounts to 3000 – 5000, while concentration of positive ions should be half as much. However, in most cases, the concentration of favorable light, NAI indoors does not exceed few dozens, while the concentration of harmful positive ions is growing rapidly, especially if there are people, TVs, computer monitors and similar devices in the room. Besides, practical field testing reveals that the somnolence, apathy, headaches, etc. ascribed to the 'dead' air in enclosed spaces can be conquered effectively by supplying moderate concentrations of negative ions (Krueger, 1985). Air ions may be healing or they may harmfully affect human health. This effect depends on the ion concentration in the air and on the proportions of positive and negative ions. These proportions are characterized by unipolarity coefficient where:

$$K = \frac{N^+}{N^-} \quad (1)$$

N^+ and N^- are mean concentrations of positive and negative cluster ions.

Sanitary-hygienic characteristics of plants include their ability to release a special volatile organic compound called phytoncides. Most plants emit different types of volatile organic compounds (Bio VOCs) and even micro-amounts of Bio VOC have a great impact on the processes of formation of cluster ions (Duddington, 1969). This effect is reinforced when volatile compounds are emitted from the plant in the ionic form, e.g., Bio VOCs emitted from the needles of conifers are ionized because of charges accumulated on the sharp tips of the needles. To a certain extent, most of the plants are air ion generators. Despite that, aeroionizers are always present in places with artificial atmosphere, e.g. in submarines, and long-term space stations. With this, the saturation of the air we breathe at work and at home with the air ions is nowadays becoming a more urgent problem (Ponomarenko, 2015). We have tried to model the artificial air ionisation using indoor plants. For this purpose, a special experimental stand was constructed (Fig. 2). It would be perspective to develop technology for artificial greening that would ensure an optimal air ion concentration for human organism and indoor microclimate conditions. In order to develop such a technology, it is necessary to assess the changes in air ion concentration depending on indoor plants and microclimate. The aim of this research is to explore the impact of indoor plants and microclimate on air ion concentration in order to find opportunities to use the plants for air quality improvement. Studies with sciophytes and carbon filters have shown that they can improve the parameters of microclimate while removing organic pollutants in buildings. This combination can be considered one of the best means for fighting the symptoms of the sick building syndrome (Wolverton *et al*., 1989).

A specially selected combination of plants and appropriate soil content can remove and absorb VOCs in the buildings, which are harmful for humans. Many scientific papers published by Dr. Wolverton confirm the effectiveness of such biosystems for improving indoor microclimate. The articles describe how the pumping action created during transpiration near the plant roots, removes air pollution to have it converted into plant food. The charges between the Earth and the ionosphere are transferred by air ions. The carriers of negative charges rush to the ionosphere, while positive air ions move to the surface, where they enter into contact with the plants. The higher the negative charge of the plant, the more positive ions it absorbs. There can be assumed that the plants react to change the electric potential of the surrounding environment (Wolverton, 1999). More than two hundred years ago, scientist Grando concluded that for the normal growth and development of plants they need constant contact with the external electric field. Chizhevskii developed the first devices for artificial generation of NAI – Chizhevsky's chandelier (Chizhevskii, 1989).

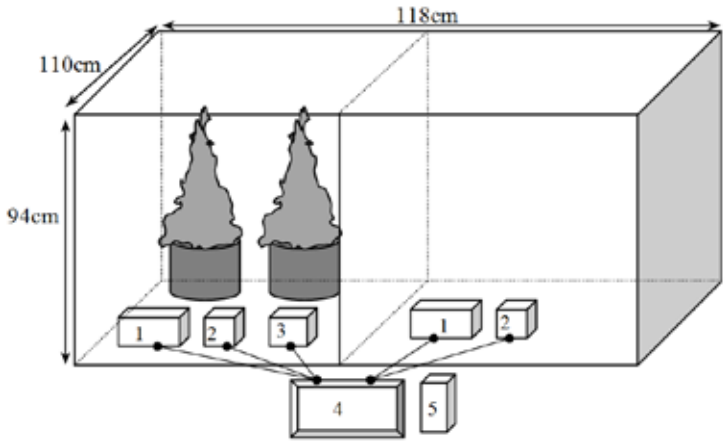

Figure 2. Schematic drawing of the experimental stand, consisting of two equal sectors. First sector houses the plants and air ionizer, the other one is empty. 1 – Air ion counter, 2 – microclimate multi-meter, 3 – air ionizer, 4 – laptop computer, 5 – radioactivity meter.

Materials and Methods

In our previous work (Sinicina *et al*., 2013; Sinicina *et al*., 2015; Sinicina *et al*., 2016) we studied the same plant samples as in the article by Tikhonov *et al*. (2004) and concluded that generation of NAI differs by the type of plant. Apparently, NAI are emitted from the pointed parts of the leaves. After conducting a series of experiments with five species of plants, it was concluded that the best among them, by the ability to generate negative ions, was *Pinus mugo*. This means that the *Pinus Mugo* could be used as a source of negative air ion. Unfortunately, this species of plants cannot grow in indoor conditions, where for them it is too warm and dry. Meanwhile, there are conifers, which are able to grow in the indoor microclimate, for example- Cypress *(Cupressus macrocarpa)* (Van der Neer, 2012), which was selected as the object of this study.

The aim of the experiments was to prove that plants are able to generate NAI to stabilize ion concentration and to reduce its fluctuations in response to the electrical stimulation. The main task was to create conditions with the least influence of external factors on the concentration of air ions (lighting, air streams *et al*.).

In order to quantify the influence of plants on the indoor ions concentrations, we have constructed an experimental stand made of 10 mm thick plywood. The general dimensions of the box are 118 cm × 110 cm × 94 cm. The box is divided by the partition into two equal compartments. A plant and air ionizer was placed in one compartment while the other one stayed without any plants. Every sector contained the devices for measuring the microclimate parameters. The box itself was placed in the basement type premises with natural air ventilation, without any windows, the walls covered with processed concrete. In addition, the radiation background was measured outside the box. During the experiment, there were no people in the premises. The premises were entered only to change plants and to retrieve the meters data. Air ion concentration was measured with the portable bipolar air ion counter 'Sapfir-3м'. This device provides simultaneous measuring of positive and NAI with minimal resolution of 10 ions per 1 cm^{-3}. The device measures air ion concentration in the air (mobility $k \geq 0.4$ $cm^2 V^{-1} s^{-1}$). This mobility interval is close to the class of cluster ions. During the measurements and according to their polarities, the air ions are channelized to positive or negative aspiration collector in aspiration chamber and, after entering into contact with the collector, the ions are discharged. Afterwards, the charge is sent to amplifiers and then the impulses are counted and displayed. The device counts the unitary charges of air ions, therefore if a ion is repeatedly charged, it is counted as several mono-ions.

Indoor climate parameters were determined using the multi-meter 'Easy Sense Q'. Systematic measurement error of this device for temperature is ± 0.3 °C, whereas error for relative humidity is ± 5%. Error for lighting is not specified. The total amount of radioactive α, β and γ radiation was measured in μSvh^{-1} with the portable device 'Gamma-Scout' with systematic measurement error less than 5%. For all devices, the average value of each measurement point was 10 minutes. Each time the measuring devices were placed in a distance of approximately 40 cm from the plants. The measurements were carried out in automatic mode for each *species* of plants individually, constantly during a 70 hrs time span.

Results and Discussion

The experimental data (Table 1) show that the number of positive air ions in the box is higher than the number of negative air ions: 41% with plants,

5% with air ionizer and 52% in an empty box, but in the sector with plants and air ionizer the number of negative air ions in the box is higher than the number of positive air ions: 110%. The maximum number of positively and negatively charged air ions was found in the sector with the ionizer: 199292 cm^{-3}/20182cm^{-3}. Whereas the minimum value of the same particles was observed with the presence of plants: 59 cm^{-3}/ 44 cm^{-3}. These data reveal that, based on the air ion concentration and unipolarity coefficient (Fig. 2), the air on the box that used to the experiments is not recommended for human health (if not ventilated), in case of if in the box houses only plants or air ionizes and empty sector.

The box in this sector has an unacceptable concentration of positiveand negative air ions and inadequate unipolarity coefficient, because, basing on the SanPin 2.2.4 1294-03, admissible concentration of positive air ions is 400-50000 cm^{-3}, concentration of negative air ions should amount to 600-50000 cm^{-3}, while admissible values of unipolarity coefficient are $0.4<K<1.0$. In the box sector with plants and air ionizer, in turn, concentration of positive and negative air ions was stabilized and was in the permissible limits. During the experiment, the temperature and relative humidity in the box increases. The measured average ambient temperature in the box with plants is about 0.5 °C higher than in the box without plants.

The average humidity is up to 15% higher in the room with plants than in the room without plants. It means that plants increase the air humidity (the water is evaporated through leaf pores). As the natural radiation level fluctuates chaotically around the average value of 0.14 μSvh^{-1} and the amplitude of these fluctuations is less than 10%, the level of radiation can be considered as constant; fluctuations of radioactive background do not affect daily changes of air ion concentration.

Table 1

Average ions concentration and Indoor climate parameters

Conditions/ Parameters	*Plants*		*Air ionizer*		*Plants and Air ionizer*		*Empty box*	
	N^+, cm^{-3}	N^-, cm^{-3}	N^+, cm^{-3}	N^-, cm^{-3}	N^+, cm^{-3}	N^-, cm^{-3}	N^+, cm^{-3}	N^-, cm^{-3}
$N^{(min)}$	59	44	166262	155521	11685	24052	124	102
$N^{(max)}$	900	646	199292	201821	19024	36335	3240	2083
$N^{(average)}$, cm^{-3}	448	317	185273	175758	15262	32198	917	602
K	1.49		1.07		0.48		1.40	
*Category of working conditions	1.level, harmful		1.level, harmful		allowable level		1.level, harmful	
T, °C	25.3-26.1		24.5-25.1		25.3-26.3		24.1-24.5	
RH, %	22.3-27.2		21.3-25.2		22.3-25.3		21.1-24.2	
Sv, μSvh^{-1}	0.10		0.14		0.12		0.10	

*16 June 2003, sanitary and epidemiological rules and regulations 'Hygienic Requirements for the Air-Ion Level of Industrial and Public Facilities SanPin 2.2.4 1294-03' (Санитарно-эпидемиологические правила и нормативы 'Гигиенические требования к аэроионному составу воздуха производственных и общественных помещений СанПин 2.2.4 1294-03').

Figure 2. Changing in the unipolarity coefficient.

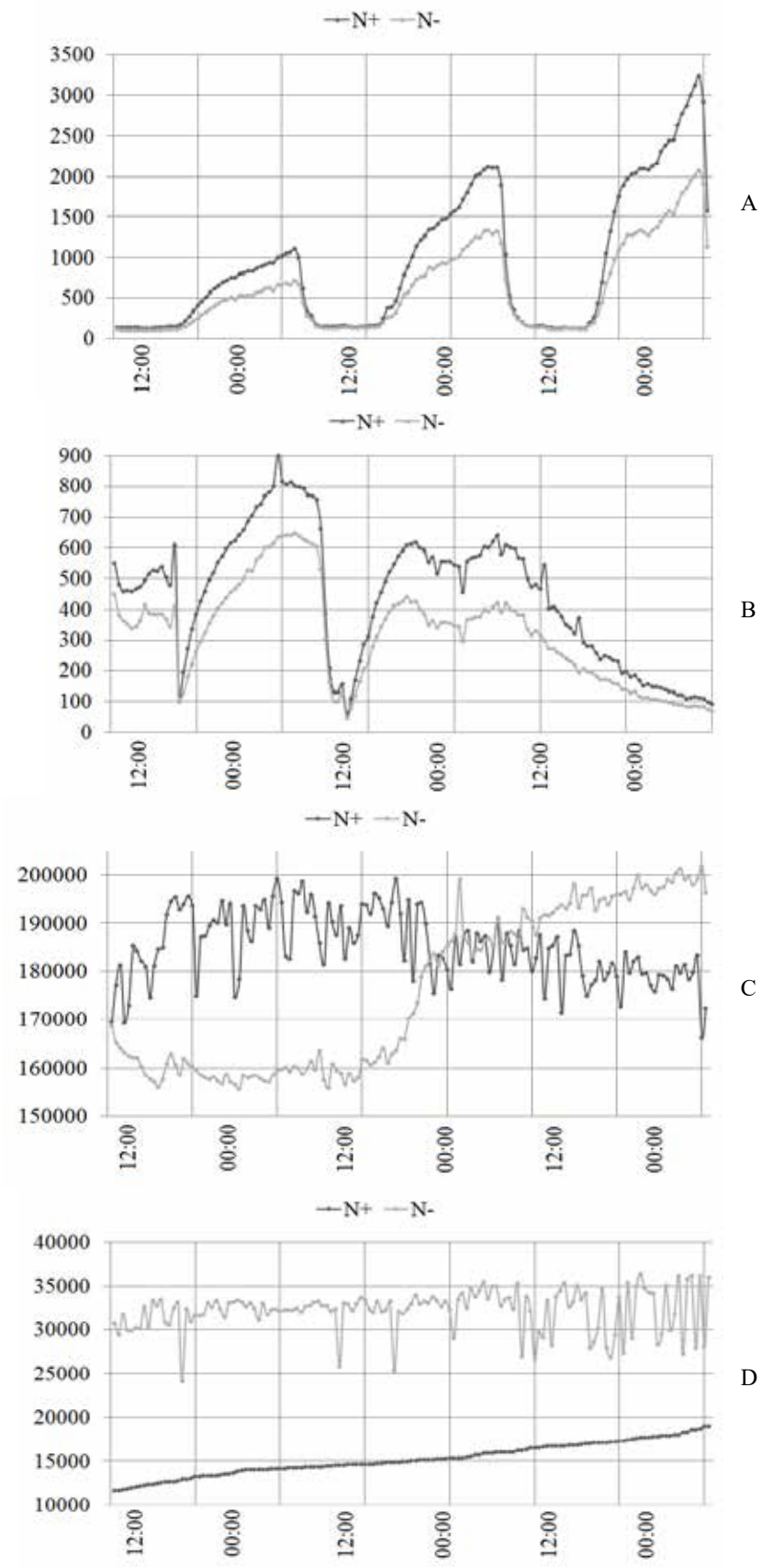

Figure 3. Concentration of negative and positive ions in the air in box cm^{-3}: A – Empty sector, B – Plants, C – Air ionizer, D – plants and air ionizer.

The indicated concentration of air ions in the presence of plants and in the empty sector (Fig. 3) allows us to conclude that fluctuation in the number of ions depends on the time of the day.

The maximum number of ions was observed in the night (01:00-05:00 a.m.), at the same time the decline of the ions was observed during the day (9:00-01:00 p.m.), although the experiment was conducted under conditions of complete darkness, i.e. without the influence of daylight or artificial light.

It can be explained as follows: negatively charged surface areas are mostly formed by free electrons flowing through dielectric adsorbent surfaces. An electron separates, leaving a positively charged area. Such positively charged surface areas lead to a change in air ion concentration. The number of air ions was decreasing during the day too. At night-time the action stops – the charged areas disappear with time and adsorption decreases, which leads to an increase of the number of air ions.

The room has a good ventilation. Air ions penetrate from the outside into the experiment room. The measurements in the box without plants points to the quantity of the ion outside air. If ventilation would be completely turned off in the room, it is possible that there would not be any cyclical changes in the graphs. Comparing the graphs with and without plants, it is possible to draw conclusions on how plants change the ion number in the diurnal period.

Conclusions

The positive and negative air ions concentration indoors varies periodically within the period of twenty-four hours – sector with plants (Fig. 3, B) and empty sector (Fig. 3, A): during the daytime it decreases, while during the nighttime it increases. In some cases, there are identified deviations from the periodicity. It is possibly related to the changes of Solar radiation activity.

In the box with plants, the concentration of positive and negative air ions is lower than in the empty sector or in the sector with air ionizer. However, at the same time, an unacceptable concentration of ions and inadequate unipolarity coefficient was experimentally recorded in these sectors.

The present study has shown that in the box with plants and air ionizer the concentration of negative ions remains almost constant during the twenty-four hour period. This means that this combination could be used as a source of support for acceptable concentration of positive and negative air ions.

Further experiments could be related to changes in air ion concentration depending on electrization of soil by high voltage pulses, thus providing for the high level of air ions.

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HEAVY METAL CONTAMINATION AND DISTRIBUTION IN THE URBAN ENVIRONMENT OF JELGAVA

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Abstract
The growing economy with following industrialization and urbanization has led to environmental contamination with trace elements worldwide. In urban environment, the large inputs of anthropogenic contaminants in atmosphere are arising from mobile and stationary sources. The snow sample analysis is one of methods to monitor air contamination with heavy metals in urban areas. The aim of this research is to analyse heavy metal contamination and distribution in urban environment of Jelgava city. The samples were collected twice in January and February. Snow samples were collected in 20 urban area sampling plots and one natural area sampling plot with three repetitions taken from 1.0 to 1.5 kg of snow. The concentration of trace elements was estimated in 126 melted and filtrated snow water using inductively coupled plasma spectrometer (ICP-OES). The average heavy metals and nutrients concentrations were calculated for each sample. The concentrations data of trace elements were analysed using agglomerative hierarchical clustering method.
The results show differences between results in January and February. The differences are related to anthropogenic impact intensity differences during the exposition periods of snow. The clustering results of snow samples taken in January show three clusters, but snow samples taken in February show four clusters.
Key words: ICP-AES; air quality; snow; pollution.

Introduction

The growing economic activity leads to more intensive exploitation of natural resources. The mining and industrial use of minerals as well as usage of different chemicals in agriculture and households lead to air pollution with trace elements (Tchounwou *et al*., 2014). The snow geochemistry has become a topic of interest since Antarctica, Greenland and Arctic snow and ice trace elements research results were published (Candelone *et al*., 1996; Gabrielli *et al*., 2005a; Barbante *et al*., 2011; Barbante *et al*., 2003; Shotyk *et al*., 2005). The anthropogenic impact on trace elements distribution are investigated in the Andes (Correia *et al*., 2003) and some studies have been done next to urbanised areas in the European Alps (Van de Velde *et al*., 2000; Schwikowski *et al*., 2004). Snow as an indicator for urban air pollution is used in several studies (Dossi *et al*., 2007; Engelhard *et al*., 2007).

Many of trace elements are hazardous (e.g. Cd, Pb, Ni) for human health and ecosystem (Wiener *et al*., 2003). The major trace elements are associated with both natural and industrial processes (Gabrielli *et al*., 2008). The sources of industrial air pollution trace elements are different: fossil and oil combustion (As, Cu, Co, Cr, V, Ni, Sb, Fe, Mn, Zn, Sn, Mn, Pb, Fe, Ni); waste water management (Pb, Zn); steel production (Cr, Mn, Ni, Co); transport emissions (Pb, Cu, Cr, Sn, Sb) and others (Pacyna & Pacyna, 2001). The concentrations of trace element in troposphere are with high temporal and spatial variability (Melaku *et al*., 2008). Snow samples with short exposition period are analysed in several studies. The trace elements from transport emissions in snow cover at roadsides and crossroads have been analysed (Loranger *et al*., 1996; Engelhard *et al*., 2007; Vasić *et al*., 2012). The significant anthropogenic emissions of trace elements related with industrial gases and energetic sector were found in long term trace element monitoring by Moreno *et al*. (2011).

The aim of this research is to analyse concentrations and distribution of trace elements in urban environment of Jelgava city. The objectives are (1) to analyze the composition of metal in snow in Jelgava city; (2) to analyze the distribution of metals in different places of Jelgava city and to conduct cluster analysis; (3) to compare the concentrations of metals in different places of Jelgava city taken in January and February.

Materials and Methods

Study area

Jelgava city has an area of 60.3 km^2 and more than 57000 inhabitants. The climate in Jelgava is cold and temperate. The yearly average temperature is 6.5 °C and average annual precipitation is 642 mm. Snow cover is normally from November till March, and the length of snow exposition period is impacted by local meteorological conditions such as city heat island influence. The main wind direction is from southwest. The research area is an urban territory of Jelgava city with different urbanisation level. Sampling plots were chosen close to transport corridors, industrial areas and living areas. The additional spot *Mežciems* was chosen in forest area in south west direction from the city centre to identify transboundary air pollution with trace elements. The sampling plots description is presented in Table 1.

Table 1

Sampling plots coordinates and anthropogenic pressure

No	Name of sampling point	X coordinate	Y coordinate	Characteristics of anthropogenic impact
1	Viskaļu str./Lietuvas str.	483086.650	6275689.140	Close to transit street and living area
2	Platones str./Lietuvas str.	482975.520	6276218.310	Close to transit street and living area
3	Savienības str./Lietuvas str.	483293.026	6276805.680	Close to transit street road and
4	Train station	483525.860	6277369.250	Jelgava train station
5	Tērvetes str./railway	481838.609	6276571.000	Transit street and railway
6	Rūpniecības str./Tērvetes str.	482043.660	6276941.400	Transit street and gasoline station
7	Tērvetes str./Pavasara str.	482380.199	6277740.316	Urban area with street canyon
8	Lielā str./Kalpaka str.	482241.609	6278438.223	Open area close to mine street
9	Lielā str./Dobeles str.	481903.940	6278612.119	Between two main streets
10	Aspazijas str./Asteru str.	481388.500	6278423.200	Open area close to school
11	Dobeles str./Satiksmes str.	481436.100	6278707.899	Main street and car roadworthiness test centre
12	Satiksmes str./Ganību str.	481507.072	6279135.466	Main street and gasoline station
13	Ausekļa str./Blaumaņa str.	482801.400	6279119.060	City market and intensive traffic
14	Pasta island	483563.400	6278804.200	Open are between rivers close to main street
15	Rīgas str./Brīvības str.	484290.526	6279236.721	Open are three main streets and gasoline station
16	Prohorova str./Neretas str.	485513.400	6278465.500	Industrial area
17	Garozas str./Rubeņu str.	485772.690	6278788.330	Near to railway
18	Aviācijas str./Lāčplēša str.	485302.390	6279209.020	Industrial area
19	Rīgas str./Loka str.	485437.330	6280722.440	Intensive traffic gasoline station
20	Institūta str./Rīgas str.	484630.350	6279806.980	Intensive traffic car parking area
21	Mežciems	486643.054	6277428.039	Natural area

Sampling strategy

The samples were collected twice in January and February with snow exposition period 7 days. The snowing was on the second of January and seventh of February, and sampling was done on 10th of January and 14th of February. Three snow samples were collected during each sampling period in each sampling plot. Plastic bags and gloves were used to avoid negative artefacts during snow sampling. The snow cover was collected via the use of a pre-cleaned Plexiglas device and a plate. Each snow column was taken from the whole depth of snow.

The concentration of trace elements was estimated in the 126 melted snow water and HNO3 solution samples using inductively coupled plasma spectroscopy (ICP-OES) method. The average concentrations of Cd, Cu, Pb, Ca, Mg, Na, Fe, Zn, Ni, Cr, Mn, K, As, Co, Li, Sr, Ti, Tl, Ba, V, Al, P and Sb were calculated for each sample. The sampling plot average concentrations standardised values for January and February concentrations data were analysed using agglomerative hierarchical clustering method.

Results and Discussion

Snow samples were collected in January and content of 26 trace elements have been analysed using ICP-OES. The concentrations of Cu, Pb, Ca, Mg, Na, Fe, Zn, Ni, Cr, Mn, K, Co, Li, Sr, Ti, Ba, Al, P were different between samples and other elements concentrations were smaller than instrument error, and they were excluded from further analysis. Snow samples taken in January, average trace elements concentration data cluster dendogram is presented in Figure 1.

There are three separate clusters with different trace element concentrations. The first cluster represents areas close to main streets with intensive traffic. The concentrations of Cu, Pb, Fe, Zn, Ni, Cr, Mn, Co, Li, Ti, Ba, Al, P are low and close to natural sample concentrations, see Table 2. The concentrations of Ca (4.5 ± 2.31 mg L^{-1}); Mg (1.6 ± 0.85 mg L^{-1}); Na (13.0 ± 1.49 mg L^{-1}); K (0.8 ± 0.6 mg L^{-1}) and Sr (7.3 ± 2.3 µg L^{-1}) are higher than second and third cluster and significantly higher than that in pristine areas (Shevchenko, 2016).

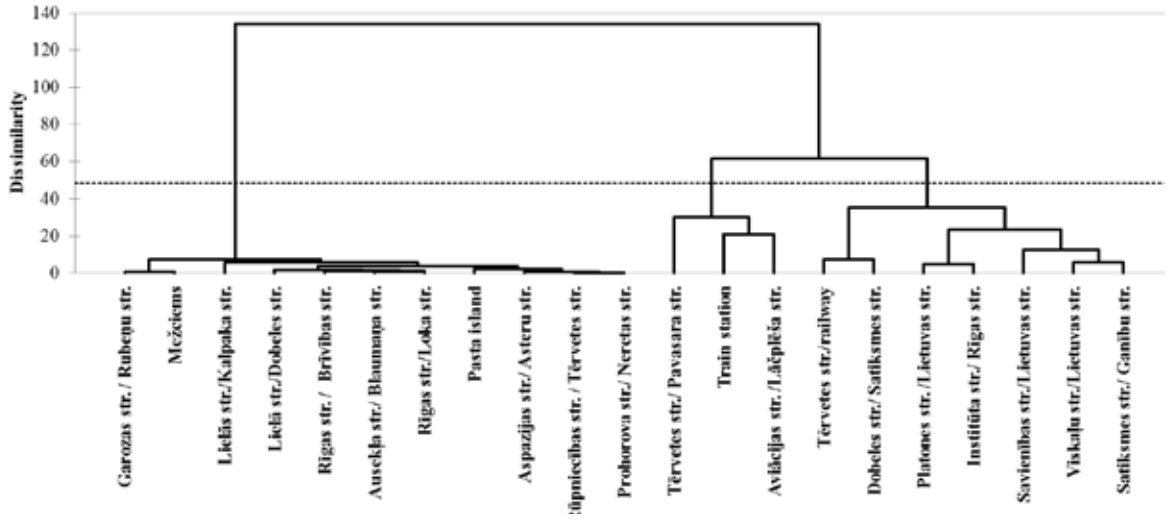

Figure 1. Dendogram of cluster analysis of snow samples taken in January.

Table 2

The mean ad standard deviation of trace elements by cluster for snow samples taken in January

Trace element	Measurement unit	1st cluster			2nd cluster			3rd cluster		
		No. of observations	Mean	Standard deviation (n-1)	No. of observations	Mean	Standard deviation (n-1)	No. of observations	Mean	Standard deviation (n-1)
Cu	µg L^{-1}	21	3.3	0.834	9	28.7	4.220	33	2.4	0.791
Pb	µg L^{-1}	21	1.2	0.669	9	7.3	0.876	33	0.9	0.608
Ca	mg L^{-1}	21	4.5	2.314	9	3.5	1.242	33	1.4	0.515
Mg	mg L^{-1}	21	1.6	0.850	9	1.2	0.531	33	0.5	0.191
Na	mg L^{-1}	21	13.0	1.493	9	10.7	7.918	33	2.7	2.584
Fe	mg L^{-1}	21	0.2	0.107	9	0.3	0.153	33	0.1	0.030
Zn	µg L^{-1}	21	44.5	11.346	9	101.2	23.963	33	32.2	12.535
Ni	µg L^{-1}	21	0.5	0.031	9	0.8	0.099	33	0.5	0.105
Cr	µg L^{-1}	21	0.3	0.103	9	0.6	0.134	33	0.3	0.003
Mn	µg L^{-1}	21	16.8	10.454	9	18.7	2.633	33	7.1	1.980
K	mg L^{-1}	21	0.8	0.643	9	0.3	0.133	33	0.2	0.010
Co	µg L^{-1}	21	0.2	0.025	9	0.2	0.021	33	0.2	0.004
Li	µg L^{-1}	21	0.2	0.033	9	0.2	0.058	33	0.1	0.020
Sr	µg L^{-1}	21	7.3	2.304	9	6.3	1.148	33	3.1	1.118
Ti	µg L^{-1}	21	2.6	0.922	9	3.8	0.994	33	1.1	0.605
Ba	µg L^{-1}	21	8.5	2.194	9	20.2	6.738	33	6.7	3.064
Al	mg L^{-1}	21	0.1	0.015	9	0.1	0.014	33	0.0	0.011
P	mg L^{-1}	21	0.0	0.009	9	0.0	0.021	33	0.0	0.005

Figure 2. Spatial distribution of cluster analysis of snow samples taken in January.

The second cluster includes three sampling plots close to railway and industrial area. This cluster characterises with relatively low concentrations of Ca, Mg, Na, K, Co, Li, Sr, Al, P. The concentrations of Cu (28.7 ± 4.22 µg L^{-1}); Pb (7.3 ± 0.85 µg L^{-1}); Fe (0.3 ± 1.5 mg L^{-1}); Zn (101.2 ± 23.96 µg L^{-1}); Ni (0.8 ± 0.1 µg L^{-1}); Cr (0.8 ± 0.1 µg L^{-1}); Mn (18.7 ± 2.6 µg L^{-1}); Ti (3.8 ± 0.99 µg L^{-1}); Ba (20.2 ± 6.74 µg L^{-1}) are higher than the first and third cluster and significantly higher than in natural areas (Shevchenko, 2016). The higher concentrations of Cu, Pb, Fe, Zn, Ni, Cr, Mn are associated with fossil and oil combustion (Pacyna & Pacyna, 2001).

The third cluster represents relatively clean areas with low concentrations of all trace elements. The snow sample plots classified in the third cluster are mostly open areas with some green infrastructure. The spatial distribution of clusters is presented in Figure 2.

Snow samples were collected in February and the content of 26 trace elements has been analysed using ICP-OES. The concentrations of Cd, Cu, Pb, Ca, Mg, Na, Fe, Zn, Ni, Cr, Mn, K, As, Co, Li, Sr, Ti, Tl, Ba, V, Al, P, Sb were different among samples and other elements' concentrations were smaller than instrument error, and they were excluded from further analysis. Snow samples taken in February average

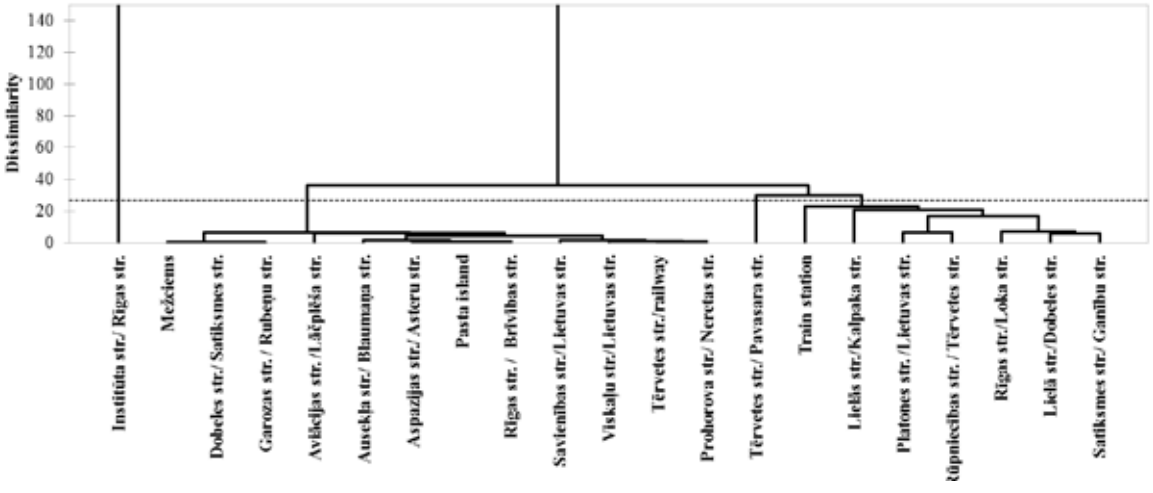

Figure 3. Dendogram of cluster analysis of snow samples taken in February.

Table 3

The mean ad standard deviation of trace elements by cluster for snow samples taken in February

Trace element	Measurement unit	1st cluster			2nd cluster			3rd cluster			4th cluster		
		No. of observations	Mean	Standard deviation (n-1)	No. of observations	Mean	Standard deviation (n-1)	No. of observations	Mean	Standard deviation (n-1)	No. of observations	Mean	Standard deviation (n-1)
Cd	µg L^{-1}	36	0.2	0.000	21	0.2	0.055	3	0.2	0.185	3	0.4	0.185
Cu	µg L^{-1}	36	15.5	16.141	21	29.9	13.677	3	10.4	0.625	3	150.4	0.625
Pb	µg L^{-1}	36	6.0	3.093	21	15.6	6.327	3	24.3	0.780	3	31.9	0.780
Ca	mg L^{-1}	36	23.0	13.693	21	45.1	34.135	3	14.4	0.025	3	259.6	0.025
Mg	mg L^{-1}	36	9.0	5.534	21	18.3	13.952	3	5.7	0.002	3	118.5	0.002
Na	mg L^{-1}	36	9.1	9.558	21	17.4	23.954	3	41.6	0.024	3	20.2	0.024
Fe	mg L^{-1}	36	1.1	0.588	21	2.7	1.523	3	0.4	0.003	3	14.6	0.003
Zn	µg L^{-1}	36	76.5	39.684	21	204.5	179.944	3	125.9	1.189	3	680.6	1.189
Ni	µg L^{-1}	36	1.4	0.570	21	3.1	1.367	3	0.9	0.309	3	11.8	0.309
Cr	µg L^{-1}	36	1.3	0.626	21	4.1	2.128	3	1.2	0.280	3	14.0	0.280
Mn	µg L^{-1}	36	97.7	51.036	21	178.8	87.448	3	62.8	0.099	3	983.6	0.099
K	mg L^{-1}	36	0.5	0.325	21	0.6	0.140	3	1.9	0.059	3	1.6	0.059
As	µg L^{-1}	36	1.9	0.000	21	2.1	0.201	3	1.9	1.912	3	3.5	1.912
Co	µg L^{-1}	36	0.7	0.262	21	1.2	0.415	3	0.4	0.216	3	7.5	0.216
Li	µg L^{-1}	36	0.7	0.331	21	1.4	0.840	3	0.4	0.021	3	9.6	0.021
Sr	µg L^{-1}	36	18.4	9.445	21	38.4	20.600	3	29.5	0.104	3	173.5	0.104
Ti	µg L^{-1}	36	16.4	7.802	21	27.9	8.458	3	4.9	0.071	3	192.0	0.071
Tl	µg L^{-1}	36	0.8	0.000	21	0.8	0.006	3	0.8	0.804	3	0.8	0.804
Ba	µg L^{-1}	36	27.4	20.499	21	87.7	83.590	3	32.0	0.581	3	222.1	0.581
V	µg L^{-1}	36	1.3	0.562	21	2.7	0.757	3	0.7	0.394	3	13.5	0.394
Al	mg L^{-1}	36	0.4	0.174	21	0.8	0.332	3	0.3	0.001	3	4.0	0.001
P	mg L^{-1}	36	0.1	0.079	21	0.3	0.064	3	1.9	0.003	3	1.2	0.003
Sb	µg L^{-1}	36	1.3	0.030	21	1.7	0.402	3	1.3	1.330	3	3.5	1.330

Figure 4. Spatial distribution of cluster analysis of snow samples taken in February**.**

trace elements concentration data cluster dendogram is presented in Figure 3.

The clustering results are quite different from January clusters, and it is related to extremely high traffic during snow exposition period. The February data are characterised with much higher concentrations of trace elements. The first cluster represents relatively clean areas and all trace elements concentrations are lower than for other clusters (see Table 3). The second cluster represents areas close to main streets; however, the third and fourth clusters consist of one sampling area.

Results show changes of snow contamination with metals affected by atmospheric deposition. Despite similar snow exposition time during winter period traffic and pyrogenic dust and aerosols reaches snow cover. These data strongly correlates with cultural and art events in the city providing increased traffic intensity and therefore emissions from transport.

Conclusions

The cluster analysis of snow samples taken in January and February show different cluster spatial and temporal distribution.

The February data clustering results show high anthropogenic impact related with fossil and oil combustion.

The future research has to concentrate on multidimensional analysis of point source and non-point source pollution impact on heavy metal spatial and temporal distribution in urban areas.

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ANALYSIS OF DAMAGED LAND CHANGES IN KLAIPEDA COUNTY OF LITHUANIA

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Abstract
The article presents the analysis of the current situation of the damaged land in Klaipeda County. The study found that the number of affected areas in the county – 266. Because the damaged areas consist of mineral quarries and territories occupied by dumps, the article contains the description and condition of these areas. It was found that mostly sand deposits (60) predominate in Klaipeda County, of which only 8 are used.
The smallest number of the deposits – the salt deposits (2). The mainly used deposits in the county are sand and gravel deposits – 29, unused – sand deposits (52).
The Klaipeda municipal waste management region comprises 7 municipalities, 39 old landfills and dumps were shut down. At present, 1 regional non-hazardous waste landfill has been arranged.
In Klaipeda County, in 2015, damaged land occupied 2,391.06 hectares and amounted to 0.46 percent of the county's area. During the period between the years 2005 and 2015 the damaged land area in Klaipeda County increased by 130.25 hectares.
The analysis of the damaged land by type of ownership showed that the damaged land areas in private land increased by 75.54 hectares or 58.52 percent. In the state land, the damaged land area decreased by 442.35 hectares or 20.04 percent.
Key words: damaged land, landfills, mineral quarries, rehabilitation.

Introduction

Article relevance. Damaged land management becomes more and more important, because due to the improperly managed and uncontrolled landfills as well as due to the damage of not rehabilitated quarries, the threat is being posed to human health and the environment. Landscape ecological stability and unity are life survival necessities.

Natural landscape is decreasing and losing the ecological and aesthetic value every year. The landscape is polluted by abandoned residential and farm buildings, reinforced concrete electric poles, illegal waste landfills, cut down forest areas, not rehabilitated quarries, innings, wasteland and others.

In the Journal of the National Land Service "Land Fund of the Republic of Lithuania by January 1, 2015" the damaged land is described in the following way: operating and depleted mineral quarries, peat bogs and landfill areas (Nacionaline, 2005 – 2015).

According to the principal purpose of land use, the damaged land is classed as land used for other purposes.

Minerals are natural materials existing in the earth's crust that can be used for substantive production or other uses (Lietuvos, 2001).

Mineral quarrying has a significant impact on nature and social environment (Burger, 2008).

The Law on Land of the Republic of Lithuania Art. 21 Item 6 (Lietuvos, 2004) states that the land owners and other users, exploiting mineral resources, must preserve the fertile soil layer and rehabilitation of damaged land.

Natural and legal persons exploiting mineral deposits and peat bogs, engaged in geological prospecting, exploration, construction and other works related to the fertile soil layer infringement must start the rehabilitation of damaged agricultural land and forest areas into agricultural land and forest, and if it is technically impossible – into bodies of water (Lietuvos, 1995).

According to the Terrestrial Law (Lietuvos, 2001 b), mineral deposits' users in the first five years of the use of geothermal resources must accumulate the funds needed for land rehabilitation after mining and other necessary measures of environmental elements to implement the recovery; however, this provision is not always carried out.

According to Virginija Atkoceviciene and Vilma Sudoniene (Sudoniene & Atkoceviciene, 2013), land owners and users, operating mineral quarries must comply with the requirements and regulations in order to preserve the fertile soil layer. In their research paper, the authors analyze the problems in the field of damaged land management and find that excavation, violation of accounting and control of the fertile layer of soil are not being carried out, which is necessary to regulate the rehabilitation of damaged lands.

Urbanization, the growth of population and generated waste in all parts of the world cause for concern and promote a sustainable and effective waste management system (Sumathi, Natesan, & Sarkar, 2008).

The Waste Management Law of the Republic of Lithuania (Lietuvos, 2002) provides that *the landfill*

is a waste disposal facility for the deposit of the waste onto or into land (i.e. underground). The landfill is assigned to a group of sources of pollution that pose a potential threat to groundwater users and other environmental objects.

15 waste disposal methods are foreseen in the European Parliament and Council Directive 2008/98/EC (The European, 2008) and the above-mentioned Lithuanian Republic Waste Management Law. Waste placement in a landfill is the least desirable option, but still the dominant method of Lithuania.

As stated in the European Parliament and the Council Directive 1999/31/EC (The European, 1999), operating and the after closing landfills should be monitored and properly managed in order to reduce or counteract adverse effects on the environment and human health. It is necessary to protect natural resources, minimize the destruction of land, to stimulate waste prevention, recycling, and disposal of used materials and energy recovery.

In planning, managing and rehabilitating damaged land areas rational, innovative models should be selected that ensure the sustainable use of natural resources and ecosystem conservation and management.

The object of the research is Klaipeda County damaged land.

The aim of the research is to carry out the analysis of the Klaipeda County damaged land area during the period between the years 2005 and 2015.

Goals of the research:

1. To describe the status quo of the damaged land in Klaipeda county.
2. To analyze and compare the damaged land change in Klaipeda county during the period between the years 2005 and 2015.

Materials and Methods

Comparative, analytical as well as statistical and logical analysis methods were used for the research.

The land fund statistics of the Republic of Lithuania (Nacionalinė žemės, 2005 – 2015), graphically depicted in figures, were used for the fulfilment of the research of the dameged land change in Klaipeda county for the years 2005 – 2015.

The following scientific methods were used in this article: the European Union directives and Lithuanian legislation, analysis of scientific literature, existing positioning and measurement, comparative method, logical analysis, analytical, filing method, graphic and generalization methods.

The article analyzed and assessed the current state of the damaged land in the city of Klaipeda, i.e. the current state of the mineral deposits' areas and dumps was analyzed, the statistics were presented, which were systemized to structure and presented graphs.

The study provides the damaged land change analysis in Klaipeda County. The ten-year period, i.e. the period between the years 2005 and 2015, was selected for the determination of the change. For the fulfilment of the analysis, statistics of the land fund and other sources of the Republic of Lithuania (Geological Survey of Lithuania, the Ministry of Environment, county and regional waste management centers) were used. They were systematized, analyzed and expression of the percentage was calculated during the preparation of the research.

General plans and solutions, regional development plans, National Waste Management Plan, regional waste management plans were considered.

Results and Discussion

Characterization of Klaipeda County.

Klaipeda region is the only region in Lithuania having access to the sea. In the north, it is bordered by Latvia and to the south with the Kaliningrad region of Russian Federation. It consists of 7 municipalities, but only 4 of them have a coastline. Neringa of them, except for tourism and the length of coastline of 60.6 km, can not fall within the scope of business activity, since it is located in a protected area. The coastline of the other three municipalities comes to the city of Klaipeda – 4.03 km, Klaipeda district – 12.98 km and Palanga city – 20.48 km. The territory from a northern pier in Klaipeda to the Lithuanian - Latvian border is 38.4 km. 3 municipalities – Šilute, Kretinga and Skuodas have no access to the sea (Markevicius, 2015).

Klaipeda county covers an area of 522220.58 hectars that makes 7.99 percent of the entire territory of Lithuania. The agricultural lands make up 53.55 percent, forest area 26.89 percent, water bodies 10.43 percent, roads 1.63 percent, other land 3.07 percent of the Klaipeda county.

According to the functional priorities of the region's territories established in the general plan of the Republic of Lithuania (Lietuvos, 2002 a) Klaipeda County area falls into the first functional area, in which the recreational use priority of the national interest, maritime shipping, offshore fishing, institutionally organized conversion, conversion set at the level of laws and sustainable forestry become apparent. Klaipeda region functional priorities include a transport development, industrial development, sustainable agriculture and forestry.

The current situation of damaged land in Klaipeda county.

In Lithuania, the areas damaged by quarries, peat bogs and landfills are several times larger than they should be according to the solid mineral mining open workings scale and the used mining technology.

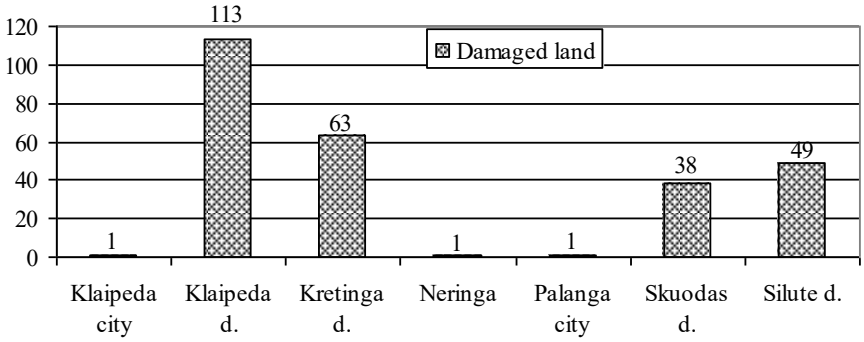

Figure 1. The number of damaged areas in Klaipeda County, in pieces
(Compiled by the author of the article).

Almost half of the damaged areas of Klaipeda County are situated in Klaipeda district (113 pcs., or 42.80 percent). The town of Palanga, Klaipeda City and the Neringa municipality (Fig. 1) each has one such area. The number of damaged areas in the county – 266.

Mineral deposits in Klaipeda County.

Lithuania has 17 species of minerals used in the world and investigated at various detail levels (Lietuvos geologijos, 2017).

Many Lithuanian minerals are on the surface, they are well researched, easily accessible – mined by open quarries. Separate kinds of mineral resources within the country are distributed unevenly. Their deposits cover only 4.3 percent of Lithuanian territory. In various layers of the deposits, there are almost all kinds of the most important non-metallic minerals (Lietuvos žemės, 2017).

Extensive spread of Lithuanian territory is characterized by gravel and sand. These resources are mainly explored not only in Klaipeda, but also in Vilnius, Kaunas, Utena, Alytus and Taurage counties. Peat deposits are located throughout the country, including Klaipeda County. Also, rock salt is found in the county. Although clay is found almost everywhere in the country, these minerals are not found in Klaipeda County.

Sand deposits are located mainly in Klaipeda County – 60, of which only 8 deposits are used. The least are salts deposits – 2 (Fig. 2).

It was found that, overall in the county, the most used are sand and gravel deposits – 29 (Fig. 3), not used – sand deposits (52).

Sand and gravel deposits are the most explored in detail ones. Currently, there are 24 peat deposits, which are preparatory explored. The prognostic 32 sand and 23 peat deposits were revealed (Fig. 4).

Landfills.

In 2006, Lithuania registered 850 municipal waste landfills (contaminated sites), of which 350 were used (Valstybės, 2013). Some of them were managed illegally, were not registered, with no permits. These landfills did not meet environmental and public health safety requirements, and their condition was poor.

Throughout Lithuania in 2009, there were about 680 small (up to 1 hectare) municipal waste landfills / dumps, about 120 medium-sized (1-5 hectare) landfills and 35 large (more than 5 hectares) landfills. In 2011, 11 regional landfills were arranged and adapted in Lithuania in accordance with the environmental requirements of the installation and use.

Klaipeda municipal waste management region comprises 7 municipalities, 39 old dumps were shut down in them, 1 regional non-hazardous waste landfill, 10 bulky waste collection sites, 7 green waste composting sites were arranged, a mechanical waste treatment plant was built, 10,000 individual composting containers were purchased.

At present, there is no complete and accurate information about the damaged areas in the country; therefore, it is necessary to systematically collect and analyze data. This should be a single institution which, according to the data available, should coordinate the land rehabilitation process.

The damaged land change.

In 2015, the damaged land in Klaipeda County covered 2391.06 hectares and amounted to 10.62 percent of all Lithuanian damaged lands and 0.46 percent of the entire county‘s area. In 2005, the analyzed land area of the county was 2,260.81 hectares or 9.14 percent of all country’s damaged lands and 0.43 percent of the Klaipeda County area (Fig. 5). During the period between the years 2005 and 2014 the analyzed area declined each year (366.81 ha, or an average of 40.76 hectares per year) and was only 1,894.00 hectares, but in 2015 the area picked up again. During the period between the years 2005 and 2015 the damaged land area in Klaipeda County increased by 130.25 hectares or 5.44 percent. It was influenced by the Klaipeda region landfill section III with the asbestos waste disposal area, the reserved

area for construction and mineral production, for example, oil extraction area businesses and 4 new drilling equipment.

The largest area of the damaged land in 2015 was fixed in the Klaipeda region municipality, accounting for 15.32 percent of the entire Klaipeda County damaged land. During the period between the years 2005 and 2015 the damaged land area in Klaipeda region decreased by 51.73 hectares or 12.38 percent.

In 2005, the largest area of the damaged land was in Kretinga district. That area decreased by 142.49 hectares or 32.55 percent. There were no damaged lands in the Neringa municipality during the period between the years 2005 and 2010. In 2011 – 2013, the area of 1.85 ha was fixed, which in 2014 decreased to 0.93 hectares. In 2015, the damaged land area in Neringa municipality occupied 1.99 ha, i.e. the smallest area of all Klaipeda district municipalities.

Damaged land can be as in the land of private property, as well as in the state land. The damaged

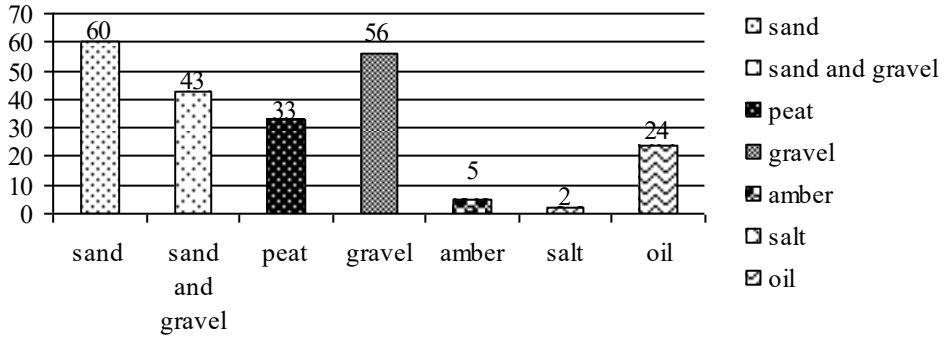

Figure 2. Mineral deposits in Klaipeda County
(Compiled by the author of the article).

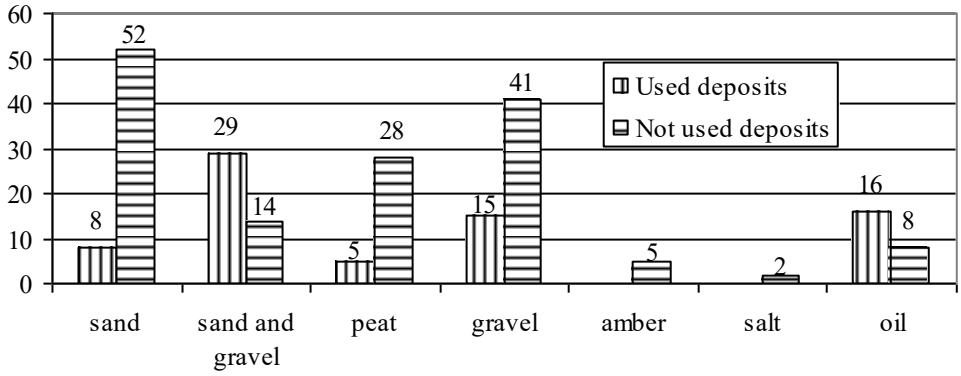

Figure 3. Condition of mineral deposits in Klaipeda County
(Compiled by the author of the article).

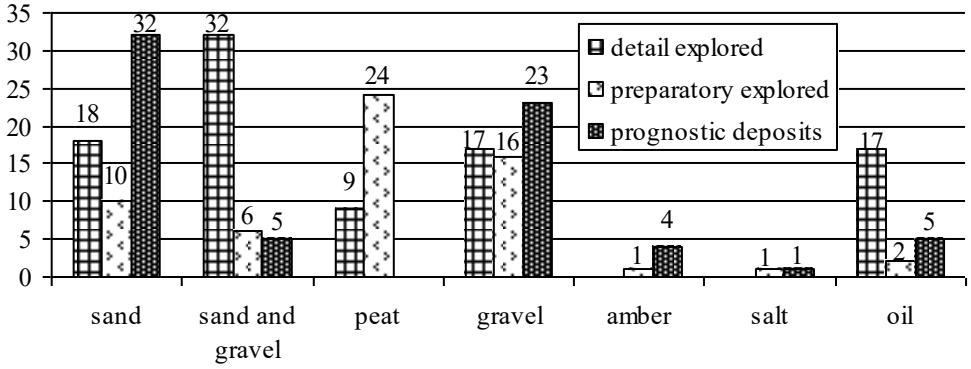

Figure 4. Deposits exploration in Klaipeda County
(Compiled by the author of the article).

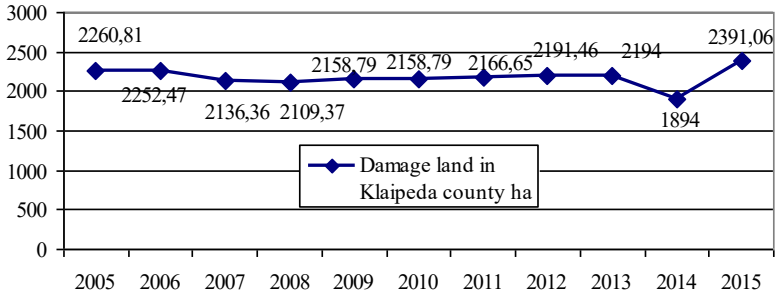

Figure 5. The damaged land change in Klaipeda County in ha during the years 2005 – 2015 (Nacionalinė, 2005 – 2015), (Compiled by the author of the article).

land analysis by type of ownership showed that the damaged land area in private land increased by 75.54 hectares or 58.52 percent. State land area decreased by 442.35 hectares or 20.04 percent (Fig. 6).

Analysis of the damaged land of Klaipeda region in planning documents.

In Klaipeda region, the waste management is carried out in accordance with approved waste management plans and programs (Klaipedos, 2013). The main aim of the regional waste management plan is to harmonize the actions of municipalities in organizing municipal waste management systems and the creation of (common for several municipalities) waste recovery or disposal facilities (Ekokonsultacijos, 2014).

Looking for the sustainable development of the Klaipeda County waste management system (for the ten-year period), it has been foreseen in the general (master) plan (Valstybės, 2012) to collect, store, sort, and manage municipal waste in the regional landfill in Dumpiai (Klaipeda district). For the landfill development the land (12.6 hectares), next to the landfill, was reserved. Rehabilitation works of Kiskenai, Joskaudai, Puodkaliai, Ankstakiai, Rumsai landfills were organized.

Klaipeda region is equipped with 937 collection sites, to be equipped with an extra 816 ones. By 2020, bulky waste collection sites (two in Klaipeda region, one – in Kretinga town and Silute district) are to be arranged and the construction of the Klaipeda region landfill section III with the asbestos waste disposal area (Ekokonsultacijos, 2014).

Thus, in order to develop an efficient municipal waste collection, sorting, storage and handling system in the county, it is planned to construct waste collection container sites in all cities and towns of the county, as well as to expand the municipal waste collection system in rural areas (Lietuvos, 2016).

A total of 439 mineral deposits (peat, oil, bulk materials and amber) are in Klaipeda region. Only 6 percent of bulk material resources are already fully exploited. Mining activities are developed by the initiative of private investors. These activities must be conducted in accordance with the principles of sustainable development.

In order to fully evaluate the mineral resources projected demand changes in the region, it is appropriate to prepare the Klaipeda County mineral exploitation feasibility study (with development potential).

The success of the county social and economic development must be based on sustainable and efficient use of sparse local minerals. In order to not affect the natural environment of the county and individual municipalities during the mineral

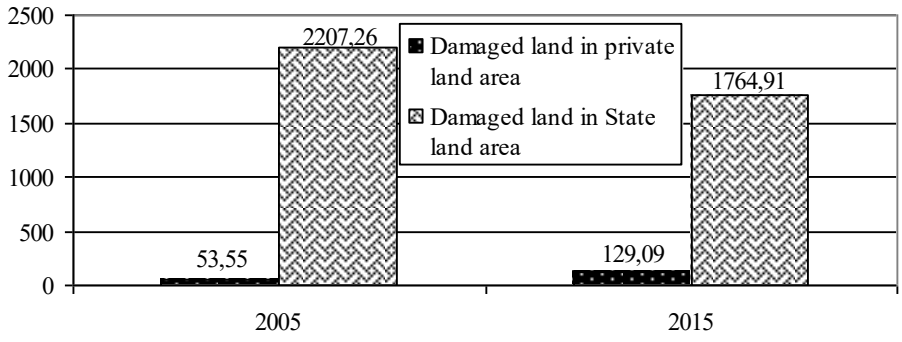

Figure 6. Distribution of the damaged land in Klaipeda County in ha by type of ownership during the years 2005 and 2015, (Compiled by the author of the article).

exploration, production and exploitation of reservoirs, the development of the mining industry and the business must be guided by the principles of sustainable development.

Mineral (prospected and prognostic) deposits to be used in accordance with the legislation, according to the county market demand. Priority is given to currently existing already prospected and at present prospected, even non-operated resources (Klaipedos, 2016).

Conclusions

1. Klaipeda county covers an area of 522220.58 ha that makes 7.99 percent of the entire territory of Lithuania. The agricultural lands make up 53.55 percent, forest area 26.89 percent, water bodies 10.43 percent, roads 1.63 percent, other land 3.07 percent of the Klaipeda county. Damage lands covers an area of 2391.06 hectares, or 0.46 percent of the county.
2. Sand deposits are located mainly in Klaipeda County – 60, of which only 8 deposits are used. The least are salts deposits –2. It was found that, overall in the county, the most used are sand and gravel deposits – 29, not used – sand deposits (52).
3. Klaipeda municipal waste management region comprises 7 municipalities, 39 old dumps were shut down in them, 1 regional non-hazardous waste landfill.
4. In 2005, the analyzed land area of the county was 2,260.81 hectares or 9.14 percent of all country's damaged lands and 0.43 percent of the Klaipeda County area.
5. During the period between the years 2005 and 2015 the damaged land area in Klaipeda County increased by 130.25 hectares or 5.44 percent. It was influenced by the Klaipeda region landfill section III with the asbestos waste disposal area, the reserved area for construction and mineral production.
6. The largest area of the damaged land in 2015 was fixed in the Klaipeda region municipality, accounting for 15.32 percent of the entire Klaipeda County damaged land. During the period between the years 2005 and 2015 the damaged land area in Klaipeda region decreased by 51.73 hectares or 12.38 percent. In 2005, the largest area of the damaged land was in Kretinga district. That area decreased by 142.49 hectares or 32.55 percent.
7. The damaged land analysis by type of ownership showed that the damaged land area in private land increased by 75.54 hectares or 58.52 percent. State land area decreased by 442.35 hectares or 20.04 percent.

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IMPORTANCE OF LANDSCAPE ELEMENTS IN PERCEPTION OF SPATIAL AESTHETICS IN RESIDENTIAL AREAS

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Abstract
Our previous research was focussed on evaluating spaces in the Soviet period housing estates in Riga according to theoretical model of spatial measurements. This model was based on assumption established *ex situ* that liking and perception of urban space is closely related to buildings. Namely, to the way walls of the buildings shape a setting in terms of its height, width, length. This current paper sets the goal to analyse the impact of landscape elements such as trees, benches, elevations of earth surface, playgrounds on the perception and preference of open spaces *in situ*. This is done to verify the hypothesis that landscape elements as well as the method of research play a role in our knowledge on how urban spaces are regarded. Semi-structured interviews in walk-along modus are used to talk to inhabitants about the ways they see and like yards and streets in three residential areas: 'Agenskalna priedes', 'Kengarags', 'Zolitude'. The interview materials are analysed discursively. The extracted information is compared to the data previously acquired by using the model of spatial measurements. The results of interviews show that trees are the landscape elements that have the largest impact on how residents perceive the size of a space and how they like a setting. There are also other elements such as benches, playgrounds, elevations that play a role. The interview material displays deviations in preference and perception of the size of space compared to the model of spatial measurements. Yet these deviations can be adapted to the model.
Key words: yards, landscape elements, spatial aesthetics, model of spatial measurements.

Introduction

Our previous work was focussed on classifying open spaces in four Soviet period residential areas in Riga according to four spatial categories, the spaces also were labelled with associated aesthetical values (Kusmane, 2016). This was done according to a model of spatial measurements that was largely informed by environmental psychology and evolutionary aesthetics. Model of spatial measurements is based on the assumption that spatial aesthetics is the shape and proportions (length, width to height ratio, angles of facades etc.) of outdoor spaces as composed by the outer walls of the buildings or sometimes also by the bordering streets. This assumption is also sustained by environmental psychology literature dealing with finding the ideal parameters expressed in meters for the streets and yards (Stamps, 2001; 2005; 2009; Alkhresheh, 2007; Lindal & Hartig, 2013). A further assumption, which is mirrored in this model, is that among four spatial categories that theoretically cover all the possible spatial shapes and proportions, the open-undefined spaces are most disliked ones. Such spaces are too large, and humans feel endangered here as the next possibility to hide is in a very far distance. Quite disliked are also enclosed scenes, they promote the feeling of extreme enclosure, and also blocked views, which restrict view in one direction. Spaces that fall under spacious, well-structured category are the most liked ones; they provide enough information to learn and sufficient places to hide for humans. This holds evidence not only for the natural, but also for the urban environments (Herzog, 1992). Yet high amount of elements termed mystery and legibility can potentially contribute to higher likability (Nasar & Cubucku, 2011). Mystery is a specific type of a prospect that promises new information if one walks into the scene, and legibility is a specific type of refuge promising a hiding place from which a scene can be observed. However, critically should be regarded the fact most but not all knowledge of environmental psychology that the model of spatial measurements is based on, is collected via quantitative surveys *ex situ,* for instance, by showing photos or computer generated images to respondents and collecting their responses on likability, safety, comfort of the spaces depicted in photos. Also, a critical reconsideration should be awarded to the fact that great many of the environmental psychology researchers when dealing with 'pure' urban spaces are not taking landscape elements into account. For instance, a research investigating urban environments and stress relief correlation asks participants of the experiment to note their reactions in a city centre in order to compare the results with the ones occurring in a city park. The city centre in above mentioned research is an environment, mostly made of concrete and asphalt. Yet, on a closer look acquired via google maps, the particular location in Helsinki possesses few trees that are ignored in the description (Tyrväinen *et al*., 2014). Ignoring trees as this and other research examples show means that landscape elements as 'minimal' greenery is perceived by scientists as irrelevant. Also, pedestrian ways, benches, pergolas, water features, relief remain overlooked in many cases.

To test the given problems acknowledged above, this research paper is devoted to two questions: to what extent model of spatial measurements that is based on the knowledge acquired *ex situ* correspond with how

inhabitants like and perceive the space *in situ* and whether humans really perceive spaces as bordered by the walls or whether elements of landscape such as greenery, relief etc. have an impact on the way people visually perceive a setting in terms of size, likability and preferability.

The aim of this research is to find evidence that the model of spatial measurements can be enhanced by adding some landscape elements to it. Accordingly, our hypothesis states that such landscape elements as trees, small elevations of earth surface, benches do influence the perception and likability of space as much as the walls of the surrounding buildings do.

Materials and Methods

Since there is a lot of theoretical knowledge (Herzog, 1992; Stamps, 2001; 2005; 2009; Alkhresheh, 2007; Lindal & Hartig, 2013) but almost no field research *in situ* on how individuals perceive and like outdoor urban spaces the most appropriate approach are semi-structured interviews (Stephens, 2010). Semi-structured walk-alongs are more flexible and can be easily adapted to the interview process (Delyser & Sui, 2012). Such interviews also encourage the partner of the conversation to talk about the spatial experiences that have never been reflected upon (Holton & Riley, 2014).

The paper utilizes walk-alongs on prechosen roots in four Soviet time housing estates in Riga that were carried out in July, 2014. The interviewees were residents representing broad age, gender and social groups who were randomly met on location and agreed to come along a certain path designed by the researcher. The model of spatial measurements that was already applied in previous research used pre-chosen routes as well. Thus, exactly the same scenes were analysed with the model of spatial measurements and evaluated by inhabitants in order to achieve comparable data sets. For working purposes each scene was ascribed a title consisting of three symbols, for instance, A13. The first symbol corresponds to the first letter of the residential area, which is 'Agenskalna priedes' in this case. The second symbol designates the number of the route – the route No. 1 in this example. The third symbol indicates the number of a scene on that route, which is the third scene in this instance.

After the interviewees had observed the scene, they were asked from the question list. Such questions included: 'How far does the scene go?' 'How often do you visit this scene?' 'How do you like the scene?' 'How could you improve the scene?' Yet since the interviews are of semi-structured type, there were questions asked that did not appear on the question list as well. For example, if the interviewee talked about his or her social background in relationship to likeability of a scene, then the interviewee followed this track in order to acquire unpredicted viewpoint on perception of spatial aesthetics.

The drawbacks of the usage of prechosen routes are that they diminish interviewee's sense of control (Kusenbach, 2003). Human geographer Jon Anderson talks about a demanding style of interviews in general and argues for more partner-like dialogue (Anderson, 2004). We tried to eliminate all the above mentioned downsides. For instance, the speed of the walk and the length of the conversation are defined by the interview.

Collected interview material is deciphered, discursive analyses performed and the narratives of interest, namely, perception and likability, are filtered. The information of liking and perception is applied to according google maps image of the scene be it a yard or a street. These images are later compared to air view images of the same areas that mirror the results acquired by model of spatial measurements earlier.

Results and Discussion

The Most Popular Space Shaping Elements

There are 72 interviews that serve as empirical source of information for this paper. Some of the interviews last as long as 10′, yet some others – 2 h. We analysed 99 scenes (equal number of scenes as when applying the model of spatial measurements). Trees and shrubs appear in the interviews as elements that affect likability and perception many times. Respondents easily explain where in a particular setting trees should be added or removed in order to actually enhance the space. Also, side-roads, benches, skate parks, playgrounds – objects of distinct function prescribed to a certain social group – are responsible for liking or disliking the space. Further, elevations (not more than 5 m high) also signify for some inhabitants the borders of a space.

A viable comparison of the achieved results to previous results in academic literature is not possible, since to the best of our knowledge, there is no relevant data collected before. There are environmental psychologists who strive to predict the preferability of urban spaces of a certain size and proportions, yet, landscape elements – the focal point of this article – are never taken into account (Stamps, 2001; 2005; 2009; Alkhresheh, 2007; Lindal & Hartig, 2013). There are some authors who try to link preferability of urban space of a certain size to a certain spatial category (Kusmane, 2016), yet also here the landscape elements as part of the aesthetics are ignored. There is a wide agreement among landscape theoreticians that natural elements and good design have a positive effect on perception and appropriation, but the metrical precision regarding the relation of the size of space and design elements is missing (Kaplan, 1995; Foster *et al*., 2013; Marzbali *et al*., 2012; Ewing & Clemente, 2013; Sutton, 2013). Also, academics active in urban

design theory are in accord when positive effects of natural elements and design elements are regarded (Gehl, 2010; Newman, 1972). Here the metrical data is given, but it is expert and not lay-man based, i.e., no relevance in the context of our paper. In short, there is no research done in comparing theoretically preferred spaces of certain size and content to the spaces and their content *in situ*.

Trees as Space Shaping Elements

In the residential area 'Agenskalna priedes' the scene with the working title A11 yard is a trapeze formed by residential buildings measuring 210 × 140 × 210 × 90 m, surrounded by houses 14 m in height. Theoretically i.e. according to the model of spatial measurements this space is ranked as open, undefined space. It possesses some mystery or bended prospects that potentially provide interesting information if one walks into this prospect, but very little legibility or places to hide, and hence is hypothetically evaluated as disliked (Fig. 1). Yet, from the utterances of some inhabitants, it became clear that they are quite satisfied with this yard space. According to some, they perceive trees as a space shaping factor. Trees for them divide the particular yard into at least two smaller spaces in comparison to analysis done by applying the model of spatial measurements. Inhabitants standing at the point A11 reported that the space that they call 'their yard' extends until the group of trees by that implying an area of ca. 70 × 140 m. This space is less than ⅓ of the original A11.

Figure 1 shows the main groups of trees visible from the standpoint A11 and referred to by interviewees. There are three separate groups of trees on this smaller space that the inhabitants are referring to as 'their yard', and all together they take up ca. 40% of the space. The largest group in the North consists of 30, 10 of which such as chestnuts (*Castanea*), maples (*Acer*), linden (*Tilia*) trees have low growing branches that one can use for climbing the tree. This cluster is composed of the oldest trees in the area, 5 of them having radius of 0.4 m and very dense canopies. The group is constantly mentioned in the walk-alongs as the 'trees' that border the yard. There are two smaller patches of trees in this 'space'. Evija, a 38 -year- old female primary school teacher and a young mother who has been living in 'Agenskalna priedes' for 15 years answered to the question how many spaces she saw in the scene A11: 'There are few spaces. Until the trees there is one space. Behind the trees – another one.'Also, other interviewees who commented on this space highlighted the same group in a similar manner. All the quoted inhabitants report that they tolerate the space. It means they indicated certain liking for this setting; however, they also desired some changes in this particular yard. To interpret the new finding back to the model of spatial measurements, the 'new' space measures ca. 70 × 140 m. These decreased measurements of size (two sides are smaller than 75 m, for more information see Kusmane A.S.) together with now increased mystery and legibility elements, which is obviously added by the presence of denser group of trees with low growing branches (possibility to rescue by climbing, possibility to acquire new information if one walks into the 'forest') allows to define this yard as an open, undefined tolerated space. In short, the space that inhabitants see at the point A11 is much smaller due trees, and at the same time 'tolerable' according to model and also inhabitants.

If we take another scene as an example, not only already existent trees can be described as space shaping elements, but also non-existent trees can be wishfully imagined in those instances where the yard seemed to be too large, both according to the model of spatial measurements in cases of open-undefined spaces, and also according to the utterances of inhabitants. A yard in 'Agenskalna priedes' temporarily called A23 which was previously ranked as open, undefined and disliked yard (145 × 55 × 160 × 80 m surrounded by 14 m high buildings), which with the exception of tree belt along the longer side of the yard (160 m) and three small trees in the central part of the yard is for 60% an empty stage. This scene has almost no legibility – possibilities to hide, yet lots of mystery formed by incoming side streets in the background. Residents pronounce disliking of the particular yard.

Figure 1. Scene A11. The white contour shows the size of theoretically open, undefined and disliked yard. White transparent field with black lines represents a significantly smaller area 'until the trees' recognized by the inhabitants as 'their yard' and is quite liked. Black torn lines indicate groups of trees visible from stand point of the interview (black point) (modified by authors).

Figure 2. Scene A13. The white contour indicates the area that was, according to the model of spatial measurements, evaluated as an enclosed one. White transparent field with black lines indicates the area 'with trees' reported by the inhabitants as a separate space. Both layers have different sizes: theoretical and reported. Both scenes, theoretical and reported, are disliked. The black point is the stand point at the time of the interview. Black torn lines indicate group of trees visible from stand points A13 (modified by authors).

Curiously, many answers indicate that more trees would play a major role in enhancing likeability of this otherwise desert like scene. Some residents think that more trees and benches would enhance such a yard, it would become more preferred and used. To interpret this information according to the model of spatial measurements – both trees and benches would provide more hiding space – legibility. Besides that as seen in the previous example, they will contribute to compartmentalizing spaces, and if planted in denser groups, also add some mystery element.

Yet the idea that trees are always a solution for enhancing space is not correct. Inhabitant utterances on other scene that bears a working title A13 show that trees can be irritating. For instance, space that according to the model of spatial measurements is estimated to be 190 × 36 m large and surrounded by the buildings of 14 m is defined as an enclosed scene. Here the mystery counts are very low – 1 point, there is no legibility (secondary refuge) in this scene, thus it was evaluated as disliked, see Fig. 2.

All the inhabitants claimed that the space closer to the viewer – the narrow corridor between two buildings is one space, but the meadow behind it is yet another space. Trees play a major role in their answers. The location of trees visible from the standpoint A13 is depicted in the Fig. 2. When Yuta was asked: 'How do you perceive this yard: as one space or as two separate spaces?' She answered: 'This is one [shows to the front area between buildings]. The trees are grown now.' In other words here, too, a respondent indicates that the trees are shaping the space, in this case compartmentalizing it.

The negative feelings in this space A13 that promotes extreme feeling of enclosure were not related to walls, but to extensive greenery. For example, when Santa, a 29 -year- old young working mother, was asked at standpoint A13: 'How does it feel as if there are two kinds of spaces, or one.' She replied: 'Surely two. One is more beautiful, the other one – more grown over, kind of shady.' Thus, also in this instance the model of spatial measurements can be corrected. In this scene, there are exclusively trees with high growing branches only, i.e., no possibility to use the tree as a refuge. Besides that their almost locked-together canopies add to the feeling of enclosure theoretically associated with this space. A similar situation (space that is theoretically defined as enclosed, disliked) where trees with almost no low-growing branches make the yard unpreferred by the inhabitants is situated in Kengarags. 20 -year- old Janis who has been living in the residential area for 11 years, in the interview defines trees in this yard as bothersome since they occupy too much space making it too small.

To summarize, the last three given examples on trees, the interviews show that trees as landscape elements do influence the perception of space in terms of its size by compartmentalizing a space or by adding to the feeling of enclosure. The positive effect of the presence of dense groups of trees in open, undefined spaces is that they apparently add to the mystery component (possibility to acquire new information if one walks further into a scene), the presence of trees with low growing branches add to the legibility (a safe hiding place with outlook possibilities). Yet not all trees are seen as enhancement to all kinds of spaces. There is evidence that trees with high growing branches in enclosed spaces make such scenes even more disliked.

Elevations of Earth Surface as Space Shaping Elements

The other landscape elements appeared in the interviews much less often. However, it can be explained by the fact, that, for example, elevations of earth surface were mentioned only in two scenes in those areas where there was some relief to refer to – 'Agenskalna priedes', 'Zolitude'. For instance, the yard that we already have seen in the examples with trees, now accessed from a different angle has demonstrated, that some inhabitants standing at the viewing point with the working title A41 see mild elevation (ca. 5 m) as the border of their yard

Figure 3. The white contour shows the size of theoretically open, undefined and disliked yard as estimated by the model of spatial measurements, but the white transparent field with black lines represents a significantly smaller area 'until the elevation' recognized by the inhabitants as 'their yard' and is liked. The black point – position from which the scene A41 is observed by the interviewer and respondent. The black rectangle represents the elevation (modified by authors).

and not the buildings at the furthermost edge of it (Fig. 3). In research this yard A41 (equal to the scene A11, but seen from a different stand point during the interview) is theoretically defined as open, undefined space which, taken into account its low legibility and mystery counts, is disliked. Yet the inhabitants are voicing different opinion. For instance, when 81 years old teacher Irina, who has been living in 'Agenskalna priedes' for 4 years, was asked to explain if the yard, at the border of which we stood, was one space, Irina said: 'One', at the same time pointing to the elevation that boarders off approximately one third of the initial space. Similarly, Laimonis, a 30 -year- old dog owner, who was walking his pet during the interview also had the opinion that place he perceives as his yard ends with the slope of the mentioned elevation. 28 -year- old Sasha chose the elevation as the demarcation of 'his' yard, too.

A surface elevation as the new border 'decreases' the size to approximately 75 × 140 m. If now the new parameters are translated back into the model of spatial measurements, then the perceived yard due to its decreased size and increased legibility becomes open, undefined and tolerated space. Legibility is increased since inhabitants seemingly perceive the elevation as a safe look-out space from which one can observe the scene but stay safe. 37 years old Sandra, a mother of two youngsters, asked if she would like to see any changes to the yard, answers positively. Sandra would place a pergola on the elevation of earth surface that we see in the yard. 'Moms could sit and observe children. Making sure that all is safe. Children are in the first place.'

In other case, there is a triangle-like yard temporarily called Z21 in 'Zolitude' that measures approximately 200 × 125 × 125 m with 26 m high buildings surrounding it (Fig. 4). Due to a very small mystery and no legibility counts, it is ranked as open undefined, disliked yard. There are almost no trees, benches or other elements of landscape in the scene, except there is an artificial elevation meant for cycling in the southern part of the space. When looking at this scene, the inhabitants propose more elevation of earth surface to enhance the feeling of likeability. A retired female Valentina asked what would make her feel more like it is actually a yard, has responded that more relief would solve the problem of attachment to this otherwise unliked scene.

Figure 4. Scene Z21. The white contour represents the open, undefined disliked scene according to the model of spatial measurements. The black point – position from which the scene Z21 is observed by the interviewer and respondent. The black area with a double white line – the imagined elevations of earth surface, which would according to some interviewees enhance the space to become a potentially liked one (modified by authors).

Figure 5. Scene A11. The white contour shows the open, undefined scene as defined by the model of spatial measurements, but the white transparent field with black lines shows the scene as perceived by the inhabitants. It stretches from the stand point during the interview until the skate park – functional object that for some people dignifies the border of the yard. The 'new' yard is liked by the inhabitants (modified by authors).

Valentina proposed two elevations in the yard, in the front part as well as in the middle of the yards Z21. If one reads her utterance under the premise of environmental psychology, this surface elevation would increase legibility and thus the information quality of the space.

In short, even small elevations (in the examples of case studies – 5 m) can be seen as a space compartmentalizing element (case of A41), especially in cases when the space is surrounded by lower buildings (14 m) that do not outcompete the effect made by the elevation. It seems that in any occasion, elevation plays a role of legibility element which being a refuge adds to the safety feeling.

Functional objects as Space Shaping Elements

For the purpose of this article we have defined functional objects as those landscape elements that are designed for usage or perceived to be used by a certain social group. For instance, a playground is intended for smaller children, whereas a skate park is for older children. Yet there are also some objects included that can be used by anyone, but perceived to be used by one group only. Benches are the most popular example of it.

Some inhabitants have revealed that their perception of the largeness and preference of space is related to such functional objects. For instance, 29 -year- old Santa, who lives in 'Agenskalna priedes' with her children has commented standing at the point A11 (Fig. 5) that the yard extends until the skate park. She said: 'From here until the skate park' This is where we (Santa has a child) walk most often.' Curiously that also 50 years old Victor, who commented on this yard, standing at the other end of it (as in the scene A51) also mentioned skate park as the border of his yards. 'Everything. The whole peace from that house till the skate park (points to the house Kristapa iela 8).' Thus, the skate park as a border of space must not necessarily include the self-use or use by other members of family to be seen as a significant landscape element that shapes the space.

Both quoted ones implied that they quite liked the space. The new 'border' makes half as large as it was theoretically assumed, now it measures 105 × 140 × 105 × 90 m. In this case, it is rather difficult to say if the skate park adds to the mystery or legibility counts since there were no slightest hints on increasing or decreasing these elements after carefully analyzing the above mentioned interviews discursively. To reinterpret the measurements back into the model of spatial measurements, it seems that a space this large (105 × 140 × 105 × 90 m) is still tolerable. Yet the difficulty is to ascertain, if people who answered in favour of the skate park, also actually perceived groups of trees and elevation as such landscape elements that increase prospects and refuges. More in depth interview would be required to do so. Yet some functional objects such as benches show the most striking metamorphosis of physically visible space.

For instance, an interview with 24 -year- old Martins who has been living in 'Kengarags' whole his life, reveals that the yard that has a working title K13 (Fig. 6) does not exist for him, even though it is situated right outside his window. He explains: 'It is not very well designed. Only drunk people are hanging out on the benches. Nothing happens there.' Martins indicated that benches (a very few pieces of furniture that take a minute part of the space) make this large yard (60 × 80 × 75 × 90 m, surrounded by houses that are 14 m in height) avoidable. Theoretically, too, this open, undefined yard that is ranked as disliked because of nonexistent legibility elements (even though there are three elements of mystery). Likewise, 20 -year- old Janis comments on the yard K23 in a relatively similar manner. 'Only alcoholics are there during the night. One cannot sit there'. The yard K13 and K23 have almost identical configuration and conversely – they are alike what regards presence of mystery and legibility elements. Also, retiree Natalija talked about the non-existent yard K23, she prefers walking to Daugava river promenade along the street instead of using a shorter pass along the yard for a similar reason.

Figure 6. Scene K13. The white contour in figure is ranked as open, undefined disliked according to the model of spatial measurements. Also, inhabitants do not like this space. The black dot marks the stand point during the interview. The double white line marks benches (modified by authors).

In many stories, the smallest landscape element benches symbolize danger – only dangerous people use them. Even though the area of the yard is impressively large (60 × 80 × 75 × 90 m), a couple of benches at one of its walls completely make the yard disappear form the mental maps. Ironically, the smallest landscape elements become the largest space shaping elements as they are able to out-blend the whole yard. If we translate the given information on this yard back into the model of spatial measurements – a square like space surrounded by almost uninterrupted houses with few mystery elements, then it becomes clear that the lack of legibility (refuge) fuelled by 'dangerous' benches has played a decisive role in dislike of this space.

In short, functional objects such as playgrounds, skate parks, benches, roads are mentioned relatively little, but they all can have either compartmentalizing effect on perceived space size or in some cases completely eliminate certain yards or streets from perception. Importantly, they are seldom mentioned in a way that can be interpreted by researcher as pointing to elements of mystery of legibility. It seems that functional objects often but not always are influencing the perception and the size of space because of their social and not form-shaping ability.

Conclusions

The development of the model of spatial measurements is far from completed. If the interview material is converted into the language of model of spatial measurements, then the later ones are meaningful addendums to the former one. It means that if environmental psychology concept of walls as space shaping elements is extended towards landscape elements, we might acquire a much more precise tool to predict perception and likeability of any space. To achieve this aim, all the involved landscape elements have to be reinterpreted as legibility or mystery elements as well as new borders of space.

Trees due to their rather frequent mentioning play an extremely important role. The position of branches and grouping of trees, and relationship of number of trees to the size of the space are crucial for positive or negative likeability as well as for increasing or decreasing legibility. Also, small elevations are good enough to compartmentalize a space and make it more likeable because of added mystery and legibility components. Interestingly, the interviews showed that such functional objects as playgrounds and skate parks can demarcate perceived border very effectively whereas functional objects such as benches regardless their small size can erase a space such as a yard from the mental map instantly because of 'dangerous' users associated with them.

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NATIONAL HEIGHT SYSTEM TESTING USING GNSS MEASUREMENTS IN LATVIA AND LITHUANIA

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Abstract
As European Union member states – Latvia and Lithuania have changed their national height systems from Baltic Normal Height System 1977 to European Vertical Reference System realizations. Performing practical Global Navigation Satellite System (GNSS) measurements and obtained data mathematical processing, there is a possibility to calculate point height difference between GNSS data and data of 1st order levelling networks. The aim of the research is to gain insight for methodology development of geodetic point height testing using GNSS measurements. To achieve the goal following tasks are set: 1) perform GNSS measurements of first order levelling network in Latvia and Lithuania; 2) do data processing to get point ellipsoidal height and compare with the point normal height; 3) detect technically feasible accuracy of geoid model for precise height measurements with GNSS method within research area. Performing GNSS measurements at the same time in the territory of Latvia and Lithuania, the experience of mutual cross-border cooperation was accumulated. The results were adjusted with academic software package Bernese and were obtained point ellipsoidal and normal heights. The height measurement accuracy using GNSS method depends on geoid model accuracy and more precise results can be obtained increasing used geoid model accuracy to 1.5 to 2 cm.
Key words: vertical network, GNSS, geoid model.

Introduction

Three Baltic states – Estonia, Latvia and Lithuania have good corporation in many fields, also in geodesy sector – in Baltic states was used united national height system – Baltic Normal Height System 1977 (BHS1977) (Celms, Helfrica, & Kronbergs, 2007) and heights between states were connected in united geodetic network. Since 1st December 2014 in Latvia as the European Union member state Cabinet of Ministers and state laws as a national height system finds the European Vertical Reference System realization in Latvia – Latvian Normal Height System 2000,5 (LHS-2000,5) (Celms, Bimane, & Reke, 2014). Lithuania changed their national height system to European Vertical Reference System realization in Lithuania – Lithuanian height system LAS07 on January 1, 2016. Approximate difference between the old (BHS1977) and new height system (LAS07) in the middle of Lithuania is about 14 cm.

Nowadays, the Global Navigation Satellite System (GNSS) offers more and more advantages. To gain insight into methodology development of geodetic point height testing using GNSS measurements, authors used GNSS method and did measurements of 13 first order levelling network points in the territory of Latvia, and 5 first order levelling network points in the territory of Lithuania were obtained. The global positioning as a method was chosen because of its simplicity – by using global positioning and calculating ellipsoidal coordinates, it is possible to see the height difference control in height system datum point and regional main geodetic points (Lazdans *et al*., 2009). On these points where direct GNSS observations are impossible to do, there is still a need for precise levelling works (Celms *et al*., 2013).

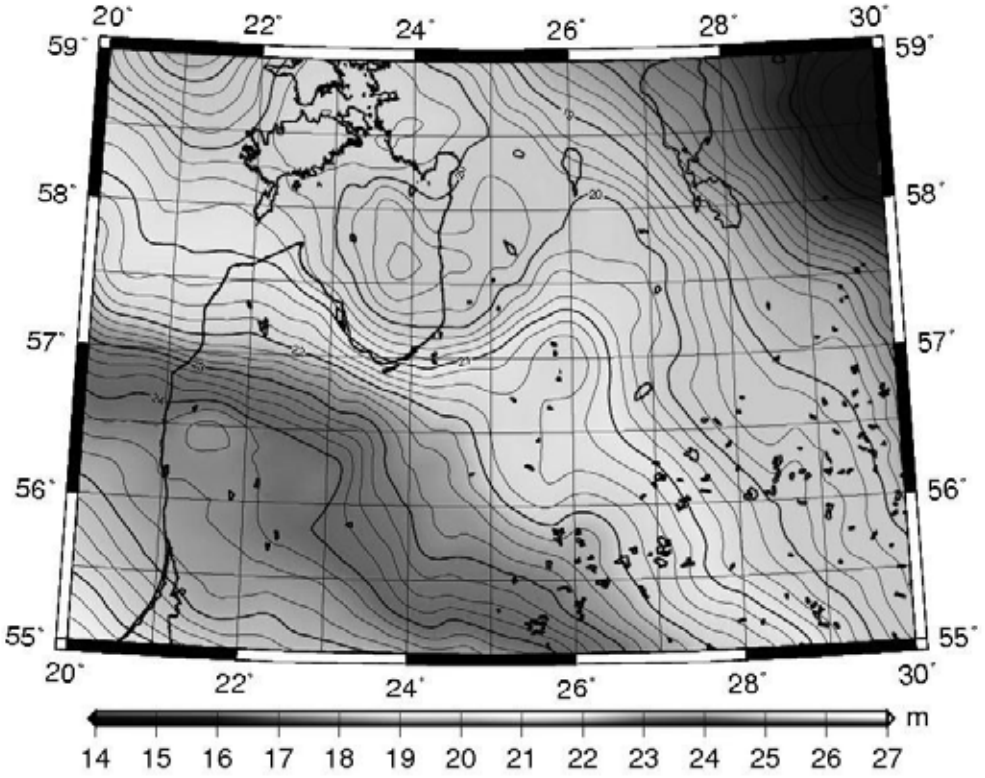
The levelling network is a national height system forming element. Levelling network ensures the realization of various functions in the national economy (Celms, Kronbergs, & Cintina, 2013).

For precise GNSS measuring, it is necessary to have a precise quasigeoid model. Since 1st December 2014 Latvian specialists have developed a new quasigeoid model from LV98 to LV'14 with 4 cm accuracies (*Latvijas kvaziģeoīda modelis*, 2015). Lithuanian digital geoid model will be improved after completing the first and second order of geodetic vertical network. This will enable to derive normal height from ellipsoidal heights determined by GNSS and replace traditional levelling of lower orders (Krikštaponis *et al*., 2014).

The aim of the research is to gain insight for methodology development of geodetic point height testing using GNSS measurements. To achieve the goal, following tasks are set: 1) perform GNSS measurements of first order levelling network in Latvia and Lithuania; 2) do data processing to get point ellipsoidal height and compare with point normal height; 3) detect technically feasible accuracy of geoid model for precise height measurements with GNSS method within research area.

Materials and Methods

Establishment of Latvian National First Order Levelling Network began in 2000 and field measurements were finished in 2010 (Celms & Kaminskis, 2005). Geodetic measurements



Source: authors' construction.

Figure 1. Geoid model LV98 for territory of Latvia and Lithuania.

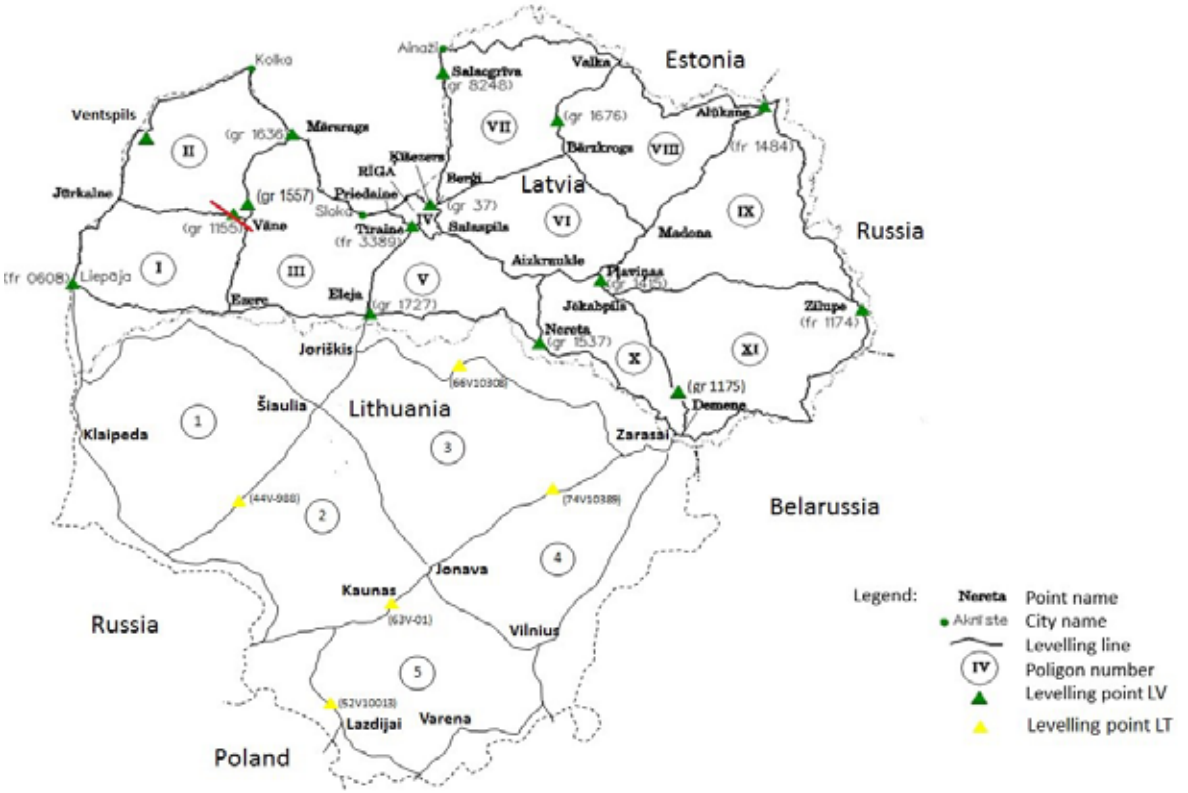
were made by the specialists of the Latvian Land Service from 2000 to 2005 and Latvian Geospatial Information agency from 2006 to 2010. Geodetic and gravimetric observation data were used for the network establishment. The general requirement for not exceeding RMS error 0.5 mm km^{-1} of the measured height differences was followed in the course of establishment of the National First Order Levelling Network (Celms, Kronbergs, & Cintina, 2012).

Lithuanian National First Order Network (NGVN) establishment was going on from 1998 to 2007. Contracting authority for network establishment was National Land Service under the Ministry of Agriculture. It consists of 5 loops of precise levelling lines (Aleksejenko *et al*., 2011).

Connection between Latvian and Lithuanian vertical networks is made in three places, so connecting lines construct the two first order levelling loops. The accuracy of Latvian and Lithuanian united levelling network in first iteration (standard deviation is 0.617 kgal×mm km^{-1}) is at the same level as that of the vertical networks of the biggest part of other participating in UELN project countries (Aleksejenko *et al*., 2011). In 2008, Lithuania also created two border connection points with Poland, thus connecting Lithuanian vertical network to European vertical network (Krikstaponis & Tumeliene, 2011). Now after national height systems' replacement from Baltic Normal Height System 1977 to European Vertical Reference System realisations in Latvia and Lithuania, the height connection between Latvia and Lithuania has been lost (Celms, Reke, & Ratkevics, 2015).

For height determination traditionally precise levelling was used and nowadays mostly for measurements and heights we also use GNSS as advanced instrument and new technology. There are many opportunities to do studies on heights and compatibility between selected geodetic points. Very suitable instrument for studies or research on geodetic consistency is homogenous quasi-geoid or geoid model over decided area. In our case, we found a suitable geoid model LV98 (Fig. 1.) for this purpose. In the future, we are planning to introduce also NKG2015 geoid for validation and verification of geodetic network (*Latvijas kvaziģeoīda modelis*, 2015).

For the GNSS measurements first of all, there was the national geodetic network point inspection done. There were some points selected and then visited on site to detect the horizon above point for each point and possibility to use GNSS methods for its height determination, the point location conformity to point sketch. Also, global positioning real time measurements were done to detect the location of satellites above point. The requirement of open area is 20 degrees and for GNSS measurement PDOP



Source: authors' construction.

Figure 2. Performed GNSS measurements in 1st class levelling network of Latvia and Lithuania.

should be at least 4 satellites. After inspection, there were thirteen1stclass levelling network points chosen, as an appropriate geodetic point for GNSS measurements – ground marks 1415, 1001, 37, 1155, 1537, 1636, 1676, 1727, 8248 and fundamental marks 1484, 0608, 3389 and 1463. In Lithuania, following first order geodetic points were measured: ground marks 44V-988, 74V10308, 63V-01, 52V10013 and 74V10389 (Fig. 2).

In this project, specialists from Latvian University of Agriculture, Riga Technical University, Latvia University, Aleksandras Stulgiskis University, Kaunas Technical University and some private companies from Latvia were involved. The main idea was to perform GNSS measurements on all selected points at the same time and use global positioning in post-processing mode providing avoidance from some errors.

In three years, 3 measurement sessions of Latvian first order levelling network – 14th December 2012, 22th November 2013 and 27th November 2014 were performed. On June 30, 2016 in the 4th session experts from Lithuania were involved and at the same time 13 first order levelling points in Latvia and 5 first order levelling points in Lithuania (Fig. 2.) were measured.

In measuring sessions from 2012 – 2014, the GNSS measurement took 4 hours in the morning about 10 to 14 o'clock in Latvia Positioning System Base Station (LatPOS) network. LatPOS is GNSS continuously operating the network of Latvia (Celms, Ratkevics, & Rusins, 2014). On each point a GNSS receiver – Leica, Trimble, Topcon or GeoMax receiver was installed – and GNSS data were collected for 4 hours. In 2016, the duration of measurements was 5 hours in the early morning from about 6 to 11 o'clock in LatPOS network and Lithuania Positioning System Base Station (LitPOS) network.

For precise data processing and adjustment after measuring, there were collected data from 3 nearest LatPOS or LitPOS base stations. The data from GNSS receivers and LatPOS/LitPOS stations were used for data adjustment and point height determination (Reiniks, Lazdans, & Ratkus, 2010).

GNSS collected data were processed and analysed using academic software package Bernese GNSS Software (Version 5.2). For data processing the following requirements were raised: blind angle – 20 degrees form horizon, precise ephemerides, NGS antenna calibration models, closest vector selection and free network adjustment.

Results and Discussion

Adjusted results from performed GNSS measurements are listed in Table 1. Using academic software package Bernese GNSS, it is possible to obtain point ellipsoidal heights.

Table 1

Point ellipsoidal and normal heights of measured points

	Point name	Point ellipsoidal height, m	Point normal height, m
Latvia	1001	155.138	138.649
	1155	105.379	82.026
	1415	100.050	76.842
	1484	137.208	156.812
	1537	104.972	80.458
	1636	21.108	6.852
	1676	84.170	58.531
	1727	58.824	32.387
	37	31.684	7.383
	8248	19.581	4.722
	0608	21.713	5.727
	3389	34.360	12.474
	1463	43.261	13.476
Lithuania	44V-988	123.787	112.608
	74V10389	153.496	125.565
	63V-01	64.268	35.731
	66V10308	45.611	34.447
	52V10013	158.414	127.085

Table 2

RMS errors of measured points

Point name	RMS error N, m	RMS error E, m	RMS error U, m
1001	0.001	0.001	0.004
1155	0.001	0.001	0.002
1415	0.001	0.001	0.003
1484	0.001	0.001	0.003
1537	0.001	0.001	0.004
1636	0.001	0.001	0.005
1676	0.001	0.001	0.004
1727	0.001	0.001	0.004
37	0.001	0.002	0.003
8248	0.001	0.001	0.004
0608	0.000	0.000	0.001
3389	0.000	0.000	0.001
1463	0.000	0.000	0.001
44V-988	0.002	0.002	0.006
74V10389	0.000	0.000	0.001
63V-01	0.000	0.000	0.001
66V10308	0.000	0.000	0.001
52V10013	0.002	0.002	0.008

Bernese is one of the most precise software programs and using GNSS measurements nowadays, it is possible to get height values with high accuracy. As seen in Table 1, ellipsoidal heights should be calculated to normal heights. Consequently, this study requires a further research.

Performed measurements have high accuracy which describes root mean square errors. The point evaluation of the North and East values is less than 2 mm. Height component average value is approximately 3 mm, and in some points it reaches 6 and 8 mm. Mean error value for axis N and E is 0.001 m, but measurement height component average error value is 0.003 m. The normal height determination accuracy with GNSS method depends on used geoid model accuracy and more precise results can be obtained increasing geoid model accuracy up to 1.5 to 2 cm which is the aim-accuracy of new geoid model of Latvia – LV'14.

Acknowledgements

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Conclusions

It was possible to successfully organize GNSS measurement works thanks to previous preparation and critical situation analysis. Performing GNSS measurements at the same time in the territory of Latvia and Lithuania, the experience of mutual cross-border cooperation has been accumulated.

It is very significant to use advanced measuring technologies and verified data processing methodology for height determination using GNSS measurements.

In connection with geoid model significance to geodetic result data precision, it is desirable to perform GNSS measurements of I class levelling networks in Latvia, Lithuania and Estonia. This way it can verify and test geoid model on larger areas thereby getting certainty about geoid model precision. Accuracy of performed measurements describes RMS errors. Average error value for axis N and E is 0.001 m, but measurement height component average error value is 0.003 m. The height measurement accuracy using GNSS method depends on used geoid model accuracy, and more precise results can be obtained by increasing geoid model accuracy from 1.5 to 2 cm.

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DETECTION AND REDUCTION OF LAND DEGRADATION IN SMARDE MUNICIPALITY RURAL TERRITORY

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Abstract
The aim of the paper is to explore the possibilities of detection and reduction of land degradation in Smarde municipality rural territory (56°57′18″ N; 23°20′17″ E). To carry out land degradation prevention measures, initially the territories of degraded land should be determined. This paper highlights the field inspection method. For territory inspection a model was used that gave the opportunity to identify degradation types with their characteristic features and possibilities to reduce the land degradation. The territory of Smarde municipality rural territory was inspected in nature and degraded territories identified. The costs of land degradation elimination depend on the type of land degradation. In territories where the land degradation reduction or elimination has been done, its control has to continue in order to stop the development of land degradation.
Key words: land degradation, field inspection, real estate.

Introduction

There are several land use and protection issues that in the existing legislation are not sufficiently regulated, but regulation is necessary to ensure the sustainable use of land, for example, land-use principles and state and local government role in the land management. Currently, laws and regulations are rather fragmented, or there does not exist an adequately defined framework for land degradation.

Experience of other countries shows that a country with a small territory or for economics usable land area (Denmark, Finland, Switzerland, Austria) rather strictly regulates land use and protection issues. (Austrian Spatial…, 2011; Finland's National…, 2002; Mugli, 2014; Spatial planning…, 2007) Up to now in Latvia the land degradation prevention issues in development planning documents, laws and regulations have been mentioned only briefly. In the National Development Plan from 2007 to 2013 (Nacionālās attīstības plāns, 2006) (approved by the Cabinet of Ministers on 4 July 2006, regulation No. 564), the country's growth prerequisites and tasks are defined. In the section "Reasonable use and preservation of natural environment" of the National Development Plan as challenges are defined – promotion of biodiversity and conservation of protected areas, as well as promotion of degraded territory remediation and revitalization.

In the European Commission's agenda and documents only in recent years there have appeared issues of land degradation. The European Commission on 22 September 2006 approved the guidelines of thematic strategy of soil protection and developed the draft of directive on soil protection 'Directive, which establishes a framework for the protection of soil and amending Directive 2004/35/EC' (Zemes politikas ...., 2008). In these documents it is stressed that soil degradation is a serious problem across Europe, and studies show that the degradation rates could further increase. The soil can be considered essentially a non-renewable resource. Any damage to the soil structure also damages other environmental elements and ecosystems. Soil biodiversity assessment and biological monitoring is required to correctly assess soil degradation and correlated risks, as well as soil quality (Aksoy *et al*., 2017; Squire *et al*., 2015).

In recent years, increasing attention is being paid to sustainable land use issues (Fischer, Klauer, & Schiller, 2013; Pašakarnis, Morley, & Malienė, 2013; Sklenicka, 2016). Not only in the world (Kosmas *et al*., 2016; Price *et al*., 2015; Smiraglia *et al*., 2016; Turner *et al*., 2016), but also in Latvia the land degradation processes are taking place. The cause of land degradation could be the formation of bushes on agricultural land, coastal erosion, lack of maintenance of drainage systems and land bogging, pollution, landslides, abandonment of built-up areas and the soil degradation. On the other hand, types of soil degradation are: soil erosion, soil compaction, decline of organic matter in the soil, loss of soil biodiversity, decrease of soil pH level, and soil pollution (Lapina & Baumane, 2015).

It is important to determine the land degradation and to take measures to prevent it. To carry out land degradation prevention measures, initially the territories of degraded land should be determined. These territories can be identified differently, for example, performing field works like field inspection or performing data analysis. This paper highlights the field inspection method.

The aim of the paper is to explore the possibilities of detection and reduction of land degradation in Smarde municipality rural territory. To achieve the aim, the following tasks were set:

- to analyse land degradation types in Smarde municipality rural territory;

- to carry out field inspection;
- to identify the approximate costs of the land degradation prevention.

Materials and Methods

The research territory which was inspected is located in Northwest side of Engure municipality and is located behind the Slokenbeka Manor (56°58′39″ N; 23°13′34″ E), about 7 km away from the Tukums city center, and is bordering with Smarde municipality rural territory borders and is located next to the Tukums airfield. Approximate area of inspected territory is ~ 3 000 ha.

For territory inspection a theoretical model was used that gave the opportunity to identify degradation types with their characteristic features and possibilities to reduce the land degradation. In Smarde municipality rural territory 7 types of degradation were observed: agricultural land overgrowing with bushes, land bogging, land pollution, excessive cutting of forests, abandonment of buildings and land, coastal erosion and soil degradation. For all of these degradation types a theoretical model of solutions of how to prevent degradation was developed.

The theoretical model for reduction of land degradation:

1. Agricultural land overgrowing with bushes

To effectively prevent this type of land degradation and prevent the loss of valuable agricultural land territories, it is necessary to:

- Make effective use of agricultural land;
- Clear the territories which are overgrown with bushes;
- Reforest territory where the scrubland amount reached significant areas and height;
- Perform deforestation on a regular basis and make regular follow up of risk areas;
- Control the territories of agricultural land which are located in the immediate vicinity of forest areas;
- Introduce sanctions against individuals who have maliciously or inadvertently contributed to the land overgrowth with bushes.

2. Land bogging

The necessary measures to stop the land bogging up process and prevent the loss of valuable land that would be used for economic activities and gain of benefits from them are:

- Afforestation;
- More efficient use of land;
- Prevent deforestation where this would contribute to land bogging up process;
- Drainage system installation;
- Elaboration of drainage systems if they are installed, but do not work;
- Introduction of sanctions against individuals who have maliciously or inadvertently contributed to the land booging up process.

3. Land pollution

Pollution is one of those things, without which in today's overpopulated world it is not possible to exist and there will always be territories that are contaminated, however, there should be some specially designated areas, intended for pollution and contamination which should be controlled and limited. Pollution may arise from abandoned buildings, industrial buildings, improper economic activities and improper use of agricultural chemicals. To prevent pollution it is necessary to:

- Terminate pollution;
- If the pollution is already formed and there is no possibility to terminate it, it should be controlled to discourage the expansion of pollution;
- Use properly the agricultural chemicals;
- Destruct the buildings and their polluting elements, or recycle them effectively;
- Introduce sanctions against individuals who have maliciously or inadvertently contributed to the land pollution.

4. Excessive cutting of forests

The necessary measures to prevent this type of land degradation effectively and to prevent the formation of bogged up territories are:

- Deforestation control;
- Deforestation only at designated places;
- Eradication of illegal felling area formation;
- Afforestation;
- Introduction of sanctions against individuals who have maliciously or inadvertently contributed to excessive cutting of forests.

5. Abandonment of buildings and land

Buildings and land abandonment is one of the basic factors that causes land degradation formation because if the land and building on it is effectively used for business purposes or just being cultivated, there does not form polluted soil, huge industrial building slums and territories that are overgrown with bushes. The necessary measures to prevent this type of land degradation effectively are:

- Demolition of buildings and the use of land for other purposes;
- Restoration of buildings and their effective reuse;

- Introduction of sanctions against abandoned land and building owners;
- Introduction of sanctions against individuals who have maliciously or inadvertently contributed to abandonment of buildings and land.

6. Coastal erosion

Latvia is bordering with the Baltic Sea, therefore it is typical in this country to have coastal erosion, which is one of the types of land degradation. Coastal erosion does not occur merely at sea coast, but also in rivers and lakes, which have frequent changes in water level and, of course, annual flooding, and less frequently ongoing floods. The necessary measures to prevent coastal erosion are:

- Strengthening of sea coastal in risk areas;
- Water level control of rivers and lakes;
- Strengthening of river and lake coasts in risk areas;
- Establishment of drainage systems in risk areas;
- Introduction of sanctions against individuals who have maliciously or inadvertently contributed to costal erosion.

7. Soil degradation

Formation of soil degradation is promoted by incorrect farming, lack of drainage systems and other factors. The necessary measures to prevent this type of degradation are:

- Efficient and proper farming;
- Regular land treatment and cultivation;
- Soil elaboration;
- Installation or improvement of drainage systems;
- Introduction of sanctions against individuals who have maliciously or inadvertently contributed to soil degradation.

These land degradation solutions for the seven causes of land degradation are the ones that would put an end, control or prevent land degradation from occurring, except for a solution to impose sanctions, which is the ultimate tool when the land degradation process has already occurred and the guilty party has to be punished.

If the landowner does not eliminate the consequences of land degradation himself, but invite such service provider, then separately for each unit of land there are costs offered for the land degradation prevention, as well as prices available currently on the market for such services. Land degradation costs are summarized by the competitive dialogue between the providers that offer this kind of services.

In nature we inspected the territory of Smarde municipality rural territory and identified 25 degraded territories. This paper highlights five of these territories.

Results and Discussion

The first territory is named Jumji, with cadastral designation 9082 004 0178 and total area of 3.89 ha from which 0.95 ha is degraded. Type of land degradation is agricultural land overgrown with bushes. This degraded territory would require clean up from the bushes for successful use of agricultural land. Scrub cutting for 0.95 ha territory, if the sawn timber is retained, would cost 200 – 250 euros, according to the price survey; if the sawn timber is not retained but returned to the service provider, it would serve as a service fee and then the costs for the owner are minimal or hardly any. Figure 1 shows the real estate Jumji, in which the degraded territory is hatched.

The second territory is named Kursedvare, with cadastral designation 9082 012 0057 and total area of 7.33 ha from which 3.53 ha is degraded. Types of land degradation are agricultural land overgrown with bushes and insufficient drainage system maintenance that have led to land bogging. This degraded territory would require clean up from the bushes and drainage system cleaning in order to successfully use the agricultural land. Scrub cutting for 3.51 ha territory

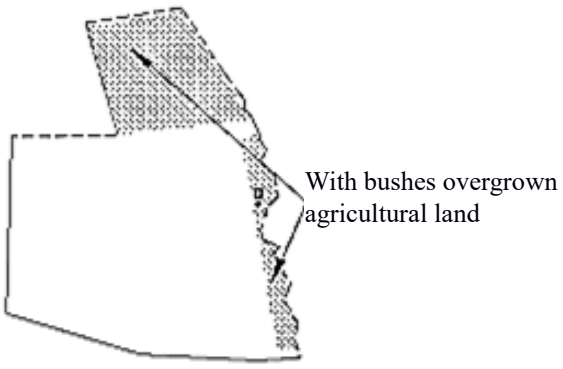

Figure 1. Agricultural land overgrown with bushes in the real estate Jumji.

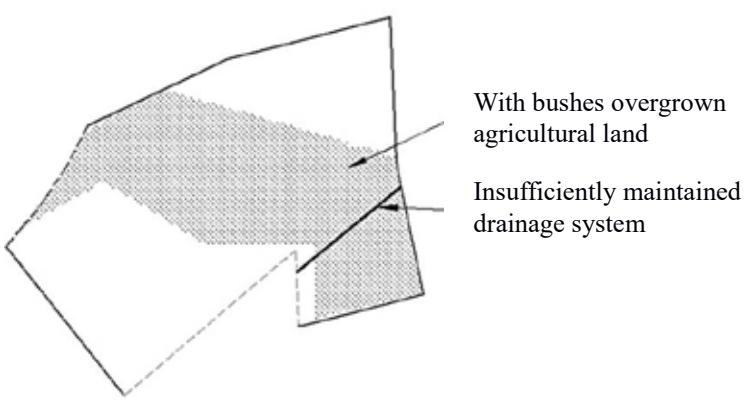

Figure 2. Agricultural land overgrown with bushes and insufficiently maintained drainage system in the real estate Kursedvare.

and drainage system cleaning for 0.02 ha territory would cost 200 – 250 euros according to the price survey, but if the sawn timber is returned to the service provider, then it would serve as a service fee and then the costs for the owner are minimal or hardly any. Figure 2 shows the real estate Kursedvare, in which the territory overgrown with bushes is hatched and the insufficiently maintained drainage system is highlighted.

The third territory is named Zemnieki, with cadastral designation 9082 004 0126 and total area of 2.62 ha from which 0.05 ha is degraded. Type of land degradation is abandonment of buildings. This degraded territory would require clean up from

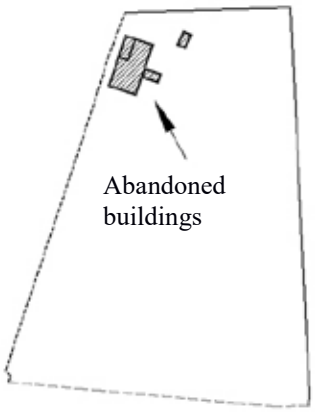

Figure 3. Abandoned buildings in the real estate Zemnieki.

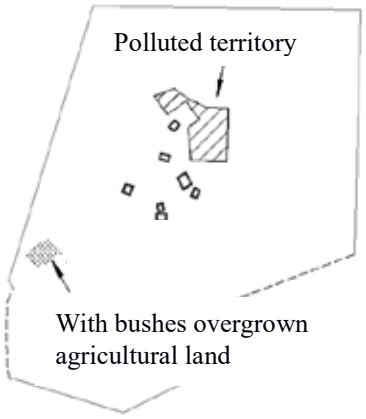

Figure 4. Agricultural land overgrown with bushes and polluted territory in the real estate Pauri.

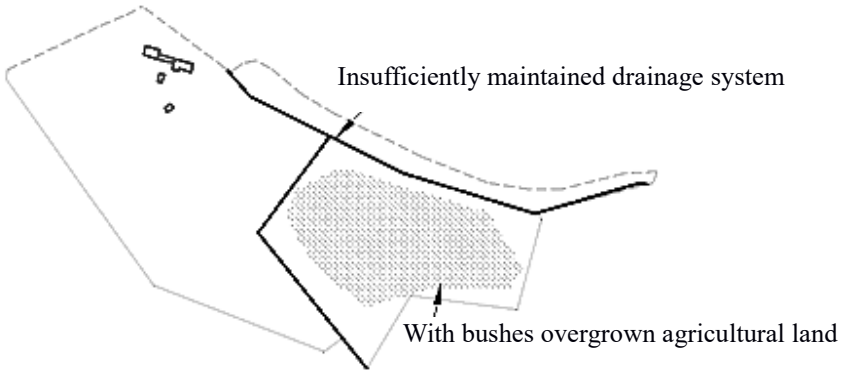

Figure 5. Agricultural land overgrown with bushes and insufficiently maintained drainage system in the real estate Eglites.

debris. Debris removal for this territory would cost approximately 10 000 euro. Figure 3 shows the real estate Zemnieki.

The fourth territory is named Pauri, with cadastral designation 9082 009 0026 and total area of 10.03 ha from which 0.27 ha is degraded. Types of land degradation are agricultural land overgrown with bushes (in this territory it is 0.04 ha) and land pollution (in this territory – 0.23 ha). This degraded territory would require clean up from the bushes for successful use of agricultural land. Scrub cutting for 0.04 ha territory, if the sawn timber is retained, according to the price survey, would cost up to 50 euros and the polluted territory clean up would cost from 5 000 – 5 500 euro. Figure 4 shows the real estate Pauri.

The fifth territory is named Eglites, with cadastral designation 9082 004 0071 and total area of 6.48 ha from which 1.67 ha is degraded. Types of land degradation are agricultural land overgrown with bushes (in this territory it is 1.55 ha) and insufficient drainage system maintenance – ditch overgrown with bushes (in this territory – 0.12 ha). This degraded territory would require clean up from the bushes and drainage system cleaning for successful use of agricultural land. Scrub cutting for 1.55 ha territory and drainage system cleaning for 0.12 ha territory according to the price survey would cost 430 – 450 euros, but if the sawn timber is returned to the service provider so that it would serve as a service fee, then the cost for owner is minimal or hardly any. Figure 5 shows the real estate Eglites, in which territory overgrown with bushes is hatched and the insufficiently maintained drainage system is highlighted.

During the territory inspection, the following degradation-promoting factors were identified:

- Scrub formation on agricultural land;
- Insufficient maintenance of drainage systems and land bogging;
- Abandonment of land, including the built-up area;
- Pollution (pesticides and fertilizers or chemicals).

As the most common problems were detected agricultural land overgrowing with bushes and lack of maintenance of drainage systems that have led to land bogging. These two types of degradation are easier to eliminate than pollution and abandonment of built-up area, and costs are significantly lower, too.

The costs of land degradation elimination were calculated per hectare for territories where scrub formation on agricultural land and lack of maintenance of drainage systems and land bogging were observed. Aaccording to the price survey, the average price of clean-up of one hectare which is overgrown with bushes is 250 euros.

Costs of land degradation elimination, in territories of built-up area abandonment and polluted territories, is estimated for 10 m^3. Construction waste and debris removal costs for 10 m^3 are 200 euros.

Pollution was detected in one unit of land. Pollutant is any substance that enters into the environment by human activities or natural processes, and which has harmful effects on living organisms. Environmental degradation means that the environment becomes unusable for its intended aims or if the living organisms and their community development are delayed. Environmental pollution and degradation can be caused by chemicals, physical agents or undesirable development of living organisms (biological factors) (Vides piesārņojums un.., [b.g].). Pollution removal would cost around 5000 – 5500 euros.

Conclusions

1. Field inspection is one of the best ways to detect the land degradation.
2. Using the field inspection method, as the most common problems were detected agricultural land overgrowing with bushes and lack of maintenance of drainage systems that lead to land bogging.

3. The costs of land degradation elimination are 250 euros per hectare, for territories where scrub formation on agricultural land and lack of maintenance of drainage systems and land bogging are observed, but costs for territories of built-up area abandonment and polluted territories are estimated to 200 euros per 10 m^3.
4. In territories where the land degradation reduction or elimination has been done, the control has to continue in order to stop the development of land degradation, because not always it is possible to completely prevent land degradation, without repetitive prevention measures.

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THE EFFECT OF VACUUM COOKING ON ENTERAL FOOD MADE FROM FRESH AND SEMI-FINISHED INGREDIENTS

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Abstract
Enteral feeding also known as enteral nutrition is nutritient delivery into the stomach. Products for enteral nutrition are a specific group of products designed to provide nutrients to the human body in case of various diseases and after surgery, when the daily intake of the product is affected. Today market offers special dietetic products, which are supplemented with synthetic vitamins and minerals, which bioavailability in the body is lower than that of natural organic complexes. Therefore it is important to develop special dietetic products from natural raw materials. The aim of this study was to evaluate the effect of vacuum cooking on the content of bioactive compounds, soluble solids, pH and shelf life of enteral food made from fresh and semi-finished (heated) fruit and vegetable juices. For this research enteral food was made using fresh or semi-finished fruit and vegetable juices. Products were vacuum cooked in 0.02 MPa pressure, with boiling point 67 °C, and 0.06 MPa pressure at 79 °C, withstander for 15 min. All samples were stored at room temperature and tested for their content of vitamin C, total carotenoids, anthocyanins, total phenols and antioxidant activity and microbiological safety, as control untreated enteral food samples were used. The obtained data showed that samples made from semi-finished juices have higher contents of vitamin C and total carotenoids and anthocyanins than samples prepared from fresh juices, but this wasn't observed with content of total phenols and antiradical scavenging activity (DPPH), where the type of ingredients used for sample preparation had no significant effect.
Key words: enteral nutrition, microbial safety, total carotenoids, phenols, anthocyanins, antiradical scavenging activity.

Introduction

Enteral nutrition using oral nutrition supplements and tube feeding gives the opportunity of increasing or ensuring nutrient intake when it is inadequate. This can only be used on patients with sufficient digestion where the food can be digested and nutrients assimilated in the body of a specially prepared diet (Ozola *et al*., 2017; Weimann *et al*., 2006). Enteral feeding is a method of supplying oral food and fluids using nasogastric, gastrostomy or jejunostomy feeding, which are also referred to as enteral tube feeding (Jones *et al*., 2011; Ozola *et al.*, 2017).

Depending on the intended use and specific needs of the patient, there are several types of enteral food, though most of them are synthetic it is possible to create enteral food using natural ingredients. To fully provide the necessary nutrients and at the same time the microbiological safety of the product it is needed to explore other product processing methods, that are lenient to bioactive compounds, vacuum cooking (cook-vide) is considered to be one of them.

The vacuum cooking treatments are aimed at improving the quality of coked products (Iborra-Bernad *et al*., 2014). Vacuum based cooking treatments may be innovative combined techniques with high potential application on an industrial scale, this could benefit microbiological, nutritional, physicochemical and sensorial quality compared to conventional cooking methods, since the partial vacuum that is created allows cooking at lower temperature (Martinez-Hernandez *et al*., 2013).

To vacuum treatments such as sous-vide (SV) and cook-vide the main advantage is the absence of oxygen and the use of temperatures under 100 °C, these two factors play a major role in protection of bioactive compounds, that are sensitive to temperature and can increase their degradation rate during food processing. The use of vacuum technologies could provide higher flavour retention , lower production of acrylamide and higher retention of pigments, where all these factors can lead to a better quality of the final product (Iborra-Bernad *et al.*, 2014; Ozola & Kampuse, 2017).

In contrast to SV treatments cook-vide consist of cooking in boiling water below 100 °C by lowering the pressure to reach the vapour pressure of water, this method of coocking has been already used in some traditional cuisine restaurants. The low pressure is maintained during the cooking time by vacuumpump. Up to this day very few scientific studies have been found in the literature about the use of this technique on fruit and vegetable or other product treatment (Iborra-Bernad, García-Segovia, & Martínez-Monzó, 2015; Martinez-Hernandez *et al*., 2013) physico-chemical and structural properties of cut and cooked purple-flesh potato, green bean pods, and carrots have been studied. Three different cooking methods have been applied: traditional cooking (boiling water at 100 \u00b0C

The aim of this research was to analyse the effect of vacuum cooking on the bioactive compound content, soluble solids, pH and shelf life of enteral food made from fresh and semi-finished (heated) fruit and vegetable juices.

Materials and Methods

Sample preparation

The research was carried out from January to February 2017 in Latvia University of Agriculture, Faculty of Food technology. For the purpose of this research samples of the same recipe enteral food were prepared ensuring 418.4 kJ intake per 161.25 g of product by using juices (97.3% of total weight) from blackcurrant (*Ribers nigrum*), beetroot (*Beta vulgaris*), pumpkin from Hubbard group (*Cucurbita maxima*), cabbage (*Brassica oleracea*) and Jerusalem artichoke (*Helianthus* tuberosus) and whey protein, canola oil made from *Brassiacaceae* family of plants, cod liver oil made from fish of the *Gadus* genus, iodized salt. For one part of the experiment fresh juices (obtained from raw fruits and vegetables grown in organic management system), for the other part juices previously vacuum cooked (heated, semi-finished) (prepared by Ltd 'KEEFA' 'Natural Food manufacturer' from the same raw material) were used (Ozola *et al.*, 2017). Semi-finished juice samples were vacuum cooked in temperatures 73 ± 5 °C under 0.05 ± 0.01 MPa pressure for 20 ± 5 min, depending on processed product, and heated up to 94 °C before packaging. Cooled juices were stored at 5 ± 2 °C.

Vacuum cocking

One average sample of each set of ingredients was made and vacuum cooked using Stephan UMC 5 vacuum kettle (Stephan Food Service Equipment GmbH, Germany) under two modes: 0.06 MPa pressure, product temperature during cooking reaches 79 ± 2 °C withstanded for 15 min; 0.02 MPa pressure, product temperature during cooking 67 ± 2 °C withstanded for 15 min (Ozola & Kampuse, 2017). The processed samples were divided between 6 individually packaged 100 mL polypropylene (PP) bottles (Kartell, Italy) from each cooking mode.

After product processing, samples were stored at room temperature (19 ± 2 °C) in direct light to observe the changes of bioactive compounds content during storage. Obtained results were compared depending on the type of used ingredients for the preparation of products and the selected vacuum cooking modes. For initial comparison of the vacuum coking impact on both group samples control tests were done with samples that were not treated (Ozola *et al*., 2017).

The quality changes of the samples during the storage were evaluated by microbial safety, tested with the determination of total plate count, coliforms, moulds, and yeasts, determination of soluble solids content, pH value, vitamin C, total carotenoids, total anthocyanins, total phenols, and antiradical activity (Ozola *et al*., 2017).

Microbiological analyses

Microbiological testing of enteral food was completed using 90 mL of 0.5% sterile peptone water solution to which 10 mL of enteral food was added and mixed. The mixture was pour-plated in duplicate for determination of: total plate count (TPC) according to standard LVS EN ISO 4833:2003 (Ref. No. 01-14, Sharlau, nutrient agar, incubation at 30 °C for 72 h); Coliforms according to standard LVS ISO 7251:2005 (Ref. No. 401460, Sharlau ENDO agar, incubation at 37 °C for 24 h); mould fungi and yeast cells according to standard ISO 21257-2:2008 (Ref. No.01-111, MRS agar, incubation at 27 °C for 48 h (yeast cells) and 5 to 7 days (mould fungi).

Microbiological safety of enteral food was evaluated according to the guidelines by Cabinet of Ministers, Latvia regulation No 461/2014 for Vegetable jams, purees and similar products which sets allowed limits for TPC at $5 \cdot 10^3$ CFU g^{-1}; presence of Coliforms per 1 g of product is not allowed; Mould fungi and yeast cells no more than 50 CFU g^{-1} (Ozola *et al*., 2017).

Soluble solids content and pH

The soluble solids content (Brix%) was measured with digital refractometer Refracto 30GS (Mettler Toledo, Japan) using standard method ISO 2173:2003.

Table 1

The identification of enteral food samples

Type and description of used juices	Vacuum cooking mode			Sample name
	temperature (°C)	pressure (MPa)	time (min)	
Fresh juices (made from raw fruits and vegetables)	untreated			fresh
	67 ± 2	0.02	15	I VS
	79 ± 2	0.06	15	II VS
Semi-finished juices (industrially vacuum cooked)	untreated			heated
	67 ± 2	0.02	15	I VP
	79 ± 2	0.06	15	II VP

Measurements were carried out in five replications. Potential of hydrogen commonly known as pH was measured with pH-meter (Lutron electronic enterprise CO., Ltd., UK) using standard method LVS ISO 5542:2010. Measurements were carried out in two replications (Ozola *et al*., 2017).

Vitamin C

Content of vitamin C was determined according to iodine method *(*Kerch *et al*., 2011). Method is based on determination of Lascorbic acid, a reduced form of ascorbic acid. A sample of 25 g of product was poured with 100 mL of 6% solution of oxalic acid and homogenized for 1 minute and filtered. Then, 2 mL of 1% solution of starch was added to10 mL of filtrate and the filtrate was titrated until change of colour, which does not disappear during 30 s. For standard-solution of ascorbic acid 20 mg of ascorbic acid were dissolved in 100 mL of the oxalic acid solution, 2 mL of the starch solution was added to 25 mL of the standard-solution and the mixture was titrated. The content of ascorbic acid mg per 100 g of product was calculated using formula (1) (Kerch *et al*., 2011; Ozola *et al*., 2017):

$$C = 5000 \cdot \frac{V_{sample}}{m \cdot V_{s\,\mathrm{tan}\,dard}} \quad (1)$$

where: V_{sample} – volume of the iodine solution titrated in a sample, mL; $V_{standard}$ – volume of the iodine solution titrated in a standard solution, mL; m – the amount of sample, g.
Measurements were carried out in four replications.

Total Carotenoids

For total carotenoid determination a spectrophotometric (UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK)) method described by (Kampuse *et al*., 2015) was used at wavelength of 440 nm. The content of carotenoids (mg 100 g^{-1}) was calculated in four replications (Ozola *et al*., 2017).

Total anthocyanins

Total anthocyanin content was determined by spectrophotometric method according to (Kerch *et al*., 2011), detected on spectrophotometer Jenway 6705 at wavelength of 540 nm. 20 g of sample was doused with 40 g of ethanol and 1.5 *M* HCl solution (85:15 by volume) and homogenized for 1 min. Then the sample was filtered, and light absorption at 540 nm was detected with spectrophotometer. Sample was diluted until absorption coefficient was between 0.6 and 0.8. Content mg per 100 g was calculated with the equation (2) (Ozola *et al*., 2017):

$$C = \frac{A \cdot v \cdot d \cdot 1000}{980 \cdot m} \quad (2)$$

where: A – absorption coefficient; v – volume of the extraction (90); d – dilution; m – sample weight in g (20).
Measurements were carried out in two replications.

Total phenol content

The detection of total phenol content was done according to the Folin- Ciocalteu method (Yu *et. al.,* 2003)2-diphenyl-1-picrylhydrazyl radical (DPPH\ u2022 with modifications: to 0.5 mL of extracted sample add 2.5 mL of 0.2 *N* Folin-Ciocalteu reagent, that has been diluted ten times with distilled water; after 5 min add 2.0 mL of 7.5% $NaCO_3$; the resulting solution was mixed and allowed to stand for 30 min at 18 ± 1 °C in a dark place; absorption was read at 760 nm using JENWAY 6300 (Banoworld Scientific Ltf., UK) spectrophotometer (Priecina & Karklina, 2014) (Ozola *et al*., 2017).

Measurements were carried out in six replications from two separately weighed samples.

Antiradical scavenging activity (DPPH)

The antiradical scavenging activity of extracts was determined according to (Yu *et al.*, 2003)2-diphenyl-1-picrylhydrazyl radical (DPPH\u2022 with modifications: to 0.5 mL of extracted sample 3.5 mL freshly made DPPH solution was added; the mixture was shaken and kept in the dark place at 18 ± 1 °C for 30 min; absorbance was measured at 517 nm using JENWAY 6300 Spectrophotometer (Ozola *et al*., 2017; Priecina & Karklina, 2014).

Measurements were carried out in six replications from two separately weighed samples.

Statistical analysis

The obtained data was processed using 'Microsoft Office Excel' 2007 version, differences between the results were analysed using ANOVA: one and two-factor with replications. The obtained results are presented as their mean with standard error. Differences among results were considered to be significant if pvalue<$\alpha_{0.05}$ (Ozola *et al*., 2017).

Results and Discussion

Effect of vacuum cooking and storage on microbial safety

All samples including control (without vacuum cooking) were microbiologically tested before storage, vacuum treated enteral food samples were also tested during storage. No coliforms and mould fungi were found in evaluated samples. The initial results (Table 2) on enteral food made from fresh juices show

that vacuum cooking can decrease the total plate count and yeast cell count, but the used treatment modes have not been successful for creating microbiologically safe products, this information does not coincide with previously made research on vacuum cooking mode application on pumpkin-guilder rose sauces (Ozola & Kampuse, 2017) and other author findings, that suggest vacuum-based cooking generally induces higher microbial reduction. Enteral food samples made from semi-finished juices show a higher microbial safety before storage however only storing them for 7 days already show some signs of spoilage for sample I VP (cooked at 67 °C, 0.02 MPa pressure) and in 14 days sample II VP (cooked at 79 °C, 0.06 MPa). Temperatures and pressure modes used in this research should be sufficient for minimizing the yeast cell count in product, but the obtained data suggest the opposite. These findings could be affected and be a direct response to contamination from air or packaging as the product filling process was done in open space.

Martinez-Hernandez *et al*., 2013 in their research vacuum boiled Kailan-hybrid broccoli (*Brassica oleracea*) at 0.9 bar (0.01 MPa) where the boiling point was reached at 54 °C and the microbial testing showed no signs of yeasts and moulds, however some mesophilic, psychrophilic and enterobacteria still had remained. The microbial testing showed that the most effective cooking method was pressure cooking and *sous vide* (Martinez-Hernandez *et al*., 2013).

NA-not analysed; Fresh- enteral food made from fresh juices, untreated; I VS- enteral food made from fresh juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from fresh juices, vacuum cooked at 0.06 MPa pressure; Heated- enteral food made from semi-finished juices, untreated; I VS- enteral food made from semi-finished juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from semi-finished juices, vacuum cooked at 0.06 MPa pressure.

Samples made from fresh juices after vacuum cooking exceed the allowed yeast cell count 50 CUF g^{-1} (Table 2) and are considered to be unsuited for further use and testing. After storing the rest of enteral food samples for 7 days sample cooked at 0.02 MPa was determined to be unsuitable accordingly to the guidelines by Cabinet of Ministers, Latvia regulation No 461/2014 for 'Vegetable jams, purees and similar products' for further testing by exceeding both total plate count $6.5 \cdot 10^3$ CFU g^{-1} (allowed TPC $5 \cdot 10^3$ CUF g^{-1}) and yeast count $1.5 \cdot 10^2$ CFU g^{-1}. Sample II VP, which was processed at 0.02 MPa, boiling point 67 °C, after storing for 14 days started to show some microbial spoilage, but signs of microbial fermentation was determined only after additional week, when this sample also was presumed to be unsuitable.

Effect of vacuum cooking and storage on soluble solids and pH

Soluble solids and product pH didn't show any significant differences and changes between the enteral foods after treatment, also no significant differences were noticed during storage or between the types of used juices for enteral food preparation. On average the soluble solids content was 11.4 ± 0.2 Brix% and the pH to samples made from fresh juices was pH 5.00 ± 0.03, but from semi-finished pH 4.61 ± 0.04.

Effect of vacuum cooking and storage on bioactive compounds (vitamin C, total carotenoids, total anthocyanins, total phenols and DPPH)

The obtained data on vitamin C, total carotenoids and anthocyanin content in enteral food samples is given in Table 3. The data show a significant difference ($p < 0.05$) in content of vitamin C between the samples depending on what type of juices were used for the preparation. Samples from fresh juices showed overall higher content of vitamin C, than samples made from semi-finished juices, but no significant difference was detected between the treatment methods or depending on the storage time.

The content of total carotenoids (Table 3) showed significant changes not only on type of juices used

Table 2

Total plate and yeast cell count in enteral food made from natural juices (CUF g^{-1})

Sample	Total plate count			Yeast cell count		
	before storage	day 7	day 14	before storage	day 7	day 14
Fresh	$1.3 \cdot 10^3$	NA	NA	$3.4 \cdot 10^2$	NA	NA
I VS	$2.9 \cdot 10^2$	NA	NA	$2.4 \cdot 10^2$	NA	NA
II VS	$1.6 \cdot 10^2$	NA	NA	$1.2 \cdot 10^2$	NA	NA
Heated	<10	NA	NA	<10	NA	NA
I VP	<10	$6.5 \cdot 10^3$	NA	<10	$1.5 \cdot 10^2$	NA
II VP	<10	<10	59	<10	<10	47

Table 3

Content of vitamin C, total carotenoids and total anthocyanins in enteral food made from natural juices (mg 100 g^{-1})

Sample	Storage time	Vitamin C	Total carotenoids	Total anthocyanins
Fresh	before storage	24.99 ± 3.46	0.44 ± 0.00	1.50 ± 0.07
I VS	before storage	24.11 ± 2.97	0.41 ± 0.01	1.59 ± 0.02
II VS	before storage	22.29 ± 1.21	0.32 ± 0.01	1.27 ± 0.01
Heated	before storage	21.37 ± 1.22	0.43 ± 0.02	2.45 ± 0.15
I VP	before storage	19.33 ± 1.12	0.43 ± 0.01	2.17 ± 0.01
	day 7	18.15 ± 2.06	0.37 ± 0.01	1.84 ± 0.04
II VP	before storage	19.13 ± 1.49	0.44 ± 0.01	1.65 ± 0.13
	day 7	18.24 ± 2.00	0.37 ± 0.01	1.49 ± 0.00
	day 14	16.25 ± 1.26	0.35 ± 0.00	1.41 ± 0.04

for enteral foods, but also samples made from fresh juices have been affected more by the vacuum cooking modes. Samples made from fresh juices show higher decrees in content of total carotenoids and anthocyanins when the vacuum cooking pressure and temperature is higher, opposite to vacuum cooking samples at 0.02 MPa pressure, when the content of total carotenoids and anthocyanins was typically closer to the control samples. Semi-finished juice enteral food samples showed a 13% decrees in content of total carotenoids during storage when it significantly dropped after storing for 7 days in sample I VP 0.43 ± 0.0 mg 100 g^{-1} before storage to 0.37 ± 0.01 mg 100 g^{-1} day 7 and in sample I VP 0.44 ± 0.01 mg 100 g^{-1} before storage to 0.35 ± 0.00 mg 100 g^{-1} day 14.

Changes of total anthocyanin content depending on the used vacuum cooking mode was also noticed in semi-finished samples, the highest total anthocyanin content was in sample I VP (0.02 MPa) made from semi-finished juices 2.17 ± 0.01 mg 100 g^{-1}, but the lowest in sample II VS (0.06 MPa) made from fresh juices 1.27 ± 0.01 mg 100 g^{-1}. These coincide with other author findings about better colour retention in products by using vacuum based treatments compared to conventional cooking which does suggest that application of higher temperatures can significantly decrees the content of carotenoids and anthocyanins that often are responsible for the natural colour of fruits and vegetables (Iborra-Bernad *et al*., 2015)physico-chemical and structural properties of cut and cooked purple-flesh potato, green bean pods, and carrots have been studied. Three different cooking methods have been applied: traditional cooking (boiling water at 100 \u00b0C.

Fresh- enteral food made from fresh juices, untreated; I VS- enteral food made from fresh juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from fresh juices, vacuum cooked at 0.06 MPa pressure; Heated- enteral food made from semi-finished juices, untreated; I VS- enteral food made from semi-finished juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from

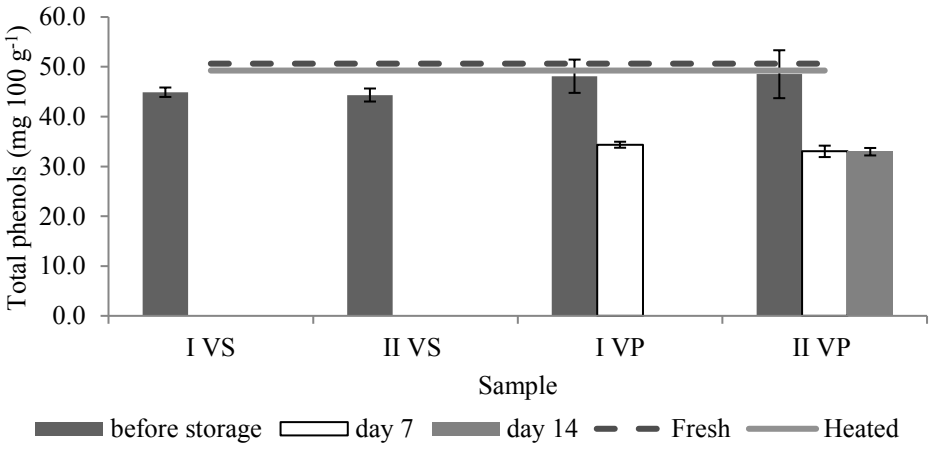

Figure 1. Content of total phenols in enteral food made from natural juices (GAE·mg 100 g^{-1}).

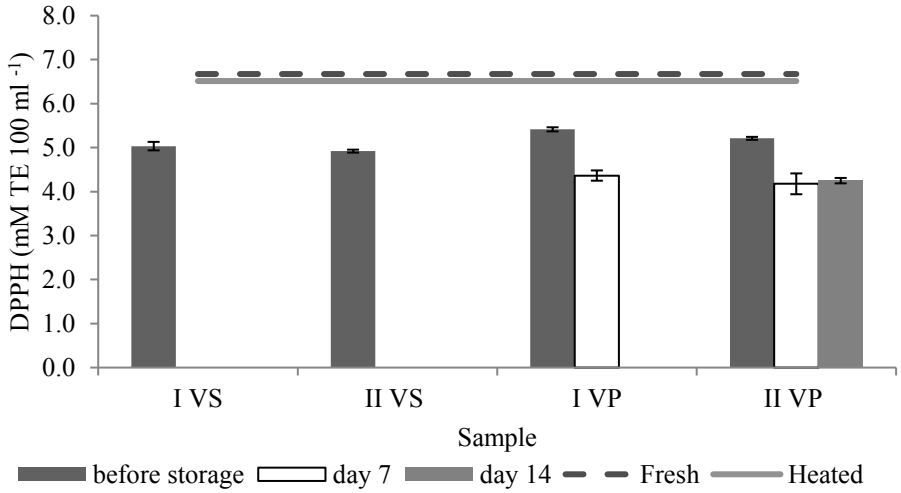

Figure 2. Contents of antiradical activity (DPPH) in enteral food made from natural juices (mM TE 100 ml^{-1}).

semi-finished juices, vacuum cooked at 0.06 MPa pressure.

Total phenol content (Figure 1) in enteral food samples show no significant difference ($p < 0.05$) between the type of juices used for sample preparation, however slightly higher total phenol content was noticed in samples made from semi-finished juices. After storing samples for 7 days a 30% loss of total phenols was detected to samples made from semi finished juices, sample I VP 48.11 ± 3.34 GAE·mg 100 g^{-1} before storage, to 34.36 ± 0.60 GAE·mg 100 g^{-1} at day 7 and to sample II VP 48.52 ± 4.81 GAE·mg 100 g^{-1} before storage, to 39.96 ± 0.75 GAE·mg 100 g^{-1} day 14.

Fresh- enteral food made from fresh juices, untreated; I VS- enteral food made from fresh juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from fresh juices, vacuum cooked at 0.06 MPa pressure; Heated- enteral food made from semi-finished juices, untreated; I VS- enteral food made from semi-finished juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from semi-finished juices, vacuum cooked at 0.06 MPa pressure.

The antiradical activity (Figure 2) of enteral food samples contrary to total phenol content showed a significant difference ($p < 0.05$) between the vacuum cooking modes used in this research and slight differences by type of juices used for sample preparation. The most noticeable changes in DPPH were detected during storage where on average a 19% decrease of DPPH was noticed after 7 days of sample storage.

Fresh- enteral food made from fresh juices, untreated; I VS- enteral food made from fresh juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from fresh juices, vacuum cooked at 0.06 MPa pressure; Heated- enteral food made from semi-finished juices, untreated; I VS- enteral food made from semi-finished juices, vacuum cooked at 0.02 MPa pressure; II VS- enteral food made from semi-finished juices, vacuum cooked at 0.06 MPa pressure.

The differences in DPPH depending on vacuum cooking mode could be impacted by the differences in content of vitamin C and total anthocyanins, which showed a bigger retention in samples cooked under lower temperature and higher pressure (67 °C, 0.02 MPa pressure) for samples made from semi-finished juices. To enteral food samples made from fresh juices also total carotenoid content was significantly different depending on the used vacuum cooking mode.

The findings of this research partly coincide with previous research done on vacuum cooking modes, where pumpkin-guelder rose sauce samples cooked under vacuum didn't show any significant differences in bioactive compound content depending on the used vacuum treatment (Ozola & Kampuse, 2017). However this research showed some coincidences with enteral food samples made from semi-finished juices.

The overall research on this particular thermal processing method is rather deficient. Due to the lack of research on this subjeckt it is difficult to make a full study analysis and to fully evaluate the efficiency of the vacuum cooking process compared to other thermal treatments.

Conclusions

The findings of this research coincide with some of the few researches that have been done on this subject, comparing to conventional cooking vacuum cooking does show a higher retention on total phenol content, DPPH and carotenoids and anthocyanins, but additional research needs to be done, to provide

sufficient amount of data on vacuum cooking modes and efficiency it has on bioactive compounds and microbial safety of products.

The obtained data also showed no significant difference in total phenol content and DPPH depending on type of juices used as ingredients, but showed better results in content of total carotenoids and anthocyanins with enteral food samples made from semi-finished juices.

Initial results show that vacuum cooking with following of hot filling wasn't sufficient enough to provide microbiologically safe products made from fresh juices, but using semi-finished ingredients the shelf life of these products cannot exceed 14 days.

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THE COMPARISON OF COMMERCIALLY AVAILABLE β-GALACTOSIDASES FOR DAIRY INDUSTRY: REVIEW

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Abstract
β-Galactosidase (EC 3.2.1.23) is one of the widely used enzymes for lactose-free milk production and whey permeate treatment. Enzymes can be obtained from microorganisms, plants and animals. Nowadays, microorganisms are becoming an important source for production of commercially available enzymes, which are of great interest and offer several advantages such as easy handling and high production yield. The aim of this review was to summarize findings of research articles on the application of commercially available β-galactosidase preparates in dairy industry, to analyse and compare the most suitable β-galactosidase commercial preparates for lactose hydrolysis. The results showed that the main factor to choose an appropriate β-galactosidase for lactose hydrolysis was reaction condition. Enzymes from microorganisms contain a wide range of optimal pH from 4.0 (*Penicillium simplicissimum* and *Aspergillus niger*) to 8.5 (*Bacillus subtilis*). The greatest commercial potential have enzymes obtained from fungi (*Aspergillus oryzae* and *Aspergillus niger*) and yeasts (*Kluyveromyces lactis* and *Kluyveromyces fragilis*). Fungal origin enzymes are more suitable for the hydrolysis of lactose in acid whey due to its acidic pH but yeasts origin enzymes for milk and sweet whey. In the study, commercial preparates from different suppliers with the purpose to analyse their lactose hydrolysis potential and give more detailed characteristics of each preparate advantages and drawbacks were also summarized.
Key words: β-Galactosidase, lactose hydrolysis, commercial preparates.

Introduction

The scientists worldwide are searching for new biotechnological tools which promote the use of dairy by-products (whey, buttermilk and other) for obtaining compounds with new perspective or products with high added value (De Jesus *et al*., 2015).

Enzymes are protein molecules which are working as catalysts for chemical reactions (Chaudhary *et al*., 2015) and are entirely involved to change and develop the nutritional, functional and sensory properties of ingredients and products, and have found extensive application in processing and production of food products (Oort, 2010). Important segment of food enzymes industry are dairy enzymes, which are used for the developing and improving sensory characteristics (aroma, flavour and colour) and functional properties of milk products (Singh *et al*., 2016). Dairy industry is one of the largest markets for commercial enzymes application (Kelly & Fox, 2012) and one of the highly used enzymes is β-galactosidase (EC 3.2.1.23) (Grosová, Rosenberg, & Rebroš, 2008). β-Galactosidase is known as lactase, which hydrolyses lactose into glucose and galactose (Panesar, Kumari, & Panesar, 2010). The enzyme is used to produce lactose-free products for people who are lactose-intolerant (Shu *et al*., 2014). Lactose is a disaccharide and major sugar in milk. This sugar may affect flavours and odours absorption and leads to many faults in refrigerated foods such as formation of crystals, deposit and sandy or gritty texture. There is another reaction where β-galactosidase can be used and it is in transglycosylation of lactose for galacto-oligosaccharides (GOSs) synthesis. These are the nondigestible oligosaccharides, which in the upper intestinal tract are not hydrolyzed or absorbed (Panesar, Kumari & Panesar, 2010). β-Galactosidase has animal, plant and microbial (bacteria, fungi, and yeasts) origin; for reducing production costs, mostly microbial enzymes, which represent a higher activity than others are used. Enzyme used in food production must come from microorganisms which have been approved by Generally Recognized as Safe (GRAS) (Dutra Rosolen *et al*., 2015). GRAS provides unhampered usage of their enzymes without any profuse and expensive purification (Carević *et al*., 2015). The greatest commercial potential shows enzymes which are obtained from fungi and yeasts (Dutra Rosolen *et al*., 2015).

The aim of this review was to summarize findings of research articles on the application of commercially available β-galactosidase preparates in dairy industry, to analyse and compare the most suitable β-galactosidase commercial preparates for dairy production.

Materials and Methods

Monographic method was used in this study. The review summarizes the research findings on commercially available β-galactosidase that is used for the hydrolysis of lactose. Enzyme properties and suitability for dairy products production, commercial preparates for industrial and research tasks, as well as lactose hydrolysis potential has been studied.

Results and Discussion

Characterisation of β-galactosidase

The commercially available β-galactosidase is derived from a variety of microorganisms which are

Table 1

Characterisation of β-galactosidase

Source	Microorganism	pH optimum	Temperature optimum, ◦C	References
Fungi	*Aspergillus niger*	4.0 – 4.5	60	Sykes *et al*., 1983
	Aspergillus oryzae	5.0	50 – 55	Park, Santi, & Pastore, 1979
	Penicillium simplicissimum	4.0 – 4.6	55 – 60	Cruz *et al*., 1999
Bacteria	*Bacillus subtilis*	8.0 – 8.5	35	El-Kader *et al*., 2012
	Bacillus licheniformis	6.5	50	Juajun *et al*., 2011
	Bacillus circulans	6.0	60	Yin *et al*., 2017
	Bacillus stearothermophilus	7.0	70	Chen *et al*., 2008
	Escherichia coli	7.0	55	Nakanishi *et al*., 1983
	Arthrobacter psychrolactophilus	8.0	10	Nakagawa *et al*., 2007
Yeast	*Kluyveromyces lactis*	7.0	40	Bosso *et al*., 2016
	Kluyveromyces fragilis	6.6	37	Jurado *et al*., 2002
	Kluyveromyces marxianus	7.5	40	Brady *et al*., 1995

of great interest and offer a number of benefits such as easy handling and high production yield (Panesar, Kumari, & Panesar, 2010).

Summary about optimal activity conditions of β-galactosidase from various research works is given in Table 1.

Feature impacts on β-galactosidase characteristics (Iliev, 2012), such as molecular properties, metal ion and substrate specificity, as well as the temperature and pH conditions necessity is very important for optimal activity of enzyme (Rampelotto, 2016). It is set that out of the total enzymes which are used for industrial use, over 50% are obtained from yeast and fungal sources and one third is extracted from bacterial sources (Panesar, Marwaha, & Chopra, 2010).

The application of β-galactosidase in industrial processes mainly depends on the hydrolysis reaction conditions (Dutra Rosolen *et al*., 2015). β-Galactosidase is used for dairy products which have pH differences (Walsh, 2007), for example, sweet whey and bovine milk pH 6.0 – 7.0 and acid whey pH have less than 5.0 (Dutra Rosolen *et al*., 2015). Enzymes are extremely sensitive to the pH of the reaction medium and therefore each β-galactosidase has an optimal pH in which it reaches maximal activity (Scopes, 2002). β-Galactosidase from *Penicillium simplicissimum* and *Aspergillus niger* have pH optimum of about 4.0 – 5.0 while from *Bacillus subtilis* has pH optimum of 8.0 – 8.5 (Table 1). This shows that β-galactosidase from microorganisms contain a wide range of optimal pH condition and are able to used for different kind of medium.

Cruz and co-authors (1999) noted that *Penicillium simplicissimum* β-galactosidase optimal pH for hydrolysis was in the range 4.0 – 4.6. The enzyme reached great results, hydrolysed 67% lactose from milk and 84% lactose from milk whey which show a potential of using for food production (Cruz *et al*., 1999). El-Kader and co-authors (2012) discovered that at pH 8.0 the activity of *Bacillus subtilis* increased rapidly till 1278 UI·mL^{-1} at 30 minutes, while enzyme activity at pH 8.5 rise to 1387 UI·mL^{-1} at 10 minutes (El-Kader *et al*., 2012). For industrial production, it is a great opportunity to choose an appropriate β-galactosidase for product with a certain pH. Another important property is enzymatic thermostability and activity at low temperature (Dutra Rosolen *et al*., 2015). Cold-active β-galactosidase is of high biotechnological interest as a food industrial enzyme, because it is intended for hydrolysis of lactose in milk and dairy products at low temperature (Pandey, Negi, & Soccol, 2017). Nakagawa and co-authors (2007) showed that the β-galactosidase which is obtained from *Arthrobacter psychrolactophilus* strain F2 could provide 70% lactose hydrolyse at 10 °C in 24 h. That shows the enzyme has a great potential as a cold-active enzyme in the industrial field (Nakagawa *et al*., 2007).

Furthermore, thermostable β-galactosidase has high reaction velocity, diminished risk of contamination, long half-lives, reduced product inhibition, high solubility and yields (Pandey, Negi, & Soccol, 2017). *Bacillus stearothermophilus* β-galactosidase has the highest enzyme activity at 70 °C (Table 1), showing that this thermostable enzyme has great industrial potential of promoting product production at high temperatures (Chen *et al*., 2008). Chen and co-authors (2008) stated that enzyme *Bacillus stearothermophilus* showed the highest activity at 70 °C and saved 80% of its activity even at 75 °C. Providing necessary condition of thermostable β-galactosidase, which optimal pH is neutral, enzyme would be a great choice for the lactose hydrolysis in

milk. This kind of enzyme would be able to be used for application in low-lactose milk production during milk pasteurization (Chen *et al*., 2008). It shows that thermostable β-galactosidase could be a potential alternative for dairy products processing, which are treated to high temperature for reducing microbial contamination.

Sources of β-galactosidase

Fungi. β-Galactosidase from fungal sources are thermostable, however, are mainly sensitive by galactose (Husain, 2010). They are usually used to hydrolyse lactose from products which has an optimum pH between 2.5 and 5.4, such as acid whey (Dutra Rosolen *et al*., 2015). β-Galactosidase from *Aspergillus, Penicillinum* and *Paecilomyces spp.* have the major interest from industrial field, they have extracellular character, higher and better thermal tolerance and lower pH of activity (Gargova, Pishtijski, & Stoilova, 1995). The most used β-galactosidases are from *Aspergillus niger* and *Aspergillus oryzae*, because of their extracellular nature, high activity, GRAS status (Hu *et al*., 2010) higher thermal stability and for the lactose hydrolysis at low pH (Hatzinikolaou *et al*., 2005).

Acidic pH is the optimal medium for *Aspergillus niger* β-galactosidase and it can be an ideal candidate for lactose hydrolysis in acid whey permeate (Hatzinikolaou *et al*., 2005). Although the enzyme can be inhibited by galactose, it is quite complicated to get complete lactose hydrolysis (Soccol *et al*., 2012).

Aspergillus oryzae is highly accepted as source of enzyme which is used for food and feeds (Nizamuddin, Sridevi, & Narasimha, 2008). The optimal temperature and pH of enzyme action at 60 °C, pH 4.75 can be used for lactose hydrolysis in whey. Benefit of this β-galactosidase from a technological viewpoint is its comparative stability at high temperature what is very important for controlling and preventing microbial risk during the hydrolysis process (Cruz *et al*., 1999).

Bacteria. Bacterial β-galactosidase has great properties of fermentation, hydrolysis activity and stability, and usually used for the lactose hydrolysis. Lactic acid bacteria (diverse group of lactococci, streptococci and lactobacilli) and bifidobacterium have been used as good sources of β-galactosidase, particularly for functional food production and more important is that organisms need to be accepted by GRAS (Princely *et al*., 2013). Lactobacilli isolated from fermented Ragi have remarkable industrial potential. They can provide better yields at high temperatures and avoid microbial contamination in milk processing (Mozumder *et al*., 2012).

Bacillus licheniformis is a soil-dwelling endospore forming microorganism. Juajun and co-authors (2011) analysed activity and stability of enzyme at numerous pH and temperature conditions, and results showed that β-galactosidase from *Bacillus licheniformis* is stable and has good perspectives compared to other industrially used enzymes. This enzyme is most active between pH 5 and 9 at 37 °C and up to 42 °C, at pH 6.5. In food manufacturing, the hydrolytic effect of β-galactosidase from *Bacillus licheniformis* opens new perspectives for lactose hydrolysis, or for improving quality and properties of dairy products by rising their solubility and sweetness level. However, it is mainly inhibited by the hydrolysis products – glucose and galactose (Juajun *et al*., 2011).

Commercial β-galactosidase from *Bacillus circulans* exerts at least four isoforms, but the most used is the so-called β-galactosidase-I (Torres & Batista-Viera, 2012). Enzyme optimal condition is at 60 °C and pH 6.0, which pH is similar to sweet whey (Yin *et al*., 2017). This enzyme also can catalyse the formation of a large amount of oligosaccharides. Enzyme optimal condition properties are suitable for the hydrolysis of lactose in milk and whey, and for oligosaccharide production (Torres & Batista-Viera, 2012).

β-Galactosidase of *Escherichia coli* have been carried out numerous times and in various ways (Huber *et al*., 1979). β-Galactosidase from *Escherichia coli* is the most extensively studied and is not suitable for industrial use, because it is regarded as not safe for food applications. Nonetheless, this enzyme is commercially available for analytical researches (Pandey, Negi, & Soccol, 2017). This enzyme hydrolyses lactose into glucose and galactose, using this sugar as a substrate for heterotrophic bacterial growth. This enzyme is as a reporter protein or molecular sensor in microbiology and molecular and cell biology (Feliu & Villaverde, 1998).

Yeast. From an industrial point of view yeast is an important source of β-galactosidase (Panesar *et al*., 2006). The food industry interest of this enzyme production by yeast might have started since this enzyme is used for the production of products with low lactose content (Husain, 2010). Yeast enzymes are commonly used for products with neutral pH which have optimum pH 6.0 – 7.0 such as milk and sweet whey (Dutra Rosolen *et al*., 2015).

Kluyveromyces lactis is one of the important sources of commercial β-galactosidase which is used in the dairy industry (Teles De Faria *et al*., 2012) because of its environmental habitat and great lactose hydrolysis productivity, enzyme main disadvantage is thermostability (Chen *et al*., 2008). Another possibility to use it is for whey processing for lactose hydrolysis to get glucose and galactose (Teles De Faria *et al*., 2012).

β-Galactosidase from *Kluyveromyces fragilis* and *Kluyveromyces lactis* are mostly the same (Jazairi,

Table 2

Commercial sources of β-galactosidase

Enzyme source	Trade name	Activity	Lactose hydrolysis, %	Supplier	References
Yeast					
Kluyveromyces sp.	Enzeco Lactase NL	NM*	95	EDC, New York, US	Horner *et al*., 2011
Kluyveromyces lactis	GODO-YNL2	5000 NLU·g^{-1}	99	Danisco A/S, Denmark	
	Maxilact® LX5000	5000 NLU·g^{-1}	100	Sedim Cedex, France	Bosso *et al*., 2016
	Maxilact-L/2000	2000 NLU·g^{-1}	90	Gist-Brocades	Jurado *et al*., 2004
	Lactozym 2600L	2600 LAU·g^{-1}	NM*	Novozymes	Reddy, Nath, & Reddy, 2016
	Lactomax Pure	NM*	75	Prozyn, Brazil	Dutra Rosolen *et al*., 2015
	Lactozym pure 6500 L	1320 U·mL^{-1}	95	Novozymes	Rodriguez-Colinas *et al*., 2014
	Ha-Lactase 5200	8040 LAU·g^{-1}	90	Chr. Hansen, Denmark	Hendriksen *et al*., 2010
Kluyveromyces fragilis	Lactozym 3000L HPG	3000 LAU·mL^{-1}	72	Novo Nordisk	Jurado *et al*., 2004; Volpato Fernanda, & Souza, 2016
Fungal					
Aspergillus oryzae	Lactomax F30	NM*	50	Prozyn, Brazil	Dutra Rosolen *et al*., 2015
	Bio-Cat	5000 NLU·g^{-1}	41	INC/USA	Bosso *et al*., 2016
Bacteria					
Bacillus circulans	Biolactase NTL	553 U·mL^{-1}	50	Biocon, Spain	Rodriguez-Colinas *et al*., 2014

*NM- not mentioned

Ghorrah, & Bakri, 2014). Matioli, Farias de Moraes, & Maria Zanin (2001) stated that the β-galactosidase enzyme from *Kluyveromyces fragilis* is highly active at 45 °C and pH 6.0 (Matioli, Farias de Moraes, & Maria Zanin, 2001). With pH 7 enzyme is well appropriate for hydrolysis of lactose in milk (Panesar *et al*., 2006). Ladero and co-authors (2002) stated that the immobilized β-galactosidase from *Kluyveromyces fragilis* can be useful for frozen dairy products production and to refrain lactose crystal formation process and to increase the intake and flavour of such products (Ladero *et al*., 2002).

Transgalactosylation activity of β-galactosidase

Oligosaccharides have attracted the attention of researchers to investigate possible transferase activities by β-galactosidase which produce a number of carbohydrates (Panesar *et al*., 2006). Research results show that the highest producer of GOS of all fungal sources is *Aspergillus oryzae* compared with *Aspergillus niger* (Prenosil, Stuker, & Bourne, 1987) from yeasts *Sterigmatomyces elviae* CBS8119 (Onishi & Tanaka, 1998). Nakanishi and co-authors (1983) showed that *Bacillus circulans* is more perspective compared to *Escherichia coli* and yeasts (Nakanishi *et al*., 1983). The proportion between hydrolysis of lactose and GOS synthesis depends significantly on the concentration of lactose, the temperature of the process and the intrinsic enzyme properties (Rodriguez-Colinas *et al*., 2016).

Commercial preparate

The main features what make enzymes appropriate catalysts in the diversity of applications are the variety of catalytic function and the capability to work under optimal conditions. Industrial enzymes can be prepared differently as partly purified or 'bulk' enzymes, and highly purified for analytical or diagnostic use (Oort, 2010). The industrial applications of β-galactosidase make use of either free enzyme or immobilized enzyme. The use of free enzyme is technically simpler, its main drawback – the enzyme

in solution cannot be reused and increases the cost of repeated production of enzyme preparations (Pandey, Negi, & Soccol, 2017). Immobilized β-galactosidase offers the possibility of repetitive and continuous use of enzyme with good operational stability (Husain, 2010). It should be noted that the company which first used the commercial production of lactose-free milk by immobilized β-galactosidase from *Kluyveromyces lactis* was Centrale del Latte of Milan (Italy) galactose (Panesar, Kumari, & Panesar, 2010). Company Drouin Cooperative Butter Factory (Australia) used immobilized *Aspergillus oryzae* β-galactosidase for manufacturing market milk and hydrolyzed whey. This enzyme was developed by Sumitomo Chemical (Japan) (Pandey, Negi, & Soccol, 2017).

Summary about commercial sources used for milk or whey lactose hydrolysis of various studies is given in the Table 2.

The main features which regulate the technology and costs of the lactose hydrolysis process is enzyme characteristics and price (Grosová, Rosenberg, & Rebroš, 2008). Now the main commercial source of β-galactosidase is *Kluyveromyces lactis* because of dairy environmental habitat (Mozumder *et al*., 2012). Studies carried out by Adalberto and co-authors (2010) identified that enzymatic preparation Lactozym 3000L HPG is poorly stable. Sensitivity to pH and heat are greater in the absence of metal ions, showing that metal ions have an influence on enzymatic stability (Adalberto *et al*., 2010).

Bosso and co-authors (2016) analysed enzyme activity in UHT and skimmed milk at variable concentration, temperature, and pH. The best results were detected at 40 °C and pH 7.0 for the enzyme Maxilact® LX5000, and at 55 °C and pH 5.0 from Bio-Cat. Bio-Cat enzyme was able to hydrolyze 41% of lactose in UHT milk and 32.7% in skimmed milk. Furthermore, Maxilact® LX5000 hydrolyses 100% of lactose in UHT milk and 92% in skimmed milk. Considering enzyme thermal stability, it showed that the enzyme from Bio-Cat had better heat resistance than Maxilact® LX5000. Knowing properties of β-galactosidase from *Kluyveromyces lactis* and *Aspergillus oryzae* allows using it more competently in many industrial productions (Bosso *et al*., 2016). Dutra Rosolen and co-authors (2015) evaluated the influence of enzyme concentration, temperature, and reaction time in milk, cheese whey, and whey permeate using two commercial preparates Lactomax F30 and Lactomax Pure. At the same enzyme condition, during 2 h of reaction, the Lactomax Pure presented higher hydrolysis efficiency 75% than the Lactomax F30 50%. The results display that a higher lactose hydrolysis of milk and whey can be achieved using the Lactomax Pure and in the permeate using Lactomax F30 (Dutra Rosolen *et al*., 2015). In the studies by Jurado and co-authors (2004) pointed out that activity of both commercial β-galactosidase preparates Lactozym 3000L HP-G and Maxilact-L/2000 have similar action in different condition and similar kinetic parameter values (Jurado *et al*., 2004). Horner and co-authors (2011) tested food-grade β-galactosidase enzymes to evaluate their potential for use in hydrolysis process. Results showed that in pasteurized whole milk in 24 h, using supplier's recommended dosage GODO-YNL2 hydrolyzed on average 99% of the lactose and Enzeco Lactase NL was able to hydrolyze 95%, added a double dose. Lactose hydrolysis can be achieved also with less enzyme if the reaction is allowed to proceed for a longer time. In pasteurized whole milk GODO-YNL2 achieved 95% average hydrolysis in 72 h while using one-fourth the recommended dosage and 99% hydrolysis under the same conditions using one-half the recommended dosage. Instead enzymes Enzeco Lactase NL accomplished approximately 99% hydrolysis on average in 72 h by using the recommended dosage. By using a smaller dose and a longer time for lactose hydrolysis, enzyme cost for a liter of lactose-free milk could be minimized, potentially reducing the cost of lactose-free milk (Horner *et al*., 2011). It shows that each preparate has advantages and drawbacks for choosing the best one for lactose hydrolysis.

Conclusions

1. The use of β-galactosidase in industrial processes mainly depends on the hydrolysis reaction conditions. Each enzyme preparate has optimal parameters in which it can reach the highest activity to hydrolyse lactose.
2. There are significant parameters such as concentration of lactose, the temperature of the reaction and the enzyme properties which impact transferase activities by β-galactosidase causing GOS synthesis.
3. A large number of scientific researches and industrial utilization showed that the greatest commercial potential has enzymes, which are gained from *Aspergillus oryzae*, *Aspergillus niger* and *Kluyveromyces lactis*, *Kluyveromyces fragilis* due to their productivity.

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EFFECT OF HIGH PRESSURE PROCESSING ON MILK COAGULATION PROPERTIES

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Abstract
Raw milk cheeses are known to have more intense and strong flavour and different texture due to natural microbiota and enzymes. Nevertheless, there are concerns about safety of these products. For microbial inactivation heat treatment of milk is used, but it can adversely affect the flavour, taste and texture of the product. Therefore, applying non-thermal technology such as high pressure processing is attracting alternative. The aim of this study was to examine the effects of high pressure treatment of cow`s milk at a wide range of pressures (400 - 600 MPa) on milk rennet coagulation time, curd firmness and curd yield. Processed milk samples were subjected to enzymatic coagulation using commercial rennet to determine rennet coagulation time, yield of coagulum and curd firmness. High pressure processing insignificantly influences coagulation properties of whole milk. However, the magnitude of changes depended on applied pressure. Rennet coagulation time and curd yield were significantly different ($p < 0.05$) among the pressure treated milk samples. The higher firmness of the curd form pressurized milk than that of raw or pasteurized milk, evaluated positively. The main effects of high pressure treatment in milk appeared to involve dissociation of casein micelles from the colloidal to the soluble phase. This study suggests that high pressure treatments of milk at 500 MPa or 550 MPa for 15 min may be beneficial for improving the coagulation properties of milk. These positive effects indicated that high pressure processing may have potential for new cheese varieties development.
Key words: high pressure, enzymatic coagulation, compression method.

Introduction

Cheese manufacture is one of the classical examples of food preservation, dating from 6 000 - 7 000 BC. There are more than 1 000 varieties of cheese produced throughout the world, created by differences in milk source, fermentation and ripening conditions as well as pressing, size and shape (Young & George, 2013). Cheese processing is a complex process involving many steps. The rennet coagulation of milk is one of the first and most important steps in a cheese making process.

The milk coagulation properties are of a great importance as they influence cheese yield and quality. The suitability of milk for cheese making is evaluated by measuring the time required for curd-firming, rennet coagulation time (RCT), the firmness, permeability, contractility, and syneresis of curd. In addition, it is very essential to obtain the maximum achievable recovery of substance from milk because the higher the recovered percentage of solids, the greater the amount of cheese obtained and therefore gain in economic terms (Chopde, Deshmukh, & Kalyankar, 2014). The yield of cheese is influenced by different factors: the composition of the milk, the species and breed of animal, milk quality and the stage of lactation. Furthermore, pre-treatment (microfiltration, homogenisation, milk maturation *et al*.) of milk can also increase the outcome of cheese (Huppertz *et al*., 2005).

The majority of cheeses are made from heat-treated or pasteurised milk (either whole, low-fat or non-fat). Thermal processing is often used to insure safety and quality of cheese milk. However, heat treatment induces many unfavourable changes in sensory properties of cheese, including modifications in texture (Grappin & Beuvier, 1997; Chawla, Patil, & Singh, 2011). High pasteurization temperature (78 - 95 °C) causes impaired clotting properties and a weaker curd; it appreciably reduces the curd firmness and increases RCT of milk (Huppertz *et al*., 2005). For this reason heat-treated milk is less suitable for cheese manufacturing. That is why during the last decades, food research has focused on the development of non-thermal technologies including high pressure processing.

The high pressure technology has emerged as an alternative to traditional thermal processing methods for foods including milk and dairy products (Muñoz-Cuevas *et al*., 2013). High hydrostatic pressure processing of milk at room temperature causes several protein (whey proteins and caseins) modifications, such as reduction in the size of casein micelles and denaturation of β-lactoglobulin and α-lactalbumin (O'Reilly *et al*., 2001). In a model of the casein micelle, colloidal calcium phosphate crosslinks the sub-micelles caseins and neutralises negatively charged phosphoserine groups, allowing the formation of hydrophobic interactions between caseins (Horne, 1998). Under pressure, hydrophobic and electrostatic interactions between proteins are disrupted and colloidal calcium phosphate is solubilised (Lopez-Fandiño & Olano, 1998). As a result, considerable changes in the size, structure and composition of the casein micelles occur (Huppertz, Fox, & Kelly, 2004a). These changes are leading to modification in the technological parameters of cheese milk (rennet

coagulation time, curd firmness) and improving milk coagulation properties and cheese yield (Huppertz *et al*., 2005; Pandey, Ramaswamy, & St-Gelais, 2003; Trujillo *et al*., 2002).

Whole milk is commonly used for cheese making and microbiological quality of raw material is a significant factor affecting cheese quality. Our previous studies about milk microbiological quality conducted that optimal regime for milk processing is at least 400 MPa for 15 min (Liepa *et al*., (2016) in press). Data about whole milk structural changes and coagulation properties in scientific literature is limited and poor. Therefore, the study of changes in coagulation properties induced by high pressure processing in whole milk is important. However, most research works in this area are focused on skim milk or heated milk. So the evaluation of influence of high pressure processing on coagulation ability of whole raw milk is necessary. The primary objective of the present study was to examine the effects of high pressure treatment of cow`s milk at a wide range of pressures (400 - 600 MPa) on milk rennet coagulation time, curd firmness and curd yield.

Materials and Methods

The study object

Bulk milk samples were collected from the morning milking according to the standard LVS 175:1999 'Sampling of raw milk procedure'. After collection, milk samples were transported to the laboratory of the Faculty of Food Technology, Latvia University of Agriculture (Liepa *et al*., (2017) in press).

High pressure and heat treatment of milk

Milk was filled in polyethylene terephthalate (PET) plastic bottles (NF2 - ∅28 mm, 120 ± 10 mL), avoiding any head space, and vacuum sealed in polyethylene pouches (70 × 200 mm sized, with 65 μm thickness) using a chamber type vacuum packaging machine Multivac C350 (MULTIVAC Sepp Haggenmüller SE & Co. KG, Germany) before being pressurized. Milk samples were pressure treated in a pressure chamber of 10 cm diameter and 23 cm length of the Iso-Lab High Pressure Pilot Food Processor S-FL-100-250-09-W (Stansted fluid power LTD, UK). Milk underwent high pressure treatment, regimes indicated in Table 1. The pressurization was completed at room temperature. Product temperature increased during pressurization up to 30 °C and dropped during pressure release to about 17 °C (Liepa *et al*., (2017) in press).

High pressure processing was compared to the high-temperature short-time (HTST) pasteurization at 78 °C for 15 - 20 s.

Totally, 18 milk samples (6 samples in triplicate) were analyzed (Table 1).

Analysis of milk coagulation parameters

The pH of milk was determined using pH meter ino-LAB pH 720 (Germany) at the temperature 20 °C before the rennet coagulation analysis in raw, pasteurized and HP treated milk samples.

Prior the assessment of milk coagulation properties, milk samples were heated to the renneting temperature 35 °C. Rennet coagulation time (RCT) is a measurement of the time elapsed between the addition of a known amount of rennet to a known volume of milk at a given temperature and the onset of clotting, assessed visually (Ciprovica, Zagorska, & Petrovska, 2016). RCT in minutes was analysed using 2 : 10 000 (v/v) of rennet (CHY-MAX 1000 IMCU mL^{-1}, Chr. Hansen, Denmark) solution into water and measuring the time until flocculation of milk was started at 35 °C. For interpretation of results, all samples were divided into four groups (fast, average, slow and non-coagulating milks) based on the time devoted for the clotting of samples. The assessment of clotting was following: fast means flocculation formation less than 10 min, average - during 10 - 15 min, slow -more than 15 min (Ciprovica, Zagorska, & Petrovska, 2016).

The same enzyme used in the analyses of curd firmness was diluted in to water 2 : 100 (v/v). The added amount of rennet, process temperature and time for curd firmness measurement were adapted from methodology using Formagraph (Foss, Denmark) unit. The curd firmness was determined after 30 min of milk renneting using Texture Analyser TA.HD

Table 1

Milk samples description

Sample abbreviation	Treatment applied to milk
Control	raw milk
PM	heat-treated at 78 °C for 15 - 20 s
HPM 400	high pressure treated at 400 MPa for 15 min
HPM 500	high pressure treated to 500 MPa for 15 min
HPM 550	high pressure treated to 550 MPa for 15 min
HPM 600	high pressure treated to 600 MPa for 15 min

Table 2

Effect of treatment on whole milk pH and coagulation properties

Sample	pH	Rennet coagulation time, min	Curd yield, g 100g-1	Curd firmness, N
Control	6.78 ± 0.02^{b}	9.21 ± 0.05^{e}	22.40 ± 0.78^{c}	1.72 ± 0.42^{b}
PM	6.80 ± 0.01^{ab}	15.21 ± 0.11^{a}	23.57 ± 0.47^{bc}	0.85 ± 0.08^{c}
HPM 400	6.80 ± 0.02^{ab}	9.27 ± 0.04^{e}	24.17 ± 0.35^{bc}	2.79 ± 0.32^{a}
HPM 500	6.81 ± 0.02^{a}	10.01 ± 0.37^{d}	24.73 ± 0.32^{b}	2.82 ± 0.18^{a}
HPM 550	6.81 ± 0.01^{a}	10.46 ± 0.10^{c}	25.60 ± 0.50^{b}	2.86 ± 0.42^{a}
HPM 600	6.82 ± 0.01^{a}	11.15 ± 0.04^{b}	33.07 ± 2.48^{a}	2.40 ± 0.03^{a}

Duncan's test: different letters (a-e) in the same row indicate significant differences among treatments ($p < 0.05$)

plus (Stable Micro Systems LTD, UK). Compression method for determination of curd firmness (disc A/BE - d45, test speed 1.0 mm s^{-1}, distance in the depth of curd sample 8 mm) was used (Ciprovica, Zagorska, & Petrovska, 2016).

Statistical analysis

Experiments were conducted triplicate and the means of the three data are presented. Duncan's multiple range test was used to determine the significance among mean values of untreated and treated samples. The obtained data were processed using Microsoft Excel (Microsoft Office Enterprise 2007, License: Shareware ***N/A***); differences among results were considered significant if p-value < 0.05. For the interpretation of the results, it was assumed that $\alpha = 0.05$ with 95% confidence.

Results and Discussion

Our previous studies showed the high pressure effect on milk microbiological quality (decrease of colony forming units) (Liepa *et al*., (2016) in press) and sensory properties (Liepa *et al*., (2017) in press), but for better understanding how pressure can influence milk properties and for which cheese variety this method of treatment would be the most appropriate, the milk coagulation properties of whole milk were evaluated during this step of research.

Effect of HP treatment on pH

pH values of analysed milk samples are shown in Table 2. This parameter ranged between 6.78 ± 0.02 and 6.82 ± 0.01. High pressure treatment due to casein micelle disaggregation alters the distribution of minerals and raises the concentration of ionic calcium in milk. As a result of an increase in the concentration of phosphate in the milk serum, an increase in milk pH occurs (Chopde *et al*., 2014; Zobrist *et al*., 2005). In the present study, high pressure increased the pH value, which was lower in raw milk. pH of pasteurized milk was similar to that of HPM 400 sample - 6.80. Heat treatment and pressurization at 400 MPa had little influence on pH of whole milk; analysed parameter values of control sample, PM and HPM 400 samples were not significantly different ($p > 0.05$). The differences in the pH also were not significant ($p > 0.05$) among all high pressure treated milk samples; however, high pressure treatment at pressure 500 MPa affected pH of milk considerably ($p < 0.05$), compared with a control sample. Other authors (Huppertz *et al*., 2005; Zobrist *et al*., 2005) also found an increase in pH that depended on treatment pressure and time.

Effect of HP treatment on rennet coagulation time of milk

Rennet coagulation time (RCT) is an index of the gelation potential of milk. In the present study, a control sample had the lowest RCT - 9.21 ± 0.05 min, whereas pasteurized milk had the highest RCT (15.21 ± 0.11 min) (Table 2). Thus, heat treatment of milk increased the RCT by 65% (Figure 1). Pasteurization induces the association of denatured whey proteins with the casein micelles, what result in deterioration of milk coagulation properties (Anema, 2008; Haq *et al*., 2014; Huppertz *et al*., 2005).

According to the recommendations for cheese processors, the best rennet coagulation time is from 10 to 15 min (Горбатова & Гунькова, 2010); HPM 500, 550, and 600 samples belong to this group. At the same time raw and pasteurized milk belongs to the groups, which are not so suitable for cheese processing – too fast or too slow, respectively.

A number of studies (Huppertz *et al*., 2005; López-Fandiño, 2006; Trujillo *et al*., 2002; Zobrist *et al*., 2005) have indicated that the magnitude and nature of the effect of high pressure treatment on RCT of milk depends on the applied pressure. In the present study, pressure treated whole milk samples obtained intermediate RCT values. As shown in Figure 1, the RCT of milk after treatment at/or above 400 MPa, is equal to/or longer than that of raw milk. Compared to the control sample, treatment at 400, 500, and 550 MPa had a little effect on RCT (increase by 0.6%,

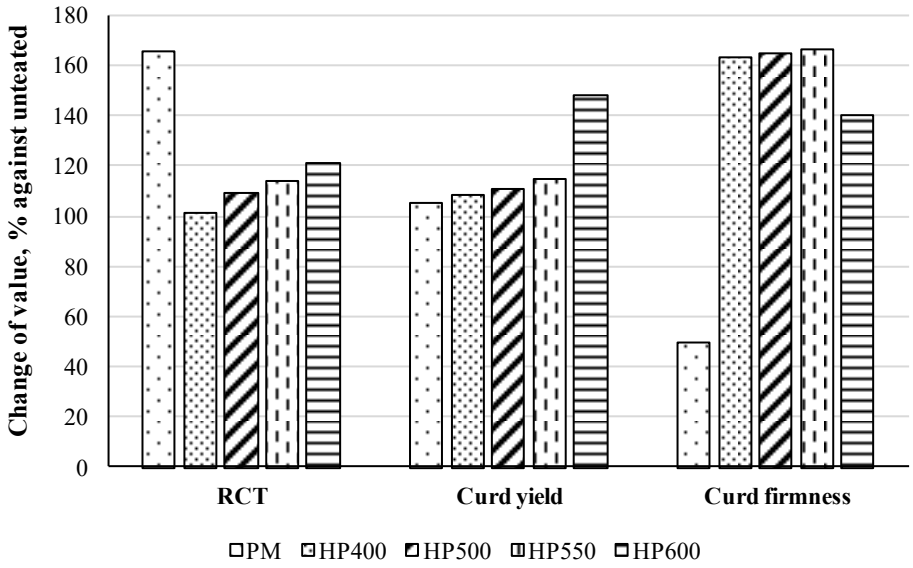

Figure 1. Effect of heat and high pressure treatments on the rennet coagulation time, curd yield and curd firmness of milk samples.

8.7% and 13.5% respectively), but treatment at 600 MPa increased this parameter significantly ($p < 0.05$) by 21.1%. An increase in the rennet coagulation time at higher pressure results from association of denatured whey proteins with the casein micelles (Huppertz & Kelly, 2005). The RCT increase after high pressure treatment was consistent with the previous reports (López-Fandiño, 2006; Lopez-Fandiño & Olano, 1998; Needs *et al*., 2000; Zobrist *et al*., 2005).

Voigt *et al.* (2010) observed reduction by 11.1% and 37.4% of RCT of at first pasteurised (63 °C / 30 min) and then pressurised at 400 and 600 MPa (20 °C / 10 min) whole milk, respectively. High pressure treatment at 400-600 MPa induced increase of RCT of unheated milk is due to the association of whey proteins with the casein micelles (Needs *et al*., 2000; Zobrist *et al*., 2005), while similar pressurization of heated milk reduces RCT greatly. This demonstrates structural differences between casein micelles of unheated, pasteurized and pressurised milk although the level of denaturation is comparable (Huppertz, Fox, & Kelly, 2004a).

Effect of HP treatment on curd firmness

One of the most important factors in manufacturing of cheese is the production of a satisfactory curd. It is required to have a sufficiently firm curd for production of good quality cheese. A heat treatment induced significant decrease ($p < 0.05$) in the firmness of the curd made from raw milk (if pasteurization temperature 78 °C was applied). In the present study, pasteurized milk had the lowest curd firmness - 0.85 ± 0.08 N (Table 2). The effect of heat treatment on the rheological parameters of milk could be attributed mainly to the denaturation of proteins and colloidal salt formation. The interactions of whey protein with casein micelles interfere with the rennet coagulation process, resulting in weak curd structures of heat treated milk (Ustunol, 1983).

Compared to pasteurized milk and control sample, high pressure treatment increased curd firmness significantly ($p < 0.05$). The rennet-induced curd formed from pressurized milk increases with pressure 400 - 550 MPa, followed by a decrease at 600 MPa (Figure 1). Notwithstanding that curd yield of HPM 600 sample was the highest, the curd firmness of this sample was the lowest (2.40 ± 0.03 N) among the high pressure treated milk samples; meanwhile HP 550 MPa sample had the highest curd firmness - 2.86 ± 0.42 N. Also, Needs et al. (2000) reported curd firmness values of pressure treated milk (at 200 - 600 MPa) was higher than values of untreated milk. The curd firmness of unheated pressurized at 600 MPa milk was lower, compared to at 400 MPa treated milk. Zobrist *et al.* (2005) suggested that this may be due to the association of denatured α-lactalbumin with casein micelles that result in obstructing of gelation process.

Effect of HP treatment on curd yield

The effect of treatment on curd yield of whole cow`s milk is shown in Table 1. The yield of curd from heat treated milk and milk treated at 400 MPa differed only slightly from that of untreated milk; however, treatment at 500, 550 or 600 MPa significantly ($p < 0.05$) increased curd yield by 10, 14 or 47%, respectively (Figure 1). High pressure induced increase of curd yield is probably due to the denaturation of whey proteins and increasing of

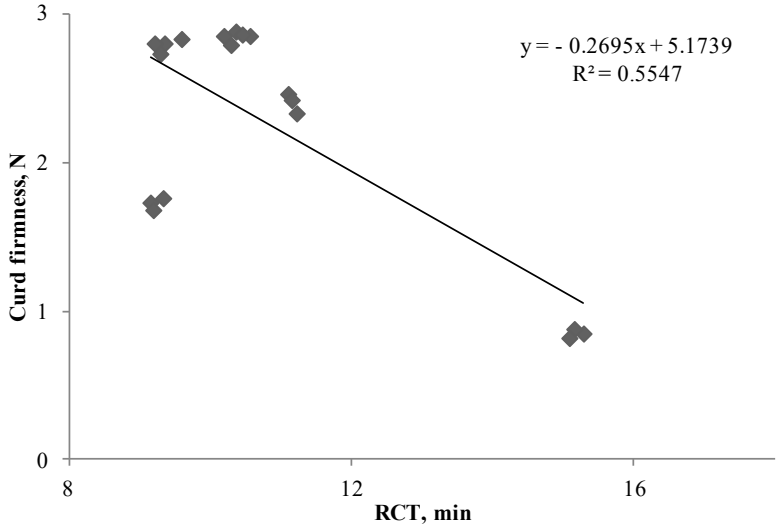

Figure 2. Correlation between RCT and curd firmness in analyzed milk samples.

moisture retention (Pandey *et al*., 2003) that can be more suitable for soft and fresh cheese production. Our previous study (Liepa *et al*., (2017) in press) found significant difference ($p < 0.05$) between milk colour before and after high pressure processing. After high pressure treatment product became more yellowish, this fact can partly prove disintegration of casein micelles during pressurizing. More detailed explanation can be given by assessing structure of milk protein after high pressure treatment, its content in whey and water content in curd; therefore, further research should be done. An increase in curd yield after treatment at a pressure ≥ 400 MPa was in agreement with previous reports (Huppertz, Fox, & Kelly, 2004b; Lopez-Fandiño & Olano, 1998).

The differences in curd firmness could be related to rennet coagulation time of milk samples. The correlation between RCT and firmness values is presented in Figure 2. In the present study, strong negative correlation ($r = 0.75$) between RCT and curd firmness was determined.

HPM 500 and HPM 550 samples belong to group, which is suitable for cheese processing (through its RCT values), and simultaneously having the highest curd firmness among all milk samples. At the same time, control sample and heat treated milk have lower suitability for cheese processing due to its undesirable RCT values and the lowest curd firmness.

Comparing coagulation properties (rennet coagulation time, curd yield, and firmness) of milk during research best results among all analysed samples were obtained from milk samples treated at 500 and 550 MPa during 15 min. Further research (cheese making process, cheese quality, maturation, economical aspects) should be done to make significant conclusions about high pressure suitability for cheese production.

Conclusions

High pressure processing insignificantly influences coagulation properties of raw milk, however, the magnitude of changes depended on treatment pressure and time.

The higher firmness of the curd from pressurized milk than that of raw or pasteurized (at 78 °C for 15 – 20 s) milk was evaluated positively. After high pressure processing the curd yield was increased by for 7 to 47 % due to a combination of incorporation of denatured whey proteins in the curd.

Average RCT values were observed in all pressure treated raw milk samples.

Results from current study suggest that treatment of milk at 500 MPa or 550 MPa for 15 min could be optimal for new cheese varieties development.

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A PRELIMINARY STUDY ON ESSENTIAL MINERALS IN HUMAN MILK: ASSOCIATION WITH DIETARY HABITS

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Abstract
Human milk provides infant with the required nutrients for growth and development. The aim of preliminary study was to determine macro- and micronutrients content in mature human milk among lactating women residing in Latvia and dietary habits affecting it. The study was carried out from November 2016 to February 2017. In total, 24 pooled diurnal milk samples were collected from mothers whose infants had reached the age of at least two months. Personal information of each participant was recorded, including mother's age, weight and height parameters, sex and age of an infant, parity, breastfeeding pattern, milk expression method used for sampling. Eating habits were obtained using Monthly Food Frequency Questionnaire. Analysed elements were determined using inductively coupled plasma mass spectrometry (ICP-MS Agilent 7700x, Japan). Concentrations ranged from 227.52 to 398.34 mg L^{-1} for calcium, 58.56 – 256.38 mg L^{-1} for sodium, 445.33 – 736.71 mg L^{-1} for potassium, 25.73 – 49.52 mg L^{-1} for magnesium, till 3.17 mg L^{-1} for iron, 0.28 – 2.77 mg L^{-1} for zinc, 0.06 – 0.43 mg L^{-1} for copper, 2.00 – 44.00 µg L^{-1} for manganese, 1.00 –10.00 µg L^{-1} for chrome which is comparable to data obtained from other studies although chrome and manganese concentration was even higher than observed in literature. Cobalt and selenium content was below detection limit. Zinc content in human milk negatively correlated with baby's age ($p < 0.05$). The content of majority of essential minerals in human milk was affected by mother's dietary habits; however, more samples need to be analysed for representative conclusions.
Key words: human milk, essential minerals, dietary habits.

Introduction

Balanced nutrition supports child's optimal development. Human milk has been recognized as the best food for an infant till six months of age and beyond (WHO, 2009; Motee & Jeewon, 2014). Breastfeeding mother eating adequate diet should provide offspring with all essential elements (Emmett & Rogers, 1997). Calcium is a mineral needed for the formation and maintenance of bone mass, as well as for normal function of nerves and muscles. Sodium and potassium support osmotic pressure in cells. Iron is an important element for synthesis of haemoglobin. Chrome potentiates insulin production. Magnesium, copper, selenium, zinc, and manganese are essential co-factors for certain enzymes, therefore needed for numerous biochemical reactions (WHO & IAEA, 1989; Soetan, Olaiya, & Oyewole, 2010). Mammary glands regulate concentrations of essential elements in milk to protect infant against deficiencies and excessive amounts (Lönnerdal, 2007, cited by Björklund *et al*., 2012). A study published jointly by World Health Organization and the International Atomic Energy Agency (WHO & IAEA, 1989) is still one of the few large-scale studies, used as a reference values for setting adequate daily intakes of minerals for infants. It seems that maternal diet has an effect on mineral composition and content in human milk, however, it depends on which element is being considered (Emmett & Rogers, 1997; Qian *et al*., 2010; Choi *et al*., 2016). There is no data about essential element composition in milk among breastfeeding women living in Latvia; therefore, the aim of preliminary study was to determine macro- and micronutrient content in mature human milk among lactating women residing in Latvia and dietary habits affecting it.

Materials and Methods

Ethical concerns

The study has obtained approval from the Riga Stradins University Ethic Committee (No. 4/28.7.2016.). Written agreement has been obtained from all the participating women.

Study design

The study took place from November 2016 to March 2017. Participant group included women from different municipalities of Latvia and babies had to be two months old prior to study. Altogether, 24 pooled diurnal milk samples were obtained. Descriptive characteristics like woman's age, weight and height ratio, sex and age of a baby, parity, breastfeeding manner (exclusive or mixed), milk expression method used during the study (by hand, breast pump or both) were recorded. Monthly Food Frequency Questionnaire (FFQ) – a transformed blank taken from guidelines developed by World Health Organisation (WHO, 2007) was used to assess mother's dietary habits during lactation. Questionnaire included information about the consumption of cereals, meat and their products, fish and shellfish, vegetables,

fruit, berries, nuts, milk and dairy products, etc. food categories over the past month.

Following five-point scale was used:

- 0 =‘I never consume this product’;
- 1 =‘I eat it less than once a week’;
- 2 =‘I eat it once a week’;
- 3 =‘two times a week’;
- 4 =‘more than two times a week but not every day’;
- 5 =‘I eat it every day’.

Information about current use of dietary supplements was also collected. Information about participants’ financial conditions was not collected although this factor may have an impact on mother’s diet during lactation.

Milk sampling and analysis

Around 100 mL of pooled diurnal milk was obtained using the most comfortable manner for the mother (hand expression, using breast pump or combining both methods). Participants were asked to express milk from different diurnal periods (including morning, mid-day and evening feeding), however, expression time was not specified (beginning (foremilk) or end of feeding (hindmilk). Information about nursing frequency was not collected. It was also not specified from which breast mothers should express milk (the breast from which baby was suckling or the opposite breast). Samples were stored at -20 °C until analysis. Following essential minerals – calcium, chrome, cobalt, copper, iron, magnesium, manganese, potassium, selenium, sodium and zinc – were determined using inductively coupled plasma mass spectrometry (ICP-MS Agilent 7700x, Japan).

Statistical analysis

Analyses were done in duplicate. Data statistical analysis was performed using software IBM SPSS Statistics, version 23.0. The Shapiro-Wilk test was used to evaluate essential element content distribution for normality but due to small sample size non-parametric tests were further used for the analysis of data. Kruskal Wallis test was used to test categorical variables and continues variables were compared using Spearman’s rank correlation coefficient (Spearman’s r). Spearman’s r was also used to analyse how mother’s dietary habits affect mineral content in milk ($\alpha = 0.05$).

Results and Discussion

The women included in the present study on average were 30 ± 4 years old. Maternal Body Mass Index was 22.11 ± 2.75. It was calculated based on given information about weight and height. Anthropometric measurements were not made during this study. Primiparas were 37% of participants. Of the babies, 54% were female but 46% – male and the average birth weight for infants was 3.54 ± 0.56 kg. The average age was 4 ± 2 months (Table 1).

Literature is inconsistent with data for many, especially ultra-trace elements (like cobalt) content in human milk. In addition, differences in sampling and analytical testing should be taken into consideration. Table 2 represents the summarization of our preliminary study results with data from literature. Despite the small sample size, our obtained results were comparable with data given in WHO & IAEA (1989) study for most elements. Concentrations of iron, manganese, chrome and zinc in milk were normally distributed. Small inter-individual variations (CV≤17) were detected for calcium, potassium, magnesium, complying with data from WHO & IAEA (1989) survey and indicating that homeostatic mechanisms are involved regarding content of those elements in human milk. Cobalt and selenium content was below detection limit. Comparing to other studies, we obtained higher results for manganese and chrome.

Plenty of elements significantly correlated with each other. Most frequently, sodium positively correlated with calcium (Spearman $r = 0.42$, $p < 0.05$),

Table 1

Descriptive characteristics of participants

Characteristics	Mean ± SD	Range
Maternal characteristics		
Age (years)	30 ± 4	23 – 37
Body Mass Index (kg m^{-2})	22.11 ± 2.75	18.25 – 28.55
Parous	37% primiparas	
Infant characteristics		
Age (months)	4 ± 2	2 – 11
Birth weight (kg)	3.54 ± 0.56	2.55 – 4.70
Sex	54% female, 46% male	

Table 2

Essential element concentration in human milk as referred in the literature and from current study

	Current study	WHO & IAEA, 1989	Yamawaki *et al*., 2005	Shi *et al*., 2011	Björklund *et al*., 2012	Andrade *et al*., 2014
mg L^{-1}						
K	445.33 – 736.71	410.00 – 550.00	437.33 ± 7.57	540.00 ± 146.00	633.00 ± 40.00	n.i.[a]
Ca	227.52 – 398.34	220.00 – 300.00	249.00 ± 16.52	334.00 ± 70.00	305.00 ± 45.00	142.30 ± 21.60
Na	58.56 – 256.38	90.00 – 130.00	120.67 ± 16.50	n.i.	217.00 ± 77	n.i.
Mg	25.73 – 49.52	29.00 – 38.00	28.33 ± 4.16	37.00 ± 10.00	28.00 ± 4.80	39.80 ± 4.20
Fe	BDL – 3.17	0.35 – 0.72	0.11 ± 0.07	0.50 ± 0.20	0.34 ± 0.13	2.70 ± 0.40
Zn	0.28 – 2.77	0.70 – 2.00	0.10 ± 0.06	2.00 ± 1.00	3.47 ± 0.98	3.60 ± 0.20
Cu	0.06 – 0.43	0.18 – 0.31	0.03 ± 0.01	0.04 ± 0.02	0.47 ± 0.08	0.40 ± 0.02
µg L^{-1}						
Mn	2.00 – 44.00	3.00 – 4.00	0.97 ± 0.21	n.i.	3.00 ± 1.40	n.i.
Cr	1.00 – 10.00	0.80 – 1.50	5.03 ± 2.55	n.i.	0.30 ± 0.27	n.i.
Se	BDL	13.00 – 24.00	1.53 ± 0.25	1.50 ± 0.60	13.00 ± 2.6	n.i.
Co	BDL[b]	0.15 – 0.35	n.i.	n.i.	0.0059 ± 0.050	n.i.

[a] No information
[b] Below detection limit

iron ($r = 0.56$, $p < 0.01$) and potassium ($r = 0.56$, $p < 0.01$) content. This is opposite to Björklund *et al.* (2012) research where a negative correlation between sodium and potassium content in milk was observed ($r = -0.39$, $p < 0.01$). Carrying on with our study, also manganese correlated with three other elements – potassium ($r = -0.42$, $p < 0.05$), magnesium ($r = -0.41$, $p < 0.05$) and iron ($r = 0.48$, $p < 0.05$). Magnesium content positively correlated with potassium ($r = 0.45$, $p < 0.05$) but chrome negatively with zinc ($r = -0.45$, $p < 0.05$) content.

Cobalt and selenium content in analysed samples were below detection limit. It is difficult to evaluate what influences cobalt content in human milk because only few studies have data about this trace mineral (WHO & IAEA, 1989; Björklund *et al*., 2012). Several studies indicate that selenium content in human milk is affected by mother's diet (Emmett & Rogers, 1997; Zachara & Pilecki, 2000). It should be emphasized that critically low selenium content in milk among lactating women residing in Latvia could be due to the fact that Latvia belongs to countries with a low selenium level in the soil and hence the food supply (Duma *et al*., 2011) but further investigations are needed.

Maternal characteristics

Arnaud and Favier (1995, cited by Choi *et al*., 2016) reported that the concentration of copper in human milk is related to parity ($r = 0.317$, $p < 0.001$) and mother's Body Mass Index (BMI) ($r = 0.324$, $p < 0.001$). Our preliminary results did not show any correlation between essential elements' content in milk and maternal BMI or parity ($p > 0.05$). However, more samples need to be analysed and potentially anthropometric measurements need to be included in the research.

Infant characteristics

There are three stages of lactation – colostrum, transitional and mature milk. Yamawaki *et al.* (2005) observed decrease for sodium and potassium content in milk comparing day 1 – 5 (colostrum) to day 165 – 181 of lactation (mature milk). Richards with co-authors (2010) observed that sodium content decreases during lactation period, reaching reduction of 33% over the first six months *post partum*. Similarly, Shi and co-authors (2011) observed that most minerals (except for sodium and potassium) in human milk remain fairly constant over the three lactation stages. In our preliminary study, babies had to be at least two months old, therefore only mature milk samples were analysed. Overall, infants were two to eleven months old and we could not demonstrate a correlation between mineral content in human milk and offspring's age, except for zinc ($r = -0.528$, $p < 0.01$). However, more samples need to be analysed to evaluate observed association.

There is evidence that infant's gender and birthweight can affect macronutrient like fat and lactose content in human milk (Altufaily, 2009; Broka *et al*., 2016) but no similar observations in any research had been made regarding essential elements content. In addition, our data marked a tendency

Table 3

Food consumption frequency points among participants

Foodstuff	Median	Minimum	Maximum
Cereals & cereal products	1.0	0	5
Bread	3.0	0	5
Eggs	3.0	0	5
Meat & meat products	2.0	0	4
Fish & shellfish	1.0	0	4
Milk	5.0	0	5
Dairy products	2.0	0	5
Fresh vegetables	3.0	0	5
Cooked vegetables	2.0	0	5
Legumes	1.0	0	3
Soups	2.0	0	4
Fresh fruits & berries	3.0	0	5
Dried fruit & berries	1.0	0	5
Nuts	1.5	0	5
Seeds	1.0	0	5
Butter	3.0	0	5
Vegetable oils (*Helianthus annuus*, *Olea europaea* oil, etc.)	4.0	1	5
Sauces & condiments	1.0	0	5
Sweets & sugary snacks	1.0	0	5
Fast food (pizza, hot dogs, etc.)	1.0	0	2
Salty snacks (chips, salted nuts)	1.0	0	2
Vegetable or fruit juices	0.0	0	5
Sugary drinks	0.0	0	1
Coffee	5.0	0	5
Other caffeine containing drinks (cappuccino, latte, hot chocolate)	2.0	0	5
Caffeine containing tea (green, black, etc.)	2.5	0	5
Herbal tea (Mint (*Mentha piperita*), Chamomile (*Matricaria recutita*), etc.)	5.0	0	5
Alcohol	0.0	0	3

that infant's birth weight or sex has no influence on mineral content in mature human milk ($p > 0.05$). However, more participants need to be acquired for representative conclusions.

Diet

Clustered foodstuff consumption frequencies are displayed in Table 3.

Most mothers consumed one or few dietary supplements during the participation in the study – vitamin D (n = 5), iron supplement (n = 4), omega fatty acids (n = 3) and complex supplements (n = 4) or functional products like pollen (n = 1), mineral water (n = 2), spirulina (n = 1) or molasses (n = 1) which could also be a source of minerals. Although Choi *et al.* (2016) observed that mothers who took daily supplements had higher iron concentration in milk, no similarity was observed in this study ($p > 0.05$).

Iron and zinc concentration in human milk is comparatively low, but their absorption is high (WHO, 2009). Although meat and offals are better dietary source of iron, dark green leafy vegetables and molasses also contribute to intake of this microelement (Soetan, Olaiya, & Oyewole, 2010). Our data from Food Frequency Questionnaire only unveiled a negative connection with cooked vegetable and soup consumption ($r = -0.49$ and $r = -0.48$, $p = 0.02$ for both, respectively) and iron content in human milk.

Most participating mothers excluded alcoholic beverages from the diet during breastfeeding (67%) but the rest – restricted consumption to less than once a week (n = 6), or 1 to 2 times per week (n = 2). Mentioned participants preferred red or white wine. Unit was

one glass, drunk in the evening, accounting the time between alcohol consumption and breastfeeding. We revealed that zinc content negatively correlates with alcoholic beverage consumption ($r = -0.42$, $p = 0.04$). Choi with co-authors (2016) observed that mothers who consumed alcohol during pregnancy tended to have lower concentration of zinc in milk, as well as less copper and iron content; however, no information about drinking habits during lactation was recorded in mentioned study (Choi *et al*., 2016). It should be noted that unlike pregnancy, when alcohol is directly passed to fetus, a lactating woman who drinks occasionally can limit exposure to offspring by considering time interval between drinking and breastfeeding (Mennella, 2001).

Our results spotted a correlation between zinc content in milk and dried fruit consumption ($r = 0.42$, $p < 0.05$). Similarly, Leotsinidis with co-authors (2005) observed that fruit consumption was positively associated with zinc level in human milk. In addition, Choi *et al.* (2016) revealed that intake of meat and meat products positively influenced zinc content.

Sodium intake in infancy may have an effect on blood pressure in later life (Geleijnse *et al*., 1997). Our study revealed a negative correlation between fresh and dried fruit as well as different seed consumption ($r = -0.42$, -0.47 and -0.59, respectively; $p < 0.05$). This maybe could be explained by the fact that fruit, especially dried fruit, are a rich source of potassium. Human milk has to be isomolar with plasma, but potassium & sodium has an antagonistic function, contributing to osmolar load (Richards *et al*., 2010).

According to our preliminary data, potassium content in milk only correlated with seed (linseed (*Linum usitatissimum*), sesame seeds (*Sesamum indicum*), etc. seeds) consumption ($r = -0.41$, $p < 0.05$).

Research done by Dagnelie and co-authors (1992) revealed that magnesium content in human milk was positively associated with meat and fish consumption. However, our study marked a tendency that magnesium concentration in mature human milk correlates with plant based product consumption. We observed a correlation between magnesium and wholemeal pasta ($r = 0.55$, $p = 0.01$) and dried berries ($r = 0.43$, $p = 0.03$) consumption. Magnesium concentration also negatively correlated with caffeine containing drink like cappuccino, latte and hot chocolate ($r = -0.49$, $p = 0.02$).

Our results marked a tendency that chrome content in mature human milk correlates with shellfish ($r = -0.45$, $p = 0.03$), sour cream ($r = 0.54$, $p = 0.01$) and vegetable oils (*Helianthus annuus*, *Olea europaea* oil, etc. vegetable oils) ($r = 0.47$, $p = 0.02$) consumption. It is difficult to explain these preliminary observations, and thus more participants need to be acquired.

Manganese content in human milk correlated with several food categories, including animal and plant based products but more data are needed to analyse acquired interconnectedness.

According to our preliminary results, calcium and copper content was not influenced by mother's diet ($p>0.05$). Dagnelie with co-authors (1992) observed that human milk from macrobiotic mothers contained less calcium. Organically grown cereals, vegetables and pulses are the dominating products in macrobiotic diet. However, also no effect of consumption of animal products on calcium was observed (Dagnelie *et al*., 1992). Choi with co-authors (2016) observed that daily intake of vitamin C, selenium and iodine were related to copper content in milk. Leotsinidis with co-authors (2005) noticed that fruit consumption was positively associated with copper level in human milk which could be related to the fact that fruit are a good source of vitamins (including, vitamin C).

Nevertheless, further conclusions should be drawn when more milk samples will be analysed.

Breastfeeding pattern

After six months of age, an infant's nutritional needs start to exceed what is ensured by human milk; therefore, a complementary feeding is started (WHO, 2009). However, breastfeeding still provides many benefits to the infant and mothers are encouraged to continue nursing till the age of 2 years and beyond (WHO, 2009). Most mothers in our preliminary study were still exclusively breastfeeding ($n = 17$), six participants had started weaning but one mother was combining breastfeeding with the use of infant formula. Results revealed that breastfeeding pattern does not influence essential mineral content in human milk ($p > 0.05$), but more participants need to be acquired for further observations.

Milk expression method

Participants were allowed to use the most convenient method for milk expression – by hand (29%), using breast pump (63%) or both techniques (8%). Milk composition can be affected by the use of breast pump because water evaporates during the process (Morton *et al*., 2009; Miller *et al*., 2013). However, we did not observe that milk expression manner impacts essential element composition in human milk ($p > 0.05$). Participants were asked to express milk from different diurnal periods (including morning, mid-day and evening feeding); however, expression time was not specified (beginning (foremilk) or end of feeding (hindmilk). Information about nursing frequency was not collected. It was also not specified from which breast mothers should express milk (the breast from which baby was suckling or the opposite breast). All the above mentioned incompleteness also could

influence the results we obtained in our preliminary study. Certainly, more samples are needed for further conclusions and continuing the research, sampling process should be more specified.

Conclusions

Preliminary results revealed that content of most elements (calcium, sodium, potassium, magnesium, iron, zinc and copper) in mature milk among respondents is comparable to data found in literature. Higher concentrations than observed from other studies were found for manganese and chrome, but lower – for cobalt and selenium. Our obtained results also marked a tendency that zinc content in mature human milk correlates negatively with baby's age. Mother's eating habits possibly influence majority of essential elements' content in human milk, but more data need to be obtained for representative conclusions. Continuing the research, sampling process should also be more specified.

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EFFECT OF HIGH PRESSURE PROCESSING ON MICROBIAL LOAD IN PORK

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Abstract
Fresh meat is a highly perishable product due to its biological composition as it serves as an ideal environment for the growth and propagation of microorganisms and common food-borne pathogens. High pressure processing (HPP) is a cold pasteurization treatment to extend shelf-life while preserving the sensory and nutritional characteristics of the product. The aim of this research was to evaluate the effects of HPP on the fresh porcine *Musculus longissimus lumborum* microbial load and related physical properties (pH, water activity a_w, and moisture content). Vacuum packed meat samples were treated at 50, 100, 200, 300, 400, and 500 MPa for 1, 5, and 15 min in a high-pressure processor ISO-Lab S-FL-100-250-09-W (Stansted Fluid Power Ltd., UK). Pressure treatment above 300 MPa resulted in a significant ($p < 0.05$) decrease of total plate count. However, the studied pressurizing time had no significant effect on microbial lethality at the same pressure applied. Other important parameters such as water activity, moisture, and pH were determined as they directly affect microorganism growth and resistance to pressure. A slight increase in pork pH was observed with increased pressure. No significant changes in water activity and moisture content were observed as a result of high pressure treatment. For future researches it would be important to evaluate the dynamics of microbial growth during storing as part of cells after pressure treatment are injured and not eliminated immediately; therefore, microbial count may further decrease during cold storage.
Key words: high pressure processing (HPP), pork quality, total plate count.

Introduction

Pork ranks first among all meat sources, and it is considered to be approximately 40% of global meat consumption (Keenan, 2016). The diverse and rich nutrient composition of meat is a good media for the growth and proliferation of both meat spoilage microorganisms and common food-borne pathogens. Regarding this fact, it is essential to use preservation methods to maintain meat safety and quality during its storage (Aymerich, Picouet, & Monfort, 2008). Consumers have growing concerns for food quality and prefer healthy, minimally processed, fresh-like food products with natural flavour and taste with extended shelf-life (Yordanov & Angelova, 2010). Therefore, the alternative non-thermal preservation technology such as high pressure processing (HPP) is considered to match these demands without compromising safety (Aymerich, Picouet, & Monfort, 2008).

HPP has got main interest as it has been demonstrated to be an effective inactivation technique for a variety of pathogenic microorganisms, spoilage microorganisms, yeasts, moulds, as well as quality-deteriorating enzymes, without heat treatment or chemical preservatives (Yordanov & Angelova, 2010; Salvi, Gosavi, & Karwe, 2016) with minimal or no changes of the sensory or nutritional characteristics of the product and potential to extend shelf-life (Sun & Holley, 2010; Zhou, Xu, & Liu, 2010; Jofré & Serra, 2016). The use of high-pressure technology in food processing has gained its popularity recently, and now is gradually being adopted for the products processing and preservation in the food industry (Huang *et al*., 2017). The number and variety of meat and meat products treated with this technology has risen dramatically worldwide especially in last years (Balasubramaniam, Barbosa-Cánovas, & Lelieveld, 2016). As a result of high demand for this technology, the recent progress in equipment design has improved access to high-pressure devices for enterprises with different capacities (Simonin, Duranton, & de Lamballerie, 2012). The basic principles that determine the behaviour of foods during HPP technology are Le Chatelier's principle, principle of microscopic ordering, and isostatic principle (Yordanov & Angelova, 2010). The isostatic transmission of pressure provides the instantaneous treatment to the processed product with no gradient, resulting in the uniform pressure distribution irrespective of the size and geometry of the material; moreover, food can be processed at ambient or even lower temperatures (Simonin, Duranton, & de Lamballerie, 2012).

The effectiveness of HPP for microbial control is affected by such factors as the process parameters, applied pressure, temperature, and dwell time, as well as intrinsic factors of the food itself, such as food composition, pH, and water activity (Zhou, Xu, & Liu, 2010; Patterson, 2014).

Moreover, microorganisms' response and resistance to pressure varies significantly among different genus, species and even strains of the same species, form, morphology of the cell and its growth stage (Hugas, Garriga, & Monfort, 2002; Garriga *et al*., 2004; Patterson, 2005; Jofré *et al*., 2010). The microbial inactivation under high pressure is mainly caused by an alteration in cellular morphology and inhibition of cell division (Tonello, 2011).

Pressure levels necessary to achieve efficient microbial inactivation is not without effects on food. Among food constituents, muscle and muscle proteins are the most responsive to pressure (Sun & Holley,

2010). For meat and meat products elevated pressure treatment induce significant changes in the quality attributes as it has been shown to induce protein denaturation and acceleration of lipid oxidation during subsequent storage. This is due to the relatively high sensitivities to pressure of muscle glycolytic processes and of the associations between myofibrillar proteins (MacFarlane, 1985). Such modifications of meat matrix lead to colour and texture changes and decreased sensory acceptability (Simonin, Duranton, & de Lamballerie, 2012).

The aim of this research was to evaluate the effects of HPP on the fresh porcine *Musculus longissimus lumborum* microbial load and related physical properties (pH, water activity a_w, and moisture content).

Materials and Methods

The research was performed at Latvia University of Agriculture, Faculty of Food Technology in 2016–2017.

Study object

Chilled pork obtained from *Musculus longissimus lumborum* (Latvia) has been purchased from the local store Ltd. 'Maxima Latvia' (unpackaged; stored in chilled condition at temperature 3 ± 1 °C; maximal expiration time 24 h). No breed, age, sex or premortal handling was recorded. Pork samples underwent testing of sensory parameters as the appearance, aroma, colour, and texture fit requirements of fresh meat before experiments.

Preparation of meat samples

The obtained chilled pork meat was cut in 1.0 ± 0.2cm thick slices across the muscle fibre and slices were divided into portions with weight of 200.0 ± 0.2 g each, packed in the vacuum pouches made from polymer film (polyamide/polyethylene film thickness 60 ± 3 μm).

High pressure processing

Samples of pork meat were treated in a high-pressure processor ISO-Lab S-FL-100-250-09-W (Stansted Fluid Power Ltd., UK) with a pressure chamber of 2 L and a maximum operating pressure of 900 MPa. The pressure transmitting medium was a propylene glycol, water mix (1:2 v/v) at room temperature. Vacuum-packed samples were randomly assigned to one of the six treatment pressures (50, 100, 200, 300, 400, and 500 MPa), while untreated sample served as the control. The HPP treatment for vacuum-packed samples at each pressure level was applied for three meat samples for durations of 1, 5, and 15 min respectively.

Microbiological analysis

Sample preparation for microbiological testing was performed in accordance with the standard method LVS EN ISO 6887-2:2004 'Microbiology of Food and Animal Feeding Stuffs – Preparation of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination – Part 2: Specific Rules for the Preparation of Meat and meat products'. Total plate count (TPC) was determined according to the standard LVS EN ISO 4833-1:2014 Microbiology of the Food Chain – Horizontal Method for the Enumeration of Microorganisms – Part 1: Colony Count at 30 Degrees C by the Pour Plate Technique using Nutrient (NA) agar (Ref. No 01–140-500), incubating at 30 °C for 48 h under aerobic conditions. After incubation, the colony forming units (CFU) were enumerated using the automated colony counter aCOLyte (Synbiosis, UK). All bacterial counts were expressed as the logarithmic (log 10) values. Microbiological analyses were completed in triplicate for all HPP treated and control samples.

Determination of pH

Measurement of pH was done with a pH meter Jenway 3520 (Jenway, UK), which was calibrated against pH standard solutions 4.00 and 7.00. Procedure was done according to LVS ISO 2917:2004 method. It was measured for five repeats of each high pressure treated pork meat sample as well as for control sample.

Determination of moisture

Moisture of pork meat samples was determined using a standard method LVS ISO 1442:1997. Triple determinations of moisture content for each sample were carried out.

Determination of water activity

Six grams of minced meat sample was filled in a cup, placed in the LabSwift-aw unit (Novasina, Switzerland), and water activity was determined in triplicate for each sample (measurement range – 0.11 to 0.99 a_w, with an accuracy of ± 0.01 a_w). Temperature during measurement was 22 ± 1 °C.

Statistical analysis

Results are presented as mean ± standard deviation. The experimental data were analysed using Microsoft Excel 2014. Single factor analysis of variance (ANOVA) was used to compare the means. For data analysis, confidence level was 95% ($\alpha = 0.05$). The factors have been evaluated as significant if p-value $< \alpha_{0.05}$.

Results and Discussion

The main purpose of meat products treatment at high pressure is to improve their microbial safety

(Patterson, 2005). The most important challenge encountered in a high pressure pasteurization of food products is that the pressure resistance of microorganisms is reinforced in nutrient-rich media such as meat. The composition of the food matrix has been shown to influence the microbial results of a high pressure treated product despite the fact that the effect of each food constituent on pressure resistance is not well known (Simonin, Duranton, & de Lamballerie, 2012).

The reduction of total plate count in pork meat as a result of the HPP treatment is presented in Figure 1. Individual species of microorganisms were not determined and evaluated in the frame of this research.

The total plate count determined in vacuum packed chilled pork was 2.99 $\log_{10}$ CFU g^{-1} for the control sample. Microorganisms in meat demonstrated resistance up to 300 MPa irrespective of treatment time. After undergoing high pressure treatment above 300 MPa, a significant ($p < 0.05$) decrease of microorganisms was observed. Also other authors reported similar results with microbial inactivation at the range of 400 – 600 MPa with short treatment duration (3 – 7 min) (Del Olmo, Calzada, & Nuñez, 2014). Similar to previous studies, it was confirmed that the TPC inactivation depends on the pressure applied. The TPC after the pressurization at 300 MPa for 1 min was at the same level as in untreated control sample, being approximately 3.14 $\log_{10}$ CFU g^{-1}, irrespective of treatment time, but it was significantly reduced after treatment at 500 MPa for 1 min to 2.17 $\log_{10}$ CFU g^{-1}, 5 min – 1.77 $\log_{10}$ CFU g^{-1}, 15 min – 2.33 $\log_{10}$ CFU g^{-1}.

Nevertheless, results of the total plate count had no significant ($p > 0.05$) difference between samples exposed to pressure for different duration of time. It shows that in the current experiment the lethality of microorganisms was more dependent on pressure applied not on the duration, the sample was exposed to pressure. However, it should be additionally noted that no significant reduction in the total plate count was found at pressures 50–300 MPa for 1–15 min. If pressure was further increased till 300 – 400 MPa, the reduction of viable microorganisms increased with the treatment time. Contrary, in meat treatment at 400 – 500 MPa for more than 5 minutes, the efficacy was lower than that in samples treated for 1 – 5 min. Bajovic, Bolumar, & Heinz (2012) stated that the protein denaturation is one of the key mechanisms for microbial inactivation, and irreversible changes in muscle proteins start at a the same level which is required for the inactivation of microorganisms.

In general terms, applying higher pressures normally results in greater levels of vegetative cells reduction. However, in many cases the inactivation curves do not follow first-order kinetics, and a plot of hold time versus log10 survivors does not give a straight line. Tailing effects are common, with plots showing an initial decrease in numbers, followed by a levelling of the curve, where there is little further inactivation as treatment time increases (Patterson, 2014).

What is not less important, other authors mention that the microbial count is not reduced to the final effect immediately after the pressure treatment but shows a significant decrease during cold storage (Jofré *et al*., 2009; Simonin, Duranton, & de Lamballerie, 2012). Analysing the information in scientific literature and experiments data, it can be concluded that the prior amount of microorganisms (contamination level) play very significant role in the meat, and the stages of microorganism development were important.

The pH values of meat and meat products generally range between 4.6 (raw fermented salami) and 6.4. The pH is an important factor in microbial load reduction, because microorganisms are the most pressure-resistant at a neutral pH and become more sensitive

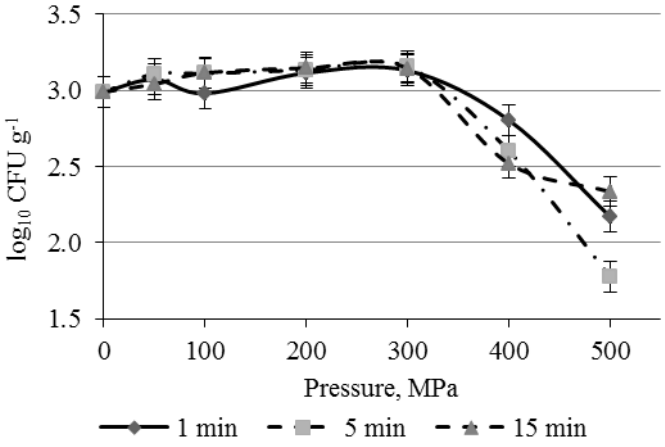

Figure 1. The total plate count (TPC) in pork meat samples depending on pressure and time of treatment.

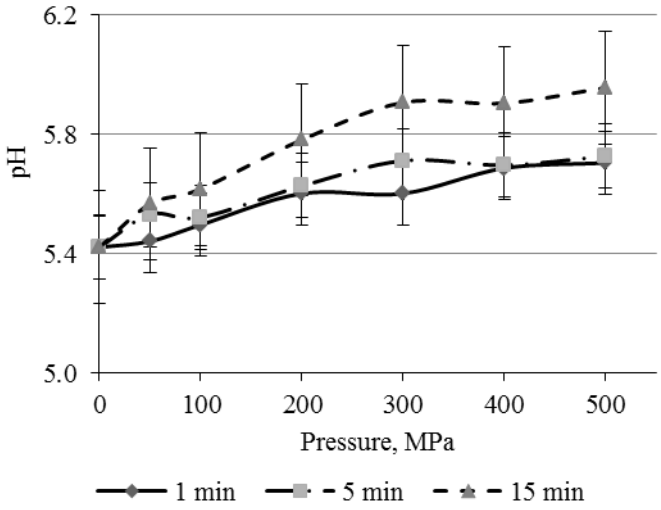

Figure 2. Effect of high pressure processing regime (pressure and time) on pH value of pork meat samples.

as the pH reduces. At a pH value of around 6.4, meat is spoiled owing to enzyme activity, which produces a large amount of metabolic by-products as well as ammonia. Sliminess, bad smell and discolouration can be seen at this point as well. According to Veģe and Bērziņa (1999), pH value of pork immediately after debleeding is in the range of 6.6 – 6.8; however, after 48 − 60 h it drops to 5.4 − 5.6. In the current research it was determined that pH of chilled pork meat immediately after purchase was 5.51 ± 0.06 (Figure 2), while this value slightly decreased for meat after it was vacuum packed and reached 5.42 ± 0.06. Typical pH value for fresh meat did not change after treatment within the pressure interval from 50 – 200 MPa and treatment time 1 – 5 min. Similarly, for 15 min treatment at 50 – 100 MPa the pH value was 5.6 ± 0.04, which corresponds to the value of fresh meat. Pressure treatment may induce reversible pH decrease as a result of the changing dissociation constants of attendant acids and bases (Stippl, Delgado, & Becker, 2004). On the other hand, Ma and Ledward (2013) mentioned that the myofibrillar protein denatures under high pressure and at the same time, pressure increases ionization, sequestering free hydrogen ions and decreasing the acidic groups which corresponds to our results.

Figure 2 shows that an increase of pressure applied led to small but significant increases in pH. Similar results were obtained by other authors who examined pressure treatment on beef meat (Ma & Ledward, 2004), ovine meat (McArdle *et al*., 2013), tuna fish (Ramirez-Suarez & Morrissey, 2006). Earlier researches have proved the slight increase in pH of post-rigor meat (by about 0.5 pH units) immediately after the pressure treatment (Hugas, Garriga, & Monfort, 2002; Sikes, Tornberg, & Tume, 2010).

Comparing the obtained results of meat samples undergoing different exposure time, there was an increase of values; however, the difference was negligible ($p > 0.05$). High pressure application of 500 MPa for 1 min increased pH to 5.70 ± 0.02, after 5 min – 5.73 ± 0.06, and after 15 min of treatment it reached 5.96 ± 0.03. Ros-Polski *et al.* (2015) also reported that the pH values of chicken meat increased with increasing pressure, and there was no significant difference ($p > 0.05$) between the samples treated at 100 and 200 MPa.

The increase of pH in the heat treated samples is attributed to a rearrangement of meat protein structures as the proteins denature and unfold resulting caused by physical and chemical interactions induced by cooking (Hamm & Deatherage, 1960). As the consequence of denaturation, the protein changes its configuration from a highly organized and native structure into a less organized (denatured) and non-native structure. Other researchers hypothesised that although the structures established by high pressure and heating may be different, the mode of unfolding is similar (Ma & Ledward, 2004). According to Messens, Van Camp, and Huyghebaert, (1997) protein unfolding is much less intense in pressurised samples in comparison to cooked samples.

The majority of water in muscle is held within the structure of the muscle itself, either within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells and between muscle bundles (groups of muscle cells) (Huff-Lonergan & Lonergan, 2005). During increased pressure application on meat its molecules get closer to each other leading to the phase transitions which are reversible after depressurization (Hugas,

Garriga, & Monfort, 2002). Chilled pork moisture content before treatment was 79.0 ± 1.0%. Pressure application for different exposure times showed no significant difference between moisture content in the studied meat samples ($p > 0.05$). True reasons of high pressure effect on water binding properties of meat and meat products are still uncertain, and those are also controversial (Hygreeva & Pandey, 2016).

The HPP can lead to the partial or total denaturation of protein structure due to the pressure induced unfolding of the protein structure and subsequent folding after pressure release (Bajovic *et al*., 2012). Increased pressure 50 – 500 MPa can cause protein denaturation, which changes the structure and decreases the number of hydrophilic groups, which in its turn can reduce water binding and holding capacity. Rastogi *et al.* (2007) stated that major changes in the tertiary structure are observed beyond 200 MPa and changes in secondary structure will take place only at a very high pressure above 700 MPa.

The results of the current study are in agreement with the research of Rastogi *et al.* (2007) who observed an increase in moisture content in the range of 100 – 200 MPa, which indicates small changes in tertiary structure of proteins. In the pressure range from 200 – 500 MPa, a decrease in moisture content was observed compared to the untreated sample. The moisture changes have been suggested as the myosin and actin of myofibrillar proteins were the major water-binding components in musculare tissue, and the rate of native myofibrillar protein aggregation and denaturation depends on pressure, temperature and ionic strength conditions (Sun *et al*., 2017).

Water activity is an important parameter affecting inactivation of microorganisms. Fresh meat exhibits an a_w of around 0.98, meaning that around 98% of the total water within meat is unbound whilst, at an a_w of 0.80, significantly less free water is present in a product.

It has been demonstrated that a low a_w decreases the efficiency of high pressure treatments and in products with $a_w \leq 0.92$ cells are protected against pressure (Garriga *et al*., 2004; Jofré *et al*., 2009; Simonin, Duranton, & de Lamballerie, 2012).

Fresh pork used in the current experiment had water activity 0.985 ± 0.005 on average. Water activity in the pressure treated pork samples at different exposure times was not considerably different ($p > 0.05$).

Conclusions

High pressure processing is an effective method for reduction of microbial load in vacuum packed pork if the applied pressure is 400 or 500 MPa. In the present research, the treatment time did not show effect on microbial changes as the exposure time to high pressure showed significant difference. Pressure treatment affects meat pH and moisture content. For future research the changes of quality parameters of HPP treated chilled pork should be studied during its shelf life.

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QUALITY EVALUATION OF POTATO AND VEGETABLE CRISPS IN LATVIAN MARKET

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Abstract
Salty snacks are popular appetizers consumed between meals and are one of favourite components of menu at different social gatherings and private celebrations. Consumers base their choice of snacks not only on flavour and smell of product, but also on different kinds of parameters like colour, texture and nutritional value as well as other information labelled on packaging. Typically salty snacks are associated with potato (*Solanum tuberosum*) crisps, but in present paper there were viewed also vegetable crisps and snacks. The aim of this research was to evaluate nutritional value and physical quality of potato and vegetable crisps and wholegrain snacks in Latvian market. From January to March 2017, 22 potato and vegetable crisps and wholegrain snack samples from Latvian market were analysed. For all samples, information on the labels was analysed as well as salt content, thickness, crispness and colour using standard methods. For 31.8% of the investigated samples, presented information on label and determined salt content do not differ significantly ($p > 0.05$). That means that 68.2% of the analysed potato, vegetable crisps and snacks on the packaging labels have represented incorrect salt content. Positive moderate correlation ($r = 0.489$) between potato and vegetable crisps thickness and crispness was found out. Raw materials and ingredients of samples directly impact colour values. If a sample contains beetroot, the colour results would indicate dark red. All potato crisps colour values point out light yellow colour.
Key words: potato and vegetable crisps, salt content, quality.

Introduction

Potato (*Solanum tuberosum*) crisps are well known as salty snacks, which make an essential part of the snack market not only in Latvian market, but also in many other countries. Potato crisps are thin slices of potato, which have been deep fried or baked. They can be used like snack, side dish or appetizer (Salvador *et al*., 2009). Lately vegetable crisps and other similar snacks like wholegrain, crisp spelt (*Triticum spelta*), corn (*Zea mays*) and potato based snacks have become very popular and have grown into significant competitors. The most important quality indicators for crisps are flavour, smell, colour and also texture or crispness. Colour is seriously assessed by consumers and can be one of their main reasons for selecting or rejecting crisps (Mendoza, Dejmek, & Aguilera, 2007). Colour of crisps is impacted by several causes – potato and vegetable type, natural defect in raw products (black spots), frying process, used oil, seasonings. Amount of absorbed oil also in post-frying process can turn colour of crisps darker and even leave oily spots on their surfaces (Mendoza, Dejmek, & Aguilera, 2007). Therefore, overall assessment of crisp colour includes colour itself, quantity, shape, allocation and intertwining of the small details distributed on crisps (Romani *et al*., 2009).

Crispness and crunchiness are terms frequently used to characterize texture of crisps. These crunchy and crispy attributes are important factors on which consumers set up their evaluation of crisps (Salvador *et al*., 2009). Crispness is not only relevant for crisps but it also makes essential impact on total consumer's appreciation level for every kind of food products. The contribution of texture of product has been studied for more than 50 years and main conclusions are that crispness is a complex group of attributes resulting from both multiple sensations and multiple physical indicators, uniting molecular, structural and production processes, and also storage conditions (Roudaut *et al*., 2002). Texture of crisps is also affected by type of oil used for frying and frying temperature (Kita, Lisińska, & Gołubowska, 2007). Efforts have been made to measure crispness of product and there are developed several techniques, one of the most popular is a puncture test using a cylindrical probe, where fracture force suggests level of crispness (Pedreschi, Segnini, & Dejmek, 2004), studies show that there is strong correlation between magnitude of force drop and the level of crispness (Vincent, 1998), force and hardness measure the power for breaking, the power and these mechanical tests can be used to rate and compare crispness of product (Rojo & Vincent, 2009), but for confirmation of this correlation, it is suggested taking into account also acoustic measurements, sounds transmitted when crisp breaks (Taniwaki & Kohyama, 2012).

Salt content and reduction of salt consumption is popular discussion object for scientific community and overall society because raised salt (sodium chloride) intake can lead to different health problems and diseases. It is proven that even small reduction of salt in dietary, would grant considerable health improvement (Bibbins-Domingo *et al*., 2010). That is why, based on recent studies, the WHO strongly recommends decrease of sodium dietary intake to less than 2 grams per day (less than 5 grams of salt per

day) for adults (WHO, 2012). It is possible to reduce amount of sodium in processed foods by applying different strategies, and in result there are not any losses of salty taste (Mueller, Koehler, & Scherf, 2016). So it is important to remind the public that salt reduction is advisable and necessary for both consumers and manufacturers. A great way to keep track of product's quality indicators is nutrition information on food labels. Although nutrition information is useful, studies show that understanding this it is one thing, but using it to make healthier choices is completely other (Grunert, Wills, & Fernández-Celemín, 2010).

The aim of this research was to evaluate nutritional value and physical quality of potato and vegetable crisps and wholegrain snacks in Latvian market.

Materials and Methods

Samples

In the research from January till March 2017 different potato and vegetable crisps and wholegrain snacks available in Latvian market were analysed. The abbreviations of analysed potato crisps (16 samples), vegetable crisps (2 samples) and snacks (4 samples) shown in Table 1 will be used in the further text. In Table 1, there is also shown information from crisp labels per 100 g – salt, fat, saturated fat content and energy value. All samples were in original packaging and kept at room temperature (21 °C), and each selection of samples for testing was in similar size. Crisps were evaluated immediately after opening the packaging.

Determination of salt content

The salt content of crisps samples was determined using Vardavas *et al*. modified Mohr method (1981) (Vardavas *et al*., 2007). Fine ground sample (2.000 ± 0.010 g) was dissolved in 100 mL of distilled water. One millilitre of 5% K_2CrO_4 solution was added and titration performed with 0.1*M* $AgNO_3$ solution to the first appearance of an orange colour. Calculation of salt

Table 1

The abbreviation and nutrition information of samples used in the research

Sample	Brand	Nutritional information, g 100 g^{-1}			Energy value, 100 g^{-1}
		Salt	Fat	Saturated fat	kJ
Potato crisps					
KC_1	The Original Taffel Snacks	1.0	32.0	3.3	2254.0
KC_2	Lorenz Snack-World	2.0	32.0	2.5	2189.0
KC_3	Adazu Istie Cipsi	1.4	25.0	1.8	1944.0
KC_4	Lay's	2.0	30.0	10.0	2139.0
KC_5	Adazu Istie Cipsi	1.6	32.0	14.0	2222.0
KC_6	Estrella	1.8	33.0	15.0	2203.0
KC_7	Rimi	1.5	36.0	16.0	2255.0
KC_8	Rimi	1.2	32.0	15.0	2245.0
KC_9	Pringles	1.4	33.0	3.4	2161.0
KC_10	Estrella	1.9	33.0	15.0	2194.0
KC_12	Trafo Bio-Organic Snacks	2.0	37.0	3.5	2247.0
KC_13	Lisa's Kartoffel-Chips	1.2	28.7	3.4	2116.0
KC_14	Biona Organic	1.2	37.0	3.5	2247.0
KC_17	Lisa's Kartoffel-Chips	2.0	26.9	3.4	2062.0
KC_18	Lisa's Kartoffel-Chips	1.7	25.8	3.4	2067.0
KC_19	Long Chips	1.5	29.3	3.2	2246.0
Vegetable crisps					
DC_15	De Rit Organics	1.6	30.0	2.6	2074.0
DC_16	De Rit Organics	1.9	36.2	2.9	1954.0
Wholegrain, crisp spelt snack					
U_11	Adazu Istie Cipsi	2.2	21.0	1.0	2074.0
U_20	Adazu Istie Cipsi	3.0	21.0	1.0	2085.0
U_21	Rosen Garten	3.0	12.8	8.5	1726.0
U_22	Rosen Garten	5.0	15.8	10.3	1690.0

content of crisps was 1 mL 0.1*M* $AgNO_3$ = 0.005844 g NaCl (Vardavas *et al*., 2007). In the article, the mean values from six measurements of each sample are given.

Determination of sample crispness

Texture analyser TA.HD plus (Stable Micro Systems, UK) was used for determination of sample crispness. For measuring a spherical probe was used; for even contact with probe samples were placed on the HDP/CFS (Crisp facture support). The test settings were: test speed – 1 mm s^{-1}, trigger force – 0.049 N and distance – 3 mm. Each kind of crisps was measured fifteen times to determine crispness. In the article, the mean values of peak force (N) from all measurements of each sample are given.

Determination of thickness

Thickness of crisps samples was measured using an electronic digital outside micrometer (Conrad Electronic, Germany), range – 0–25.000 ± 0.001 mm. Ten crisps of each sample at two random locations on the surface were measured. In the article, the mean values from all measurements of each sample are given.

Determination of colour

Objective crisps sample colour (CIE L*a* b*) measurement was carried out using a Tristimulus Colorimeter (ColorTec Associates, Inc., USA). Crisps sample colour was measured for 10 crisps at three random locations on the surface of each sample. Colour values were recorded as L *– lightness, a* – redness or greenness, and b* – represents blueness or yellowness values (Liu *et al*., 2016). In the article, the mean values from all measurements of each sample are given.

Statistical analysis

All obtained results were processed by statistical methods – mean and standard deviations, Pearson correlations coefficient, hierarchical clusters. For interpretation of results, it was assumed that p = 0.05 with credibility of 95% (significance was defined at $p < 0.05$).

Results and Discussion

Crisps are the most popular products obtained from potatoes, but they are with high salt (2.0 – 5.0 g 100 g^{-1}) and fat content (30.0 – 40.0 g 100 g^{-1}). In Figure 1, summarised data (salt and fat content and energy value) from packaging labels about commercial potato and vegetable crisps and wholegrain snacks from Latvia market are presented.

Dendrogram (Figure 1) shows that samples can be divided into three clusters based on the information (salt and fat content and energy value) on their labels: the first cluster includes

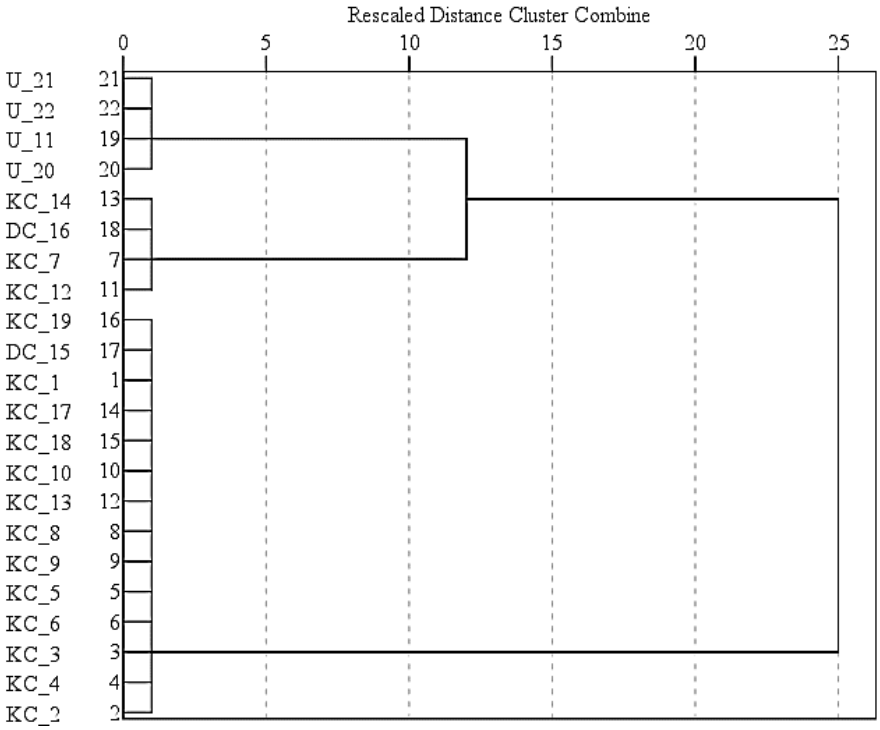

Figure 1. Division of analysed samples in clusters, based on their labels – salt, fat content and energy value. KC – potato crisps, DC – vegetable crisps, U – wholegrain, crisp spelt snacks.

Table 2

Determined and labelled salt content, g 100 g^{-1}

Sample	Salt content, g 100 g^{-1}		Difference, %
	determined	labelled	
KC_1	1.178 ± 0.008	1.000	17.80
KC_2	2.709 ± 0.049	2.000	35.45
KC_3	1.400 ± 0.117	1.400	0.00
KC_4	2.124 ± 0.051	2.000	6.20
KC_5	1.549 ± 0.153	1.600	-3.19
KC_6	1.927 ± 0.045	1.800	7.06
KC_7	1.446 ± 0.186	1.500	-3.60
KC_8	1.303 ± 0.058	1.200	8.58
KC_9	1.187 ± 0.059	1.400	-15.21
KC_10	2.107 ± 0.047	1.900	10.89
KC_12	0.722 ± 0.158	2.000	-63.90
KC_13	1.469 ± 0.064	1.200	22.42
KC_14	1.203 ± 0.041	1.200	0.25
KC_17	2.209 ± 0.140	2.000	10.45
KC_18	1.152 ± 0.128	1.700	-32.24
KC_19	1.951 ± 0.079	1.500	30.07
DC_15	1.514 ± 0.081	1.590	-4.78
DC_16	1.720 ± 0.090	1.900	-9.47
U_11	2.144 ± 0.035	2.200	-2.55
U_20	3.820 ± 0.216	3.000	27.33
U_21	1.343 ± 0.221	3.000	-55.23
U_22	5.289 ± 0.062	5.000	5.78

KC – potato crisps, DC – vegetable crisps, U – wholegrain, crisp spelt snacks

14 samples, second cluster – 4 samples, third cluster – 4 samples.

In the first cluster, there are samples with moderate fat content (25.0 – 33.0 g 100 g^{-1}), avarage salt content (1.5 – 2.0 g 100 g^{-1}) and average energy value (2022 – 2252 kJ 100 g^{-1}), second cluster includes samples with high fat content (36.0 – 37.0 g 100 g^{-1}), but salt content (1.2 – 2.0 g 100 g^{-1}) and energy value (1964 – 2260 kJ 100 g^{-1}) are similar to the first cluster. In the third cluster, there are combined samples with low fat content (12.8 – 21.0 g 100 g^{-1}), higher salt content (2.2 – 5.0 g 100 g^{-1}) and lower energy value (1680 – 2031 kJ 100 g^{-1}). The first cluster contains both potato and vegetable crisps, just as the second cluster. Snack samples group does not really fit with crisps samples, and all four samples make the third cluster. As products from the third cluster show good results in low fat content and energy value, manufacturers should focus on salt content reduction in order to produce better and healthier product. Regarding potato and vegetable crisps, producers should pay attention to reducing fat content as first two clusters show higher values of fat and energy.

For more than million years our ancestors, like other mammals, ate food that contained less than 0.25 g of salt per day and, when there were discovered salt preservation properties, intake of salt considerably increased, and then again after invention and development of refrigerators and similar devices, salt intake started decreasing until recent decades (He & MacGregor, 2009). A significant rise of highly salted food consumption like processed food, snacks, soft drinks etc., has led to an increase of salt intake (He & MacGregor, 2009). In this research, the salt content of potato and vegetable crisps and wholegrain snacks was determined, and results were compared with information on the packaging label about salt content (Table 2). Analysing information on the potato and vegetable crisps labels, it was observed that salt content was from 1.0 g 100 g^{-1} (sample KC_1) to 5.0 g 100 g^{-1} (sample U_22), where 81.8% of samples contained salt between 1.0 g and 2 g 100 g^{-1}. However, determined salt content was from 0.722 ± 0.158 g 100 g^{-1} (samples KC_12) to 5.289 ± 0.062 g 100 g^{-1} for sample U_22 (Table 2).

A significant error limit of 5% considered that statistically is displayed as the first significant, thus it can be concluded that 31.8% of the investigated samples presented on label and determined salt content does not differ significantly ($p > 0.05$). That means that 68.2% of the analysed potato and vegetable crisps on the packaging labels have represented incorrect salt content. For 22.7% of all analysed samples salt content was determined lower than indicated on packaging, the highest negative differences between measured and labelled salt content were for samples KC_12 (-63.9%), where determined salt content was 0.722 ± 0.158 g 100 g^{-1} but labeled – 2.0 g 100 g^{-1}, U_21 (-55.2%) and DC_15 (-32.3%). However, the significant positive differences were for samples KC_2 (35.4%), where determined salt content was 2.709 ± 0.049 g 100 g^{-1} but labelled – 2.0 g 100 g^{-1}, DC_16 (30.1%) and U_20 (27.3%). Results show that the highest salt content is for snacks' samples group (from 2.2g to 5g 100 g^{-1}).

Nutrition information on food labels should promote consumer comprehension about food quality and it should encourage people make healthier choices, reduce salt intake, help identify and seek solutions to obesity problems (Cheftel, 2005). If a

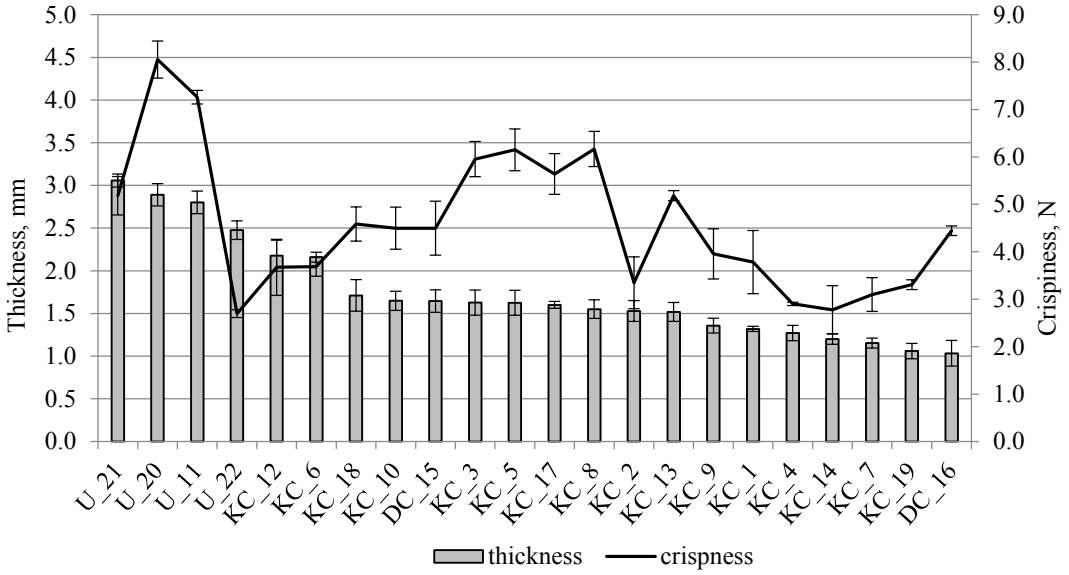

Figure 2. Thickness and crispness of analysed crisps samples.
KC – potato crisps, DC – vegetable crisps, U – wholegrain, crisp spelt snacks.

person consumes 100 g of salty snacks a day, then salt intake is already more than 7 times higher than evolutionary intake of WHO suggested salt intake amount (less than 5 g). If a person eats 100 g of KC_1 sample crisps, salt intake by crisps would make 20% of total daily intake of salt, whereas if a person eats 100 g of U_22 samples, then salt intake would make 100% of suggested daily intake only by snack consumption. Salty snacks typically contain a lot of salt and, it is essentially important to find ways to decrease amount of it. Even if there are consumed crisps with low salt content, it still makes a large part of recommended daily amount of salt.

Crisps are a very good example of consumer acceptance reasons, as in this case consumer relies more on texture than flavour (Rojo & Vincent, 2009). That is why there have been different researches with the aim to determine crispness, factors, which impact it, and measurement possibilities. As crispness is one of key causes that defines consumer's choice, food producers need to develop their recipes and technologies in order to deliver product that meets consumer expectations. The most important for the producer is to choose appropriate variety of raw product (potato, vegetable variety). Regarding potato crisps, it is confirmed that texture mainly depends on starch contents of potato tubers and after that on the sum of nitrogen substances and non-starch polysaccharides (Kita, 2002).

There was measured thickness and crispness (hardness) of all samples (Figure 2). Results show that the thickest samples were revealed in the snack group (U_21, U_20, U_11 and U_22), from 2.48 to 3.06 mm, the crisps were thinner than snacks – from 1.03 to 2.18 mm, and the thickest were samples KC_12 (2.18 mm) and KC_6 (2.16 mm), while the thinnest samples were DC_16 (1.03 mm) and KC_19 (1.06 mm). Although vegetable crisps sample was the thinnest, fat content for this exact sample is second highest. For better combination of product quality indicators, manufacturers should reassess their technological process in order to improve finished product. The hardest samples also mainly were from the snack group – U_20 (8.1 N), U_11 (7.3 N) and then KC_8 (6.2 N), but the most fragile samples were U_22 (2.7 N), KC_14 (2.8 N) and KC_4 (2.9 N). It can be explained by the number of different producers (together 13 brands for 22 samples), where every manufacturer has their own recipe, ingredient's proportion, selected oil, varieties of raw materials and production process special nuances and parameters.

Average crispness for potato crisps was 4.3 N, for vegetable crisps – 4.5 N and for snacks – 5.8 N. If mean values are compared by product groups, then the hardest are snacks, while potato and vegetable crisps have more similar results.

Positive moderate correlation between thickness and crispness, $r = 0.489$ ($p < 0.05$), which means positive moderate correlation was found out. This result confirms that these indicators are connected, but this connection does not have high correlation level.

The first thing consumers evaluate when choosing a food product is its colour, one of the most important characteristics, which is often also connected with the notion of quality. Table 3 represents colour values of the potato and vegetable crisps samples.

Value L* of the potato crisps colour component, characterising the light and dark colour nuances of the

Table 3

Characterization of potato and vegetable crisps colour

Samples	Colour value		
	L*	a*	b*
KC_1	71.34 ± 1.34	-5.80 ± 1.29	24.35 ± 1.15
KC_2	60.39 ± 4.97	-2.87 ± 1.19	18.48 ± 2.90
KC_3	62.18 ± 5.99	-4.81 ± 1.59	28.44 ± 3.14
KC_4	60.61 ± 5.66	-6.40 ± 4.14	26.21 ± 1.53
KC_5	63.82 ± 3.68	-5.2 0± 1.06	23.11 ± 2.36
KC_6	70.02 ± 2.13	-6.51 ± 1.02	28.40 ± 1.89
KC_7	71.20 ± 2.16	-7.76 ± 1.63	26.27 ± 3.91
KC_8	67.24 ± 1.26	-5.04 ± 1.61	24.60 ± 1.35
KC_9	68.75 ± 1.50	-6.54 ± 1.84	17.22 ± 3.61
KC_10	63.56 ± 4.33	-6.56 ± 1.92	28.13 ± 3.03
KC_12	60.25 ± 5.90	-5.40 ± 1.40	29.55 ± 5.99
KC_13	61.24 ± 4.61	-4.54 ± 1.94	22.94 ± 4.24
KC_14	67.69 ± 5.84	-5.08 ± 1.12	27.38 ± 4.51
KC_17	60.34 ± 5.25	-6.50 ± 1.80	24.55 ± 3.11
KC_18	55.84 ± 4.71	-1.62 ± 0.27	24.12 ± 6.64
KC_19	79.42 ± 1.38	-4.68 ± 0.40	23.22 ± 1.75
DC_15	20.60 ± 3.99	5.56 ± 1.61	-1.16 ± 0.77
DC_16	39.40± 5.43	5.10 ± 1.48	22.40 ± 6.94
U_11	50.24 ± 5.24	0.43 ± 0.11	22.50 ± 5.97
U_20	52.25 ± 5.14	1.12 ± 1.01	17.67 ± 5.41
U_21	40.32 ± 0.59	16.49 ± 0.85	9.85 ± 1.38
U_22	56.83 ± 1.35	-0.51 ± 0.76	20.37 ± 1.03

samples, varies from 55.84 ± 4.71 (sample KC_18) to 79.42 ± 1.38 (sample KC_19). KC_18 samples have darker results in terms of colour, as these were crisps with tomato flavour, thus the colour L* and a* values stand out. For potato crisps b* values are between 17.22 ± 3.61 (sample KC_9) and 29.55 ± 5.99 (sample KC_12), which means that the colour of the samples is light yellow. Both vegetable crisps samples are different from each other regarding b* and L* colour values, but have similar values for a* colour value. DC_15 vegetable crisps were from beetroot, which explains low L* value (20.60 ± 3.99) and also other two colour indicators, which points out dark red colour, while DC_16 contained vegetables like carrots, potatoes and beetroots and that accords to results that these samples are between yellow and red. Snack samples do not have significant differences ($p > 0.05$) in the colour values; the results are similar, except the sample U_21, which has different values for all three colour indicators, and it has a solid explanation, as the sample's U_21 one of ingredients is beetroot concentrate (13%).

Conclusions

All analysed samples can be divided into three clusters, based on the information (salt and fat content and energy value) on their labels: the first cluster includes samples with moderate fat content, average salt content and average energy value, second cluster – samples with high fat content, average salt content and energy value similar to first cluster, third cluster – samples with low fat content, higher salt content and lower energy value. The first cluster contains both potato and vegetable crisps, just as the second cluster, while the third cluster includes snack samples. For 31.8% of the investigated samples, information presented on label and determined salt content does not differ significantly ($p > 0.05$). That means that 68.2% of the analysed potato and vegetable crisps and wholegrain snacks on the packaging labels have represented incorrect salt content.

Positive moderate correlation ($r = 0.489$) between potato and vegetable crisps thickness and crispness was found out.

Potato crisps, vegetable crisps and snacks colour affects the used raw materials – carrots, potatoes and beetroots or other.

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EFFECT OF PROBIOTICS AND HERBALS ON HEALTH AND SHEDDING OF RESISTANT *ESCHERICHIA COLI* IN PIGLETS

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Abstract
The purpose of this study was to evaluate the effect of probiotics, herbals and buckwheat bran (*Fagopyrum esculentum* L.*)* on growth, profile of blood, gut microbiota, profile of fatty acid in meat and shedding of resistant *Escherichia coli (E. coli)* in piglets. A total of 44 piglets *(Sus scrofa domesticus)* from age of day 14 to 56 were divided into 4 groups. Control received basal diet (group C), basal diet + probiotics (group P), basal diet + 3% buckwheat bran (group PB) and basal diet + 1.5% herbals (group H). No effect was observed in growth in all groups. The count of *Lactobacillus* spp. increased ($p < 0.05$) in jejunum in group P. In the faeces, *Enterobacteriaceae* decreased in the group P ($p < 0.05$) of 35 days old piglets, but *Enterobacteriaceae* and *E. coli* decreased in the group H ($p < 0.05$) of 56 days old piglets. The prevalence of resistance to at least one antibiotic class was 66.7% before and 50% after the experiment in all groups. Multidrug resistance of *E. coli* was not observed in 14 days old piglets, but was observed in 50% and more in all of study groups of 56 days old piglets. The fatty acid composition of *Longissimus thoracis* muscle had higher levels of α-linolenic acid and palmitoleic acid ($p < 0.05$), but lower level of stearic acid ($p < 0.05$) in group P. In conclusion, probiotics and herbals improved gut microbiota, fatty acid profile and affected shedding of resistant *E. coli*, but not growth performance.
Key words: buckwheat bran, gut microbiota, fatty acids, growth performance, blood profile.

Introduction

Nowadays typical is an intensive pig *(Sus scrofa domesticus)* farming. Pigs are commonly housed at high densities that can promote the spread of diseases. Antibiotics are used for treatment and prevention of diseases, and promoting of growth. The heightened use or misuse of antibiotics is contributing to the alarming increase of antibiotic resistance in bacteria, therefore it is an increasingly serious threat to global public health (WHO, 2014). In the European Union the use of antibiotics as growth promoters have been banned since 2006. Following feed supplementation with heavy metals has increased in pig breeding, but it might promote the spread of antimicrobial resistance too (Liedtke & Vahjen, 2012). There is an increasing interest in finding alternative means to improve the health of pigs and reduce the use of antibiotics. Probiotics have been studied and used as an in-feed supplement. Probiotics promote development of healthy microbiota, reduce enteric pathogens, luminal pH and improve mucosal immunity in piglets (de Lange *et al*., 2010). Buckwheat (*Fagopyrum esculentum* L.*)* seed is nutritionally important and flour of it is used as the prebiotic (Coman *et al*., 2013). Herbs have a number of beneficial effects, of which antimicrobial and antioxidant activity is the most important (Windisch *et al*., 2007). It is not enough to focus on alternative means to influence the spread of resistance. The aim of our study was to find out the impact of probiotics, buckwheat bran and composition of herbals on growth performance, blood and fatty acid characteristics, gut microbiota and shedding of resistant *E. coli* in piglets.

Materials and Methods

Feed additives

As feed additives were used buckwheat bran, herbal powder, and commercially available probiotics 'ProbioHelp'. Buckwheat (*Fagopyrum esculentum* L.*)* bran was chosen from an organic farm. Composition of herbals was made by authors: the aerial parts of St. John's wort (*Hypericum perforatum*) and leaves of greater plantain (*Plantago major*) and nettle (*Urtica dioica*) were collected in the Livani and Dobele districts of Latvia in 2015 and authenticated by the Institute of Horticulture of the LLU.

Experimental design and collection of samples

The experiment (6 weeks) was conducted in a conventional pig fattening farm in Latvia, in 2015. A total 44 Duroc – Landrace crossbred piglets were used. The age of piglets was 14 days with an average weight of 4.96 ± 0.13 kg. Piglets were divided into 4 treatment groups (11 pigs / pen): group C received basal diet, group P received basal diet + 'ProbioHelp', group PB received basal diet + 'ProbioHelp' + 3% buckwheat bran and group H received basal diet + 1.5% composition of herbals. Buckwheat bran and herbals were mixed into basal diet, but 'ProbioHelp' was added to drinking water, depending on the age of piglets: 14, 28, 35, 42 and 49 days old ones received the concentration of 1%, 0.75%, 0.45%, 0.34%, and 0.32%, respectively. All piglets were given *ad libitum* access to feed and water through local feeder and nipple drinker. The piglets were weighed individually at the beginning of the experiment (on day 14) and on days 35, 49 and 56. Offered and refused feed was weighed daily to calculate the growth parameters -

average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) (Willems, Miller, & Wood, 2013). Blood samples (n = 38) were obtained via jugular vein puncture and sampled into 5 mL EDTAK2 tubes and into 10 mL tubes without anticoagulant at the end of experiment. Blood samples were sent directly to the laboratory of LLU veterinary clinic. The faecal samples (n = 77) were collected from the first defecation after the daily cleaning of the pens on days 14, 35 and 56. On day 56, piglets (four from each treatment group and two from control) were slaughtered. The gastrointestinal tract was removed and digestive contents (n = 14) from jejunum were collected. *Longissimus thoracis* muscles were collected from both sides of the pig carcass for detection of fatty acid profile and transported to the laboratory shortly afterwards.

Haematological and serum biochemical measurements

Count of white blood cells (WBC), red blood cells (RBC), granulocytes (GRAN), lymphocytes (LYM), haematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin, (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured using the blood counter (Exigo eos, Sweden) by photometric method. The levels of serum glucose (GLU), calcium (Ca), phosphorus (P), gammaglutamintransferase (GGT), alkaline phosphatase (ALP) and aspartate aminotransferase (ASAT) were measured using the chemical analyser (Mindray BS-380) by absorbance photometry method.

Microbiological investigation

Faeces were used for enumeration of *Enterobacteriaceae*, *E. coli*, but digestive contents were used for enumeration of *Enterobacteriaceae*, *E. coli* and *Lactobacillus* spp. Preparation of test samples, initial suspension and decimal dilutions for microbiological examination were done according to LVS ISO 6887-1:1999. The count of *Enterobacteriaceae* was carried out based on LVS ISO 21528-2:2007, *E. coli* - on LVS ISO 16649-2:2007, but the count of *Lactobacillus* spp. according to MRS (Biolife) manufacturer's instructions with little modification. Briefly, the faecal, digestive contents (10 g) were diluted with 90 mL of 1% peptone broth (Maximum Recovery Diluent, Biolife) and then homogenized. Counts of bacteria in the faecal samples were conducted by plating serial 10-fold dilutions (in 1% peptone solution) in Violet Red Bile Glucose agar (Biolife), Tryptone Bile X- Gglucuronide Medium - TBX (Oxoid), and MRS Agar with Tween® 80 (Biolife) to isolate the *Enterobacteriaceae*, *E. coli* and *Lactobacillus*, respectively. Colonies were expressed as log10 cfu g^{-1}.

Confirmation of E. coli and antimicrobial susceptibility testing

Two of *E. coli* isolates from TBX agar (from each faecal sample) were streaked with a sterile bacterial loop onto Levine EMB Blue Agar (Biolife) and incubated at 37 °C for 24 h. Isolates were assumed to be *E. coli* if showed typical blue black colonies with green metallic shine and additional basic biochemistry confirmed (oxidase test, citrate utilization and indole production tests).

E. coli isolates were phenotypically tested via agar disk diffusion to 12 antibiotics using BD BBL Antimicrobial Susceptibility Disks (ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefazolin (30 µg), cefotaxime (30 µg), imipenem (10 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), trimethoprim (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), enrofloxacin (5 µg) according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2008). Inhibition zones were measured on the Mueller-Hinton agar plates (Biolife) and interpreted according to the manufacturer's recommendations. If *E. coli* isolates were resistant to cefotaxime (30 µg), phenotypic confirmatory test for ESBL producing *E. coli* was used according to CLSI recommendation using both cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) disks alone and in combination with clavulanic acid (CA, 10 µg) (BD BBL).

Analysis of fatty acid profile

The fatty acids (n = 36) were detected in muscle *Longissimus thoracis* samples by gas chromatography method (BIOR-T-012-131-2011) and analyses were performed in the accredited laboratory - BIOR.

Data statistical analysis

Data were analyzed using R version 3.3.2. All results were presented as the mean ± SEM (standard error of the mean). Duncan`s multiple range test was used to compare the means at level of significance of $p < 0.05$.

Results and Discussion

There is a wide interest in the improvement of feeding strategies using different feed additives that stimulate growth performance and minimize the use of antibiotics. In our study no differences were observed in average daily feed intake (ADFI), average daily gain (ADG) or feed conversion ratio (FCR) in all treatment groups. However, the weight gain of group PB was significantly higher than in other groups ($p < 0.05$) (Table 1). The initial weight of group PB was

Table 1

Effects of treatment on performance of 14 - 56 days old piglets

Traits	Group C	Group P	Group PB	Group H
Initial weight (kg)	4.500 ± 0.141^{b}	4.673 ± 0.208^{b}	5.836 ± 0.255^{a}	4.827 ± 0.236^{b}
Final weight (kg)	19.982 ± 1.063^{b}	19.482 ± 0.614^{b}	23.255 ± 0.465^{a}	20.273 ± 0.864^{b}
Weight gain (kg)	15.482 ± 1.138^{b}	14.809 ± 0.630^{b}	17.418 ± 0.501^{a}	15.445 ± 0.681^{b}
ADG (g d^{-1})	0.369 ± 0.027^{a}	0.353 ± 0.015^{a}	0.415 ± 0.012^{a}	0.368 ± 0.016^{a}
ADFI (g d^{-1})	0.580 ± 0.082^{a}	0.620 ± 0.088^{a}	0.658 ± 0.087^{a}	0.577 ± 0.082^{a}
FCR	1.681 ± 0.148^{a}	1.795 ± 0.085^{a}	1.599 ± 0.044^{a}	1.606 ± 0.083^{a}

ADG – average daily gain; ADFI – average daily feed intake; FCR – feed conversion ratio; a and b – means in the same row with different letters are significantly different ($p < 0.05$).

significantly higher compared to the other groups. Effect of probiotic bacteria has been studied before. Some researchers observed that probiotic bacteria in piglet diet produced a positive effect on growth (Liu *et al*., 2015). Whereas, other researchers have shown that probiotics have no positive effect of growth performance on piglets (Speiser *et al*., 2015), similar to our results. The factors which could affect ADG are different: species of probiotics, method of administration, age of pigs, components of basal diet and others (Zimmermann *et al*., 2016). A beneficial effect of probiotics on ADG was observed when the basal diet contained maize (*Zea mays* L.) as the principal feed ingredient, but not barley (*Hordeum vulgare* L.) or wheat (*Triticum aestivum* L.) (Zimmermann *et al*., 2016), which were principal feed ingredients in basal diet in our experiment. The lowest ADFI was observed in group H, but it was not significant. That might be attributed to the special organoleptic properties of herbs which may reduce diet palatability (Yan, Meng, & Kim, 2012).

All serum biochemical and haematological parameters after feeding trial were within the reference intervals (Klem *et al*., 2010), except ALP, which was slightly higher compared to the results in groups P, H and C, respectively, - higher by 1.0%, 1.5%, 11.9%. A higher level of ALP (without ASAT) is always an indicator of intense bone formation in the growth phase, particularly, if the level of phosphorus in diet is lower (Luiz *et al*., 2012). The RBC was lower ($p <$

Table 2

Effects of treatment on blood parameters in 56 days old piglets

Parameter	Group C	Group P	Group PB	Group H
WBC 10^{9} L^{-1}	23.780 ± 0.530^{a}	24.373 ± 1.175^{a}	21.336 ± 1.360^{a}	22.600 ± 1.274^{a}
GRAN 10^{9} L^{-1}	9.740 ± 0.464^{a}	11.027 ± 0.949^{a}	8.664 ± 0.703^{a}	9.664 ± 0.625^{a}
LYM 10^{9} L^{-1}	11.900 ± 0.539^{a}	11.055 ± 0.447^{a}	10.936 ± 0.720^{a}	11.064 ± 0.666^{a}
RBC 10^{12} L^{-1}	6.712 ± 0.159^{a}	6.670 ± 0.107^{a}	6.995 ± 0.095^{a}	6.288 ± 0.120^{b}
HCT %	34.900 ± 0.460^{a}	35.418 ± 0.641^{a}	35.145 ± 0.757^{a}	33.818 ± 0.627^{a}
HGB g dL^{-1}	10.960 ± 0.147^{a}	10.882 ± 0.133^{a}	10.927 ± 0.201^{a}	10.418 ± 0.195^{a}
MCV fl	$52.040 \pm 0.830^{a,b}$	53.112 ± 0.772^{a}	50.218 ± 0.201^{b}	53.827 ± 0.754^{a}
MCH pg	16.320 ± 0.252^{a}	16.327 ± 0.141^{a}	15.628 ± 0.220^{b}	16.618 ± 0.192^{a}
MCHC g dL^{-1}	31.400 ± 0.145^{a}	30.818 ± 0.270^{a}	31.145 ± 0.142^{a}	30.864 ± 0.192^{a}
ASAT U L^{-1}	75.240 ± 8.367^{a}	48.009 ± 4.606^{b}	$60.018 \pm 3.306^{a,b}$	68.373 ± 6.707^{a}
ALP U L^{-1}	$\uparrow 335.560 \pm 36.074^{a}$	$\uparrow 304.381 \pm 1.029^{a}$	260.327 ± 7.018^{b}	$\uparrow 303.082 \pm 10.044^{a}$
Ca mmol L^{-1}	2.928 ± 0.068^{a}	2.918 ± 0.036^{a}	2.728 ± 0.031^{b}	2.756 ± 0.053^{b}
P mmol L^{-1}	2.890 ± 0.059^{a}	2.810 ± 0.064^{a}	2.975 ± 0.028^{a}	2.871 ± 0.044^{a}
GGT U L^{-1}	45.200 ± 2.172^{a}	49.345 ± 8.201^{a}	37.682 ± 2.670^{a}	46.009 ± 2.487^{a}
GLU mmol L^{-1}	4.560 ± 0.093^{a}	4.832 ± 0.241^{a}	4.665 ± 0.119^{a}	4.715 ± 0.139^{a}

WBC – white blood cells; GRAN – granulocytes; LYM – lymphocytes; RBC – red blood cells; HCT – haematocrit; HGB – haemoglobin; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; ASAT – aspartate aminotransferase; ALP – alkaline phosphatase; Ca – calcium; P – phosphorus; GGT – gammaglutamintransferase; GLU – glucose; a and b means in the same row with different letters are significantly different ($p < 0.05$). ↑ the level is higher compared to the reference interval (Klem *et al*., 2010).

Table 3

Effects of treatment on digestive microbial populations of 35 and 56 days old piglets

Item (log_{10} cfu g^{-1})	Group C	Group P	Group PB	Group H
faecal 35 d:				
Enterobacteriaceae	3.37 ± 0.47[a]	1.73 ± 0.38[b]	2.78 ± 0.43[a,b]	2.95 ± 0.47[a,b]
E. coli	2.91 ± 0.29[a]	1.66 ± 0.38[a]	2.05 ± 0.35[a]	2.92 ± 0.44[a]
faecal 56 d:				
Enterobacteriaceae	5.19 ± 0.70[a,b]	5.41 ± 0.23[a]	3.93 ± 0.52[b,c]	3.18 ± 0.37[c]
E. coli	4.94 ± 0.60[a]	5.02 ± 0.22[a]	3.85 ± 0.49[a,b]	3.16 ± 0.33[b]
jejunum 56 d:				
Enterobacteriaceae	5.29 ± 2.69[a]	3.92 ± 0.56[a]	3.04 ± 0.85[a]	2.99 ± 0.67[a]
E. coli	4.74 ± 2.21[a]	3.54 ± 0.46[a]	2.96 ± 0.83[a]	2.98 ± 0.67[a]
Lactobacillus spp.	6.27 ± 0.82[b]	7.75 ± 0.24[a]	6.62 ± 0.14[a,b]	5.98 ± 0.51[b]

a, b and c – means in the same row with different letters are significantly different ($p < 0.05$).

0.05) in group H, compared with group C. ASAT was lower in group P ($p < 0.05$) and group PB ($p > 0.05$), compared with group C. The level of phosphorus was higher in group BP (Table 2).

Group H received a diet supplemented with composition of herbals, wherein one of the three components was nettle. In traditional medicine, nettle is used for treatment of anemia. The beneficial effect of nettle on the erythropoiesis was similar to that of iron-containing preparations (Upton, 2013). The mean of MCV and MCH was the highest in group H, therefore reduced count of RBC cannot be associated with iron deficiency. In our study probiotics decreased the level of ASAT; it coincided with other studies about the effect of probiotics on layer chicks (*Gallus gallus domesticus* L.) (Hatab, Elsayed, & Ibrahim, 2016). At the same time, there are other authors who have not observed this tendency (Liu *et al*., 2015). Group PB had the highest level of phosphorus, it could be explained with the fact that group PB received basal diet supplemented with buckwheat bran, which is an additional source of phosphorus, and therefore resulted in a lower level of ALP in group PB ($p < 0.05$).

Compared with the group C, diet supplementation with probiotics increased the count of *Lactobacillus* spp. in jejunum in both group P ($p < 0.05$) and group PB ($p > 0.05$). In the faeces of 35 days old piglets, the count of *Enterobacteriaceae* decreased in the group P ($p < 0.05$) compared to the group C. In the faeces of 56 days old piglets, the count of *Enterobacteriaceae* and *E. coli* decreased in the group H ($p < 0.05$) compared to the group C, but the total count of *Enterobacteriacea* in group PB compared to the group P ($p < 0.05$) and group C was decreased ($p > 0.05$) (Table 3).

We observed that diet supplemented with probiotics increased the count of *Lactobacillus* spp. and decreased count of *Enterobacteriaceae* and *E. coli*, which coincides with other authors (Liu *et al*., 2015)**.** Probiotic bacteria increased the production of short chain fatty acids in an *in vitro* study and these help to reduce digest pH and growth of pathogenic bacteria (Gibson, 1999). We observed beneficial effect of herbal supplementation on shedding of faecal microbial, particularly, at the age of 56 days. Similar to other authors (Yan, Meng, & Kim, 2012), in our study, herbals decreased the count of *Enterobacteriaiaceae* and *E. coli* in faeces of piglets. Some herbals contain essential oils with strong antimicrobial activity, particularly, phenolic structures, which damage cell membrane of bacteria (Lambert *et al*., 2001), but with little inhibition towards *Lactobacillus* spp. (Si *et al.,* 2006), that can be seen in our study, too. Buckwheat flour has demonstrated the potential of prebiotic. It has also been reported that extracts of methanol from buckwheat inhibited *E. coli* (Coman *et al*., 2013). Buckweat bran did not increase count of *Lactobacillus* spp. in jejunum, but reduce count of *Enterobacteriaceae* and *E. coli* in our study. After weaning, piglets pass through acute and adaptive phases. Primarily the effect of acute phase is on the reduced feed intake. It could take seven days, when weaned piglets learn to eat dry feed and the intake of dry matter content reaches volume as it was before weaning (Pluske, Hampson, & Williams, 1997). Considering this fact, we assumed that piglets did not ingest enough feed that was supplemented with herbals and therefore we did not observe the beneficial effects of herbals to faecal microflora. The effect of probiotics in acute phase is higher if consumed with water.

During the study, a total of 80 *E. coli* were isolated from faeces of 14 and 56 days old piglets. Before experiment, higher prevalence of resistant *E. coli* was observed to ampicillin (42%) and trimethoprim (33%). After experiment, higher resistance was observed to tetracycline in groups P (50%), PB (50%)

Table 4

Antibiotic resistant *E. coli* isolates from 14 and 56 days old piglets

Antibiotic	14 days old piglet (n = 12)	56 days old piglet			
		Group C (n = 16)	Group P (n = 20)	Group PB (n = 16)	Group H (n = 16)
Ampicillin	5 (42%)	4 (25%)	5 (25%)	6 (38%)	5 (31%)
Amoxicillin-clavulanic acid	0	2 (13%)	1 (5%)	0	0
Cefazolin	0	1 (6%)	1 (5%)	3 (19%)	4 (25%)
Cefotaxime	0	1 (6%)	0	0	0
Imipenem	0	0	0	0	0
Gentamicin	0	0	0	0	0
Tetracycline	2 (17%)	5 (31%)	10 (50%)	8 (50%)	7 (44%)
Ciprofloxacin	0	0	0	0	0
Trimethoprim	4 (33%)	4 (25%)	10 (50%)	7 (44%)	6 (38%)
Trimethoprim-sulfamethoxazole	1 (8%)	3 (19%)	9 (45%)	6 (38%)	5 (31%)
Chloramphenicol	0	6 (38%)	0	0	0
Enrofloxacin	0	0	0	0	0

and H (44%), and to trimethoprim – in groups P (50%) and PB (44%). Resistance to chloramphenicol and cefotaxime was observed only in group C (38% and 6%, respectively) (Table 4).

The prevalence of resistance to at least one antibiotic class was observed – 66.7% before and 50% after the experiment in all groups. Most of *E. coli* isolates were resistant only against one antibiotic class in 14 days old piglets (62.5%). Multidrug resistance (resistant to three or more antimicrobial classes) of *E. coli* isolates was not observed in 14 days old piglets, but was observed 50% and more in all of study groups of 56 days old piglets. Resistance against 5 antibiotic classes was observed only in group C (12.5%) (Figure 1).

High prevalence of resistant *E. coli* to amoxicillin in 14 days old piglets and to tetracycline and trimethoprim / trimethoprim-sulfamethoxazole in 56 days old piglets could be explained with antibiotic usage habits in this farm. Resistance to chloramphenicol, and it was of high level, was observed only in the group C. Chloramphenicol has been banned in EU since 1994 for livestock treatment. Despite the fact that some specific antibiotics have not been used in animals for a long time, some resistant genes can be maintained due to a link with the other genes coding resistance to other antibiotics, which are allowed for use in food-producing animals (Diarra *et al.*, 2007). Suckling and weaned piglets are highly sensitive to bacterial infections, therefore more often are pressured of therapeutic antibiotics, as a result, a high prevalence of resistant *E.coli* is observed (Akwar *et al.*, 2008). During the experiment, none of treatment groups received antibiotics additionally and this could

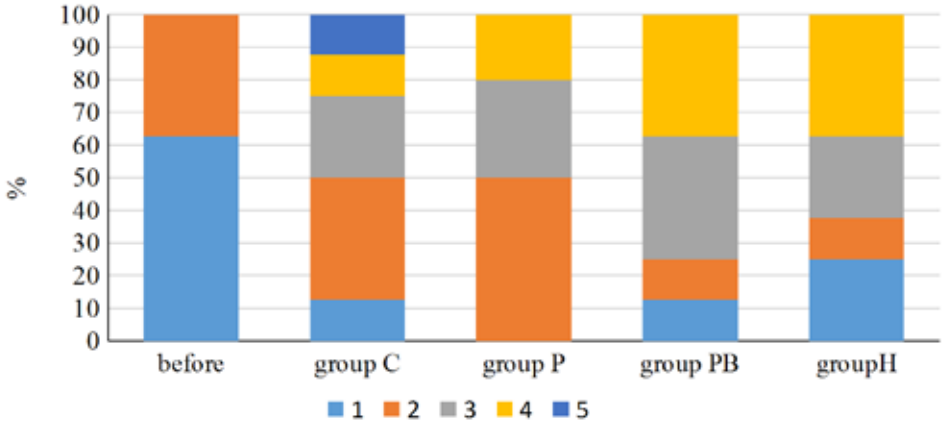

Figure 1. Proportion of resistant *E. coli* before (14 days old piglets) and after experiment (56 days old piglets) against 1, 2, 3, 4 and 5 antibiotic classes.

Table 5

Fatty acid* composition (% of detected fatty acids) of *Longissimus thoracis* muscle in 56 days old piglets

Fatty acid	Group C	Group P	Group PB	Group H
C18:3 n3 α-linolenic acid	0.90 ± 0.12^{b}	1.80 ± 0.10^{a}	1.60 ± 0.10^{a}	1.50 ± 0.10^{a}
C20:4 n6 arachidonic acid	1.40 ± 0.2^{a}	$0.75 \pm 0.05^{a,b}$	$1.05 \pm 0.15^{a,b}$	0.50 ± 0.10^{b}
C18:2 n6 linoleic acid	10.37 ± 0.70^{a}	11.60 ± 0.40^{a}	11.85 ± 0.75^{a}	9.65 ± 0.75^{a}
C18:1 oleic acid	$41.63 \pm 0.49^{a,b}$	42.55 ± 0.95^{a}	39.60 ± 0.60^{b}	42.55 ± 0.25^{a}
C16:1 palmitoleic acid	3.07 ± 0.26^{b}	4.55 ± 0.25^{a}	$4.25 \pm 0.55^{a,b}$	3.25 ± 0.15^{b}
C16:0 palmitic acid	24.50 ± 0.61^{a}	24.25 ± 0.15^{a}	25.75 ± 0.05^{a}	24.65 ± 0.45^{a}
C18:0 stearic acid	13.13 ± 0.13^{a}	9.40 ± 0.60^{b}	$11.20 \pm 1.30^{a,b}$	13.10 ± 0.70^{a}
SFA	40.22 ± 0.49^{a}	36.70 ± 0.25^{b}	40.08 ± 1.23^{a}	40.53 ± 1.13^{a}
MUFA	$47.15 \pm 0.63^{a,b}$	49.03 ± 1.08^{a}	45.65 ± 0.75^{b}	$48.05 \pm 0.20^{a,b}$
PUFA	13.40 ± 0.85^{a}	14.90 ± 0.60^{a}	15.10 ± 0.80^{a}	12.25 ± 0.95^{a}
PUFA/SFA	0.33 ± 0.02^{a}	0.41 ± 0.01^{a}	0.38 ± 0.03^{a}	0.30 ± 0.03^{a}

* - not all detected fatty acids are showed in table; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids.; PUFA: SFA ratio of polyunsaturated fatty acids and saturated fatty acids; a and b means in the same row with different letters are significantly different ($p < 0.05$).

explain the fact that the prevalence of resistant *E. coli* in weaned piglets (56 days old) was lower compared to 14 days old piglets. Large proportion of multi-drug resistant *E. coli* in all treatment groups was explained by the uptake of resistant genes from the environment as well as diet received by piglets, supplemented with zinc oxide. According to previous studies, feed supplementation with heavy metals, particularly zinc, increase proportion of multi-drug resistant *E.coli* in piglets (Bednorz *et al*., 2013). Clay minerals also could have selection effect on uptake of resistant genes (Jahanbakhsh *et al*., 2015), but they do not give any information about the impact of different herbals. Probiotics have demonstrated to leave impact on the transfer of antibiotic resistance genes, but information about the strains with higher effect and mechanism of this effect is not known (Moubareck *et al*., 2007). Differences of proportion of resistant *E. coli* between treatment groups indicate that feed supplementation with natural feed additives (herbals and probiotics) could have selective effect on resistant bacteria or uptake of resistance genes.

The fatty acid composition of *Longissimus thoracis* muscle showed that piglets fed with basal diet supplemented with probiotics had higher levels of α-linolenic acid and palmitoleic acid ($p < 0.05$), but lower level of stearic acid ($p < 0.05$) compared to group C. Only level of α-linolenic acid was higher in groups H and PB compared to group C. SFA was lower ($p < 0.05$) and PUFA/SFA rations were higher ($p > 0.05$) in group P (Table 5).

SFA have a negative effect on cardiovascular system of humans. According to Department of Health (1994), the recommended PUFA/SFA rations to reduce the risk of cardiovascular diseases is greater than 0.4 (Wood *et al*., 2008). Pigs are monogastric animals and some of PUFA (linoleic acid, α-linolenic acid) pass through the stomach unchanged. They are absorbed into the blood flow through small intestine and get into tissues (Wood *et al.,* 2008). As basal diet was the same for all treatments groups, differences of fatty acid composition in group P proved that probiotics can improve the fatty acid profile of pork. Effect of probiotics on reduction of SFA and increased level of MUFA and PUFA in pigs has been reported before (Ross, Nieuwenhove & González, 2012).

Conclusions

Dietary supplementation with probiotics, buckwheat bran or herbals did not affect the growth performance. Probiotics increased *Lactobacillus* spp. in jejunum. Probiotics consumed with water more effectively reduced *Enterobacteriaceae* in acute phase, but herbals were more effective in reduction of *Enterobacteriaceae* and *E. coli* in adaptive phase of post weaning. Buckwheat bran did not exert the effect of prebiotic, but more effectively reduced *Enterobacteriaceae* and *E. coli in vivo.* Differences of proportion of resistant *E. coli* between treatment groups indicated that feed supplementation with probiotics and herbals could have selective effect on resistant bacteria or uptake of resistance genes. These results confirmed that probiotics can improve the fatty acid profile of pork. Probiotics reduced SFA and increased the level of MUFA and PUFA in pigs.

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CELL PROLIFIRATION ACTIVITY IN LYMPH NODES INFECTED BY PORCINE CIRCOVIRUS-2

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Abstract
High economic losses in agriculture can be caused by pig disease like Postweaning Multisystemic Wasting Syndrome (PMWS). Porcine circovirus-2 (PCV2) is the primary agent of PMWS. It is known that PMWS causes cellular and humoral immunity disorders, therefore it is considered a general immune deficiency disease in piglets. It is still unknown, why piglets with PMWS have severe loss of lymphocytes in lymphoid tissues and in the blood. Thus, the hypothesis of the study was that lymphocyte depletion occurs because of lymphocyte life-cycle delay in pigs infected by PCV2. The activity of cell life-cycle is attributed to protein Ki67. This protein is found in all active phases of the cell like interphase G1, S, G2 and mitoses. However, protein Ki67 is not observed at all in the cell's "quiet" phase G0. The aim of our work was to investigate the lymph node cell proliferation activity of pigs infected by PCV2. In total, 42 pig carcasses were collected from 5 to 15 weeks old pigs with PMWS clinical manifestation. Lymph nodes were selected from each pig carcass for histological and imunohistochemical testing. We concluded that B lymphocyte population was reduced first in the lymph node with PCV2, but the number of T lymphocytes decreased later. PCV2 decreased B lymphocyte prolifiration activity.
Key words: Ki67, CD79α, CD3, lymphocyte, pig.

Introduction

Pig breeding is one of the main agricultural sectors in Latvia. According to the data of the Latvian Statistic Center, 305,521 pigs were registered in 2016. The swine production comprises 8% of the total agricultural production in Latvia. High economic losses in agriculture can be caused by pig disease like Postweaning Multisystemic Wasting Syndrome (PMWS). This disease develops in 10-30% cases of naturally infected pigs (Darwich, Segales, & Mateu, 2004). Mortality of PMWS is within very variable range of 4 – 30% and 70 – 80%, depending on the underlying health status of animals from the affected farm (Segales & Domingo, 2002).

Porcine circovirus-2 (PCV2) is etiological agent of PMWS. PCV2 infection is distributed worldwide throughout the domestic pig population. The first report about PCV2 infection in Lithuania and Latvia was published in 2007 (Stankevicius *et al.,* 2007).

Clinical confirmation of PMWS is difficult, because this disease has non-specific clinical features and non-specific gross pathology. Manifestations of the disease are weight loss, diarrhea or respiratory disorder, pale or icteric mucouses (Rosell *et al*., 1999; Segales *et al*., 2004). Specific changes of PCV2 appear only in the microscopic structure of the lymph nodes (Rosell *et al.*, 1999). This virus replicates in macrophages, therefore, lymph node lymphocytes are "substituted" by the macrophages (Sanchez *et al.,* 2003). It is known that PMWS causes cellular and humoral immunity disorders, therefore, it is considered as a general immune deficiency disease in piglets (Segales *et al*., 2004; Ferrari *et al*., 2014).

It is still unknown why piglets with PMWS have severe loss of lymphocytes in lymphoid tissues and in the blood. Some human and animal viruses can cause lymphoid tissue apoptosis (Benedict, Banks, & Ware, 2003; Irusta, Chen, & Hardwick, 2003). Apoptosis is a programmed cell death and is developed by activation of specific enzyme caspases (Hengartner, 2000; Lockshin & Zakeri, 2004). However, the latest results reveal that apoptosis has no significant impact on lymphocyte depletion in lymph nodes of pigs infected by PCV2 (Kiupel *et al*., 2005; Resendes *et al*., 2004; Resendes *et al*., 2011).

Thus, the hypothesis of the study was: lymphocyte depletion occurs because of lymphocyte life-cycle delay in pigs infected by PCV2. The activity of cell life-cycle is attributed to protein Ki67, which is found in all active phases of the cell, like interphase G1, S, G2 and mitoses. However, protein Ki67 is not observed at all in the cell's "quiet" phase G0. The aim of our work was to investigate the lymph node cell proliferation activity of pigs infected by PCV2.

Materials and Methods

Five pig farms were investigated in Latvia from 2008 to 2011. The clinical manifestation of PMWS and mortality of piglets was reported in all investigated farms at different mortality ranges. Such PMWS clinical manifestations as weight loss, diarrhea or respiratory disorder, pale or icteric mucouses were observed before the death of piglets. In total, 42 pig carcasses were collected; the age of investigated pigs being that of 5-15 weeks. Two *lnn. inguinales superficiales,* three *lnn. jejunales* and three *lnn. tracheobronchales* were selected from each animal for histological and imunohistochemical testing.

Lymph node samples were fixed by neutral-buffered 10% formalin for 24 h at room temperature

and were processed using a Leica TP1020 automated tissue processor with standart protocol, after that embeded into parafine. Four micron thick sections were cut and floated onto Plus slides. Standart deparaffinization protocol was run for all slides. One slide from each lymph node was stained by hematoxylin and eosin. Four slides from each lymph node were stained by imunohistochemistry for PCV2, CD3, CD79α and Ki67 detection.

PCV2 detection protocol included tissue blocking with 3% hydrogen peroxide for 30 min, after that proteinase K was used for epitope demasking; primary antibody (Ingenasa 36A9) was diluted 1:250 with TBS and albumin; primary antibody was incubated for 45 min at + 4 °C, for 15 hours. Secondary antibody Policlonal Goat-Anti Mouse Immunoglobulin Biotinylated was diluted 1:200 with TBS and applied at room temperature for one hour. Then avidin peroxidase conjugated kit was applied to the tissue slide and incubated for two hours at room temperature. After that the chromogen-substrate (AEC) was added, stained by hematoxylin and covered by mounting media with coverslip.

Microwave was used for CD3, CD79α imunohistochemical staining and Ki67 detection for epitope demasking (incubation for 15 minutes by 350 watts and 7 minutes by 750 watts); after that endogene peroxidase of tissues was blocked with 3% hydrogen peroxide for 10 min. Each slide from one lymph node was applied to by three different primary antibodies. One slide was applied to by primary antibody Monoclonal Mouse Anti-Rat Ki-67Antigen, Clone MIB-5 for Ki67 protein detection, which is a specific protein of cell life activity (Polaček *et al*., 2007; Debeer *et al*., 2013); to the second slide a primary antibody Monoclonal Mouse Anti-Human CD3, Clone F7.2.38 was added for CD3 positive cell visualization, which is a T lymphocyte (Polaček *et al*., 2007; García-Nicolás *et al*., 2015); the third slide was applied to by primary antibody Monoclonal Mouse Anti-Human CD79α, Clone JCB117 for CD79α protein, which is B lymphocyte specific protein (Polaček *et al*., 2007; Debeer *et al*., 2013; García-Nicolás *et al*., 2015). After that all slides were incubated in DakoEnVision polymer-HRP (Policlonal Goat–Anti Mouse Immunoglobulins) at room temperature for 45 minutes. At the end, they were washed by TBS, added chromogen-substrate (DAB), stained by hematoxylin and covered by mounting media with coverslip.

The tissue evaluation was done according to the common system, quantity of PCV2 antigen was expressed by plus system:

- mild quantity of PCV2 antigen (+) was observed in less than 10% of the follicles (Opriessnig, Meng, & Halbur, 2007) or lymph node parenchyma;
- moderate quantity of PCV2 antigen (++) was observed in 10% to 50% of the follicles (Opriessnig, Meng, & Halbur, 2007) or lymph node parenchyma;
- severe quantity of PCV2 antigen (+++) was observed in more than 50% of the follicles (Opriessnig, Meng, & Halbur, 2007) or lymph node parenchyma.

CD3, CD79α and Ki67 positive cells were evaluated in different layers of the lymph node: in the follicles, in paracortex near the follicles, in paracortex near sinuses and in medulla of lymph node. In each layer of lymph node, five randomly selected fields were analyzed. In each field, 100 cells with magnification x400 were counted. A microscope Zeiss Axiolab with cell counting program Zeiss version 4.10 was used.

The average quantity and standard deviation was used for CD3, CD79α and Ki67 positive cells. *T*-test was used to detect significant differences for CD3, CD79α and Ki67 positive cell quantity in different layers of the lymph node with and without PCV2.

To determine the correlation between number of CD3, CD79α and Ki67 positive cells in different layers of the lymph node and quantity of PCV2 antigen, Spearman's rank correlation coefficient of SPSS Statistics version 22 (IBM Corporation, Chicago, Illinois) was used.

Results and Discussion

The study results are summarized in Table 1, where CD3, CD79α and Ki67 positive cell quantity in different layers of the lymph node is with and without PCV2. The correlation between the increasing quantity of PCV2 in lymph node and number of CD79α, CD3 and Ki67 positive cells in the various layers of the lymph node is examined additionally (Table 2).

These results reveal that CD79α positive cells are significantly different in all layers of the lymph node with and without PCV2 antigen (Table 1). Negative correlation of CD79α positive cells was observed with increasing quantity of PCV2 in lymph node, but it is statistically significant only in cortex (r_s = -0.587) and paracortex near follicle (r_s = -0.339) (Table 2). It means that CD79α positive cells are significantly reduced in cortex and paracortex near follicle with increasing quantity of PCV2 in the lymph nodes.

The number of CD3 positive cells in all layers of the lymph node with moderate and severe PCV2 quantity was significantly lower than in the lymph node without PCV2 antigen (Table 1). Besides that, the number of CD3 positive cells in all layers of the lymph node with mild quantity of PCV2 and moderate changes of follicle visualization or unvisible follicles in lymph node was significantly lower than in the lymph node without PCV2 antigen. In lymph nodes without and with PCV2 where were mild changes in

Table 1

Average number of CD79α, CD3 and Ki67 positive cells in lymph nodes with and without PCV2

PCV2 in lymph node	Layers of lymph node	CD79α positive cells ± standard deviation	CD3 positive cells ± standard deviation	Ki67 positive cells ± standard deviation
PCV2 negative	cortex	48.7 ± 8.0 [a]	0.0 ± 0.0[e]	92.8 ± 1.9 [k]
	paracortex near follicule	18.6 ± 5.4 [b]	97.1 ± 1.3[f]	15.2 ± 4.0[l]
	paracortex near sinus	42.9 ± 10.4 [b]	96.7 ± 1.1[g]	0.0 ± 0.0[n]
	medulla	21.9 ± 10.0 [d]	64.8 ± 3.1[h]	19.0 ± 8.9 [m]
PCV2 (+) and mild changes of follicle visualization in lymph node	cortex	32.8 ± 4.3	0.0 ± 0.0	76.3 ± 6.8
	paracortex near follicule	7.9 ± 3.7	100.0 ± 0.0	44.6 ± 3.0 [l]
	paracortex near sinus	0.0 ± 0.0	100.0 ± 0.0	43.3 ± 2.7 [n]
	medulla	1.4 ± 2.2	48.9 ± 3.3	15.4 ± 1.2
PCV2 (+) and moderate changes of follicle visualization in lymph node	cortex	7.4 ± 15.2	31.0 ± 15.7 [e]	52.3 ± 4.2
	paracortex near follicule	5.5 ± 5.3	62.7 ± 19.1 [f]	42.4 ± 2.9[l]
	paracortex near sinus	10.1 ± 5.3	55.3 ± 22.2[g]	40.6 ± 0.7 [n]
	medulla	5.4 ± 1.8	29.3 ± 5.0 [h]	26.3 ± 6.3
PCV2 (+) and follicles were not visible in lymph node	cortex	0.0 ± 0.0	-	-
	paracortex near follicule	3.9 ± 3.9	53.3 ± 7.5 [f]	17.2 ± 3.3
	paracortex near sinus	7.7 ± 1.5	45.6 ± 5.8 [g]	33.7 ± 5.4[n]
	medulla	1.5 ± 1.8	22.0 ± 11.7[h]	17.3 ± 5.8
PCV2 (++)	cortex	3.4 ± 9.1	39.8 ± 17.0[e]	51.2 ± 7.1[k]
	paracortex near follicule	4.3 ± 4.8	58.3 ± 16.5 [f]	25.9 ± 5.7[l]
	paracortex near sinus	7.9 ± 4.8	39.3 ± 19.5 [g]	45.3 ± 12.3[n]
	medulla	0.9 ± 1.6	23.1 ± 9.3 [h]	21.4 ± 3.5
PCV2 (+++)	cortex	0.0 ± 0.0	48.9 ± 9.1 [e]	68.2 ± 7.0[k]
	paracortex near follicule	2.3 ± 4.4	45.9 ± 10.9 [f]	58.1 ± 16.6[l]
	paracortex near sinus	4.0 ± 4.4	32.7 ± 8.7 [g]	71.5 ± 9.0[n]
	medulla	1.7 ± 2.9	17.2 ± 5.1 [h]	32.8 ± 12.6[m]

[a] significant difference between quantity of CD79α positive cells in cortex with and without PCV2 ($p < 0.05$)
[b] significant difference between quantity of CD79α positive cells in paracortex near follicle with and without PCV2 ($p < 0.05$)
[c] significant difference between quantity of CD79α positive cells in paracortex near sinus with and without PCV2 ($p < 0.05$)
[d] significant difference between quantity of CD79α positive cells in medulla with and without PCV2 ($p < 0.05$)
[e] significant difference between quantity of CD3 positive cells in cortex with and without PCV2 ($p < 0.05$)
[f] significant difference between quantity of CD3 positive cells in paracortex near follicle with and without PCV2 ($p < 0.05$)
[g] significant difference between quantity of CD3 positive cells in paracortex near sinus with and without PCV2 ($p < 0.05$)
[h] significant difference between quantity of CD3 positive cells in medulla with and without PCV2 ($p < 0.05$)
[k] significant difference between quantity of Ki67 positive cells in cortex with and without PCV2 ($p < 0.05$)
[l] significant difference between quantity of Ki67 positive cells in paracortex near follicle with and without PCV2 ($p < 0.05$)
[n] significant difference between quantity of Ki67 positive cells in paracortex near sinus with and without PCV2 ($p < 0.05$)
[m] significant difference between quantity of Ki67 positive cells in medulla with and without PCV2 ($p < 0.05$)

follicle visualization, was observed similar quantity of CD3 positive cells and significantly differenet quantity of CD79α positive cells (Table 1). These results show that B lymphocyte population was reduced at first in the lymph node with PCV2, but the number of T lymphocytes decreased later. B lymphocyte provides a humoral immunity, but T lymphocyte – cellular immunity (Zachary & McGavin, 2012). These results reveal that PCV2 impact on the cellular immunity at first and after that causes changes in humoral immunity.

A significant correlation (Table 2) between the increasing number of PCV2 and number of CD3 positive cells in lymph node was detected (in cortex $r_s = 0.737$, in paracortex near follicle $r_s = -0.633$, in paracortex near sinus $r_s = -0.685$, in medulla $r_s = -0.617$). The decrease of CD3 positive cells in paracortex and medulla was caused by the increasing

Table 2

Correlation between the quantity of CD3, CD79α and Ki67 positive cells in different layers of the lymph node and the quantity of PCV2 antigen

Specific cell protein	Correlation parameters	Cortex	Paracortex near follicle	Paracortex near sinus	Medulla
CD79α	r_s	-0.587[b]	-0.339[a]	-0.074	-0.192
	p	0.004	0.018	0.618	0.192
	n	22	48	48	48
CD3	r_s	0.737[b]	-0.633[b]	-0.685[b]	-0.617[b]
	p	0	0	0	0
	n	22	48	48	48
Ki67	r_s	-0.403	0.367[a]	0.711[b]	0.536[b]
	p	0.063	0.010	0	0
	n	22	48	48	48

[a] – correlation significance less than 0.05
[b] – correlation significance less than 0.01

number of PCV2. Furthermore, the increasing number of PCV2 caused the increase of CD3 positive cells in cortex (Table 2). However, the total number of CD3 positive cells was lower in lymph nodes with PCV2 than in lympn nodes without PCV2 (Table 1). T lymphocyte infiltration in cortex depends on follicle damage in cortex. Klausmann *et al*. (2015) study demonstrates that PCV2 strongly impacts T-cell selection processes in other lymphoid organ, – in the thymus.

Ki67 positive cells were of a very high number (92.8 cells) in cortex of lymph nodes without PCV2 (Table 1). The reason for this is a germinal centre in cortex, where cells divide all the time (Brūveris, 2015). The obtained results show that the number of Ki67 positive cells is significantly different in cortex of lymph nodes without PCV2 and with moderate or severe quantity of PCV2, its cells are approximately two times less in lymph nodes with PCV2 (Table 1). It is known that cortex is a B lymphocyte depending area (Brūveris, 2015), therefore, it can be considered that Ki67 positive cells in cortex are mainly B lymphocytes. These results revealed that PCV2 presence in the lymph node significantly decreased B lymphocytes. Other authors report a similar tendency for cell proliferation index in the lymph nodes with and without PCV2 (Lin *et al*., 2011). However, our results show that it does not have a significant correlation between the number of Ki67 positive cells and PCV2 quantity in lymph node (Table 2). It means that PCV2 presence in lymph node significantly decreases B lymphocyte proliferation, but B lymphocyte proliferation does not depend on PCV2 quantity in lymph nodes.

Ki67 positive cells significantly increased in paracortex and medulla depending on PCV2 increase in lymph node (Table 2). Ki67 protein contains all cells, which are active for dividing or are in the dividing process (Booth *et al*., 2014). It is known that paracortex is a T lymphocyte depending area (Brūveris, 2015). Previous study showed that PCV2 increased histiocyte number (Segales *et al*., 2004). Simultaneously, PCV2 decreased T lymphocytes in lymph node (Table 1, Table 2). It could be concluded that histiocytes were Ki67 positive cells in paracortex and medulla with PCV2. In another study it was found that the proliferation activity of blood monocytes and monocyte-derived macrophages (some transformation form of histiocyte) was significantly enhanced by PCV2 (Tsai *et al*., 2010).

Ki67 protein is present in all active phases of cell life cycle: interphase G1, S, G2 and mitosis. However, protein Ki67 is not observed at all in the cell's "quiet" phase G0 (Scholzen & Gerdes, 2000). Thereby, our study showed that PCV2 did not stop B lymphocyte life cycle, but PCV2 stimulated these cells to come directly to inactive form (G0 phase). A cell in G0 phase can stay for a long time, but if it is necessary, the cell is able to return again in the active phases. However, cells in G0 phase may also become old and never renew their life cycle (Scholzen & Gerdes, 2000). Quan *et al.* (2016) *in vitro* study shows that G0/G1 cell cycle arrest induced by PCV2 may provide favourable conditions for viral protein expression and virus replication.

Currently, the question remains: does PCV2 delay cell cycle in G0 phase, or does PCV2 cause DNA damage, thereby, accelerating aging process of cells? Therefore, investigations *in vitro* by cell culture are necessary to understand PCV2 impact on the cell life cycle. It is not clear how PCV2 infection involve PCV2-mediated cell cycle arrest and contributes to virus replication.

In vitro study shows, that PCV2 impacts the increase of cytosolic Ca^{+2} in cells (Gu *et al*., 2016). This means that PCV2 possibly causes cell necrosis because of Ca^{+2} ion increase in the cytoplasm to activate several enzymes (endonuclease, protease, ATPase), which leads to cell death (Zachary & McGavin, 2012). If PCV2 simultaneously impacts the ability of cell proliferation and cell necrosis, this can explain such a severe immunodeficiency and lymphopenia.

The pathogenesis of the virus still remains unresolved question. For example, why one of pigs remains healthy in the presence of PCV2 and others go on to develop clinical disease that typically results in death.

Conclusion

Morphological changes were detected in all layers of lymph nodes in pigs with PMWS. Analyzing the loss of lymphocytes in the lymph nodes in piglets with PMWS, we concluded that B lymphocyte population had been reduced at first in the lymph node with PCV2, but the number of T lymphocytes decreased later. PCV2 decreased B lymphocyte prolifiration activity. However, PCV2 increased cells' prolifiration activity in paracortex and medulla of lymph nodes, - it is could be associated with histiocytes infiltration in lymph nodes.

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METHANE MITIGATION POSSIBILITIES AND WEIGHT GAIN IN CALVES FED WITH PREBIOTIC INULIN

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Abstract
Methane is considered to be the main greenhouse gas (GHG) emitted by livestock. One method for reducing methane emissions from ruminants is to improve production efficiency, which reduces methane emissions per unit of product (FAO, 2010; Gworgwor, Mbahi, & Yakubu, 2006). There are many researches about prebiotics which can reduce methane production in livestock, for example, galacto-oligosaccharides reduced methane emission up to 11% (liters/day) (Zhou *et al*., 2004). There is almost no information about prebiotic inulin, so the aim of this research was to determine the impact of different dosages of inulin concentrate (50%) on the increase of calves' body weight and its impact on methane emission, as well as to find out how the results change if it is added to barley flour not to milk as in our previous research. Approximately fifty days old, clinically healthy, different Holstein Friesian crossbreed calves kept in groups of 8, in a partly closed space with a natural ventilation through windows were included in this research. Eight calves were in the control group (CoG) and sixteen received inulin (Pre12 (n = 8), Pre24 (n = 8)). At the beginning of the experiment – the 28th and 56th day - we determined each calf's weight and measured the methane level in the rumen by using the PICARROG-2508 gas analyser (Fleck, 2013). We concluded that inulin supplement significantly ($p \leq 0.05$) increased the live weight gain comparing Pre24 and CoG. The highest methane production on 1 kg of body weight at the end of the research was detected in Pre24 – 1.24 mg m^{-3} and the lowest in CoG – 0.99 mg m^{-3}.
Key words: calves, greenhouse gas emission, inulin, weight gain.

Introduction

Climate changes come along with new challenges that are critical in agricultural production both in Latvia and the whole world. After regaining independence, Latvia takes an active part in reducing the negative climate changes in the world. However, since the year 2005 due to the rise of economic activities in Latvia, the greenhouse gas (GHG) production in the agricultural sector shows a progressive tendency, furthermore, the agricultural sector is the second largest source of GHG, creating 20% of all GHG in the country. (Bērziņa *et al*., 2014) According to the collected GHG data about the situation in Latvia in the agricultural sector, the main sources of GHG are: 1) agricultural soil management nitric oxide (N_2O); 2) ruminant enteric fermentation where methane (CH_4) is produced due to biological food processing and fermentative processes; 3) methane (CH_4) and nitric oxide (N_2O) production from manure (Bērziņa *et al*., 2014; Ilgaza *et al*., 2016).

Globally, 50-60% of methane emissions are from the agricultural sector, specifically from livestock production operations; the principal source of methane is from ruminant animals (McMichael *et al*., 2007). Digestive processes happen mainly in anaerobic conditions, and microorganisms have a huge role in these processes. Enteric fermentation from livestock is a large source of methane, which has a global warming potential 23 times of carbon dioxide (Bhatta *et al*., 2007; Loh *et al*., 2008). The ruminant stomach consists of 4 compartments and the rumen is the main place of methane production. Approximately 90% of the whole methane are produced there, the rest of it is produced in the large intestines. Almost all methane (89%) emitted from ruminants is produced in the rumen and exhaled through the mouth and nose (Murray *et al*., 1999).

One way of decreasing GHG is to use a specific methane inhibitor 3NOP (3-nitrooxypropanol) that has no significant impact on cows: feed intake, fibre digestibility and milk production. Reseachers have also noticed the increase in milk protein and lactose level in milk. The emitted methane was reduced by 30% (Hristov *et al*., 2015). In other studies, researchers reached the methane reduction by 20%, at the same time, the weight gain increased by additional 75g/day, and milk yield increased by 1l/day in dairy cattle (Bruinenberg *et al*., 2002; Ilgaza *et al*., 2016; Nkrumah *et al*., 2006). It means that with a significant reduction of CH_4 emission the animal productivity can be improved, but we need to find the optimal feeding recipe which would be compatible with conditions and available feed sources in Latvia, taking into account economic factors and the obtained production. The other way to decrease GHG is to reduce the use of dairy cattle for beef production. Therefore, it is advisable to slaughter those calves which are not used for herd reproduction before they have become full ruminants, that is - before the emission of gases from the rumen into the surrounding environment has not significantly increased. However, producers in order to get more valuable production from one animal grow animals longer till they reach a higher live weight. We consider that a shorter animal growing period can be achieved by making corrections in the animal feeding strategy and by using natural feed supplements which

can promote animal interior body reserve usage. Prebiotics can increase daily weight gain in calves and are used along with nitrate, probiotics, and yeasts and have a potential to reduce methane production (Mwenya *et al*., 2004; Hasunuma *et al*., 2011; Ilgaza *et al*., 2016). Such prebiotics as fructo-oligosaccharides (FOS), inulin and galacto-oligosaccharides (GOS) can be used to reduce methane production in ruminants (Zhou *et al*., 2004; Charalampopoulos & Rastall, 2009). But there are fewer studies done about FOS and inulin (both are components of Jerusalem artichoke) and their impact on methane reduction in ruminants (Roonal, 2015), therefore we used inulin for our research. In the previous study by Ilgaza *et al*. (2016), milk supplementation with inulin had very promising results regarding weight gain when animals in the experimental group reached 90kg weight 3 weeks earlier than the control group; also the live weight gain was higher in calves fed with additional supplement of inulin.

In newborn ruminants the oesophageal groove is present. It is a channel that takes milk from the oesophagus into the abomasum, bypassing the rumen, reticulum and omasum. Oesophageal groove practically disappears in the fifth month of the calf's life (Millen *et al*., 2016).

In this research, the way of inulin supplementation was changed to see if the results differ from our previous study. If inulin is added to fodder, it passes directly to the rumen and not abomasum because the oesophageal groove facilitates only the movement of liquid. The aim of this study was to repeat the research with inulin and evaluate how it impacts weight gain in a dairy farm of intensive production by using two doses of inulin added to barley flour which showed the best results in the previous research, and also how this inulin supplementation impacts methane production in calves' rumen.

Materials and Methods

The research was conducted with clinically healthy different Holstein Friesian crossbreed calves. Their average age was 49 ± 10 days and average weight was 79.6 ± 12.82 kg. They were kept in groups of 8 calves in a partly closed space with a natural ventilation through windows. Before the research, they all had received the same feed and were kept in the same conditions.

Eight calves were in the control group (CoG) and sixteen were fed with an additional prebiotic – specially produced flour of Jerusalem artichoke, organized in groups Pre12 (n = 8), Pre24 (n = 8). The study included prebiotic inulin concentrate flour of Jerusalem artichoke (50%) produced in Latvia by using special technologies. The flour of Jerusalem artichoke usually contains 10% of inulin, but it is possible to increase its amount up to 48.5% – 50% (Fleming *et al*., 1979; Valdovska *et al*., 2012; Ilgaza *et al*., 2016). Calves from group Pre12 were fed with additional 12 g flour of Jerusalem artichoke (containing 6 g of inulin), group Pre24 – 24g of flour (containing 12 g of inulin). The prebiotic was added to barley flour once a day in the evenings. All calves had free access to water and hay and they also got whole milk and a calf starter meal (Ilgaza *et al*., 2016).

The length of the research was 56 days. On the 1st, 28th and 56th day we determined each calf's weight by using the weight measuring tape and determined the amount of methane in the rumen by using a mobile PICARROG-2508 gas analyser (Fleck, 2013). We acquired the gas from the rumen by puncturing it through the abdominal wall by using a 14G needle and a 20 ml syringe. The gas was evaluated as soon as it was obtained to reduce the possibility that it can mix with the surrounding air. For statistical analysis of all obtained data we used the computer programme MS Excel 2013. The analysis was conducted by applying the statistical methods to calculate mean values and

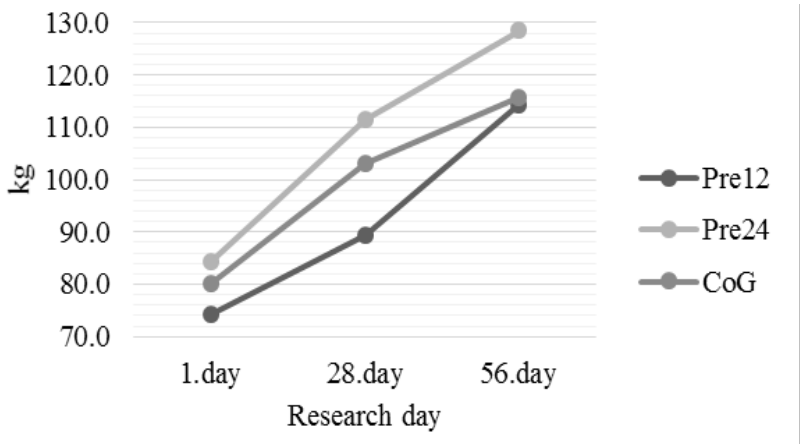

Figure 1. The increase of live weight of calves during the research.

Table 1

Growth Performance of Calves

Groups	Average live weight gain (kg and %), 1st – 28th research day	Average live weight gain (kg and %), 28th – 56th research day	Average live weight gain (kg and %), 1st – 56th research day	Average daily weight gain (kg d^{-1})
CoG	22.9 ± 3.80 (+ 28.4%)	12.5 ± 9.77 (+ 12.1%)	35.4 ± 7.73 (+ 44.0%)	0.6 ± 0.14
Pre12	15.1 ± 4.19 (+ 20.3%)	24.9 ± 6.92 (+ 27.8%)	40 ± 6.39 (+ 53.8%)	0.7 ± 0.11
Pre24	27.4 ± 10.60 (+ 32.4%)	16.8 ± 6.36 (+ 15.1%)	44.1 ± 11.15 (+ 52.3%)	0.8 ± 0.20

standard deviation. The significance was determined by the t-test; $p \leq 0.05$ means that difference is significant.

Results and Discussion

Our first aim of this research was to find out if the inulin supplement, which is added to barley flour, can speed up the growth rate of calves. On the first day of the research, the median weight of all calves was without significant (p value > 0.05) differences. On the 28th day of the research, the median weight of Pre12 calves was significantly lower ($p \leq 0.01$) than the median weight of Pre24 calves (89.5 ± 7.29 kg and 111.6 ± 19.91 kg, respectively) (Fig. 1).

On the 28th day of the research, the median weight of Pre12 calves was lower than in CoG calves. We cannot explain this slower weight gain in group Pre12, but it could be due to some health problems like diarrhoea and respiratory diseases as these problems occurred time after time in calves during the research. On the 56th day of the research, the median weights between the groups were very similar, in Pre12 it was 114.4 ± 12.37 kg and in CoG – 115.6 ± 7.11 kg. The final weight of Pre12 was similar to CoG weight because of better daily weight gain, and the prebiotic could positively impact this increase of daily weight in Pre12.

At the end of the research, on the 56th day, there was a significant difference in the average weight between Pre12 and Pre24 ($p \leq 0.05$), as well as between Pre24 and CoG ($p \leq 0.05$) (respectively 114.4 ± 12.37 kg; 128.4 ± 18.8 kg and 128.4 ± 18.8 kg; 115.6 ± 7.11 kg).

It was essential to determine the average live weight gain in each period of our research (1st – 28th day; 28th – 56th day and 1st – 56th day). The results in kilograms and percentages are summarized in Table 1.

There was a significant difference in the average live weight gain between Pre12 and Pre24 and between Pre12 and CoG in both research periods: 1st-28th day ($p \leq 0.01$) and 28th-56th day ($p \leq 0.05$). The average live weight gain at the end of the research was the highest in Pre24 (44.1 ± 11.15 kg), but the lowest in CoG (35.4 ± 7.73 kg). The increase of the live weigh expressed in percentage at the end of the research was in CoG - 44.0%, in Pre12 – 53.8% and in Pre24 – 52.3%. We can conclude that groups fed with inulin supplement showed much better increase of the live weight compared to the control group. These results conform to the results of other researchers who found that inclusion of inulin in the milk replacer of pre-ruminant calves leads to significantly higher live weight gains and better faecal consistency (Kaufhold *et al*., 2000; Verdonk &Van Leeuwen, 2004).

The foregut and hindgut houses millions of diverse groups of microflora, namely, bacteria, fungi, yeasts, phage particles, archaea etc. Prebiotics are fermented by a number of rumen bacteria for its utilization as a source of energy (Öztürk, 2008; Kaufhold *et al*., 2000; Cota &Whitefield, 1998; Samanta *et al*., 2013). Also anaerobic fungi (*Neocallimastix* stains) can metabolize inulin in the rumen (Fonty, 1991).

The lowest average daily weight gain was noticed in CoG (0.6 ± 0.14 kg d^{-1}). It was higher in groups fed with inulin supplement, in group Pre12 0.7 ± 0.11 kg d^{-1} and in group Pre24 0.8 ± 0.20 kg d^{-1}, respectively. The results of t-test showed that calves of group Pre24 fed with 24 g of prebiotics did not give a significantly higher average daily weight gain compared to group Pre12 (12 g of prebiotics) and CoG. Our results showed that the double dose of inulin did not give a significantly higher average daily weight gain.

In the study by Ilgaza *et al*. (2016) where inulin was added to milk (12 g and 24 g), the results showed that inulin increased the average daily weight gain. The highest average live weight gain during the whole study was in Pre24 group, and similar results were obtained also in this experiment.

Our second aim of this research was to determine if inulin supplement reduces methane production. We set this aim because many researchers have investigated the possible impact of prebiotics on reducing GHE. For example, the administration of galacto-oligosaccharides (GOS) supplementation decreased nitrite accumulation in the rumen and plasma and nitrate-induced methaemoglobin, while retaining low methane production. 11% reduction in methane emission (litres/day) in GOS supplemented diet compared to the control diet has been reported (Zhou *et al*., 2004). Other research also indicates that GOS are efficacious in reducing methane production in

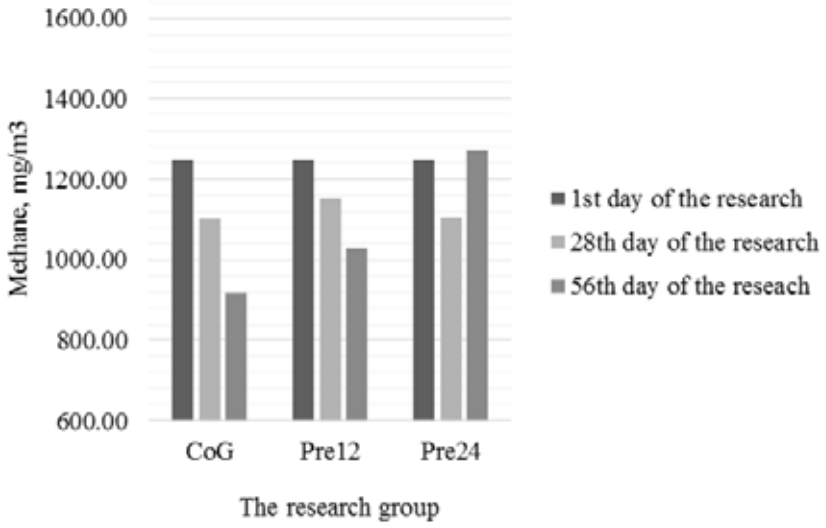

Figure 2. The concentration of methane in the rumen by groups during the whole research.

dairy cows. Supplementation of dairy cows with GOS resulted in an 11% reduction in methane production (Charalampopoulos & Rastall, 2009). Inulin, FOS and GOS are called prebiotics and can reduce methane production, but there are fewer studies done about inulin and FOS (both are components of Jerusalem artichoke) and their impact on methane reduction in ruminants (Roonal, 2015), therefore we used inulin for our research. To reduce greenhouse gas emission, researchers also propose to shorten the period of breeding calves of dairy breeds (Mirzaei-Aghsaghali *et al*., 2015; FAO, 2010; Ilgaza *et al*., 2016).

In our research, we noticed that in CoG calves the median amount of methane in the rumen showed a tendency to get lower, and the same process was noticed in calves of group Pre12, while in group Pre24 the methane concentration in the rumen at the end of the research increased (Fig. 2).

The amount of methane in the calves' rumen at the end of the research in group Pre24 was the highest – 1,273.0 ± 317.60 mg m^{-3}, but in group CoG the lowest – 917.5 ± 217.30 mg m^{-3}. By using t-test we found out that this difference was significant ($p \leq 0.05$). We can conclude that feeding the chosen concentrations of inulin supplement promote the production of methane in the rumen. This did not match to our thoughts and to results of some other authors about inulin and FOS that these prebiotics can reduce methane production in ruminants (Zhou *et al*., 2004; Charalampopoulos & Rastall, 2009; Roonal, 2015). The efficiency of prebiotics of other group (GOS) has been proved in many studies mentioned before. More research is needed about inulin and FOS and their possible impact on reduction of methane emission in ruminants.

The highest methane production on 1 kg body weight at the end of the research on day 56 was noticed in Pre24 – 1.24 mg m^{-3}, less in Pre12 – 1.12 mg m^{-3}, and the lowest it was in CoG – 0.99 mg m^{-3}.

Although other authors have reported that prebiotics can impact methane production by lowering it, we did not find any reduction of methane production in the rumen in calves fed with barley flour and inulin (Zhou *et al*., 2004; Charalampopoulos & Rastall, 2009). We noticed that the higher the dose of inulin, the higher the methane production on 1kg of body weight.

Our next aim is to repeat the research in one farm with calves which receive inulin with whole milk and calves which receive this prebiotic with fodder, and to compare the results since in these feeding strategies inulin gets into different stomach compartments.

Conclusions

1. Calves of group Pre24 fed with 24 g of prebiotics did not show a significantly higher average daily weight gain in comparison with Pre12 (12 g of prebiotics) and CoG. But at the end of the research, on the 56th day there was a significant difference in the average weight between Pre12 and Pre24 ($p \leq 0.05$), as well as between Pre24 and CoG ($p \leq 0.05$).
2. The amount of methane in the calves' rumen at the end of the research was the highest in group Pre24 – 1,273.0 ± 317.60 mg m^{-3}, but the lowest in group CoG – 917.5 ± 217.30 mg m^{-3}; this difference was significant ($p \leq 0.05$). We can conclude that feeding the chosen concentrations of inulin supplement promote the production of methane in the rumen.
3. The highest methane production on 1 kg body weight at the end of the research was found in Pre24, but the lowest was in CoG, so the higher the chosen concentration of inulin, the higher the methane production on 1kg of body weight.

Acknowledgements

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ANTIMICROBIAL RESISTANCE OF *AEROMONAS SPP.* ISOLATED FROM THE SEA TROUT (*SALMO TRUTTA* L.) IN LATVIA

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Abstract
The aim of this study was to investigate the patterns of antibiotic resistance of *Aeromonas spp.* bacteria isolated from the sea trout (*Salmo trutta*) from the state fish hatcheries of the Institute of Food Safety, Animal Health and Environment 'BIOR', Latvia.
Bacteriological investigations were performed at four state fish hatcheries located in the drainage basins of the main Latvian rivers – Daugava, Venta and Gauja, during the five-year period (2012 – 2016). In fish with visible clinical signs, bacteriological samples were collected from heart, liver, spleen, kidney and ulcer surfaces.
Aeromonas hydrophila and *Aeromonas salmonicida* were isolated from sea trouts. A total of 52 individual sea trouts were examined.
Resistance to amoxicillin, ampicillin, cephalexin, colistin, doxycycline, enrofloxacin, erythromycin, florfenicol, gentamycin, kanamycin, lincomycin, neomycin, oxytetracycline, spectinomycin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole was tested.
The results of this study suggest a multi-drug resistance pattern among the *A. hydrophila* isolates. All the isolates were resistant to amoxicillin (100%), ampicillin (100%), cephalexin (100%) and erythromycin (100%). The lowest level of resistance was found against florfenicol (4.55%), gentamycin (4.55%), kanamycin (4.55%), but susceptibility was recorded to enrofloxacin, neomycin and trimethoprim/sulfamethoxazole. *A. salmonicida* isolates were resistant to oxytetracycline (9.38%) and tetracycline (9.38%). For other antibiotics *A. salmonicida* isolates were susceptible.
Key words: *Aeromonas spp*., antibiotics resistance, sea trout, aquaculture, fish pathogen.

Introduction

Aquaculture is one of the fastest developing sectors in the global food industry (FAO, 2016). A new record was reached on the quantity of fish consumption - it has risen to above 20 kg per capita in 2014. Currently, aquaculture produces half of the all fish consumed by humans (FAO, 2016).

The most significant diseases in fish hatcheries are caused by bacteria (Briede, 2010). The *Aeromonas spp.* are the dominant fish bacterial infectious agents in Europe, as well as in Latvia, which causes economically significant losses in cultivated salmonids in fresh and marine waters (Briede, 2010; Toranzo, Magarinos, & Romalde, 2005). It also affects a variety of non-salmonid fish and shows a widespread distribution (Briede, 2010; Toranzo, Magarinos, & Romalde, 2005). Due to inadequate personal hygiene and poor quality of water, mainly in developing countries in different parts of the world, *Aeromonas spp*. have been associated both to food-borne and water-borne diseases (Ahmad & Odeyemi, 2014; Odeyemi & Ahmad, 2017).

Aeromonas spp. are ubiquitous bacteria in terrestrial and aquatic milieus (Igbinosa *et al*., 2012). In salmonids, they are renowned as enteric pathogens causing hemorrhagic septicemia, fin rot, soft tissue rot and furunculosis resulting in major die-offs and fish kills (Fečkaninová *et al*., 2017).

A. salmonicida mainly affects salmonid fish, causing high mortality rate in the Atlantic salmon (*Salmo salar)* and the rainbow trout (*Oncorhynchus mykis)* (Fernández-Álvareza *et al*., 2016). In salmonids, different clinical presentations of the disease, varying from acute to chronic as well as subclinical form, have been described (Coscelli *et al*., 2014). According to statistics, the mortality caused by this disease can reach a high of nearly 100%, resulting in heavy marked-economic losses (Liu, 2017).

Motile aeromonad infection is probably the most common bacterial disease of freshwater fish (Fečkaninová *et al*., 2017). By far the most important fish pathogen is *A. hydrophila.* Clinical signs of *A. hydrophila* infection range from superficial to deep skin lesions, to a typical, gram-negative bacterial septicemia, with or without skin lesions. The disease is often associated with serious damage and economic losses in rainbow trout farming industry (Fečkaninová *et al*., 2017).

Indiscriminate and comprehensive use of antibiotics to prevent and treat bacterial diseases and the application of subtherapeutic dose of antibiotics have induced a global increase in antibiotic resistance among pathogenic bacteria (Hernández Serrano, 2005; Vivekanandhan *et al*., 2002). Therefore, the aim of this study was to investigate antibiotic resistance patterns of *Aeromonas spp.* isolated from the sea trout (*Salmo trutta*) from the state fish hatcheries.

Materials and Methods

Study site and sampling

Investigations were performed in four fish hatcheries of the Institute of Food Safety, Animal

Health and Environment 'BIOR' in the main Latvian river drainage basins of the Daugava, Venta and Gauja during the five year period (2012 – 2016). In all these hatcheries fish are reared in the flow-through systems. Sea trout of different age groups were used in our research: fry, one year old parr and smolt (n = 52). Live sea trout was brought to the Animal Disease Diagnostic Laboratory of the Institute of Food Safety, Animal Health and Environment 'BIOR'. Sampling was done 9 times during the 5-year period: in May 2012, 2 times in June 2014, August 2015, 2 times in April, June, August and September 2016.

For processing the received data, the percentage deductions were used.

Isolation and identification of the bacteria

The material for bacteriological investigations from ulcers of the skin and from the internal organs: liver, kidney, heart and spleen, aseptically were sampled from fishes with the clinical signs. Samples were cultured, according to standard bacteriological method by direct cultivation on the double set of plates with a culture medium consisting of Tryptone soya agar, Blood agar and MacConkey agar (Biolife, Italy). The plates were incubated in aerobic conditions: one set of plates at 22 °C and the other set at 37 °C for 24 – 48 h.

The microorganisms that showed typical colony morphology of *Aeromonas spp*. were sub-cultured on double set of plates with Tryptone soya agar, Blood agar and MacConkey agar, respectively, incubated at 22 °C and 37 °C in the aerobic conditions for 24 – 48 h, until the pure culture with homogenous colonies was obtained.

Identification of *Aeromonas* species was based on the colony morphology, microscopic appearance, a positive oxidase and catalase reactions, growth on MacConkey agar and fermentation of carbohydrates. For confirmation and species identification of *Aeromonas spp.,* a standardized system for the identification of non-fastidious and non-enteric Gram-negative rods – API 20NE – was applied (BioMérieux, France).

Antimicrobial susceptibility testing

One isolate of *Aeromonas spp.* from each sample that had been identified to the species level was selected for the determination of antimicrobial susceptibility. The antimicrobial susceptibility testing was performed according to the standard of Clinical and Laboratory Standards Institute (Miller *et al*., 2010) using the disk diffusion method. The following antimicrobial paper disks (Bio-Rad, USA) impregnated with a defined concentration of antimicrobial agent were used to determine the antimicrobial susceptibility of the isolates: Amoxicillin (25µg), Doxycycline (30µg), Erythromycin (15µg), Florfenicol (30µg), Gentamycin (10µg), Kanamycin (30µg), Lincomycin (15µg), Neomycin (30 IU), Oxytetracycline (30µg), Spectinomycin (100µg), Streptomycin (10µg) and Tetracycline (30µg).

For antimicrobial susceptibility testing the 2-3 bacterial colonies from Tryptone soya agar with a loopful (1µl) were transferred and suspended in 5 ml Tryptic-soya broth (Biolife, Italy). The density of the bacterial suspension, according to MacFarland 0.5, was controlled using a densitometer (Sensititre, TREK Diagnostic Systems Ltd.). The adjusted inoculum suspension was streaked on Mueller Hinton agar plates (Ø90 mm) using a sterile cotton swab on an applicator. After 3-5 minutes, when excess surface moisture had been absorbed by agar, the antimicrobial disks were positioned on the inoculated agar surface using 6-Disk dispenser (Bio-Rad, USA). The plates were incubated for 24 h, respectively, for the detection of antimicrobial resistance of *A. salmonicida* at 22 °C for and for *A. hydrophila* at 37 °C. The diameter of the inhibition zones was measured (mm), after the incubation and expounded as susceptible, intermediate or resistant according to the CLSI breakpoints and the manufacturer instructions.

Results and Discussion

The investigations from 2012 to 2016 demonstrated that the sea trouts from the free river drainage basins have been exposed to *Aeromonas spp.* (Table 1). Most often we isolated *A. salmonicida* (42.31%, Figure 1). In the case of *A. hydrophila,* the prevalence was 23.08%. The mixed infections by *A. hydrophila* and *A. salmonicida* were observed in 19.23% of the examined fish. In 15.38% *Aeromonas spp*. were not detected.

Briede (2010) reported *Flexibacter spp., A. salmonicida* and *A. hydrophila,* as the main isolated bacteria groups from fishes with the clinical manifestations of disease in Latvia. Similar results were obtained by Medne & Liepins (2004). Aeromonosis of the sea trout has been ascertained in the state fish hatcheries on the Daugava and Gauja river drainage basins. The bacteriological studies demonstrated that fish sampled from the Gauja and Daugava river drainage basins showed more affirmative results for Aeromonads. It may be occasionally that the Daugava and Gauja river drainage basins were more polluted by bacteria that can induce the disease (Briede, 2010). In the Daugava river there were three salmonid fish hatcheries downstream one another which supposedly allowed the infection to travel between hatcheries, causing the disease outbreaks even in the most distant hatchery (Briede, 2010).

The disease outbreaks occurred more often in the spring – summer period when the water temperature

Table 1

Positive results for *A. hydrophila* and *A. salmonicida* in sea trout from river drainage basins

	River basin					
	Daugava	Venta		Gauja		
Year	*A. salmonicida* (the number of examined fish, percentage)	*A. hydrophila*	*A. salmonicida*	*A. hydrophila*	*A. hydrophila*+ *A. salmonicida*	No *Aeromonas spp.* detected
2012					1 (n = 10, 19.23%)	
2014	2 (n = 14, 26.92%)					
2015		1 (n = 1, 1.92%)				
2016	1 (n = 3, 5.76%)		1 (n = 5, 9.62%)	2 (n = 11, 21.15%)		1 (n = 8, 15.38%)
Total	9 samplings (n = 52, 100%)					

was above + 20 °C (Medne & Liepins, 2004; Briede, 2010).

The percentage of *A. hydrophila* isolates demonstrating resistance against specific antibiotic is given in Table 2. The obtained result revealed a high level of multi antibiotic resistance among the isolates. *A. hydrophila* were resistant to amoxicillin, ampicillin, cephalexin and erythromycin.

Resistance to β-lactam antibiotics also was reported by Odeyemi & Ahmad (2017) and Vivekanandhan *et al*. (2002). They claimed that all *A. hydrophila* isolates of fish were resistant to ampicillin and methicillin. Resistance to these drugs is chromosomally mediated, but is sometimes caused by plasmids or integrons. The frequent use of antibiotics to treat *Aeromonas* infections often results in the increased levels of antimicrobial drug resistance (Strateva, Olumide, & Odeyemi, 2016).

In our research more than 90% of the *A. hydrophila* isolates were resistant to lincomycin and oxytetracycline. Half of the isolates were resistant to tetracycline. Different authors reported about *A. hydrophila* tetracycline resistant strains (Vivekanandhan, 2002; Penders & Stobberingh, 2008). In some previous studies on antibiotic resistance in aquaculture found frequencies of oxytetracycline-resistant *Aeromonas spp*. similar to our results: rainbow trout in Denmark (oxytetracycline resistance found in 69% cases); catfish in the USA (tetracycline resistance found in 46% cases); and catfish in the USA (oxytetracycline resistance as high as 62%; tetracycline resistance 43%) (Penders & Stobberingh, 2008).

Kanamycin and neomycin were the other antibiotics, to which a low frequency of resistance was noticed (Table 2). In contrast, Vivekanandhan *et al*. (2002) observed a high frequency of *Aeromonas spp.* antibiotic resistant strains.

4.55% of *A. hydrophila* strains from the sea trout were established resistant to gentamycin.

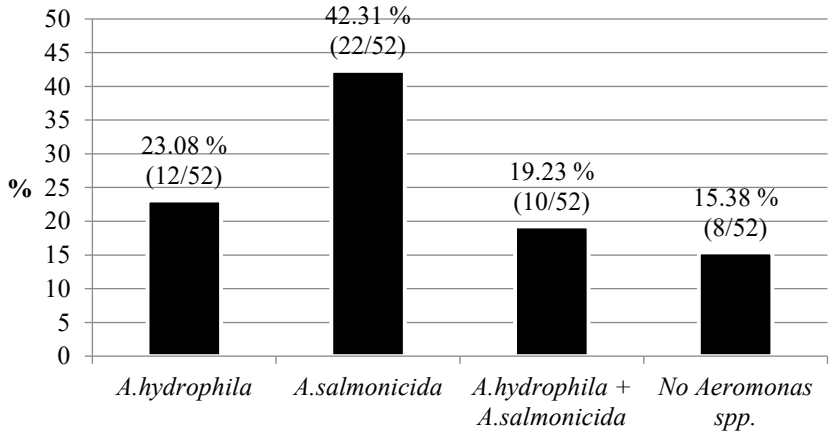

Figure 1. Relative occurrence of *Aeromonas spp*.

Table 2

Frequency of antibiotic resistant *A. hydrophila* and *A. salmonicida* isolates from the sea trout

Antibiotics	*A. hydrophila* (n = 22)	*A. salmonicida* (n = 32)
Amoxicillin	100.0	-
Ampicillin	100.0	0
Cephalexin	100.0	0
Colistin	-	0
Doxycycline	50.0	0
Enrofloxacin	0	0
Erythromycin	100.0	-
Florfenicol	4.55	0
Gentamycin	4.55	0
Kanamycin	4.55	0
Lincomycin	91.67	-
Neomycin	0	0
Oxytetracycline	91.67	9.38
Spectinomycin	9.09	0
Streptomycin	10.0	0
Tetracycline	50.0	9.38
Trimethoprim-sulfamethoxazole	0	0

Vivekanandhan *et al*. (2002) reported that 8.2% of the *A. hydrophila* strains isolated from fish with the clinical signs and healthy fish demonstrated resistance to this antibiotic, which was not considerably higher than the resistance found in our research (Vivekanandhan *et al*., 2002).

The least resistance was observed to florfenicol (4.55%), gentamycin (4.55%), kanamycin (4.55%) spectinomycin (9.09%) and streptomycin (10%). Similar results were obtained by Odeyemi *et al.* (2017), who reported that the least resistance was to aminoglycosides. All of *A. hydrophila* isolates were susceptible to enrofloxacin, neomycin, trimethoprim/sulfamethoxazole.

The frequency of *A. salmonicida* resistance against each antibiotic is shown in Table 2. *A. salmonicida* showed the prevalence of resistance to oxytetracycline (9.38%) and tetracycline (9.38%). In regard to other antibiotics *A. salmonicida* isolates were susceptible.

Nowadays an increase in the levels of resistance of *A. hydrophila* strains to commonly used antibacterial agents (penicillins and cephalosporins) has been observed (Vivekanandhan *et al*., 2002). It is believed that the greatest potential risk to public health associated with the use of antimicrobial agents in aquaculture is the creation of a reservoir of transferable resistant genes in bacteria in aquatic environments from which such genes can be spread by horizontal transfer of genes to other bacteria and ultimately reach human pathogens (Smith, 2008). This is confirmed by other studies on antibiotic resistance of *Aeromonas spp*. (Hernández Serrano, 2005).

As a result of the growing realization that antibiotics should be used with greater caution, there is a trend towards stricter antibiotic regulations in the aquaculture sector and the presence of antibiotic residues in aquaculture products. In some countries (specifically Europe, North America and Japan), regulations on the use of antibiotics are strict and only a few antibiotics are licensed for the use in aquaculture (Defoirdt, 2011).

It is therefore essential to conduct further surveillance and control on bacterial resistance in fish. There is a need for further study on antibiotic resistance to Aeromonads from other salmonids.

Conclusions

The results of the research show that in the state fish hatcheries of the Institute of Food Safety, Animal Health and Environment 'BIOR', Latvia *Aeromonas spp*. are present. *A. hydrophila* 23.08% and *A. salmonicida* 42.31% were isolated most often. The results confirm the presence of multi antibiotic resistant *Aeromonas spp*. isolated from the sea trout in Latvia. *A. hydrophila* were resistant to amoxicillin, ampicillin, cephalexin and erythromycin. *A. salmonicida* showed resistance to oxytetracycline (9.38%) and tetracycline (9.38%).

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CLINICAL SYMPTOMS AND SEX STEROID MEASUREMENTS IN DOMESTIC FERRETS *(MUSTELA PUTORIUS FURO)* WITH HYPERADRENOCORTICISM

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Abstract
Hyperadrenocorticism (excessive adrenal production of sex steroids related to adrenocortical adenomas and carcinomas) is a common disease in neutered male and female pet ferrets (*Mustela putorius furo*) and causes significant morbidity. Incidence of ferret adrenal disease in Latvia is high because the majority of them are gonadectomized. The most common clinical symptoms include symmetrical alopecia and recurrence of sexual behavior. Sex steroids used to diagnose disease are estradiol, androstenedione and 17α-OH progesterone. It is mentioned that androstenedione is the most sensitive during development of the disease. The aim of this study was to sum up, evaluate and compare clinical findings and blood hormone measurements in ferrets diagnosed with hyperadrenocorticism. Forty two ferrets (n = 20 female, n = 22 male) with various clinical symptoms related to hyperadrenocorticism were included in this research. Clinical examination based on clinical symptom protocol was performed and blood samples were taken in all cases. There is a high correlation between the age of neutering and onset of clinical signs in female ferrets. In both sexes, there is no correlation between clinical signs and levels of sex steroids. In male and female group, symmetrical alopecia, pruritus, scaling and fragility of skin and lethargy are the most common findings. During this research, a ranking system (from 1 to 4) for the severity of alopecia was developed. All ferrets had one or two elevated sex steroid levels. In our research, androstenedione was elevated in 25 cases out of 42. Clinical signs and sex steroid measurements are important in diagnosing hyperadrenocorticism in ferrets.
Key words: Adrenal Disease, Alopecia, Lethargy, Pruritus, Sex steroids, Androstenedione.

Introduction

Adrenocortical disease has been recognized in many countries all over the world. It is seen in middle-aged to older ferrets (Carpenter & Quesenberry, 2012). Adrenocortical neoplasms are common in certain gonadectomized animals, including ferrets. These tumors may produce ectopic sex steroids that cause significant morbidity (Bielinska *et al*., 2006; Bielinska *et al*., 2009; Schillebeeck *et al*., 2015; Beuschlein, Galac, & Wilson, 2012). Chronic elevation in circulating luteinizing hormone (LH) that follows gonadectomy is a reason for neoplastic transformation in the adrenal glands (de Jong *et al*., 2014). Furthermore, this transformation indicates abnormal hormonal levels causing the appearance of various clinical symptoms. In adult ferrets, LH receptors have been found not only in gonadal organ testicles and ovaries, but also in adrenal gland cortical part, which means that adrenal glands are taking part in the heat cycle regulation (Schoemeker *et al*., 2002). Adrenal weight is higher in animals in late proestrus or estrus than in animals in anestrus or early proestrus (Fox, 1988).

The most obvious symptoms of hyperadrenocorticism in ferrets are symmetrical alopecia, vulvar swelling in neutered jills and mating aggression in neutered males (Fox, 1988; Rosenthal & Peterson, 2000). Alopecia is the most common clinical manifestation of adrenocortical disease and develops in both male and female ferrets. Alopecia is usually symmetrical, beginning on the rump, the tail, or the flanks with progression to the lateral trunk, dorsum, and ventrum. Hair epilates easily. Pruritusmay also be present, most frequently observed on the dorsum between the shoulder blades. Skin erosions can occur due to pruritus and fragility of skin (Hnilica, 2010; Keeble & Meredith, 2009). More than 70% of female ferrets with adrenal gland disease have an enlarged vulva (Rosenthal *et al*., 1993). It is also proven that there is no sex predilection (Schoemeker *et al*., 2000). Perivulvar region may be hyperemic and covered with erosions (Hnilica, 2010). Mammary gland enlargement in female ferrets is also seen (Keeble & Meredith, 2009). Partial or complete urinary blockage in male ferrets is occasionally associated with adrenocortical disease. Periurethral cysts develop in the region of the prostate (originating from hormone responsive cells) and cause urethral narrowing (Coleman, Chavez, & Williams, 1998). Polyuria and polydipsia arealso reported in ferrets with hyperadrenocorticism, but it is not clear if the adrenal hormones are responsible for these symptoms. Enlarged abdomen may be seen in ferrets with adrenal disease as well (Fox, 1988; Banks *et al*., 2010).

At the beginning, clinical signs tend to appear in the breeding season but eventually they become persistent. Median time interval between neutering and diagnosis of hyperadrenocorticism is 3.5 years. A significant linear correlation between the age at neutering and age at time of diagnosis is detected. It is concluded that age at neutering may be associated with age at development of hyperadrenocorticism in ferrets (Schoemaker *et al*., 2000; Bielinska *et al*., 2006).

Diagnosing is also based on increased plasma concentrations of androstenedione, 17α-OH progesterone, dehydroepiandrosterone sulfate, and/or estradiol (Rosenthal & Petherson, 1996). Reference ranges have been set by Clinical Endocrinology Laboratory, College of Veterinary Medicine, University of Tennessee, Knoxville: Androstenedione (nmol L^{-1}) 0.1-15, Estradiol (pmol L^{-1}) 30-108, 17α-OH progesterone (nmol L^{-1}) 0.1-0.8. If one or more of these results are raised in a neutered ferret, this confirms hyperadrenocorticism. Androstenedione is the most sensitive of the above hormones (Meredith & Redrobe, 2002). Cortisol should be in normal range (Mitchell & Tully, 2009).

In the Baltic States, there are no research papers about this condition in domestic ferrets. There is no ranking system for severity of alopecia, when ferrets are brought in for clinical examination. Also, here is no research describing if there is any correlation between clinical symptoms and sex steroid levels.

The aim of this study was to sum up, evaluate and compare clinical findings and blood hormone measurements in ferrets diagnosed with hyperadrenocorticism.

Materials and Methods

The research was carried out in three veterinary clinics in Latvia during the period from March 2013 till February 2016. Twenty two castrated male ferrets and twenty neutered female ferrets were included in this research at age of 36 to 66 months. All ferrets were under the owners' supervision and all of them had various clinical signs related to adrenal disease. All ferrets were held indoors and had feed and water ad libidum. They all were kept under the owners' supervision. Ferrets were randomly numbered from 1 to 42 for the owner data safety and privacy. Legal and ethical requirements had been met. Ferret owners were informed about their animals being included in the research under terms of privacy.

All ferrets were clinically examined. Animals were assessed by one examination protocol which included basic information (age, age of neutering/castration and gender) and questions about clinical symptoms observed in each animal.

During research, the ranking system for description of alopecia was developed. Severity of alopecia was ranked from 1 to 4, where 1 – mild seasonal shedding on tail and dorsum, 2 – seasonal symmetrical alopecia on dorsum, ventrum and tail, 3 – persistent symmetrical alopecia on dorsum, ventrum, chest and tail, 4 – animal has lost all fur, except on head and paws. Blood samples were collected from each animal, sent to the Central laboratory (Riga, Šarlotes street 1B) and levels of estradiol, androstenedione and 17α-OH progesterone were detected. Blood sampling was performed once in each animal, during the first clinical examination. Statistical analyses were carried out using MS Excel.

Results and Discussion

Mean age of neutering of ferrets included in this research is 11.3 months (SD 3.7). There is no significance between gender – male ferrets are castrated at 12 months of age (min. 8 months, max. 18 months) and female ferrets are neutered at 10.6 months of age (min. 7 months, max. 18 months) $p < 0.05$.

The age at which the first clinical signs appear vary between 24 and 66 months and it depends on the age of neutering. Mean age at which the clinical symptoms appear is 43.2 months (SD 8.0). Mean age at which the first clinical signs appear among genders is not significant $p < 0.05$. It is 44.5 months in male ferrets and 41.6 months in female ferrets. There is an average correlation $r = 0.50$ between the age of male castration and appearance of clinical signs and a high correlation $r = 0.82$ in the female ferret group.

Table 1

Occurrence of clinical symptoms in ferrets included in the research

Clinical symptom	Male		Female	
	n	%	n	%
Alopecia	22	100	20	100
Signs of oestrus (swollen vulva in females, mating aggression in males)	8	36	16	80
Pruritus	16	73	19	95
Scaling and fragility of skin	15	68	17	85
Lethargy	13	59	13	65
Poliuria, polidipsia	10	55	4	20
Abdominal enlargement	7	32	3	15
Weight loss	11	50	11	55

Table 2

Estradiol, androstenedione and 17α-OH progesterone levels in female and male ferrets

Nr.	Male			Female		
	Estradiol (pmol L^{-1} 103.2-180)	Androstenedione (nmol L^{-1} 0.1-15)	17α-OH progesterone (0.1-0.8)	Estradiol (pmol L^{-1} 103.2-180)	Androstenedione (nmol L^{-1} 0.1-15)	17α-OH progesterone (0.1-0.8)
1	73.42	17.16*	0.93*	286.71*	10.50	0.05
2	279.23*	4.82	4.14*	175.11*	59.44*	0.13
3	205.89*	1.68	0.16	90.67	133.92*	0.27*
4	81.49	10.50	2.61*	30.60	79.79*	0.03*
5	111.23	59.44*	3.60*	154.56	59.44*	0.77*
6	114.90	25.70*	1.34*	218.06*	10.50	0.03
7	122.98	20.48*	0.16	85.53	72.73*	0.03
8	108.29	14.68	1.21*	40.01	10.48	0.47*
9	200.44*	10.49	0.10	45.50	16.40*	0.30*
10	370.78*	0.30	1.30*	253.56*	14.69	0.25*
11	106.83	13.64	1.05*	56.32	16.28*	0.08
12	105.74	94.41*	0.84*	32.58	11.22	0.82*
13	237.15*	10.48	0.10	106.87	71.45*	0.06
14	36.70	20.34*	0.22	302.65*	23.76*	0.14
15	62.04	17.49*	0.26	103.56	63.42*	0.20*
16	93.24	32.01*	0.12	108.96	17.05*	0.03
17	82.26	21.13*	0.20	193.62*	13.47	0.03
18	258.27*	17.92*	0.10	64.73	71.68*	0.03
19	221.36*	12.70	0.84*	113.12	17.06*	0.20*
20	300.23*	20.97*	0.45	195.44*	11.86	0.03
21	202.24*	10.48	0.96*	-	-	-
22	261.72*	78.43*	0.10	-	-	-

* – hormonal level is increased.

At the evaluation of clinical symptoms, the most significant sign was symmetrical alopecia observed in all ferrets in various degrees included in this research (Tab. 1).

By our ranking system, most of ferrets had stage 3 (n = 19) and stage 2 (n = 17) alopecia, meaning they had permanent symmetrical alopecia on tail, back, extremities and chest. It is significant p=0.05, in male ferrets alopecia was more severe than in female. Third stage alopecia was observed in 55% of male ferrets whereas it was only 25% in the female group.

Signs of estrus – swollen vulva in female and mating aggression in male ferrets were observed in 24 animals. Abdominal enlargement was observed in 10 animals. Polyuria and polydipsia were observed in 14 animals. Pruritus was observed in 36 animals. Lethargy was observed in 26 animals. Scaling and fragility of skin was observed in 32 animals. Decreased weight was observed in 22 ferrets.

Out of 8 clinical symptoms listed in examination form, animals had min. 2 max. 7 of the mentioned. One male ferret had 2 clinical signs, other animals, male and female combined, had more. Two animals (n = 1 female, n = 1 male) had 7 symptoms. Four animals (n = 2 female, n = 2 male) had 3 symptoms. Nine animals (n = 2 female, n = 7 male) had 4 symptoms. Sixteen animals (n = 10 female, n = 6 male) had 5 symptoms resulting in majority of animals included in this research. Ten animals (n = 5 female, n = 5 male) had 6 symptoms. Thirty five animals - 83.3% (n = 17 female, n = 18 male) - had 4 to 6 clinical symptoms.

Sex steroid levels were compared with Adrenal panel. Results indicated that all ferrets had one or two elevated hormone levels (Tab. 2).

In the female group, 9 animals had increased one hormone and 11 had increased two hormones (p = 0.05). Androstenedione was elevated in 13 cases.

In the male group, one hormone was increased in 8 cases and two hormones were elevated in 14 cases. Androstenedione was increased in 12 cases in the male group. Also in male group in 11 cases the level of 17α-OH progesterone was increased. In our research, there was no case when all three sex steroids would be increased.

There was no correlation found between the clinical symptoms and hormone mesurements.

There is high possibility of estrogen induced bone marrow suppression if the female remains intact and is not fertilized. If jill is not brought out of the estrus, it may result in nongenerative anemia and death (Chen *et al*., 2014).

High prevalence (r = 0.82) in female group related to age at the castration and onset of clinical signs may be referred to the education of pet owners. Majority of jills in Latvia were neutered, before there was enough information about subcutaneous implants containing desloreline acetate which is manufactured for heat cycle regulation in various animal species. Implant also prevents the development of adrenal disease in ferrets. In Latvia, neutering is still more popular than desloreline acetate implants due to the price. For example, in England most of the ferrets are intact and prevalence of adrenal disease is low (Schoemeker *et al*., 2002).

The most often mentioned clinical symptom is alopecia. In our research it was observed in all animals. Ranking of alopecia for ferret adrenal disease has not been reported in research papers. It is mentioned that over years it gets more severe, eventually becoming persistent (Carpenter *et al*., 2012). It would be useful to rank the severity of alopecia when the ferret is for the first time brought for examination and further on as well.

Pruritus (n = 36 animals), scaling and fragility of skin (n = 32), lethargy (n = 26), signs of estrus (n = 24), weight loss (n = 22) were observed in more than half of the animals forming majority of clinical symptoms. Signs of estrus (swollen vulva in female and mating aggression in male ferret) is mentioned as one of the most common clinical symptoms in ferrets with hyperadrenocorticism (Rosenthal *et al*., 1993). In our research, there are some symptoms that appear more often (pruritus, fragility of skin, lethargy).

Polyuria and polydipsia (n = 16), abdominal enlargement (n = 10) were also observed. Various clinical symptoms may refer to other underlying conditions in these animals.

It is mentioned that in one animal numerous clinical symptoms may be noticed (Keeble *et al*., 2009).

In 16 animals (n = 10 female, n = 6 male) 5 symptoms were observed and only in 1 male ferret 2 symptoms were present.

Androstenedione is mentioned as the most sensitive hormone for the diagnosis of hyperadrenocorticism (Meredith & Redrobe, 2002). There are research papers where only the level of androstenedione is detected (de Jong *et al*., 2014). In our research it was elevated in 25 cases out of 42, which indicates that it is significant to detect other hormones as well.

Conclusions

1. Ferret clinical examination and sex steroid measurements are essential in the diagnosis of hyperadrenocorticism in domestic ferrets.
2. In our research, the female ferrets tend to develop adrenal disease earlier than male ferrets due to neutering at a young age.
3. Symmetrical alopecia, pruritus, scaling and fragility of skin, lethargy and signs of estrus are the most frequent clinical symptoms observed in our research.
4. Ranking of alopecia allows to evaluate the severity of it every time the ferret is brought for examination.
5. One animal had two clinical signs, two animals had seven clinical signs. The majority of ferrets had five (n = 16) or six (n = 10) clinical symptoms of hyperadrenocorticism.
6. Level of androstenedione must be measured together with other sex steroids.

Conflict of interest statement

All ferrets were selected in various veterinary clinics. Owners and other veterinarians played no role in the study design nor in the collection, analysis and interpretation of data, nor in the decision to submit the manuscript for publication. None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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