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The studies of the False darkling beetle *Phryganophilus ruficollis* (Fabricius, 1798) in Latvia 2012-2013

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Key words: Coleoptera, Melandryidae, *Phryganophilus ruficollis*, saproxylic beetles.

The *Phryganophilus ruficollis* is included in European Council Directive on the Conservation of natural habitats and of wild fauna and flora as a priority species. The species has a wide distribution area, which includes Palaearctic forest zone from Japan and Far East of Russia to Atlantic Ocean (Nikitsky, Pollock 2008). Until now the species was found in Latvia twice, in 1861 and in 1998, when two specimens were caught (Kawall 1867; Barševskis 2001).

The aim of the study was to clarify the status of the population in the known locality in Slītere National park and search for species through at least 10 potential localities in Latvia.

The window trap was used as the main method. From April till July 68 and 57 traps were exposed in 2012 and 2013 respectively in Slītere National park. 110 traps (10 traps per locality) were exposed from April till July 2013 in potential localities in Latvia.

Consequently *Phryganophilus ruficollis* was neither found in the known locality, nor in the potential localities. Other mycetophagous and saproxylomycetophagous beetles, like Erotylidae, Mycetophagidae, Melandryidae, Pyrochroidae families were found in all localities. Several species were caught as 1 to 3 specimens per one or several localities [e.g. *Dolotarsus lividus* and *Phloiotrya rufipes* (Melandryidae), *Orchesia micans* (Orchesiidae), *Leiesthes seminigra* (Endomychidae), *Triplax rufipes* (Erotylidae)]. *Cryptophagus reflexus* (Cryptophagidae) was caught in one locality, which is a new species of the fauna of Latvia. A very rare species *Leiesthes seminigra* and *Mycetina cruciata* (Endomychidae) were found in two and five new localities respectively.

The fact that the target species was not found during the two field seasons does not mean that the searching methods were unsuitable. The populations of the *Phryganophilus ruficollis* are not multitudinous in Central and Northern parts of Europe and commonly the beetles can be found by one or in small groups. Even in localities, were species made permanent populations and traps were exposed all vegetation season, only one or several beetles were caught per year (Gutowski 2009).

It is most likely that the population of the *Phryganophilus ruficollis* in the known locality in Slītere National park is dispersal and/or small-numbered. Substantially bigger effort is necessary for establishing *Phryganophilus ruficollis*, than it is usually used in other studies or principally new methods for studies of species should be introduced.

Acknowledgements

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Potential of FT-IR spectroscopy for studies of herbicide MCPA leaching from soil

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Key words: FT-IR spectroscopy; herbicide MCPA; soil.

Today more and more new herbicides are offered to the market. In spite of the decreasing concentrations of applied herbicides, eventually there are several environmental risks including pollution of ground and over ground waters, soil. In 2012, the selective herbicide methyl chlorophenoxy acetic acid (MCPA) was the third among applied active substances in Latvia. MCPA is classified as medium toxic for water plants, fish, phyto- and zooplankton, but it iss mostly harmless for insects and mammals in concentration under 2 μg L^{-1} (PANNA 2013). The solubility of MCPA in water is low; the adsorption strongly depends on the type of soil (Hiller et al. 2010) and thus the pollution by leaching increases with the volume of flush water (Wofford, Lee 1995). As strong herbicides are used it is necessary to assess the environmental risks of specific active substance. Thus there is a need for quick and simple herbicide detection methods. Fourier transform infrared (FT-IR) spectroscopy can serve as time-saving and informative method replacing chromatography methods.

In this study 100 μL of 5% MCPA (Nufarm, Austria) were added to 550 mg each of five different soils: three sandy soils with organic carbon content (C_{org}) of 0.04, 0.16 and 0.23%, loamy sand (C_{org} 1.32%) and peat substrate ($_{Corg}$ ≈38%), shaked, kept in backwater for 1 to 6 days. Mixtures were spinned at 13000 rpm. FT-IR spectra of MCPA, soil water extracts and MCPA water extracts (10 μL) were recorded on a VERTEX 70 coupled with the microplate reader HTS-XT (Bruker, Germany). Absorption spectra were collected over the range of 4000 to 600 cm^{-1}, at a resolution of 4 cm^{-1}, and 64 spectra were coadded. Baseline was corrected by the rubber band method, CO_2 bands excluded.

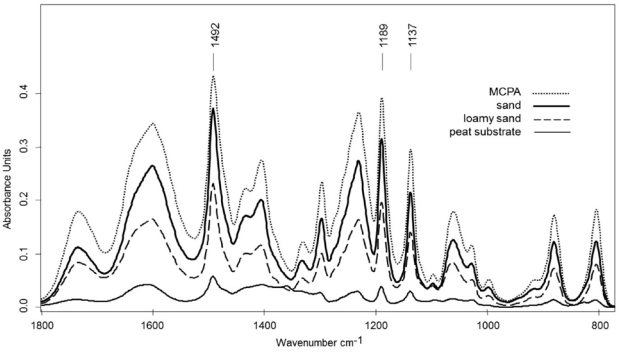

Fig. 1. FT-IR spectra of MCPA (0.70%) and its water extracts from different soils after 1 day: MCPA – 0.70% MCPA; Sand – C_{org} 0.04%; loamy sand C_{org} 1.32%; peat substrate C_{org} ≈38%.

Evaluation of FT-IR spectra of MCPA, soil water extracts and MCPA water extracts showed that MCPA can be identified by a specific absorption bands at 1492, 1189 and 1137 cm^{-1}. Thus by changes of the MCPA specific band intensity (in our case directly proportional to the MCPA concentration) it was possible to evaluate the amount of washed out MCPA. It was shown that the amount of MCPA in water extracts strongly depends on the soil type. MCPA was not adsorbed on pure sand because all was washed out. The highest adsorption was detected on peat substrate sample and less on loamy sand, respectively. Our results showed that higher organic component concentration in soil is related with lower MCPA content in water extract (Fig. 1) thus indicating to the adsorption of MCPA by organic compounds which is in agreement with literature. Storage of MCPA with soil for 1 to 6 days at room temperature showed that binding occurs within first 24 h. Storage of MCPA and soil mixtures for 5 days in backwater resulted in higher MCPA concentrations in extracts.

This study proved FT-IR spectroscopy as a quick method for monitoring of the herbicide leaching.

Acknowledgements

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Low-penetrance melanoma risk gene polymorphisms in Latvian population

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Key words: melanoma; *MC1R*; *MDM2*; *MITF*; *TP53*.

Cutaneous melanoma is cancer that develops from melanocytes and arises through the interaction of environmental, individual's pigmentation phenotype and genotype factors. The aim of this study was to analyze association between polymorphisms in medium (*MITF*) and low (*TP53, MDM2, MC1R*) penetrance melanoma risk genes and melanoma in Latvian population.

The microphthalmia-associated transcription factor gene (*MITF*) polymorphism Glu318Lys (rs149617956) has shown association with melanoma both in melanoma families and general population therefore is classified as a medium penetrance melanoma risk polymorphism (Yokoyama et al. 2011).

TP53 is tumor suppressor that is negatively regulated by mouse double minute 2 homolog (MDM2). TP53 among the other targets activates melanocortin 1 receptor (MC1R) signaling pathway subsequently leading to the synthesis of UV-protective pigment. Many studies demonstrate association between *MC1R* gene polymorphisms and melanoma (Williams et al. 2011). *MC1R* polymorphisms with the highest melanoma risk are designated as RHC (red hair color) polymorphisms. There are also several studies with regard to association between TP53 polymorphism Pro72Arg c.215C>G (rs1042522), *MDM2* gene promoter polymorphism c.14+309T>G (rs2279744) and melanoma risk, however results are conflicting (Cotignola et al. 2012; Ye et al. 2013).

MITF gene region with polymorphism Glu318Lys was sequenced in 490 melanoma patients and 377 healthy controls however Glu318Lys was found in none of them.

In the analysis of *TP53* and *MDM2* polymorphisms altogether 490 melanoma patients and 356 controls were included. *TP53* Pro72Arg analysis was performed using RFLP method with endonuclease Bsh1236l and *MDM2* gene promoter region with c.14+309T>G was sequenced. When allele and genotype frequencies of polymorphisms Pro72Arg and c.14+309T>G were examined individually, none of them was associated with melanoma (OR 1.07, 95% CI 0.87–1.32, P = 0.517 and OR 1.18, 95% CI 0.95–1.45, P = 0.131, for allele frequencies respectively). None of Pro72Arg and c.14+309T>G genotype combinations were associated with melanoma risk. When the presence of *MC1R* gene RHC polymorphisms were taken into consideration, there were more melanoma patients than controls with *TP53* Pro72Arg GG genotype and *MC1R* polymorphisms (OR 2.76, 95% CI 1.02–7.52, P = 0.040) (Table 1). There was no

Table 1. Association between *TP53* polymorphism Pro72Arg and melanoma depending on the presence of MC1R polymorphisms. WT, wild type (including synonymous polymorphisms); RHC, red hair color polymorphisms; NRHC, non-red hair color (all other nonsynonymous) polymorphisms; OR, odds ratio; CI, confidence interval; *P*, *P* value

TP53 Pro72Arg	MC1R polymorphisms	Patients (*n*) 279	%	Controls (*n*) 217	%	OR	95% CI	*P*
CC	WT	7	2.5	11	5.1	1	–	
	RHC	13	4.7	6	2.8	3.40	0.88 – 13.19	0.072
	RHC/NRHC	24	8.6	15	6.9	2.51	0.80 – 7.91	0.111
CG	WT	33	11.8	36	16.6	1.44	0.50 – 4.15	0.498
	RHC	38	13.6	25	11.5	2.39	0.82 – 6.99	0.107
	RHC/NRHC	72	25.8	61	28.1	1.85	0.68 – 5.08	0.224
GG	WT	41	14.7	36	16.6	1.79	0.63 – 5.10	0.273
	RHC	52	18.6	29	13.4	2.82	0.99 – 8.06	0.048
	RHC/NRHC	102	36.6	58	26.7	2.76	1.02 – 7.52	0.040

such an association in the presence of *TP53* Pro72Arg CC or CG genotype leading to the conclusion that Pro72Arg GG genotype in combination with *MC1R* polymorphisms has additional impact on melanoma risk.

Acknowledgements
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Description of the purifying effect of the microorganisms isolated from a highly loaded pharmaceutical waste water pre-treatment plant

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Key words: batch tests; COD reduction; microorganisms; waste water pre-treatment.

The performance of a biological waste water treatment process depends on microbial diversity, activity and their ability to degrade specific pollutants (Gerardi 2006). The aim of this study was to isolate the microorganisms associated with pharmaceutical waste water treatment process and to identify those which are showing the highest reduction ability of chemical oxygen demand for JSC "Grindeks" industrial waste waters.

There were 33 bacteria, 32 yeast and filamentous fungi strains isolated and subsequently identified from the activated sludge of the waste water pre-treatment process within the framework of this study. Screening of the biodegradation potentials for the isolates were done in batch experiments followed by evaluation of each isolate's ability to reduce a chemical oxygen demand (COD) of the batch sample.

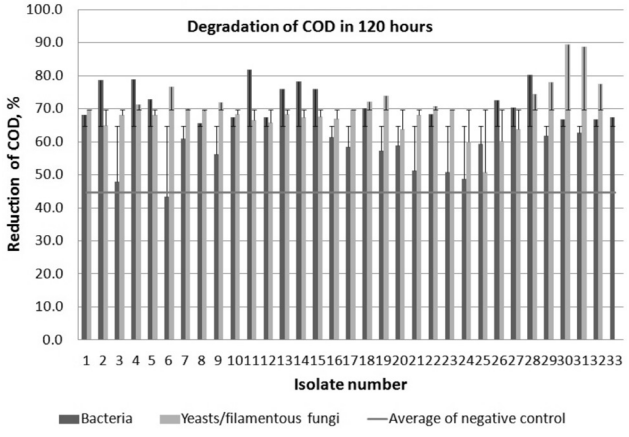

Fig. 1. Individual ability to reduce COD for all bacteria, yeast and filamentous fungi isolates compared to average negative control result.

A specific individual reduction of COD in 120 h for each isolate varied from 43.4 to 81.9% for bacteria strains and from 50.7 to 89.4% for yeast and filamentous fungi strains with no statistical significance between reductions of those two microorganism groups. Each batch test was provided with a negative control sample with no microorganisms added to the waste water. Those negative control samples showed an average COD degradation of 51.2% in the same 120 h. All individual reductions are shown in Fig. 1.

A COD reduction of more than 50% in the negative control samples can be explained by the high concentration of volatile organic compounds present in the tested waste water (Stephenson, Blackburn 1998), as sterility of the negative control was monitored using turbidity measurements during the incubation, but the huge variation of the results around mean value is due to individual characteristics of each isolate (Saraswathi, Saseetharan 2010) as well as variations in a waste water composition in each batch test.

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Characterization of the microorganisms associated with honey bees in apiaries of Latvia and an insight into bee functional food research

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Key words: American foulbrood disease; honey bee; intestinal tract; microflora; probiotics

The microflora associated with honey bees can be divided into three groups according to the interaction with host: pathogenic, neutral, and beneficial. The pathogenic microflora is the most studied due to the economical significance for the bee keeping industry. However, during the last few decades studies of natural and beneficial microflora in honey bee intestinal tracts have become more relevant due to the need for improvement of bee health (Wu et al. 2013).

In order to characterize neutral and potentialy beneficial microflora associated with intestinal tract of honey bees, nine apiaries in Latvia were visited and several hundreds of honey bee samples were taken. After preparation of intestinal tracts, aerobic cultivation of heterotrophic microorganisms as well as bacterial 16S rRNA amplification and screening in order to describe total colony-forming units (CFU) per bee intestine and presence/absence for becteria of specific genera were performed.

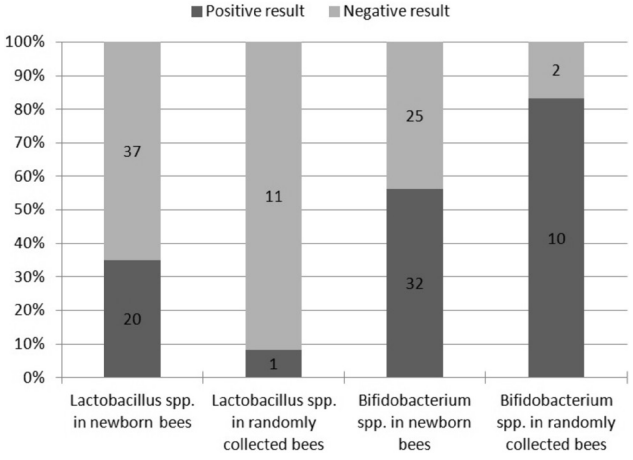

Fig. 1. Comparison of *Lactobacillus* and *Bifidobacterium* in newborn and randomly-selected honey bees within the same hives after 16S rRNA screening.

It was found out that CFU of aerobically grown heterotrophic bacteria were highly variable among randomly collected bees. There was a statistically significant difference between CFU in randomly collected and newborn bees. The 16S rRNA screening determined the presence and absence of the lactic acid bacteria from genera *Lactobacillus* and *Bifidobacterium*, and both are compared in Fig. 1.

Several lactic acid bacteria have a probiotic effect on other animals and bees (Vasquez et al. 2012), so there was an attempt for their isolation on a MRS agar medium (Nikita, Hemangi 2012) with a successful result of 28 isolates, 10 of which showed antagonistic action on *Paenibacillus larvae*, the causative of American foulbrood disease.

As the first results indicated on the isolates being capable to suppress *P. larvae* growth *in vitro*, the next step will be field studies for determination of *in vivo* effect of the isolates on the honey bees. For this purpose a liquid carrier media will be prepared.

Acknowledgements

The study was done in collaboration with the Faculty of Biology of the University of Latvia and the Latvian Beekeepers Association and several volunteer beekeepers.

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Distribution of species among suspension and ceramic granules after incubation of bacterial association in anaerobic conditions

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Key words: anaerobic conditions; bacterial association; biofilms; ceramic granules.

Microorganisms have abilities to remain in suspension or they attach themselves to the different surfaces, become immobile and form biofilms. In biofilms, microorganisms have a better chance of survival, especially during periods of stress, because they are protected from predators, dehydration and biocides and can use the benefits from community collaboration within the matrix (Bhinu 2005; Singh et al. 2006).

Adhesion and subsequent biofilm formation is influenced by the chemical composition of environment and surface properties of microorganisms. Bacterial surface undergo changes in response to changes in the environment, for example, by adsorption of ions and macromolecules. Studies have shown that immobilization of bacterial associations is also influenced by the composition of the population and that it varies greatly from immobilization of individual cultures (McEldowney, Fletcher 1987).

In this study, ceramic granules made from Planči deposit of Devonian clay and sintered at 1200 °C were used. The granules have diameter 1.2 cm, length 1.5 to 1.7 cm and density 1.33 g cm^{-1}. Bacterial association consisted from six bacterial species: Gram-positive *Bacillus mycoides* MSCL 1010, *Clostridium butyricum* MSCL 1019 and *Clostridium paraputrificum* MSCL 1171, and Gram-negative *Enterobacter asburiae* MSCL 899, *Enterobacter cloacae* MSCL 1166 and *Tissierella praeacuta* MSCL 1160. Immobilization of bacterial association on the ceramic supports was studied in sterile serum bottles and Müller-Hinton broth in anaerobic conditions under inert atmosphere of argon gas at temperature of 37 °C.

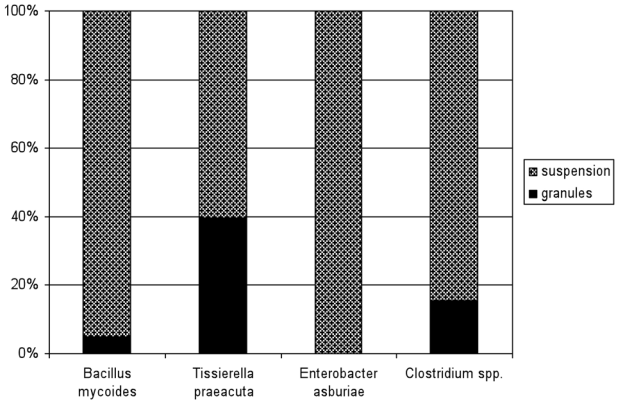

Fig. 1. Distribution of colony-forming units of bacterial species on the granules and in the suspension after five days of incubation in anaerobic conditions.

The results showed that the selected ceramic material and environmental conditions are suitable for immobilization of Gram-positive and Gram-negative bacteria as well as for anaerobic and relatively anaerobic bacteria. However, the difference appeared between bacterial species composition on the ceramic granules and in the suspension (Fig. 1).

Ceramic granules selected and modified the composition of bacterial population. Some species multiplied mainly on the surface of granules and formed biofilms while other species remained in the suspension. We would like to believe that this applies not only to artificially formed associations, but also to the natural microbial associations.

Acknowledgements

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Phytoplankton communities as indicators of ecological quality of Latvian – Estonian transboundary lakes

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Key words: ecological quality; phytoplankton; transboundary lakes.

The aim of the study was to detect ecological quality of transboundary lakes situated in the basin of Gauja River. Phytoplankton samples were gathered three times in growing season of 2011 (May, July and September) in the lakes Mazais Baltiņš (Väiku Palkna), Ilgājs (Kikkajärv) and Muratu (Murati). In deep, clear water lakes (Mazais Baltiņš and Ilgājs) samples were collected from surface (0.3 to 0.5 m), metalimnion (5.0 m), hypolimnion (10, 15, 20 m) and bottom water layers. Phytoplankton samples from shallow lake Muratu were collected from surface and bottom water layers. For phytoplankton counting procedure Utermohl's methods and technique was used (Utermöhl 1958). Phytoplankton ecological quality was determined by use of Estonian multimetric method (Anonymous 2009).

In Lake Mazais Baltiņš (pH 7.47; electrical conductivity 58.9 µS; total hardness 1.07 mgEk L^{-1}; color 27 CoPt units) phytoplankton biomass was low (0.2 to 0.6 mg L^{-1}) except midsummer hypolimnion biomass (1.37 mg L^{-1}) in the depth of 10.0 m and 15.0 m at the end of July. Early summer phytoplankton was dominated by *Dynobryon suecicum, Peridinium* sp., *Cryptomonas* spp. and *Rhodomonas* spp. as well as unidentified planktonic algae. Midsummer phytoplankton was dominated by *Dinobryon* spp., *Cosmarium* sp. and *Tetraedron* sp. Late summer phytoplankton showed minimal dominance of *Cruccigenia* sp., small amount of diatoms, dinophytes and unidentified planktonic algae. Phytoplankton parameters allow to evaluate the ecological quality of the lake as high.

In Lake Ilgājs (pH 8.9; electrical conductivity 245 µS; total hardness 1.95 mgEk L^{-1}; color 54 CoPt units) most of the phytoplankton taxonomical groups were represented. Phytoplankton community was rich with high number of taxa (32 to 49). Early summer phytoplankton was characterised by *Dinobryon sertularia* and *Cyclotella* sp. Highest biomass (4.06 mg L^{-1}) was observed at the end of July in hypolimnion dominated by *Dinobryon sertularia, Peridinium* spp. and *Ceratium hirudinella*. Late summer phytoplankton showed dominance by *Ceratium hirudinella* and small amounts of cyanobacteria. Main phytoplankton parameters allows to evaluate the ecological quality of the lake as good.

Lake Muratu (pH 8.17, electrical conductivity 174.3 µS, total hardness 1.95 mgEk L^{-1}; color 170 CoPt units) phytoplankton community was rich with high number of taxa (41 to 58). Early summer phytoplankton was dominated by *Aulacoseira* spp., *Rhizosolenia* sp. Midsummer phytoplankton was dominated by diatoms and cyanobacteria *Woronichinia* sp. In September high phytoplankton biomass (19.4 to 20.1 mg L^{-1}) at surface and bottom layers was formed by cyanobacteria *Aphanizomenon flos-aquae* and raphidophyte *Gonyostomum semen* was observed at the surface and bottom layers. Main phytoplankton parameters allow to evaluate ecological quality as moderate till poor. September phytoplankton samples shows bad ecological quality due to „algal blooms".

Acknowledgements
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Ephemeroptera and Plecoptera fauna survey of lotic and lentic waters in Krustkalni Nature Reserve

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Key words: entomofauna, Ephemeroptera, lakes, Plecoptera, ponds, springs, streams.

The aim of this study was to detect the biological diversity of Ephemeroptera and Plecoptera fauna in five lakes (Lake Dreimaņu, Lake Lielais Plencis, Lake Mazais Plencis, Lake Graulīšu and Lake Raganacis), two streams (Svētupe, Niedruška), two ponds (Kaļķu dīķi), and six springs. Qualitative samples were obtained according standard methods (APHA 1992). Sieves with a mesh size of 0.5 mm were used, and samples were preserved in 4% formaldechyde solution. Samples were collected and analysed in detritus, sand, gravel, pebble, cobble, wood and macrophyte substrate. Due to this survey 10 Ephemeroptera and six Plecoptera species were found in the inspected freshwaters (Fig. 1).

Observed species were *Baetis rhodani* (Pictet,1843), *Baetis niger* (Linnaeus, 1761), *Baetis* sp. juv., *Centroptilum luteolum* (Muller, 1776), *Cloeon dipterum* (Linnaeus, 1761), *Caenis horaria* (Linnaeus, 1758), *Caenis robusta* (Eaton, 1884), *Caenis* sp. juv., *Ephemera vulgata* (Linnaeus, 1758), *Ephemera lineata* Eaton 1870, *Leuctra fusca* (Linnaeus, 1758), *Leuctra digitata* Kempny, 1899, *Isoperla difformis* Klapalek, 1909, *Isoperla grammatica* Poda, 1761, *Nemoura flexuosa* Aubert, 1949, *Nemoura cinerea* Retzius, 1783.

Highest numbers of Ephemeroptera and Plecoptera species of was found in running waters (stream Niedruška, stream Svētupe,„Krākas" springs and in the Lake Dreimaņu due to impact of cold, oxygen and calcium rich waters from "Krākas" springs which flows into the lake. Lentic waters (observed lakes and ponds) shows very low numbers of Ephemeroptera and Plecoptera species.

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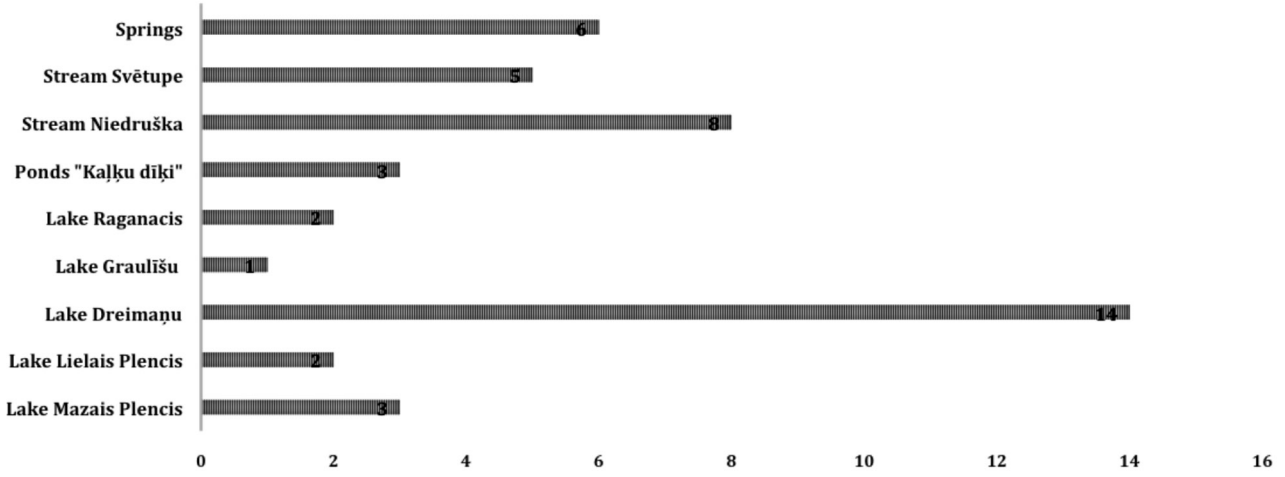

Fig. 1. Number of Ephemeroptera and Plecoptera species observed in freshwaters of Krustkalni Nature Reserve.

Characterization of cosmetic product plant extract ingredients in human skin cell culture

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Key words: oxidative stress, plant extracts, proliferation, skin mesenchymal stem cells.

It has been shown that the human skin has the remarkable ability to absorb applied products into the bloodstream (Roberts 2013), therefore concerns about possible long term effects due to the combination of synthetically derived chemicals used in cosmetics, are revealed. This causes the increase of using and investigating new natural raw materials in cosmetics, which is a healthier alternative than absorbing petroleum by-products and synthetic chemicals without harming both the skin and the environment. Since EU Cosmetic Directive prohibits animal testing it is necessary to develop alternative methods such as *in vitro* testing model based on primary cell cultures.

The aim of this study was to determine effects of six different plant extracts extracted with water-ethanol-glycerin (WEG) (45:49:6) solution as vehicle on skin mesenchymal stem cell proliferation and oxidative stress level. Plant extracts tested were from *Hippophaë rhamnoides, Galium verum, Alchemilla vulgaris, Equisetum arvense, Humulus lupulus, Trifolium pratense*.

Cell proliferation rate and population doubling time was determined in the presence of WEG and multiple (eight in total, ranging from 0.125 to 3%; v/v) concentrations of plant extracts. The effect of extracts and WEG solution on the oxidative stress of cells was determined by method of reactive oxygen species detection, cell proliferation rate was determined by counting stained cells in the plot while population doubling time was measured by performing the real-time xCELLigence analysis. Two controls were used, one was cell culture medium and the other was cell culture medium-vehicle solution.

Stimulatory effect on cell proliferation was observed with 0.5, 1, 1.5 and 2% *Hippophaë rhamnoides* extract. Anti-oxidative properties were observed for all of the extracts tested (0.125 to 1%), with the exception of *Humulus lupulus* (Table 1). xCELLigence analysis convincingly revealed *Hippophaë rhamnoides* as cell doubling time decreasing extract confirming its skin renewal properties (Survakumar, Gupta 2011). Population doubling time of cells was also decreased by the 2% vehicle-medium solution, which could be explained by ethanol ability to increase permeability of cell plasma membrane thereby increasing the cell size (Shireman 1983) and the cell index value as measured by electrical impedance.

Table 1. Effect of different extracts in concentrations ranging from 0.125 to 1% and 2% vehicle-medium solution on human skin mesenchymal stem cell proliferation and level of oxidative stress

Extract/control	Stimulates cell proliferation	Decreases oxidative stress
Hippophaë rhamnoides	+	+
Galium verum	0	+
Alchemilla vulgaris	0	+
Equisetum arvense	0	+
Humulus lupulus	0	0
Trifolium pratense	0	+
Vehicle-medium solution	0	0

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Benthic macroinvertebrates and their affecting environmental factors in Latvian bog lakes

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Key words: benthic macroinvertebrates, bog lakes, diversity, water chemistry.

In bog lakes as well as in other aquatic habitats diversity of benthic macroinvertebrates is mainly affected by the heterogeneity of bottom substrate and hydrochemistry (Ward 1992). For the most part bog lakes are highly humic with rather high acidity causing low biodiversity, density and biomass in all trophic levels (Druvietis et al. 2010). Study area covers typical bog lakes in Ramsar site Teiči Nature Reserve.

The aim of the study was to establish the main environmental factors affecting macroinvertebrates of bog lakes using historical data. Quantitative benthic data and chemical data from Lake Islienas, Lake Siksalas and Lake Tolkovas from July 1996 were used for analysis. Lake Islienas and Lake Siksalas are dyseutrophic lakes with littoral zone situated on the border of fen and mineral soils (Druvietis et al. 2010). Lake Tolkovas is a typical bog lake lacking littoral zone. In each lake macroinvertebrate samples were collected using Ekman-Berge grab sampler from the coastal and profundal zones. Redundancy Analysis (RDA) was used to determine the connection between bog lake chemistry and species data. Data analyses were performed using Canoco for Windows Software 4.5 (Braak, Šmilauer 2002; Fig. 1).

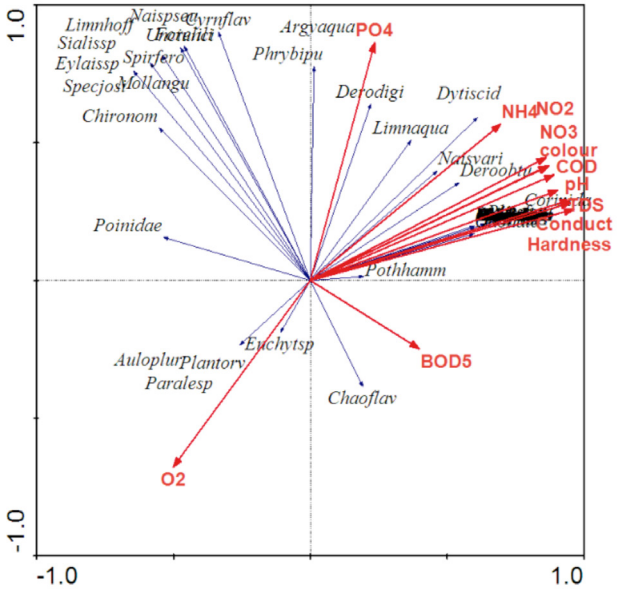

Fig. 1. Redundancy Analysis (RDA) ordination showing the relationship between species and water chemistry in Islienas, Siksalas and Tolkovas lakes (length of gradient 1,783<3).

Oligochaetes are the most diverse group in species richness in studied bog lakes, most of them showing positive correlation with increasing water hardness, conductivity, total dissolved solids (TDS), pH, chemical oxygen demand (COD), colour and nitrogen compounds. The upper site of the graph represents littoral zone while the lower part shows profundal zone (Fig. 1). Profundal is represented with lesser species, higher oxygen concentration in epilimnion and higher biological oxygen demand (BOD) than in littoral. Dyseutrophic bog lakes are more diverse in macroinvertebrate taxa than distrophic lakes because of emerged, floating and submerged and macrophytes.

We assume that there is no use to collect macroinvertebrates from profundal zone in bog lakes concerning future applied studies on biodiversity. Our suggestion is to refine the macroinvertebrate sampling method by collecting using kick sampling technique from coastal vegetation overhangs.

Acknowledgements

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Impact of pine (*Pinus sylvestris*) and spruce (*Picea abies*) biomass extracts on *Colletotrichum acutatum*, causal agent of strawberry anthracnose, *in vitro*

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Key words: phytopathogenic fungi; radial growth test; sporulation inhibition.

Phytopathogenic microorganisms cause serious economic losses in horticultural production and post-harvest diseases in fruits and vegetables. In strawberry industry, fungi are the main agent inducing diseases and reducing yields. Main protection against fungi is use of synthetic fungicides. Wide use of synthetic fungicides have lead to environmental pollution and resistance of pathogens so alternative plant protection products are required. Plant extracts have shown antifungal properties and could potentially be used as biofungicide. The aim of this study was to estimate an inhibition effect of pine (*Pinus sylvestris* L.) and spruce [*Picea abies* (L.) H. Karst.] bark ethanol extracts on mycelial growth and sporulation of *Colletotrichum acutatum* Simmonds to evaluate potencial use of the extracts as biofungicides.

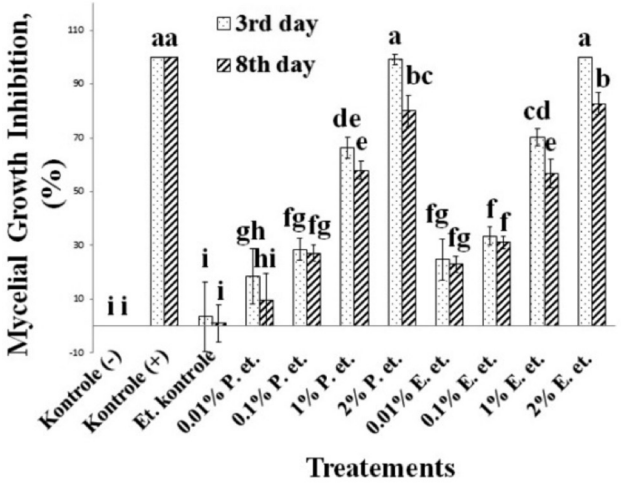

Fig. 1. Mycelial growth inhibition of *Colletotrichum acutatum* by pine (P) and spruce (S) biomass ethanol extracts at concentrations 0.1, 1, 10 and 20 g L^{-1} at third and eighth day of incubation. Data shown are mean values of five replicates with standard error. C (–), control without fungicide; C (+), chemical fungicide Signum® 1 g L^{-1}. Bars with different letters indicate significant ($P < 0.05$) difference according to the Tukey's honest significant difference multiple comparison test.

Poisonous food technique and radial growth test of inhibition of mycelial growth and hemocitometer for reduction of sporulation was used previously to evaluate impact of plant extracts on *C. acutatum* (Islam et al. 2003). Effect of extracts on mycelial growth on strawberry leaves *in vitro* was measured according to the method described by Pretorius et al. (2002).

Pine and bark extracts significantly inhibited mycelial growth of *C. acutatum* according to Tukey's HSD test ($P < 0.05$) (Fig. 1). Mycelial inhibition coefficient increased at higher extract concentrations. During longer fungal incubation period inhibition coefficient decreased. Sporulation of *C. acutatum* was reduced both by pine and spruce extracts. No significant differences between control and extract treatments on lesion development strawberry leaves induced by *C. acutatum* was observed. However, reduced formation of acervuli on leaves treated by extract was observed.

Investigation show that pine and spruce extracts reduce mycelial growth and sporulation of *C. acutatum* in vitro, however further investigation of extract effect on fungal phytopathogens *in vivo* is necessary to evaluate the potential use of extracts as biofungicides.

Acknowledgements
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Cyclic triads in groups of 'Konik polski' horses in nature park „Dvietes paliene"

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Key words: cyclic triads, horses 'Konik polski', nature park "Dvietes paliene".

First 'Konik polski' horses were introduced in Pape from the Netherlands in 1999. Currently, there are about 22 'Konik polski' herds in Latvia, which live in the number of protected and private areas. Exchange of horses should occur between the protected areas to increase genetic potential of herds and limit population density. Group hierarchy, horses rank and group cyclic triads must be considered during horses exchange, because after two or even one horse exclusion usually follows the whole group division. The horse, which are involved in great number of cyclic triads, get a central place in cyclic triads. In that case, their disappearance or removal could result in a reduction in cyclicity, as well as in major changes in network structure and even function (McDonald, Shizuka 2012).

Within a strictly linear hierarchy, all dyads have a dominant-subordinate relation, and dominance relation for every set of three players (triads) are 'transitive': when individual A dominantes B and B dominantes C, then A also dominantes C (Shizuka, McDonald 2012). Cyclic triads occur when A dominates B, B dominates C, and C dominantes A. Cyclic triad results in dominance relations that are unresolved and prevents the linear arrangements of rank (Shizuka, McDonald 2012).

Observations have been made from April until October in 2012 in nature park "Dvietes paliene". Behavior of 27 horses were recorded in the observation. These horses form five groups. In total, 17 agonistic behavior elements were selected as related to dominant/subordinate or winner/looser interactions. The data were collected at randomly distributed times and weather conditions during daylight period. The total time of observation was 148 h. The linearity of hierarchies was established by calculating Kendall index *K*. The derivation of *K* being based on the calculation of the number of circular triads (de Vries 1995). $K = 1$ when no cyclic triads *d* exist. $K = 0$ when the number of cyclic triads *d* is maximal.

$K = 1$ was calculated in a group, which consisted of one stallion and three mares. $K = 0.88$ was calculated in another group, which also consisted of one stallion and three mares. *K* was a little smaller because no agonistic interactions were observed between two middle ranked mares. Individuals may avoid interacting when two similarly ranked indiviaduals gain little benefit from outranking each other (Shizuka, McDonald 2012). *K* was relatively small (0.47 and 0.64) in multiple male and female groups, and amount of cyclic triads were higher (4.25 and 7.5). In the bachelor group, which consist of five stallions, *K* was 0.75 and cyclic triads d was 1.25.

The increase in the number of stallions in the harem, probably will increase the chance of success in conflicts with another groups of horses, but, because of large number of stallions, the mutual competition within the group will also increase. As a result it is difficult to establish transitive triads (also linear hierarchy) and cyclic triads occur constantly.

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Health-related physical fitness and physical activity of women of different age

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Key words: health-related physical fitness; physical activity; women age groups.

Health-related physical fitness has been defined as components of physical fitness associated with some aspects of good health and/or disease prevention (McArdle 2007). An important public health research topic is the age-related rate of declining of physical fitness. Cardiorespiratory fitness (CRF) in adults decreases with age and is influenced by lifestyle. Men and women become gradually less fit with age, with declines accelerating after age 45. Low CRF in particular is associated with risk of diseases and the ability of older persons to function independently (Jackson et al. 2009). However Green et al. (2014) report that sedentary behavior and light physical activity were independently associated with markers of cardiometabolic health in young, adult women.

The aim of the research was to determine the difference of physical activity and health-related physical fitness parameters in women of two age groups: 20 to 39 and 40 to 60 years.

Health-related physical fitness tests and physical activity was evaluated in 106 relatively healthy women (mean age of 20 to 39 years age group was 24.91 ± 0.7 years, mean age of 40 to 60 years age group was 46.51 ± 0.9 years). Health related physical fitness tests included body composition analysis, testing of abdominal muscles strength and handgrip strength, elasticity of hamstrings and m. quadratus lumborum muscles and submaximal veloergometry testing (aerobic fitness). Physical activity was measured using International Physical Activity Questionnaire Long Form and continuous physical activity scores were obtained (METs.min per week).

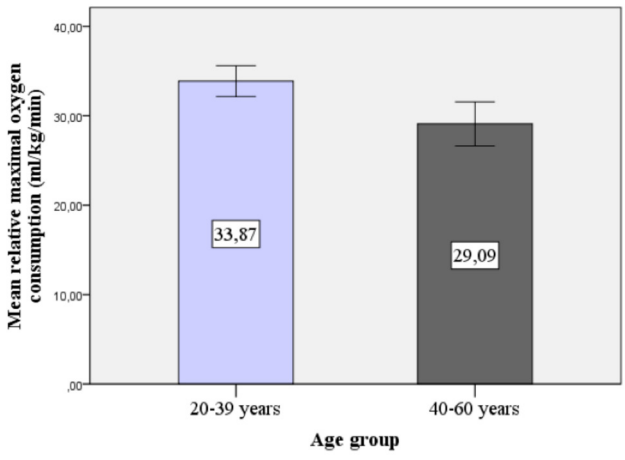

Fig. 1. Relative maximal oxygen consumption (mean ± SE) in two age groups of women. Asterisk indicate significant difference between groups (p = 0.002).

Comparing two groups, results showed that women of younger age group had healthier body composition parameters (body mass index, body fat, body water), higher relative maximal oxygen consumption (Fig. 1) and higher abdominal muscles strength.

In total, 55.66% of women (35.85% women aged 20 to 39 and 19.81% aged 40 to 60 years) had moderate physical activity level ($p > 0.05$ between groups). However, 44.56% of women of both age groups had prevalence of average level of aerobic fitness ($p > 0.05$ between groups). No significant differences were found analyzing results of elasticity of hamstrings and m. quadratus lumborum muscles and handgrip strength.

Healthier body mass index, body fat and higher vigorous-intensity physical activity amount in a week was associated with better abdominal muscles strength and higher relative maximal consumption in women aged 20 to 39, and vigorous physical activity amount was independent predictor of higher handgrip strength in both age groups.

In conclusion, women older than 40 years have fairer body composition, aerobic fitness parameters and abdominal muscles strength that younger women. However, there are no significant differences in other health-related physical fitness parameters and physical activity.

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Optimisation of growth of *Pseudomonas* and *Stenotrophomonas* genus cultures in the presence of hydrocarbons

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Key words: hydrocarbons; molasses; nitrogen sources; *Pseudomonas* spp.; *Stenotrophomonas* spp.

Environmental pollution with hydrocarbons is one of the biggest environmental problems. Use of bacterial consortia instead of separate bacterial strains is often more productive because of the interaction between strains. Improving bacterial growth and their biodegradation activity by altering nutrient supply, pH, temperature and other conditions leads to an enhanced efficiency of bioremediation process as a whole (Speight, Arjoon 2012).

The aim of this study was to improve the hydrocarbon degrading potential of bacteria consortium via step-by-step strategy.

The bacteria consortium and eight separate strains of genera *Pseudomonas* and *Stenotrophomonas* were tested. Bushnell-Haas broth was used as a base of experimental medium supplemented with 0.05, 1, 3% (w/v) of molasses, both with added diesel oil and without it. Eight different nitrogen-containing compounds were tested for their influence on bacterial growth. Experiments were conducted in 96-well microplates.

The presence of diesel oil influenced the growth activity of individual strains and consortia differently, in dependence of the concentration of molasses in medium. Diesel (1%) inhibited or had no impact on bacterial growth in the presence of 3% molasses. Conversely, in the presence of 0.05% molasses in medium, diesel oil stimulated the growth of both, individual bacterial strains and the consortium.

Among nitrogen sources tested in this study, $NaNO_2$ and $(NH_4)_6Mo_7O_{24} \times 4H_2O$ were shown to be inefficient for bacterial growth in the presence of diesel oil. NH_4Cl was found to be an appropriate source of nitrogen for most of the bacterial strains, and for the consortium. Urea was found to be one of the most efficient nitrogen sources among the tested nitrogen sources for most of individual strains. However, urea was inefficient in the sets with consortium tested. Lee and Silva (1994) have proved that urea is a suitable nitrogen source for petroleum degrading consortia. The results of our research can be explained by the fact that microbial end products and extracellular enzymes can act as antagonists and inhibit growth of other bacteria (Barton, Northup 2011).

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Bacteria response to zinc, copper and lead in the presence of diesel oil

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Key words: biosurfactants; ecotoxicology; heavy metals; hydrocarbons.

Soil remediation in oil-polluted sites in a great extent depends on the ability of bacteria to degrade hydrocarbons in the presence of other contaminants, in particular, heavy metals. Bacteria produce biosurfactants, promoting the emulsification of hydrophobic chemicals, in particular, hydrocarbons. As was shown by Mulligan et al. (1999) and Jayabarath et al. (2009), surfactants promote the removal of metals.

Our study was focused on the hydrocarbon-degrading activity of bacteria consortium consisting of *Pseudomonas* spp. and *Stenotrophomonas* spp., in the presence of Zn, Cu and Pb in different concentrations. Bacteria response to multi-compound contamination was assessed also from the ecotoxicological point of view. Degradation assays, as well as growth kinetics, disk diffusion test, microbial respiration, and vegetation experiments were used in this study.

Growth of bacteria consortium in Bushnell-Haas medium was stimulated by addition of 25 g L^{-1} diesel oil. Among three metals tested, Zn (2.5 mg L^{-1}) demonstrated the strongest inhibition effect to the growth of bacteria, both, alone and in the presence of diesel oil. This effect was shown aslo in disc diffusion test. Bacteria respiration in loamy sand soil spiked with 1.0 g kg^{-1} diesel was considerabely inhibited by Zn and Pb (2.5 mg L^{-1}).

Further study will be focused on the mechanisms of bacteria response to hydrocarbons and heavy metals in different combinations, with emphasis on biodegradation activity.

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Zooplankton of the Middle Daugava during the drainage phase of the spring floods

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Key words: drainage phase; Lagrangian method; spring floods; the Middle Daugava River; zooplankton.

On April 10, 2012, the 4th drift expedition on the Middle Daugava River was conducted. It coincided with the drainage phase of the spring floods. The drift was performed by applying a manned drifting research platform constructed from a marine life-raft and an inflatable boat (Gruberts et al. 2012). The platform was equipped with the measurement and sampling equipment (a multiparameter Hach DS5 Sonde, the Apshtein-type zooplankton sampling net, an echo sounding device, a GPS antena etc.) in order to perform the in situ water quality measurements and sampling every hour according to the Lagrangian method (Doyle, Ensign 2009).

The aim of this drift experiment was to examine the zooplankton communities of the Middle Daugava River by applying the Lagrangian sampling strategy, and to identify main factors which impact longitudinal distribution of the zooplankton.

The drift continued uninterruptedly from 09:00 till 19:30, and approximately 40 km long reach of the Middle Daugava River (from Krauja to Nīcgale) was covered.

During the drift, the river depth and drift velocity decreased whereas the water temperature and conductivity increased along the river channel. Within this reach of the river, the average depth was 6.1 m and the average drift velocity 4.1 km h^{-1}. It was less than recorded during the two previous drift expeditions in 2010 and 2011 (the average depths 10.4 m and 9.7 m, respectively), because in 2012, the peak flood discharge and water level in the Daugava River at Daugavpils were much lower than usually.

In total, 33 zooplankton taxa were recorded during this study (on average 14 taxa per sample). The average zooplankton abundance was 24936 individuals m^{-3}. Rotifera was dominant group by abundance (on average 21845 individuals m^{-3}). It was the largest abundance of the zooplankton recorded during all drift expeditions conducted on the Middle Daugava River since 2007.

The most frequently recorded taxa (found at 90 to 100% of the sampling sites) were *Polyarthra* sp., *Synchaeta*

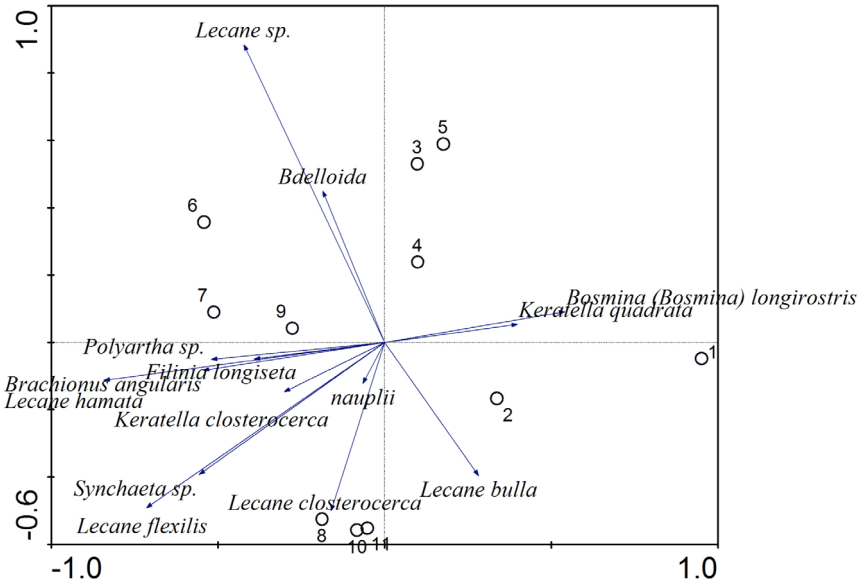

Fig. 1. Comparison of sampling places in the Middle Daugava by abundance of zooplankton.

sp., *Lecane bulla, Lecane closterocerca, Lecane hamata, Keratella cochlearis, Keratella quadrata, Filinia longiseta* and Cyclopinae development stage nauplii. The less occurring taxa (found at 55 to 64% of the sampling sites) were *Lecane flexilis, Lecane* sp., *Brachionus angularis, Bdelloida, Bosmina* (*Bosmina*) *longirostris*.

In comparison to the other drift expeditions, taxonomic composition of the zooplankton was characterised by the taxa which can inhabit both planktonic and benthic (or littoral) environment, for example, Lecanidae (Ricci, Balsamo 2000). Total abundance of the zooplankton and rotifers as well as the most abundant Rotifera taxa were obviously related to the availability of the flood water storage zones, where the stream velocity and the river depth is reduced (7, 8, 9, 10, 11; Fig. 1). Probably, the slowly moving or still waters and small depths of the adjacent storage zones and floodplain areas could promote zooplankton development and transport to the main channel of the Middle Daugava River during the drainage phase of the floods. Such conclusion is supported by the recent studies on the River Danube, where an import of the zooplankton from the adjacent lentic areas and riparian floodplains have been proposed as possible source of the river zooplankton (Reckendorfer et al. 1999).

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Antifungal activity of water extracts of vermicompost

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Key words: *Beauveria*; fulvic acids; *Fusarium; Nectria; Pseudeurotium*; vermicompost.

Only few studies exist about the antifungal characteristics of vermicompost and its products. For example, it is reported that aqueous extracts of vermicompost inhibited spore germination of several fungi from *Alternaria, Curvularia* and *Helmintosporium* genera and development of powdery mildews on balsam and pea in India (Singh et al. 2003). In other study water extracts of vermicompost that was produced from paper sludge and dairy sludge inhibited spore germination of Fusarium moniliforme (Yasir et al. 2009).

In present study 12 vermicompost samples from five producers from Latvia were used. These vermicomposts have been produced using cow manure, sewage sludge and starchless potato pulp together with composted grass. Samples were mixed with water in the ratio 1:1 (v/v) and incubated at room temperature for 4 h. The resulting slurry was filtered through a paper filter and used as 50% extract. pH of the extracts was measured. Using spectrophotometer the absorbance at 250 and 365 nm was measured of the extracts in dilution 1:10 and ratio E2/E3 calculated. High E2/E3 ratios have been related to low aromaticity percentages of natural humic matter as well as high content of low molecular weight substances fulvic acid solutions (Carvalho et al. 2008).

Antimicrobial activity was determined by the agar well diffusion method (Perez et al. 1990). The test was performed on Rose Bengal agar with chloramphenicol (Biolife Italiana S.r.l., Milan, Italy). Fresh inoculums of approximately 106 CFU (colony-forming units) mL^{-1} of tested fungi were used. Aliquots of 70 µL of each extract were applied into 6.0 mm diameter wells. After incubation at 20 ± 2 °C for five days, the width of the inhibition zone around the well was measured in millimeters and used to express the antifungal activity. The test with every fungal culture was done in two replicates.

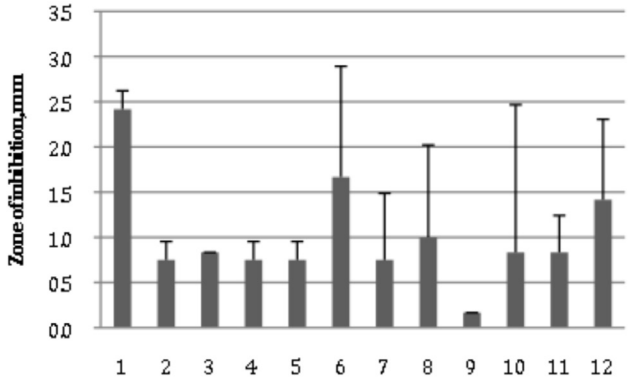

Fig. 1. The antifungal activity of all tested vermicompost extracts (1 to 12) against fungi from the genus *Pseudeurotium*. Error bars indicate standard deviations (±SD), $n = 6$.

Correlation analysis was performed with Excel (Microsoft, USA). Pearson correlation coefficients (r) were determined.

Altogether 84 fungal isolates were used in the tests from following genera representing plant pathogenic fungi, fungi associated with seeds or plant growth promoting fungi: *Acrostalagmus* (1 isolate), *Alternaria* (1), *Amylomyces* (1), *Arthrinium* (1), *Aspergillus* (1), *Beauveria* (8), *Bionectria* (6), *Cladosporium* (2), *Fusarium* (4), *Gibberella* (1), *Humicola* (1), *Hypocrea*/*Trichoderma* (14), *Ilyonectria* (1), *Isaria* (1), *Kernia* (1), *Metarhizium* (3), *Mortierella* (12), *Nectria* (4), *Neonectria* (3), *Penicillium* (1), *Podospora* (2), *Pseudeurotium* (3), *Rhizosphaera* (1), *Stephanonectria* (1), *Talaromyces* (4), *Tolypocladium* (3), *Umbelopsis* (1) and *Verticillium* (2).

The highest antifungal activity was observed against fungi from genera *Pseudeurotium* (the average width of the inhibition zone 1.0 mm [Fig. 1]), *Beauveria* (0.8 mm), *Nectria* (0.5 mm), and *Fusarium* (0.1 mm). The width of the inhibition zone positively correlated with the pH of extracts ($r = 0.54$ in the case of *Nectria* spp. isolates) and negatively with the ratio E2/E3 ($r = –0.55$ and $–0.62$ in the case of *Nectria* and *Pseudeurotium* isolates). pH ranged from 4.67 to 7.22, and the ratio E2/E3 ranged from 1.16 to 17.18. Extracts with the highest pH and lowest ratio E2/E3 had the largest antifungal activity and they were produced from starchless potato pulp and composted grass.

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