ABSTRACTS
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Analysis of biologically produced hydrogen in activated metal hydride alloys

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Key words: biohydrogen, *Enterobacter aerogenes, Escherichia col*i, glycerol, metal hydride alloys.

Hydrogen gas is seen as a future energy carrier not only because it is renewable, but also because it is environmentally friendly. It can be produced by chemical reactions, but it can also be obtained by bacteria from renewable resources such as organic waste or biomass (Demirbas 2009). One of the biggest problems with hydrogen is its storage. Currently it is mainly stored as liquid at –253 °C and at low pressure or in high pressure cylinders. Since these techniques are not very convenient, the search for new storage methods is necessary. More and more attention is paid to hydrogen storage in a form of metal hydrides. It is a very energy-efficient method and hydrogen in many metal hydrides can be stored at near-ambient temperature and normal atmospheric pressure (Broom 2011).

The present study is one of the attempts for biologically produced hydrogen storage in metal hydrides directly in nutritional broth.

The aim of this study was to investigate the amount of sorbed hydrogen, enzymatically produced by bacteria, in typical hydride forming metals and alloys (Pd, $LaNi_5$, AB_5, AB_2) by using thermogravimetric weight loss method.

Hydrogen producers *Enterobacter aerogenes* from Microbial Strain Collection of Latvia (MSCL 758) and *Escherichia coli* BW25113 *hyaB hybC hycA fdoG frdC aceE ldhA::kan* (kindly provided by prof. T.K. Wood, Texas A&M University, USA) were used. Strains were grown on AB medium containg (g L^{-1}) tryptone 1.0, yeast extract 2.5, cysteine 0.5. Crude glycerol 5.0 g L^{-1} (by-product of biodiesel) was also added. For hydrogen absorption hydride-forming powdered metals and alloys (Pd, $LaNi_5$, AB_5, AB_2) were used. To measure the amount of absorbed hydrogen simultaneous thermogravimetry/differential thermal analyzer DTG-60 (Shimadzu, Japan) was used.

It was possible to use hydride-forming metals and alloys and hydrogen absorption directly from nutritional broth with hydrogen producing bacteria (Fig. 1). Absorption effect was stronger with Pd, AB_5 and AB_2 than with $LaNi_5$.

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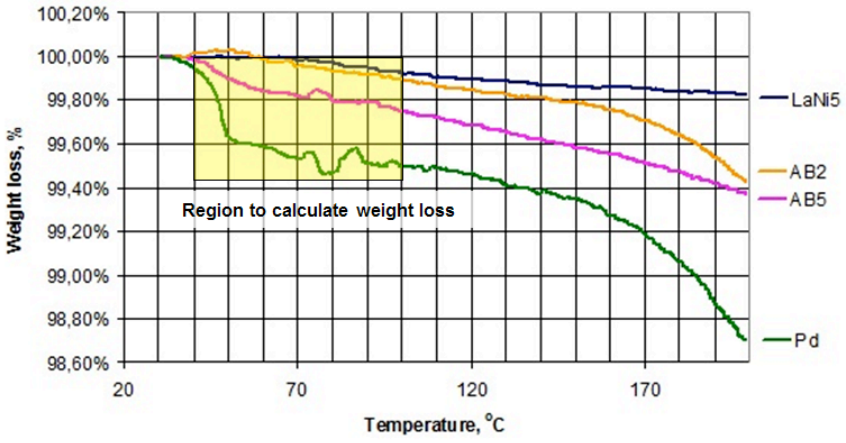

Fig. 1. Thermogravimetric release of hydrogen from hydrides of different metals after contact with *E. coli* fermentation medium..

Relationship between avian malaria distribution and water body proximity in the vicinity of the lake Duneklis in Kraslava county

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Key words: avian malaria, disease distribution, abiotic factors, hydrographic network, proximity to water bodies, tits, disease vectors.

Malaria is an infectious disease caused by parasitic protozoan order Haemospororida. Humans and various animals, including birds, are infected by blood-sucking insects. Studies on the blood parasites of birds have been often used as a model to explore the links between vector-borne infectious diseases and environment. However, a few studies have been carried out to determine the prevalence of avian malaria outside the breeding season. The hypothesis of this study is the assumption that there may be a negative correlation between the prevalence of avian malaria infection in mixed-species tit groups, and the distance between home-ranges of the flocks and water bodies. The objective of the study is to clarify the relationships between prevalence of avian blood parasite infections and the distance between bird flocks and water bodies outside the breeding season.

During the study, tits were attracted to permanent feeders. Blood smears were collected from the birds captured in mist-nets. The blood smears were analysed in the laboratory by using light microscopy. Coordinates of the feeders were detected by an handheld GPS receiver. Maps of the study area were produced and the distance between the feeders and water bodies were determined using ArcGIS software. Mathematical and statistical processing, and graphical output of the data on the prevalence and intensity of the blood parasite infections were done by SPSS software.

As the result of this study, a significant negative correlation between the distance from the water bodies to territories of flocks and blood parasite prevalence in crested tits (*Lophophanes cristatus*) and willow tits (*Poecile montanus*) was found (Pearson correlation coefficient $r = -0.65$, significance level $p = 0.022$). The prevalence of infection decreased with the distance from water bodies in birds under this study.

The distance to water bodies is an important abiotic factor that determines the blood parasite infection prevalence, i.e. the distribution of vector-born disease in territorial parid species.

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Chromosome structural changes as the cause of development and progression of cancer

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Key words: cancer, chromothripsis, chromoanagenesis, microcells, micronucleus.

Nowadays cancer is the most common disease in human population. There are many reasons causing cancer as described in the literature. Cancer is a failure of tissue control over cellular proliferation and death (Frank 2007). Genomic rearrangements as a cell single catastrophic event termed chromothripsis (from Greek, *chromos* from chromosome and *thripsis* for shattering into pieces) was for the first time described by Stephen et al. (2011). Other researchers suggest that during the time of genomic arrangements a cell can survive and as a result could be a possible mutation of tumour suppressor genes, activation of oncogenes, or generate fusion proteins that can promote tumour progression (Christopher et al. 2012).

Chromosome shattering is a result of a mistake in chromosome segregation in mitosis that leads to the formation of a micronucleus (Crasta et al. 2012). Reduced nuclear import of some vital components has an impact on the chromatin sequestered inside micronuclei First, micronuclei exhibit defective DNA damage response signalling, resulting in defective or delayed repair of induced DNA damage (Holland et al. 2012). Second, while micronuclei are still replicating DNA, the major nucleus passes through the G2 phase. Third, when macronucleus is entering into mitosis the micronucleus is undergoing DNA replication producing DNA double strand breaks in micronuclear DNA (Crasta et al. 2012).

In our study we have observed phenomena of microcell development. In the previous study with human sarcoma cell line HT-1080 after treatment with thiophosphamidum, we found that near the projection of the nucleolus damaged macrocells may form one or several microcells (Buiķis et al. 1999). Microcells are representative by their roundish or oval form, scanty cytoplasm, and homogeneously and intensively stained nuclei. The relative numbers of microcells in tumour tissue markedly increase after chemotherapy, irradiation or immunotherapy (Buiķis et al. 1999). Microcells development from damaged tumour cells via sporosis suggests the new cytological mechanism of cancer cell population immortality (Buiķis et al. 1999). These microcells could proliferate, migrate and differentiating. Through sporosis genome reconstruction is adventitious process and genetic material is collated in random order (Buiķis et al. 1999).

In the recent study the microcells was observed in luminal and triple negative breast cancer histological samples stained using Feulgen method. Microcells are commonly observed in triple negative breast cancer, which is more aggressive than luminal breast cancer. Through theory of sporosis the genetic instability of a tumour cell population and cell chromotripsis can be explained more easily (Buiķis et al. 1999).

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Nuisance Raphidophyte *Gonyostomum semen* (Ehr.) Diensig – common algae in bog water bodies of Latvia

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Key words: algal blooms, *Gonyostomum semen*, humic substances.

In the end of July and in August till September in bog water bodies of Latvia it is possible to observe "algal blooms" of nuisance Raphidophyte algae *Gonyostomum semen* which causes an slimy coating on the skin after swimming in the bog waters. Cells of these algae are very fragile and very easy explode. For some people it could cause irritation or allergy (Cronberg et al. 1988). The species is widespread in dystrophic and dyseutrophic lakes located in raised sphagnum bogs. *G. semen* is common also in neighbouring countries of Latvia (Korneva 2000; Laugaste et al. 2005; Pęczuła 2007; Karosiene et al. 2014). Our permanent observations performed in very clean bog environments, Teichi Bog reserve and Ziemeļvidzeme Biosphaere reserve, showed that the highest values of *G. semen* density and biomass occurred in the lakes characterised by the highest values of water color and lowest values of water transparency as well as low values of pH. Obviously dark brown water color and low pH of studied lakes indicates high ammount of humic substances, acidic environment and consequently a chance for "algal blooms" of *G. semen.*

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Composition of natural substances with uroantiseptic effect

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Key words: antibacterial activity, essential oils, ethanol extract, UTI, *E. coli*, experimental model on rats.

Urinary tract infection (UTI) is the second most common bacterial infection following the upper respiratory tract infection. In most cases UTI is more common among woman, but after age of 50, men become more susceptible to this infection. Due to a frequency of UTI and increase of bacterial resistance against antibiotics, it is significant to develop new composition, based on natural substances, for use in cases of UTI (Head 2008).

In the present study the antibacterial activity of 13 commercially produced plant substances were assessed. Six were essential oils (EO) from *Ocimum basilicum, Cymbopogon citratus, Mentha piperita, Abies sibirica, Pelargonium graveolens* and *Salvia officinalis*, and seven were ethanol extracts (EE) from *Oxycoccus quadripetalus, Syzygium aromaticum, Piper nigrum, Humulus lupulus, Origanum vulgare, Nasturtium officinale* and *Armoracia rusticana* from two manufacturers (from Latvia and France). The effect of single EO and EE, and their combinations was determined against four bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Proteus mirabilis* (LMKK590) and *Staphylococcus epidermidis* (LMKK992). After finding a composition with the highest antibacterial activity, its effectiveness was determined in vivo by experimental model of UTI in rats.

Essential oils have been obtained by stem distillation, ethanol extracts have been extracted in the ratio of 1:1 or 1:5 by 30 or 70% ethanol as a solvent.

Antibacterial activity was determined by agar well (Chauhan et al. 2010), disc diffusion (Muthaiyan et al. 2012) and minimum inhibitory concentration (MIC; Cardozo et al. 2013) methods with modifications. The tests were performed on Plate count agar or in Nutrient broth (Oxoid Ltd., UK). Fresh inoculums of approximately 10^6 CFU mL^{-1} of tested bacteria were used. After overnight incubation at 37 ± 1 °C the diameter of inhibition zone around the well was measured in millimeters and used as indication of antibacterial activity. The assays with all plant substances and their combinations were carried out on three independent experiments conducted in duplicate.

Statistical analysis was performed with Excel (Microsoft, USA). Data were expressed as a mean ± standard deviation (SD). For representing statistical analysis Student's t-test was performed with statistical significance at $p < 0.05$.

EO of *C. citratus* and EE of *O. quadripetalus* showed the highest antibacterial activity against *E. coli, S. epidermidis* and *P. mirabilis*. Based on antibacterial activity of individual plant substances and their combinations out of two and more, several versions were made and combination with the highest antibacterial activity was selected to become part of ingredients of the new composition for use in case of UTI.

Ingredients of the new composition were essential oils of *Ocimum basilicum, Cymbopogon citratus*, ethanol extracts of *Oxycoccus quadripetalus, Syzygium aromaticum* and emulsifier polysorbate 20.

The MIC of new composition against *S. epidermidis, E. coli, P. mirabilis* and *P. aeruginosa* was 0.67, 1.33, 1.33 and 5.34%, respectively.

Effectiveness of the new composition *in vivo* was tested using the experimental urinary tract infection model on rats (Hung et al. 2009). Lab animals were inoculated with bacterial suspension (*E. coli* 3.9 10^8 CFU mL^{-1}) or sterile saline. On the sixth day after inoculation, rats were treated for 10 days per os with the new composition with daily dose of 350 mg kg^{-1} body weight or placebo. At the end of the treatment, rats were euthanized; kidneys and urinary tract organs were obtained for histological analysis.

Histopathological examination showed UTI caused pathological changes in rat kidneys as well as bladder and urethra. Treatment with new composition reduced desquamation and hemorrhage in animals kidneys and prevented epithelial necrosis and desquamation in bladder and urethra.

These results suggest that the composition of plant substances is a potential alternative for treatment of lower urinary tract infections.

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Immobilization of root nodule bacterium *Rhizobium leguminosarum* biovar *viciae*

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Key words: clay, immobilization, peat, *Rhizobium leguminosarum*.

Rhizobium leguminosarum biovar *viciae* is a soil bacterium that can establish nodules on roots of peas, faba beans, vetches and lentils, and fixes atmospheric nitrogen (N_2) in symbiosis with host plants (Kuykendall et al. 2005). Selected rhizobial strains can be used as plant growth-promoting biofertilizers.

The carrier is the delivery vehicle for living bacteria. A sustainable carrier should deliver the right number of viable bacteria, support bacterial survival, should be non-toxic, non-polluting etc. The inoculant should have sufficient shelf life. The raw materials of most commercial carriers are cheap and naturally abundant (peat and soil fractions; for review, see Bashan 1998). It is believed that peat formulations have been developed into effective and accepted carriers but their development has almost reached its limits.

The objective of this study was to determine the survival of *R. leguminosarum* MSCL 802 in liquid and different carrier materials with goal to develop improved formulations for rhizobia.

Five materials were tested: peat (from "Bioefekts" Ltd.); clay powder ("Ceplis" Ltd.); oval aggregates of expanded clay ("Fibo S" from Maxit, Germany, and "Kano-p" from "Kurzemes Sēklas" Ltd.); and cylindrical (on average 5 × 10 mm) ceramic granules with an apparent porosity of 17.8%, a specific surface area of 4.30 $m^2\ g^{-1}$ and bulk density 1.58 $g\ cm^{-3}$ made from Planči deposit of devonian clay, sintered at 1200 °C and characterized in the Institute of Silicate Materials, Riga Technical University.

Fifteen grams of sterile carrier material were watered with 50 mL of suspension of *R. leguminosaru*m (Fig. 1). The excess of liquid was decanted after 2.5 h. Inoculated carriers were stored at three temperatures, 20, 4, and –18 °C, respectively. The number of CFU per g of carrier was determined after mechanical detachment of bacteria from their surface.

Studies showed that both the carrier material and maintenance temperature influence the success of immobilization and survival of bacteria. The best results were achieved during the maintenance of bacteria in suspension and with immobilization on the peat. We suggest to keep *R. leguminosarum* products at the temperature of 4 or –18 °C.

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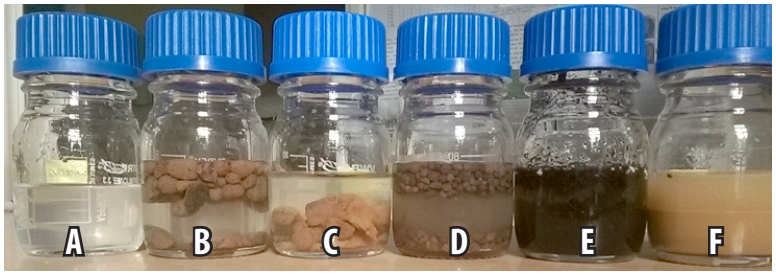

Fig. 1. Suspension of *R. leguminosarum* without (A) and with carrier materials. B, "Kano-p"; C, cylindrical ceramic granules; D, "Fibo S"; E, peat; F, clay powder.

Evaluation of spoilage yeasts causing chalk mould defects on rye bread

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Key words: chalk mould, rye bread, yeast.

Chalk moulds are one of the kinds of defects on bread. They are most often seen in the soft part of sliced bread as visible white dust-type spots. Chalk moulds are caused by approximately 24 types of yeasts, which, based on their morphological and biochemical properties, can be classified into 11 groups. The most widespread yeast strains causing chalk moulds are *Endomyces fibuliger, Zygosaccharomyces bailii, Hyphopichia burtonii* and *Saccharomyces cerevisiae* (Spicher 1986).

The aim of this study was to identify the potential agents of chalk disease. Rye flour, a knife for bread-slicing and a sponge, vegetable oil, an oil pipe and rye bread from three bakeries in Latvia were analysed.

The present study used a modified Cenis method (1992) for DNA extraction. The rDNA ITS1-5.8S-ITS2 region of fungi was amplified with primers ITS1F (Gardes, Bruns 1993) and ITS4 (White et al. 1990). The DNA fragments were treated with FastAP™ Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Scientific Fermentas Molecular Biology Solutions, Lithuania). PCR products were sent for sequencing to Macrogen Europe (Amsterdam, the Netherlands) and multiple comparisons of the sequences were performed (http://tcoffee.crg.ca).

By means of the biochemical and molecular methods, microscopic fungi, which were isolated from spoiled bread, vegetable oil, oil pipes, sponges and knives as well from rye flour and the air of packing rooms, were identified with the purpose of tracing the paths of the spread of infection as much as possible. On the whole, five different yeast fungi, chalk disease agents, were detected. The chalk disease agents were detected in the samples of the spoiled bread, a different one in each sample. – *Saccharomycopsis fibuligera* and *Trichosporon mucoides*. *S. fibuligera* was found in the tested vegetable oil, too, while *T. mucoides* was detected nowhere else. *Wickerhamomyces anomalus* was found in the vegetable oil, inside the oil pipe and on the sponge. Another disease agent, *Hyphopichia pseudoburtonii*, was detected in the vegetable oil and inside the oil pipe and on the knife, while *Hyphopichia burtonii* was found in the coarsely ground rye flour.

Data are lacked to convincingly prove the origin of microorganisms causing the chalk disease and the paths of this infection; yet, one can see that these fungi may be found in flour and in the vegetable oil used for lubricating bread-slicing knives but not in the air of packing rooms. The infectious flour in dough cannot be a source of infection, as the fungi causing the chalk disease have no high thermal resistance in order to remain alive during baking. One can conclude that in bakeries, the primary infection source of the chalk disease is flour and/or vegetable oil. The infection agents with dust or directly with vegetable oil used in lubricating the bread-slicing knife reach the sponge and bread and contaminate bread slices by means of a knife.

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Characteristics of macroinvertebrate communities in small waterbodies of Melnais Lake mire

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Key words: benthic macroinvertebrates, mire, small waterbodies.

Bog lakes and pools are unique aquatic habitats with naturally low biodiversity due to acidity and low nutrient availability (Desrochers, van Duinen 2006; Druvietis et al. 2010). However, some macroinvertebrate species are considered as bog lake specialists (Mazerolle et al. 2005). Melnais Lake mire is a nature reserve established in 2004 with an area of 317 ha. The major part of the nature reserve consists of territories protected by the EC Habitats Directive, such as dystrophic ponds and lakes. Studied waterbodies are small, with low pH values and high concentration of humic substances. Two out of the five investigated waterbodies were located at the drained part of the Melnais Lake mire, three others at the restored part of the bog.

Our objectives were to modify sweep-sampling method for mire waterbodies and to establish their characteristic macroinvertebrate communities.

Macroinvertebrate samples were collected in May 14th, 2014 from coastal vegetation overhangs with sweep-sampling net (frame size of the net 0.25 × 0.25 m, mesh size 0.5 mm). Four replicates were taken at each waterbody. Samples were preserved in 96% ethanol; benthic invertebrates were sorted by taxa in laboratory. Adult macroinvertebrates were identified to the nearest taxa while juvenile and early life cycle stage specimens – to the family or genus level.

Larvae of non-biting midges Chironomidae and ghost-midges *Chaoborus* sp. dominate the waterbodies of Melnais Lake mire. In addition, larvae of biting midges Ceratopogonidae, water mites Hydrachnidia, larvae of dragonflies Odonata, water bugs Heteroptera and all life cycle stages of diving beetles Dytiscidae were present at all surveyed waterbodies. Rare species protected by Cabinet of Ministers Regulation No. 396, such as *Anax imperator, Leucorrhinia albifrons* and *L. pectoralis*, were found in the Melnais Lake mire. The abundance of macroinvertebrates at the investigated waterbodies varies from 2000 to 8000 individuals per m^2. We assume that vegetation overhangs and depth affect differences of taxa richness and abundance in the bog waterbodies. The highest diversity of macroinvertebrates occur on submerged vegetation. Values of Shannon-Wiener biodiversity index were generally low at all sampling sites caused by high numbers of chironomids. Our results suggest caddisfly species *Agrypnia obsoleta* and dragonflies *Leucorrhinia* sp. as bog specialists.

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Impact of river restoration on macrozoobenthoss communities of two lowland rivers in Latvia

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Key words: macrozoobenthoss, river restoration.

The aim of the study was to detect ecological responses on structure of macrozoobenthoss communities caused by river restoration works. The terms restoration have been used to describe various activities meant to restore ecological proceses or improve aquatic habitats (Roni, Beechie 2013). The study was performed in Mūsa and Salaca rivers in summer low water period of 2013 (before river restoration works) and in 2014 (after one year following river restoration works). Macrozoobenthoss sampling was carried out in five sampling sites each of investigated rivers. After zoobenthoss sampling in 2013 macrophytes were removed from the investigated river section.

Macrozoobenthoss of Mūsa River was characterised by high biomass, which was 48.2 g m^{-2} before restoration works in 2013 and 52.8 g m^{-2} after restoration works in 2014. Dominated groups were Mollusca, Trichoptera and Ephemeroptera. In total 53 nacrozoobenthoss species were detected in Mūsa river. Number of individuals was high: 5450 individuals per m^2 before restoration works in 2013 and 6960 individuals per m^2 after restoration works in 2014. Similarly after restoration works zoobenthoss communities were dominated by the same groups (Ephemeroptera, Trichoptera and Mollusca), but in different order. Macrozoobenthoss of Salaca river was characterized by high medium biomass: 49.0 g m^{-2} in 2013, dominated by Mollusca (82.8% from total zoobenthoss biomass), and 28.9 g m-2 in 2014, dominated by Trichoptera (14.2 g m^{-2}) and Mollusca (5.19 g m^{-2}). In total, 56 macrozoobenthoss species were documented in sampling sites of Salaca river. Number of zoobenthoss individuals in 2013 was high (1662 individuals per m^2), but in 2014 – (8225 individuals per m^2). In both years of invesigation dominated group of zoobenthoss was Varia (7625 and 2175 individuals per m^2, respectively). Thanks to restoration works increase in zoobenthos biomass was observed. Also, replacement of species charasteristic for potamal environment to species characteristic for rhitral such as *Hydropsyche angustipennis, Baetis niger, Heptagenia sulphyrea, Theodoxus fluviatilis, Sphaerium corneum, Ancylus fluviatilis* and *Unio tumidus* was found. Such species as *Radix ovata, Bithynia tentaculata* and *Caenis* sp. were common in overgrown riffles with macrophytes. After sampling of zoobenthos, macrophytes were removed from riffles and optimal conditions for salmonide fish spawn and feeding were developed. More than one year after restoration works in both rivers overgrowth with macrophytes occurred showing stepwise regeneration of the restored river.

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Benthic macroinvertebrate community structure in the small waterbodies of Cena Mire

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Key words: benthic macroinvertebrates, mire ecosystem, small dystrophic waterbodies.

Pools in raised bogs belong to dystrophic waterbodies, which are characterised by low productivity, high content of humic organic matter, heavily stained brown water and reduced pH. The littoral zone plants may drive the metabolism of these ecosystems as source of organic matter and energy (Wetzel 2001). Pools that have minerotrophic input may differ from this pattern (e.g., with higher pH; Beadle et al. 2015).

The study was performed in the restricted Nature Reserve Cena Mire (established in 1999; area of 2133 ha), which belongs also to the Natura 2000 network of protected areas. The greatest part of the mire is covered with especially protected habitats, such as intact raised bog, transition mire and complex of pools and ridges. Raised bog habitats were impacted due to drainage and extraction of peat in S, W and SE side of the mire. Restoration measures were implemented in 2006-2007, filling up drainage ditches (Pakalne 2008).

Five small waterbodies were surveyed in the insignificantly impacted raised bog habitat with typical vegetation and pool complex in SW part of the Cena Mire in the end of May 2014.

Benthic macroinvertebrate samples were taken using standard hand net applying sweeping technique; measurements of physical and chemical parameters were performed using multi-parameter zonde; littoral zone vegetation was described and additionally phytoplankton samples were taken from epilimnion.

Studied waterbodies had characteristic low conductivity and pH (4.52 to 5.05), and high concentration of dissolved oxygen. Shoreline was covered with bryophyte *Sphagnum* spp. overhangs, in the littoral zone red algae *Batrachospermum* sp. and green algae *Mougeotia* sp. filaments covered the peat substratum.

In total 21 phytoplankton taxa were found in the studied pools of Cena Mire. In general, poor spring period phytoplankton was found, dominated by cryptophytes *Cryptomonas* sp., which are characteristic to bog waterbody flora. Second dominant group was represented by typical oligotrophic waterbody species, such as golden algae *Dinobryon sertularia* and *Dinobryon divergens*, which were found in four pools of Cena Mire. Frequently boat-shaped diatoms *Navicula* sp. were found.

In the littoral zone of the pools the most abundant benthic macroinvertebrate taxa were juvenile nymphs of damselflies (*Lestes* sp.) and dragonflies (Libellulidae Gen. sp., whitefaces *Leucorrhinia* spp.). Nymphs of EU especially protected species, dark whiteface *L. albifrons* were found.

High abundance of juvenile water beetle Dytiscidae larvae and diverse aquatic bug fauna (water boatmens Corixidae and backswimmers Notonectidae) was characteristic. Significant role in the food webs has Diptera – non-biting midge Chironomidae and phantom midge *Chaoborus* spp. larvae.

Low abundance and taxa diversity was characteristic for caddisfly Trichoptera larvae (e.g. Phryganeidae and Polycentropodidae) and water mites Hydrachnidia.

It may be concluded that studied small dystrophic waterbodies with near natural hydrological regime were represented by similar benthic macroinvertebrate communities, which are tolerant to reduced pH and depends on the littoral zone habitat patchiness. Predators were the most dominant functional feeding group.

Acknowledgements
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Characterization of macrophyte vegetation in very shallow and shallow brownwater lakes in Latvia

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Key words: colonization depth, bioindicators, macrophytes, species composition, vegetation zones.

Macrophytes are valuable biological indicators that are used to assess ecological quality of waterbodies (Penning et al. 2008), as they integrate information about environmental conditions and reflect it through taxonomic composition and abundance (Schaumburg et al. 2004).

Altogether 52 macrophyte taxa were found in the studied lakes. Most common species were *Nuphar lutea, Phragmites australis, Potamogeton perfoliatus* and *Scirpus lacustris*. Most widely represented with eight species was Potamogetonaceae family.

Found species indicates wide range of environmental conditions. For example, *Potamogeton praelongus*, found in Lielais Virānes Lake and *Potamogeton gramineus*, found in Burtnieku Lake, both are indicators of mesotrophic waters. Contrary to above mentioned *Potamogeton pectinatus*, *Ceratophyllum demersum* and *Myriophyllum spicatum* are nutrient-demanding species, which have replaced slowly growing isoetids and charophytes in many European water bodiesdue to eutrophication. In Burtnieku Lake and Mariznejas Lake *Elodea canadensis*, invasive species for Latvian waters, was found; it indicates anthropogenic impact.

Highest number of species (26) was observed in Pērkonu Lake, lowest – in Sargovas Lake (12) and Juglas Lake (13). It should be noted that low number of species not always indicate lower ecological quality, as in general higher richness of species is found in mesotrophic and eutrophic conditions (Penning et al. 2008).

According to the classification by Arber (1920) and Sculthorpe (1967) (Wetzel 1983) helophyte vegetation zone dominates by number of taxa in most of studied lakes, forming more than a half of a total number of found taxa. The least found were freely-floating macrophytes.

Average macrophyte colonization depth in very shallow lakes was 1.6 m, in shallow lakes – 1.8 m; average colonization depth of submerged macrophytes was lower – 1.4 m in very shallow and 1.6 m in shallow lakes. Submerged macrophytes usually occur deepest in lakes, but in the studied lakes lower colonization depth as well as low number of submerged macrophyte species was found. Humic substances in brownwater lakes reduces availability of light, therefore limiting occurrence of submerged macrophytes (Kirk 1994), which existence primary depends on availability of light (Sand-Jensen, Madsen 1991).

Acknowledgements
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Influence of cultivation conditions on the formation of exopolysaccharides in *Gluconobacter* sp. culture

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Key words: acetic acid bacteria, exopolysaccharides, fructans, *Gluconobacter nephelii*.

Acetic acid bacteria (AAB) possess important applications in production of food, beverages and industrial chemicals (Raspor, Goranovič 2008). Besides, certain cultures of AAB can produce fructan-type exopolysaccharides (EPS) which can be used in food production (Jakob et al. 2013).

In the present study *Gluconobacter nephelii* strains A10 and A14 were tested. Firstly, in accordance with the Plackett-Burman design of experiments (DoE), the significance of varied experimental factors was assessed under conditions of shake flask cultivation. Most important of them (substrate and yeast extract concentrations, agitation intensity, strain) were examined in the separate 2^3 factor experiment for each strain under study. Subsequently, cultivation of *G. nephelii* A14 was performed using the block of multiple fermenters (Biostat®, Q-plus, Sartorius). Obtained biotechnological indexes are shown in Table 1.

The preparations of fructan-type EPS from *G. nephelii* A14 were obtained and analyzed by means of size exclusive chromatography [ACQUITY Advanced Polymer Chromatography System, Waters (MA, USA)] to estimate the number average molecular weight (Mn), the weight average molecular weight (Mw) and polydispersity (Mw/Mn) specified as 53513 ± 1056; 1186.28 ± 138.11 and 22.15 ± 3.03, respectively.

The statistical conclusions obtained using DoE and multivariate regression analysis methods confirm that the sucrose and yeast extract concentration in the culture medium and agitation intensity are the most important factors affecting biomass and products (fructan-type EPS, gluconic acid) formation by AAB - *G. nephelii* A14.

Besides, a significant factor interaction was confirmed where the effect of sucrose and yeast extract concentration depends on the agitation intensity in the medium. Negative correlation was established between the biomass and EPS concentration during *G. nephelii* A14 cultivation.

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Table 1. Summary of *G. nephelii* A14 biotechnological indexes

Variant Index	120/5 X1/X2 Sucr/YE	170/5	220/5	120/10	170/10	220/10
S0-S1 g/L	56.02	78.90	128.35	51.28	76.01	67.59
Qp g/(L × h)	2.334	3.288	5.348	2.137	3.167	2.816
Yp/s g/g	0.359	0.337	0.219	0.380	0.531	0.426
Qx g/(L × h)	0.005304	0.005383	0.004829	0.0057	0.005146	0.004592
Yx/s g/g	0.002272	0.001637	0.000903	0.002668	0.001625	0.00163
qp g/(g × h)	6.578	8.570	10.104	5.931	13.617	10.890
qs g/(g × h)	18.336	25.445	46.141	15.619	25.643	25.557

Study on physiological response of *Pseudomonas fluorescens* AM11 to cleaning compositions with added disinfectant

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Key words: disinfectants, synergy, *Pseudomonas fluorescens*, specific growth rate.

A broad spectrum of surface cleaning preparations (SCPs) is available worldwide. Nevertheless many problems remain unsolved in the context of efficiency of these preparations. SCPs with disinfection properties require additional testing of surface active compounds and disinfection agents, both separately and in a mixture, because of their possible synergistic effect. SCPs are applied in different areas, e. g., health care, house keeping, car washing, etc. Because many humans spend a large amount of time in closed environments (including cars), it can be expected that the microbial ecology of these environments will influence the human microbiome (Stephenson et al. 2014).

The aim of this study was to compare the effect of N,N-dimethyldodecylamine N-oxide (DDAO) addition to different chemical compositions of cleaning agents.

Disinfecting properties of two different preparations (A and B) amended with DDAO were tested on bacteria *Pseudomonas fluorescens* AM11. *P. fluorescens* is a commonly used test organism. Minimal inhibitory concentration (MIC) of DDAO for *P. fluorescens* AM11 was 0.03%. This concentration was used in the present experiments. Both preparations were tested in the concentration range from 0.025 to 100%. The cleaning preparation A contained propanol, wax emulsion, mineral oil and quaternary ammonium compounds and exhibited strong disinfecting properties. Conversely, the cleaning preparation B contained silicone emulsion, isopropanol and three aromatic compounds and did not show any inhibition of microbial growth. Optical density measurements and disc diffusion tests were used to characterize the effect of cleaning preparations and DDAO on bacteria. Experiments were carried out in microplates with overnight *P. fluorescens* AM11 culture in minimal nutrient broth with 1% glucose. Optical density was measured in Tecan Infinite 200 PRO series microplate reader for 22 h every 11 min. Optical density measurements were used for calculating the specific growth rate of *P. fluorescens* AM11. Tryptone glucose agar (TGA) was used for disc diffusion method. Bacteria culture (50 µL, 10^8 CFU mL^{-1}) was spread on the surface of TGA, after that a well (4 mm in diameter) was made in the center of TGA plate, which was filled with 40 µL of preparation. Inhibition zones were measured after 24 h incubation at 30 °C.

A physiological response of *P. fluorescens* AM11 was detected only in the sets with highly diluted samples of preparation A and DDAO. In particular, 0.025% preparation A inhibited bacterial growth up to 33% as compared to control. Addition of 0.03% DDAO to this mixture resulted in a total inhibition of bacterial growth. It was taken into consideration that preparation A and DDAO have strong disinfecting properties. In turn, preparation B showed growth stimulation effect for *P. fluorescens* AM11 in a concentration-dependent manner. The results obtained with the disc diffusion method were in a good agreement with those obtained by batch cultivation. Further experiments are needed to better understand chemical interactions occurring in complex cleaning compositions which influence microbial susceptibility to disinfectants.

Acknowledgements

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Seasonal temperature adaptation of boreonemoral bryophytes: problems and perspectives

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Key words: bryophytes; chlorophyll fluorescence; temperature adaptation.

Physiological adaptations of boreonemoral bryophyte species to winter conditions are only poorly understood. The aim of the present study was to analyze seasonal temperature adaptation in different epigeic bryophyte spacies from the same site by means of chlorophyll *a* fluorescence analysis, possibly reflecting changes in photochemical activity of photosystem II.

Bryophyte samples from four epigeic species from pine forest (ectohydric *Hylocomium splendens*, *Pleurosium schreberi, Ptilium crista-castrensis*, endohydric *Rhytidiadelphus triquetrus*) were collected on February 19, March 31, May 7, and June 11. Samples were allowed to take up distilled water, blotted dry and subjected to chlorophyll a fluorescence measurement using Handy PEA (Hansatech, UK). Chlorophyll a fluorescence was measured in controlled conditions within a range of natural temperature changes exhibited by boreonemroal bryophytes, from –20 to 30 °C. Tissue samples together with positioned darkening clips were placed in a freezer (–20 and –10 °C), refrigerator (4 °C) or growth chamber (15, 23 and 30 °C) in black polyethylene bags for 1 h. After incubation, chlorophyll *a* fluorescence was immediately measured.

Significant differences were found between various fluorescence parameters in respect to temperature dependence even for the same samples. Maximum value for F_v/F_m and F_v/F_0 was at 4 °C from February to May, as average air temperatures were relatively low. Only in June 11, optimum temperature for F_v/F_m shifted to 15 °C (Fig. 1). For Performance Index, the maximum intensity was at somehow borader range, but the first maximum changed from 4 to 15 °C only in June. The character of temperature dependence changed from winter to spring, but these changes depended not on absolute level of the average temperature but rather on nature of temperature fluctuations within the previous days. On February 19, when temperature was relatively stable at 1.4 °C, as well as on May 7, when there was a period of relatively low temperature after significant decrease, variation between the species was more pronounced in comparison to March 31 and June 11, which coincided with the peak of temperature irrespective of its absolute value (Fig. 1). It is interesting to note that the absolute values of photochemical activity of photosystem II did not change significantly during the course of the experiment, indicating that the photosynthetic apparatus is well adapted to functioning at both low and moderate temperature.

It is possible that other factors besides temperature control intensity of photochemical reactions of photosystem II in boreonemoral bryophytes during winter and spring, e.g., native water content at the time of sampling and light intensity in native conditions.

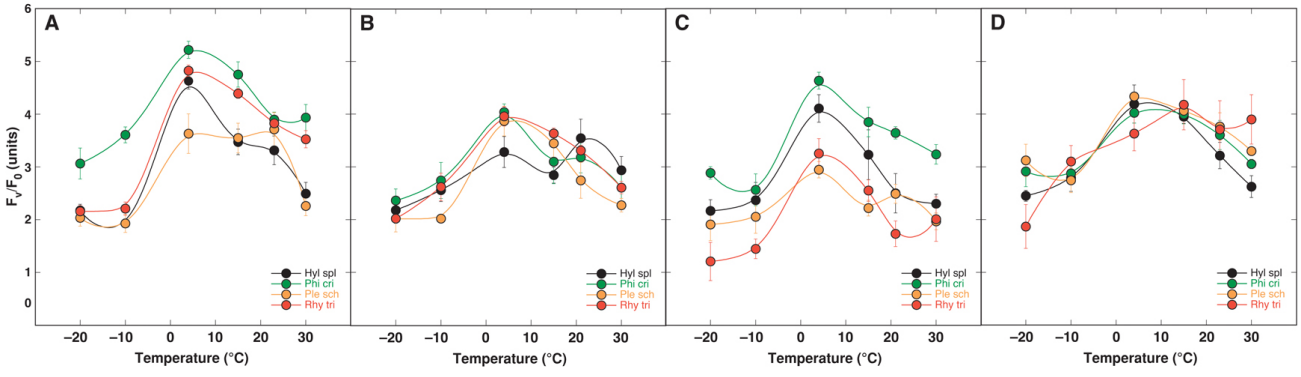

Fig. 1. Temperature dependence of fluorescence parameter F_v/F_0 in bryophyte samples of different species collected on February 19 (A), March 31 (B), May 7 (C) and June 11 (D). Data are means from 5 samples ±SE at each time point.

Leaf and fruit diseases in plum orchards in Latvia

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Key words: brown rot, *Diaporthe eres*, latent infections, *Monilia fructigena*, plums, *Wilsonomyces carpophilus*.

Several leaf and fruit diseases have been described on plums: plum pockets caused by *Taphrina pruni*, rust (*Tranzschelia pruni-spinosae*), shot-hole disease (*Wilsonomyces carpophilus*), brown rot (*Monilinia fructigena, Monilinia laxa*), bacterial spot (*Xanthomonas campestris* pv. *pruni*) (Ogawa et al. 1995). *X. campestris* pv. *pruni* can cause leaf spots, lesions on twigs and fruits (Hetherington 2005). In Lithuania the plum scab caused by *Venturia carpophila* has been detected also (Valiuškaite 2002). Considering the brown rot it has been described that infections with fungi from the genus *Monilinia* can be latent (Michailides et al. 2007). In Lithuania in the assessment of stone fruit trees during 1995 – 1999 it was observed that the most harmful diseases were moniliosis, cherry leaf spot caused by *Blumeriella jaapii*, silver leaf disease caused by *Chondrostereum purpureum*, and anthracnose (*Colletotrichum* spp.) (Valiuškaite 2002).

In the present study six plum orchards in Latvia were examined in July and August 2014. One orchard was commercial with integrated pest management practices, one was with organic management, two orchards were scientific collections and in two orchards plums were grown as a minor crop, using integrated pest management practices. The shot-hole disease, silver leaf disease and fruit rot were monitored in the field. Samples of twigs and leaves were taken for further examination in laboratory if some other disease symptoms were observed. From 10 varieties in three orchards 100 immature fruits were taken to the laboratory to assess the latent moniliosis according to the methodology of Michailides et al. (2007). *M. fructigena* isolates from all orchards were subjected to fungicide sensitivity tests.

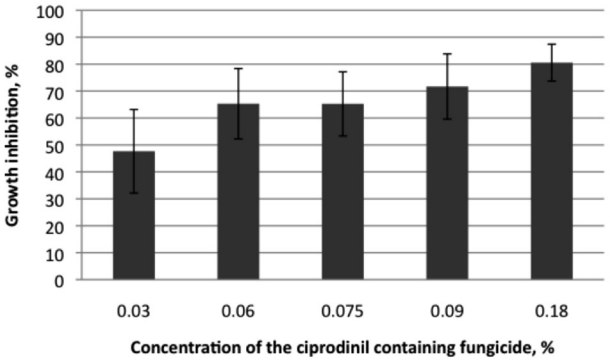

Fig. 1. Growth inhibition of *M. fructigena* in the presence of the ciprodinil. Error bars indicate standard deviations (±SD), n = 17.

In total 50 European plum (*Prunus domestica*) and six diploid plum cultivars were inspected: one Japanese plum (*Prunus salicina*) cultivar, one myrobalan plum (*Prunus cerasifera*) cultivar, four Japanese plum hybrids *P. salicina* × *P. cerasifera*.

The silver leaf disease was observed in two orchards. Incidence and severity of shot-hole disease was significantly different among various orchards comparing the same cultivar, as well as between diploid and European plum cultivars. The average incidence of shot-hole disease was 41% in diploid plums and 80% in European plums, while the average severity was 9 and 15%, respectively. In the field fruit rot caused only by *M. fructigena* was detected. The average incidence of brown rot on diploid plums was less than 1% but on European plums it was 3.6%.

In the fungicide sensitivity tests dithianon, penkonazole, mankoceb and boscalid with piraclostrobin inhibited the growth of *M. fructigena* isolates by 92 to 100% but lower sensitivity was observed against ciprodinil (Fig. 1).

The latent infection tests showed that plum fruits had higher incidence of brown rot than was observed in the field, up to 44%. Additionally from the fruits subjected to these tests *Botrytis cinerea, Diaporthe eres* and *Colletotrichum* spp. were isolated.

Examinations in the laboratory showed the presence of *D. eres* in samples from all orchards. In one of the scientific collections *D. eres* was isolated from twigs, leaves and fruits. In other orchards this fungus was isolated only from fruits, and mainly from the latent infection tests.

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Cloudberry *Rubus chamaemorus* in Latvia – a new perspective crop

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Key words: cloudberry, *Rubus chamaemorus*.

Cloudberry (*Rubus chamaemorus* L.) is a perennial species with a boreal circumpolar distribution. It is widespread in North America, in Europe it is found mainly in Russia, Norway, Finland and in Asia (Thiem 2003). Cloudberries grow mostly in peaty moors and bogs in mountains, in generally acidic and nutrient-poor soils. In Latvia cloudberry is a wild-harvested fruit, traditionally used as a healthy food, considered as a delicacy with very high price in the local market. The natural cloudberry stands are only seldom found in peat bogs. Cloudberry has a low and variable productivity. The yield is limited by many factors, including climate conditions, mineral nutrition, sensitivity to late frosts. One of the factors limiting cloudberry yield is complicated reproduction system. Cloudberry is dioecious and fruit production by a female plant requires pollination from a male plant, a right sex ratio is necessary to ensure sufficient pollination (Thiem 2003).

There are two ways how to produce cloudberries. The first is using natural stands increasing yield by fertilisation. This method of production is unsuitable for Latvia because of rare distribution of cloudberry plants. The second method involves artificial cultivation in appropriate conditions. Over the past few years, a stable commercial hermaphrodite variety has been produced in Norway and is propagated in Finland for cultivation under controlled conditions. Hermophrodite cultivars have been selected in order to increase plant productivity and berry yield. The best variety for the production so far is 'Nyby' (Rapp 2004).

A substantial economical value of cloudberry cultivation in Latvia is the same as for other acid-loving commercial horticultural crops. It could be associated with the geographical situation and climate of Latvia providing all of the necessary cultivation conditions for cranberries: (1) vast high peat bog territories, (2) sufficient availability of freshwater, (3) a moderate, moist summer climate, as well as unfulfilled market (Osvalde et al. 2010). Cloudberry cultivation could also be an interesting option for the after-use of decommissioned peat extraction areas, adding value to the reclamation approach (Rancourt et al. 2009).

To our knowledge there are no studies on the cloudberry biology in Latvia. Therefore, the goal of the future study will be to explore mineral nutrition characteristics of cloudberry in natural conditions, as well as to assess soil conditions that determine their growth and reproductive success in a given habitat. This information will form a basis for futher studies in controlled conditions ensuring optimal status for cultivated cloudberry plantations.

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Assessment of muscle microcirculation using correlation spectroscopy technique

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The mechanisms governing non-exercised muscle vascular response during local exercise, involving a small muscle mass are poorly understood to date. Manifestation of such responses, largely depend on exercising muscle mass, location and type of exercise and its intensity (Duprez et al. 1989; Yoshizawa et al. 2008). Contractions involving small muscle mass can induce different (even opposite) responses of peripheral resistance in vascular bed of non-exercised muscle (Taylor et al. 1989; Tokizawa et al. 2006; Tschakovsky et al. 2006).

Currently there is no widely available non-invasive method for assessment of perfusion at multiple muscle sites.

The promising technique for such a measurement is near infrared diffuse correlation spectroscopy (NIR-DCS) which can be utilized in different types of tissue.

The aim of this study was to reveal blood perfusion changes in exercising and non-exercising muscle during unilateral static contraction using NIR-DCS. All procedures were approved by local ethics committee, five male volunteers were recruited and gave their informed consent.

The protocol comprised two parts – for forearm and thigh muscle microcirculation recording, exploiting two light source-detector distances: 1.5 cm for superficial and 2.5 cm for signal acquisition from deeper tissue layer. In order to calibrate signal baseline, arterial occlusion test has been accomplished prior to exercise.

Static handgrip test was performed to assess blood perfusion of m. extensor digitorum in active and contralateral forearm. NIR-DCS signal was recorded during rest (4 min), unilateral handgrip contraction (50% MVC, 80 s) and following post-exercise recovery period (5 min).

Knee extension exercise was performed to evaluate blood perfusion of m. vastus lateralis. NIR-DCS signal was recorded during rest (4 min), unilateral static knee-extension (50% MVC, 80 s) and following post-exercise recovery period (5 min).

Static exercise induced larger increase of perfusion in deep muscle tissues (distance 2.5 cm) in comparison to superficial. In contralateral extremity a small increase of perfusion during contraction has been observed, possibly due to increased systemic cardiovascular parameters. In active limb, in contrary to contralateral, post-exercise hyperaemia following active hyperaemia has been observed.

We conclude that NIR-DCS technique utilizing HemoFloMo device can be used for non-invasive evaluation of skeletal muscle microcirculation during static exercise from different muscle parts at different depths.

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