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# ABSTRACTS
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# The antimicrobial effect of modified clay materials

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**Key words:** clay, antimicrobial effect, fungi.

Clay is one of the most common sedimentary rocks in world and also in Latvia. The spectrum of clay application is very wide, it is used in construction, cosmetics and other production sectors, but only a few clay types have natural anti-microbial activity (Haydel et al. 2008). So the research of new antimicrobial additives is important because it would not only enhance the application of clay in the areas where it is already used, but would help to discover new aspects of use.

*Cladosporium herbarum* and *Alternaria alternata* are saprophytic fungi. These species are very common and often contaminate air. In natural conditions they are found in many substrates – in composites, plant debris, soil, in wood materials and they are also plant pathogens. In indoor conditions they develop on foodstuffs, textiles, walls and elsewhere (Schubert et al. 2007; Bagherabadi et al. 2015).

Fungi from both *Cladosporium* and *Alternaria* genera are allergic agents. Since they are able to grow on the walls, their elimination is important not only from a medical point of view, but also from the point of view of preservation of cultural heritage. The major fungal growth is observed in buildings with high relative humidity. Nowadays 15 to 40% of North Americans and Northern Europeans encounter mould growth in indoor conditions (Andersen et al. 2011).

Malt extract agar medium was prepared for this study. Four different modified clay powders from LLC Baltic Clay Minerals where added to 0.2 and 2.0% concentration. One half of media were infected with *Cladosporium herbarum*, other half with *Alternaria alternata*. Plates were incubated at room temperature and results were assessed after seven days. Antimicrobial effect was observed in three of the four tested samples. (Fig. 1).

*A. alternata* and *C. herbarum* growth analyses were also performed on potential composite construction materials, containing modified additives.

**Acknowledgements**
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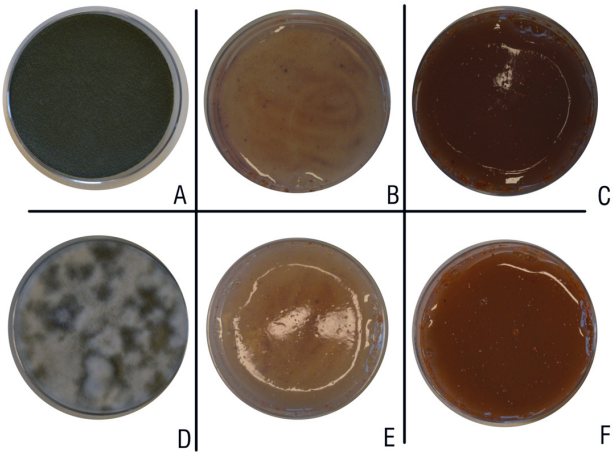


**Fig. 1.** Control plates and plates with modified clay powder number 2. A, B, C – *Cladosporium herbarum*; D, E, F – *Alternaria alternata*; A, D – control samples without clay powder; B, E – medium with modified clay powder, contains 0.2% clay; C, F – medium with modified clay powder contains 2.0% clay.

# First results of research on cloudberry *Rubus chamaemorus* in Latvia

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**Key words:** cloudberry, cultivation, mineral nutrition, *Rubus chamaemorus*.

Cloudberry (*Rubus chamaemorus* L.) is a perennial species with a boreal circumpolar distribution. It is widespread in North America, and in Europe it is found mainly in Russia, Norway, Finland (Thiem 2003). Cloudberries grow mostly in peaty moors and bogs in mountains, in acidic and nutrient-poor soils. Specific species biology (Korpelainen 1994) is the main challenge for scientists as well as for growers: (1) about 95% of plant biomass is allocated to underground and only 5% to sexual reproduction, (2) complicated pollination related to unisexuality in cloudberries is serious obstacle for cultivation due to different time of flowering and unfavourable proportion of female and male plants.

The significant turning point in the cloudberry cultivation could be associated with propagation of stable hermaphrodite individuals (having both male and female sexual characteristics and organs) cloned from a wild population in Finland. After observations and trials, this clone was named as a cultivar 'Nyby'. This cultivar is commercially available from 2005, with high self-pollination ability and successful results in the greenhouse and open field trials (Uosukainen 2010).

Our first task was 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 particular habitat. Leaf and soil samples were collected from different cloudberry sites in woodlands (Olaine, Mālpils, Dobele, Līvāni district) of Latvia. Concentrations of 12 biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) were determined in all soil and leaf samples.

Our first results showed especially high heterogeneity of plant-available nutrient concentrations in soils from natural cloudberry stands. The results of our previous studies on different wild berries in Latvia were used to evaluate nutrient status of cloudberry. In comparison to wild blueberries (*Vaccinium myrtillus*), wild cranberries (*Vaccinium oxycoccos*) and lingonberries (*Vaccinium vitis-idaea*), cloudberries showed especially high rate of N, P, K, Mg, Zn accumulation in leaves. Probably cloudberries need higher level of N for successful growth and berry production. In general, the level of plant-available nutrients in cloudberry soil was more similar to cranberry growing medium, but a level of nutrients in leaves was similar to that in blueberries. Further studies are necessary to reveal specificity of cloudberry mineral nutrition in natural populations.

Our second task was to start the development of basic principles for cultivation technology of hermaphroditic cloudberry cultivar 'Nyby', corresponding to soil and climate conditions in Latvia. Field experiment was established in farm Strelnieki, autumn 2014, with seedlings of cloudberry cultivar 'Nyby'. Different fertilization treatments were used according to the previous experience on acidophilic species, as highbush blueberries (*Vaccinium corymbosum*) and American cranberries (*Vaccinium macrocarpon*). To our knowledge there is not much publications on mineral nutrition of cloudberries. Cloudberry grew much better in less-decomposed fibric peat (Bussieres 2015).

The results of the first study years revealed that there are many external factors important in cloudberry cultivation, including soil type, light, temperature, water availability, and quality, as well as management practices. The main factors that seriously affected cloudberry survival were insect pests, weeds, water quality, and high light intensity.

To better understand specificity of cloudberry physiology and promote growing success, further research should focus on possibilities to eliminate the adverse impact of the limiting factors. Vegetation experiments with cloudberry seedlings in controlled or partly controlled conditions are crucial to perform this task.

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# Effect of different salts on growth and physiological charateristics of *Secale cereale* seedlings: a possible relationship with ethylene

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**Key words:** cereal seedlings, chlorophyll *a* fluorescence, ethylene, ethephon, ions, 1-methylcyclopropene, polyethylene glycol, salinity, *Secale cereale*.

NaCl is often regarded as an extremely toxic compund for plants. Altered water status, ion imbalance and hyperosmotic stress resulting from NaCl treatment are usually reported as causing further growth inhibition and molecular damage through production of reactive oxygen species (Munns, Tester 2008). Limited experimental evidence suggest that cellular toxicity of $Na^+$ is comparable to that of $K^+$ and $Mg^{2+}$ (Kinraide 1999; Tobe et al. 2002). It has been shown that modulation of plant ethylene responses affects salinity tolerance suggesting involvement of ethylene in the respective regulatory mechanisms (Cao et al. 2007).

The aim of the present study was to compare physiological effect of different non-heavy metal salts on winter rye seedlings. It was tested if treatment with different combinations of metal cations ($Na^+$, $K^+$, $Mg^{2+}$) with anions ($Cl^-$, $SO_4^{2-}$) in salt could differentially affect physiological responses in a glycophyte species. For comparison, osmotic stress effect was assessed using treatment with polyethylene glycol (PEG) at osmomolar concentrations. It was also hypothesized that ethylene is involved in regulation of both low salt-induced growth stimulation and high salt-induced growth inhibition of winter rye seedlings, therefore, ethylene receptor blocker 1-methylcyclopropene (MCP) was used.

Winter rye (*Secale cereale* L.) seedlings were cultivated in hydroponics system using filter paper rolls (10 germinated seeds per roll, with five replicates per treatment). Rolls were placed in plastic tissue culture containers accomodated in 50 L transparent plastix boxes, closed with lids and cultivated under 150 μmol $m^{-2}$ $s^{-1}$ light with 16 h photoperiod (22 ± 2 °C). Containers with rolls contained 100 mL 200% Knop' s macronutrient solution with Murashige and Skoog micronutrients plus 100 mL of an appropriate test solution (200%). For salt treatment, NaCl, KCl, $MgCl_2$, $Na_2SO_4$, $K_2SO_4$ and $MgSO_4$ was used in a range of final concentration 10, 25, 50, 100, 200 mM. For polyethylene glycol treatment, final concentration of PEG 4000 was 8, 16, 32, 64, 128 mM. In some experiments a glass vial with MCP or ethephon solution was placed inside boxes. Chlorophyll *a* fluorescence parameters were analyzed in dark-adapted leaves by Handy PEA fluorometer (Hansatech Instruments). Electrical conductivity and $Na^+$, $K^+$, $NO_3^-$ concentration in plant water extracts were analyzed by LAQUAtwin compact meters (Horiba, Japan).

Seedling growth was activated at low salt and PEG concentration (10 and 25 mM) and the effect decreased in an order KCl > PEG > $Na_2SO_4$ > $K_2SO_4$ > NaCl > $MgSO_4$ > $MgCl_2$ (Fig. 1). Growth was inhibited at high salt and PEG concentration (200 mM) and the effect increased in an order $Na_2SO_4$ < $K_2SO_4$ < KCl < NaCl < $MgSO_4$ < $MgCl_2$ < PEG. Photochemistry of photosystem II was activated in an order $K_2SO_4$ > $MgSO_4$ > KCl > $Na_2SO_4$ > NaCl > $MgCl_2$.

In control seedlings MCP significantly stimulated elongation growth of the first leaf and tended to stimulate growth of the second leaf as well as fresh mass accumulation, indicating that perception of endogenously produced ethylene lead to growth inhibition in rye seedlings. Stimulative effect of 25 mM NaCl and KCl on elongation of the first leaf was completely abolished in MCP-treated seedlings, showing ethylene-dependent effect of low salinity treatment. However, this effect can be also interpreted as an inability of low salt treatment to induce further increase in first leaf elongation in MCP-treated seedlings. All salts at high concentration (200 mM) inhibited elongation of the first leaf of MCP-treated seedlings, but for NaCl and KCl it can be interpreted also as MCP-dependent growth stimulation, as leaf height in 200 mM treated seedlings was higher in comparison to control seedlings grown without MCP. In contrast, MCP treatment resulted in more pronounced growth inhibition of the first leaf due to 200 mM $MgCl_2$ treatment.

In NaCl-treated seedlings, $Na^+$ accumulated in a diminishing concentration in root > coleoptile > 1st leaf > 2nd leaf. MCP treatment stimulated $Na^+$ accumulation only at low NaCl concentration (10 and 25 mM). $NO_3^-$ concentration significantly increased in tissues of all parts of rye seedlings.

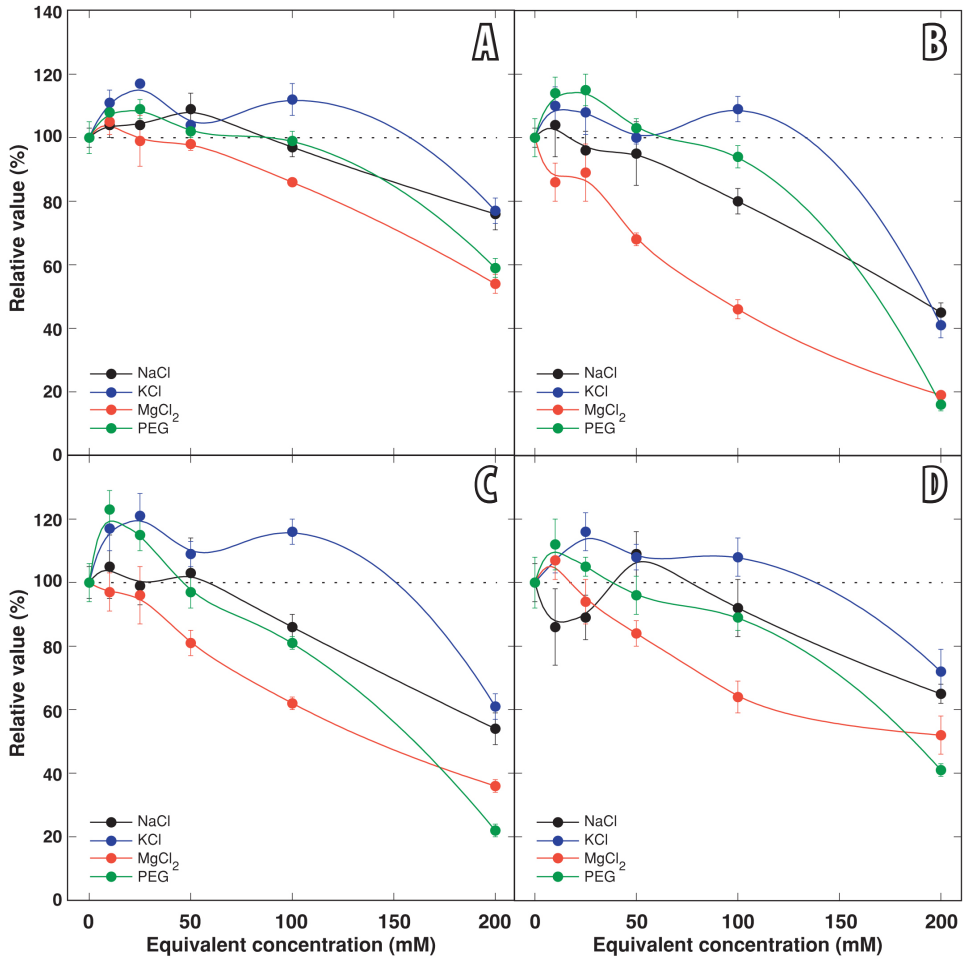


**Fig. 1.** Relative effect of different salts and polyethylene glycol (PEG) on height of 1st leaf (A), height of 2nd leaf (B), fresh mass of shoot (C), and dry mass of shoot (D) of 8-day-old winter rye seedlings.

Inhibitory effect of salts on growth of winter rye seedlings is due to involvement of specific regulatory systems rather than results from a direct osmotic or toxic effect. It appears that in winter rye seedlings ethylene is involved in regulation of low salt-induced growth stimulation but not in high salt-induced growth inhibition. It is possible that MCP has additional physiological effect(s) in the present experimental conditions besides blocking of ethylene perception.

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# Response of European protected species *Dianthus arenarius* subsp. *arenarius* to trampling and sand burial in controlled conditions

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**Key words:** chlorophyll, chlorophyll *a* fluorescence, *Dianthus arenarius*, ions, protected species, sand burial, trampling.

*Dianthus arenarius* subsp. *arenarius* L. (sand pink, Caryophyllaceae) is protected by the EU Habitat Directive 92/43 EEC (Annex II and IV) as well as Annex I of Resolution 6 of the Bern Convention. It is calcareous dry grassland and gray dune species growing along coasts of the Baltic Sea in Sweden, Estonia, Latvia and Lithuania. *D. arenarius* subsp. *arenarius* has been characterized as positively responding to trampling in natural conditions, together with intermediate disturbance preference, but the number of individuals responded negatively to mechanical soil disturbance showing re-establishment during the second year (Schnoor, Olsson 2010). Factors affecting distribution and physiological performance of *D. arenarius* subsp. *arenarius* is far from clear. It is reasonable to predict that, similar to other typical grey dune species, *D. arenarius* subsp. *arenarius* are affected by light availability, sand burial, trampling and other types of disturbance (Ievinsh 2006). The aim of the present experiment was to study the effect of sand burial and trampling as well as interactions between these factors in controlled conditions. It was hyopthesized that burial of plants by sand at different levels can affect the following physiological response to trampling.

Plants for experiments were propagated vegetatively from a small sample collected in nature. One week after final transplanting in 1 L plastic containers containing 7 cm of quartz sand (Saulkalne) and 2 cm of garden soil (Biolan), plants were asigned to the following treatments: (i) control, (ii) trampling 1 – five impacts by a mechanical foot per week for 4 weeks, (ii) trampling 2 – ten impacts by a mechanical foot per week (on two successive days), (iii) burial 1 – burial by 1.3 cm of quartz sand, (iv) burial 2 – burial by 2.7 cm of quartz sand, (v) burial 1 plus trampling 1, (vi) burial 1 plus trampling 2. The mechanical foot was designed to simulate human trampling and consisted of a metal cilinder filled with metal rods (total weight 10 kg), cork-covered base in a form of soil surface of a growth container, and a block system allowing for easy and uniform impact. Five individual plants per treatment were used. One week after the final trampling non-destructive analysis of photosynthesis-related parameters were started for the following eight weeks during the recovery period. Leaf chlorophyll concentration was analyzed by a chlorophyll meter CCM-300 (Opti-Sciences). Chlorophyll *a* fluorescence parameters were analyzed in dark-adapted leaves by Handy PEA fluorometer (Hansatech Instruments). After eight weeks, plants were harvested, separated in roots and shoots, and both fresh and dry mass were measured. Electrical conductivity and $Na^+$, $K^+$, $NO_3^-$ concentration in leaf water extracts were analyzed by LAQUAtwin compact meters (Horiba, Japan).

Immediately after the end of the 4-week-long trampling treatment, plant growth was significantly depressed in comparison to that of control plants. Higher trampling intensity resulted in more depressed growth. There were no visual differences in morphology of trampled plants and those buried by sand before trampling. These observations were supported also by the data from non-destructive physiological analysis, showing significant inhibition of activity of photosystem II as well as decreased leaf chlorophyll concentration. Interestingly, burial itself had more pronounced negative effect on physiological performance. Recovery of physiological parameters was evident already two weeks after the end of the treatment in trampled-only plants. When the experiment was terminated eight weeks after the end of the treatment, trampled plants were still significantly smaller in comparison to control (in average, 49 and 41%, for trampling 1 and 2, respectively; Fig. 1). However, moderately buried plants had reached control level and even had significantly higher dry mass of roots. Plants buried by 2.7 cm of sand still had lower mass (in average, 75% from that in control). Burial of plants before trampling had positive effect on growth recovery in the case of lower trampling intensity (in average, 67% from control), but it was slightly negative in the case of higher trampling intensity (in average, 38% from control). Trampling had negative effect on solute concnetration in leaves and lowered $K^+$ concentration, but burial by sand (which itself had no significant effect on these parameters),

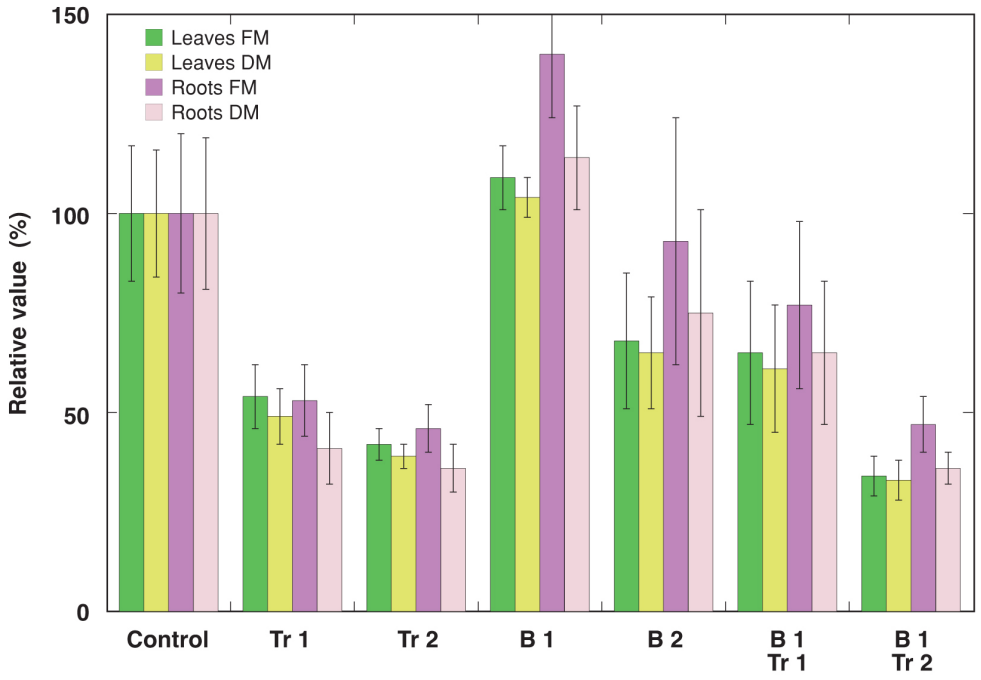


**Fig. 1.** Effect of different intensity of simulated trampling (Tr 1, Tr 2), sand burial (B , B 2) and combination of factors on relative fresh and dry mass of leaves and roots of *Dianthus arenarius* subsp. *arenarius* 8 weeks after the end of the trampling. Data are means ±SE from five replicates.

resulted in recovery of the parameters in trampled plants.

It can be concluded that *D. arenarius* subsp. *arenarius* plants are relativelyt tolerant to sand burial and, to a lesser extent, to moderate trampling, but burial can lead to lesser damage or faster recovery after moderate level of trampling. However, physiological performance is more negatively affected by sand burial in comparison to trampling.

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# Possible effect of storage conditions on concentration of photosynthetic pigments in bryophyte samples

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**Key words:** bryophytes, carotenoids, chlorophyll, photosynthetic pigments, spectrophotometric assay.

Measurement of concentration of photosynthetic pigments in bryophyte tissues represents an important method both in ecological and environmental studies. Methodology of sampling, manipulations before analysis as well as nature of extrahant used can affect results of the study. It has been shown that 95% ethanol is a most reliable solvent for spectrophotometric determination of photosynthetic pigment concentration in bryophytes (Bao, Leng 2005). Sometimes it is necessary to store sampled bryophyte tissues before analysis. The aim of the present experiment was to compare possible effect of different storage conditions on results of photosynthetic pigment assay using three common boreonemoral bryophyte species.

Samples from *Rhytidiadelphus triquetrus, Hylocomium splendens, Pleurosium schreberi* were collected in pine forest on November 21 when daily air temperature was near 0 °C. The samples were transfered to laboratory within less than 24 h. Control samples were immediately analyzed for pigment concentration. Part of the tissues were used for determination of field water content after incubation at 60 °C for 48 h. The rest of the tissues were divided in five samples stored for 50 days at different conditions. The treatments included: storage at –20 °C in a tightly closed polyethylene bag, storage at 4 °C in a tightly closed polyethylene bag, storage at 4 °C after 1 week drying at room temperature, drying and storage at room temperature, storgae at room temperature after drying for 48 h at 60 °C. For analysis of photosynthetic pigments, 10 mg of dried tissues or 50 mg of wet tissues were ground in 10 mL of 96% ethanol and centrifuged. Absorbtion was measured spectrophotometrically at three wavelengths (665, 649, 440 nm) and pigment concentration was calculated. Three subsamples from every sample were analyzed in three replicates. Results were expressed on dry mass basis.

There were no significant differences in pigment concentration between samples stored at –20 °C and control samples except carotenoids in *H. splendens*, as well as samples stored at room temperature except carotenoids in *R. triquetrus* (Table 1). Four cases of significant differences from control samples were seen both for samples stored at 4 °C after 1 week at room temperature as well as for samples stored at room temperature after 48 h at 60 °C. In contrast, six cases of significant differences were seen for bryophyte samples stored wet at 4 °C. Thus, both storage at –20 °C and storage at room temperature of bryophyte samples for at least 50 days are appropriate leading to minimum changes in photosynthetic pigment concentration.

**Acknowledgements**
We thank Dr. biol. Ligita Liepiņa for providing bryophyte samples.

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**Table 1.** Effect of storage conditions on concentration of photosynthetic pigments in bryophyte samples. Data are means from 3 replications (subsamples) ± SE. Identical letters within a column indicate non-significant differences between the means. T, temperature

| Conditions | *Rhytidiadelphus triquetrus* | | | *Hylocomium splendens* | | | *Pleurosium schreberi* | | |
|---|---|---|---|---|---|---|---|---|---|
| | Chl *a* | Chl *b* | Carot | Chl *a* | Chl *b* | Carot | Chl *a* | Chl *b* | Carot |
| Control | 100 ± 16 a | 100 ± 16 a | 100 ± 10 a | 100 ± 17 a | 100 ± 17 a | 100 ± 14 a | 100 ± 10 a | 100 ± 12 a | 100 ± 6 a |
| –20 °C | 94 ± 12 ab | 92 ± 8 a | 105 ± 9 a | 103 ± 12 a | 111 ± 6 a | 138 ± 21 b | 90 ± 5 a | 92 ± 4 a | 103 ± 4 a |
| 4 °C | 82 ± 12 b | 50 ± 8 b | 77 ± 9 b | 60 ± 17 b | 41 ± 6 b | 91 ± 5 a | 108 ± 10 a | 92 ± 8 a | 179 ± 30 b |
| 4 °C (after 1 week at room T) | 94 ± 9 ab | 84 ± 6 c | 96 ± 9 a | 81 ± 11 c | 76 ± 9 c | 73 ± 11 c | 90 ± 17 a | 92 ± 4 a | 104 ± 3 a |
| Room T | 96 ± 9 a | 104 ± 11 a | 85 ± 11 b | 98 ± 15 a | 86 ± 12 a | 91 ± 4 a | 92 ± 9 a | 95 ± 11 a | 102 ± 8 a |
| Room T (after 48 h at 60 °C) | 74 ± 10 bc | 111 ± 4 a | 62 ± 17 bc | 109 ± 16 a | 140 ± 9 d | 93 ± 6 a | 98 ± 6 a | 122 ± 15 b | 101 ± 4 a |

# Physiological changes of rare coastal species *Plantago maritima* due to extreme substrate salinity

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**Key words:** chlorophyll, chlorophyll *a* fluorescence, ions, *Plantago maritima*, proline, rare coastal species, salinity.

*Plantago maritima* is a relatively widely used model species in studies of halophytic plants native to temperate zone (Sekmen et al. 2007; Sleimi et al. 2015). It is extremely rare species in Latvia, occuring only at wo sites, the major being wet salt-water affected meadow in the territory of city Liepāja on shore of Lake Liepājas. In natural conditions, *P. maritima* is subjected to transient episodes of increased soil salinity due to soil lodging with saline water early in the season followed by freshwater inundation. The aim of the present study was to examine physiological response of *P. maritima* plants to extreme substrate salinity following treatment with NaCl solution with higher than sea water salinity level.

Plants for experiments were propagated from seeds collected in nature and transplanted in 1 L plastic containers with garden soil (Biolan). Treatments were started four weeks after final transplanting. Initially plants were watered with 250 mM NaCl, and one week later with a final concentration. The resulting treatments were 0, 250, 500, 1000, and 2000 mM NaCl. Leaf chlorophyll concentration was analyzed by a chlorophyll meter CCM-300 (Opti-Sciences). Chlorophyll *a* fluorescence parameters were analyzed in dark-adapted leaves by Handy PEA fluorometer (Hansatech Instruments). Plants were harvested 9 weeks after the start of the treatment.

Plant growth was gradually decreased in parallel with increasing NaCl concentration. Four weeks after the start of the treatment 2000 mM NaCl-watered plants started to perish and after two weeks they all were dead. This was clearly reflected by changes in chlorophyll a fluorescence parameters (Fig. 1). Growth of large leaves was less negatively affected by salinity, showing some increase of fresh mass (7%) and dry mass (22%), followed by small leaves, roots, flowers and flower petioles. Leaf chlorophyll concentration was increased in all treatments except 2000 mM NaCl. Photochemical efficiency of photosystem II gradually recovered 3, 4 and 6 weeks after the treatment for 250, 500, and 1000 mM NaCl-treated plants, respectively. $Na^+$ accumulated in a decreasing concentration in old leaves < young leaves < flower petioles < flowers, and were excluded from roots, but $K^+$ concentration in roots increased linearly with increasing NaCl concentration. There was a significant increase in leaf proline concentration in NaCl-treated plants.

In conclusion, *P. maritima* can maintain active physiological processes together with reduced growth under extreme events of salinity up to 1000 mM NaCl in watering solution. Exclusion of $Na^+$ from roots and accumulation in leaves together with protective substances (proline) could constitute physiological salinity tolerance in this species.

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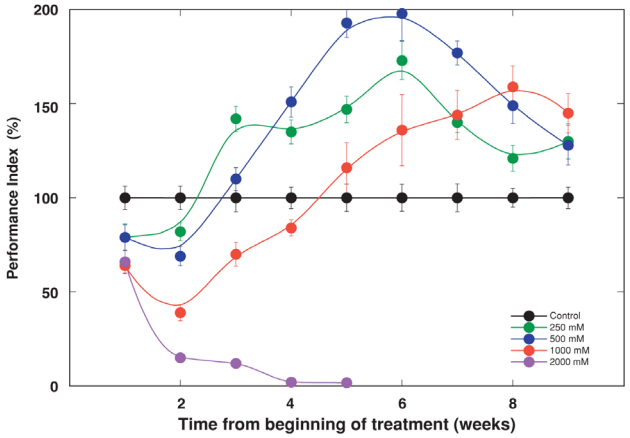


**Fig. 1.** Recovery of photochemical efficiency of photosystem II in leaves of *Plantago maritima* after treatment with different concentration of NaCl. Plants treated with 2000 mM NaCl ultimately perished on week 6.

# Effect of cocultivation of related species on abiotic and biotic responses: the first results from the *Trifolium* study

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**Key words:** chlorophyll, chlorophyll *a* fluorescence, cocultivation, competition, ions, rare coastal species, symbiotic N-fixing bacteria, *Trifolium fragiferum, Trifolium repens*.

Competition between different plant species is usually a neglected aspect in vegetation experiments in controlled conditions. However, it is reasonable to suggest that cocultivation can affect plant responses to changes in both abiotic and biotic environmental factors (Reader, Bonser 1993; Howard, Goldberg 2001). *Trifolium fragiferum* and *Trifolium repens* are economically important legume species. In Latvia these species grow together natively in a wet salt-water affected meadow in the territory of city Liepāja on shore of Lake Liepājas. Both species are characterized by intensive clonal growth, therefore, it is difficult to predict the outcome of competition between them. Episodes of high soil salinity due to sea water inundation in conditions of coastal marsh can affect the competition through differences in salinity and flooding tolerance: *T. fragiferum* (relatively rare) is known as intermediate tolerant species while *T. repens* (relatively common) is recognized as more salt sensitive species. In addition, symbiotic nitrogen-fixing bacteria could have effect on *T. fragiferum*-*T. repens* interaction in natural conditions (van der Heijden et al. 2006). There is no information available on this type of interaction between rare and common taxonomically and functionally related species. The aim of the present study was to assess possible effect of symbiotic bacteria on interaction between the both species grown at elevated substrate salinity in controlled conditions. During the first stage of the study, it was specifically asked whether (i) cocultivation of different species affects their physiological responses to bacterial symbiosis and salinity, and (ii) different bacterial inoculants affect response to salinity.

Seeds of *T. fragiferum* and *T. repens* were collected in natural conditions at Lake Liepajas. Seeds were germinated aseptically and initially grown in autoclaved garden soil. For the experiment, 90 plastic containers (1 L) filled with autoclaved garden soil (Biolan) with two plants per container were used. One plant of eiher species from natural conditions was used for isolation of symbiotic bacteria from root nodules. Competition between plants/species was assessed by planting either two *T. fragiferum* or *T. repens* individuals per container, or each individual from different species. As a biological factor, N-fixing bacteria isolated from *T. fragiferum* and *T. repens* from wild plants were used as a stock for inoculation of experimental plants. As an abiotic factor, plants were watered with increasing concentration of NaCl weekly, finally reaching 400 mM. In total, 18 treatments in five replications were used. Measurement of ion concentration in leaves and petioles as well as non-destructive analysis of physiological performance were assessed every week during the experiment. Leaf chlorophyll concentration was analyzed by a chlorophyll meter CCM-300 (Opti-Sciences). Chlorophyll *a* fluorescence parameters were analyzed in dark-adapted leaves by Handy PEA fluorometer (Hansatech Instruments).

It was evident 4 weeks after the start of cultivation that inoculants were biologically efficient in N-fixation as control plants without inoculation had significantly lower leaf chlorophyll concentration showing clear signs of mineral deficiency (Fig. 1). Both inoculants were active for either species, but some specificity was evident in respect to physiological responses. Thus, $Na^+$ concentration increased in leaves of NaCl-treated *T. fragiferum* plants only in the case of inoculation with the own bacteria. In addition, only inoculation of *T. repens* with the own inoculum significantly increased leaf $NO_3^-$ concentration during cocultivation with *T. fragiferum*. In general, effects of inoculation were more pronounced in conditions of cocultivation. Cocultivation of both species itself significnatly changed physiological responses of plants. Thus, cocultivation with *T. repens* resulted in significant increase of Performance Index in leaves of *T. fragiferum* in the presence of own inoculant, but significant decrease was seen in the presence of inoculant from *T. repens*. Similar changes were evident for $K^+$ concentration. However, cocultivation resulted in increase of $NO_3^-$ concentration in *T. fragiferum* tissues and decrease in that in *T. repens* tissues independently on the type of inoculant used, while the effect of cocultivation was negative for both species

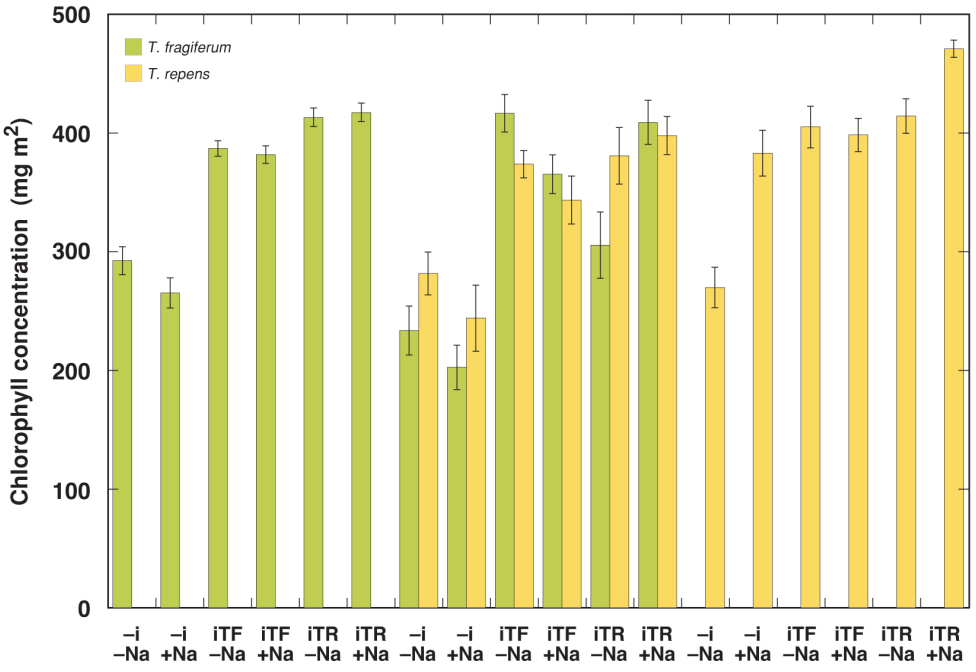


**Fig. 1.** Effect of cocultivation, inoculation with N-fixing bacteria and NaCl on chlorophyll concentration in leaves of *Trifolium fragiferum* and *Trifolium repens* 4 weeks after the start of the experiment. –i, without inoculant,; iTF, inoculant from nodules of *T. fragiferum*; iTR, inoculant from nodules of *T. repens*. Data are means from 18 (separate species) or 9 (cocultivation) individual measurements per treatment ±SE.

cultivated without inoculation.

In conclusion, cocultivation of individuals of *T. fragiferum* and *T. repens* affects plant responses to inoculation with N-fixing bacteria and NaCl. Effect of cocultivation is more pronounced in the presence of bacterial inoculum. Both inoculants are biologically active with more intense physiological effect in the case of cocultivation. Physiological reactions of *T. fragiferum* are more responsive to inoculation and cocultivation in comparison to those of *T. repens*.

**Acknowledgements**

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# Expression, purification and immunological properties of influenza hemagglutinin peptides

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**Key words:** hemagglutinin, influenza, peptides..

The lack of universal influenza vaccine is a global health problem. The protective properties of current influenza vaccines are limited as the globular head domain of hemagglutinin (HA) to which the immune response is mainly targeted is highly variable across the influenza virus strains. However, the membrane proximal stalk domain of HA has a relatively conserved sequence and structure. Including such evolutionary conserved virus protein domains in influenza vaccine could elicit broad spectrum protection against multiple subtypes of influenza virus. Here, we selected two stalk domain fragments corresponding to HA amino acids 418-474 (peptide 1) and 403-474 (peptide 2). Both peptides were derived from the most widespread influenza virus subtypes – H1N1 and H3N2 – and contain so-called long alpha helix domain, with peptide 2 extended by the unstructured loop region at the N terminus.

Expression and purification of the peptides 1 and 2 from both subtypes were accomplished in the study. T7 promoter-directed expression was performed in BL21 (DE3) cells according to standard protocols. Synthesis of target proteins and their solubility was easily detectable in Coomassie-stained acrylamide gels. For the first step of purification, we exploited thermal stability of our peptides since it was expected that they might form native HA-like α-helical trimers. Indeed, after the thermal treatment majority of target proteins remained in solution while a lot of contaminants had precipitated. Next the protein mixture was applied to weak anion exchange HiPrep 16/10 DEAE FF column and column-bind proteins were eluted with increasing salt gradient. Selected fractions were further purified on strong anion exchange column MonoQ 5/50 GL. For final polishing, peptides were subjected to size exclusion Superdex 200 10/300 GL column. Two peaks were observed here – major peak corresponding to trimer size and minor peak corresponding to monomer size. All the runs were monitored using the AKTA FPLC chromatography system. The purified peptides from trimer peak reached at least 95% purity and were further used to immunize naive 10-week-old BALB/c mice. Mice were vaccinated via the intranasal route with 30 μg of antigen with MF59 and CpG adjuvant mix and boosted two and four weeks later with 15 μg of antigen with MF59 and CpG adjuvant mix. Negative control animals were vaccinated only with adjuvant mix. Five weeks after the first immunization a blood sample was taken and six weeks after the first immunization mice were challenged with 2-2.5 MLD50 of H1N1 or H3N1 virus.

First, we tested the peptide 1 vaccinated mice. To characterize the stalk specific antibody response the mice sera were analyzed by ELISA using peptide 1 and full-length recombinant HA from H1 and H3 subtypes as a substrate. As a result, immunization with peptide 1 (either H1, or H3 subtype) elicited a strong antibody response against homologous antigens, but not to any heterologous antigen. To assess if the stalk specific antibodies can induce protection against influenza virus infection, mice were challenged with a native influenza virus. All mice immunized with peptide 1 (H3 genotype) showed rapid weight loss and succumbed to infection by day 5 post infection in both cases. Similarly, mice immunized with peptide 1 from genotype H1 showed 100% mortality by day 4 after the heterosubtypic H3N2 virus challenge, while infection with the H1N1 virus led to the death of 80% of animals by day 6 (Fig. 1A). Thus, vaccination with peptide 1 in current conditions could not provide sufficient protection against influenza virus challenge.

Next, we focused on mice immunization with N-terminally prolonged peptide 2. Similarly as with peptide 1, immunization with peptide 2 induced antibodies with high reactivity only to homologous full-length HA antigens. Interestingly, sera from mice immunized with peptide 2 from subtype H1 reacted in ELISA equally well with peptide 2 from both H1 and H3 subtypes, while sera from the peptide 2 (H3) group showed an antibody response only to homologous peptide 2. After virus challenge, mice immunized with peptide 2 (H3 subtype) lost weight rapidly and showed 100% mortality by day 6. In contrast, mice immunized with peptide 2 from subtype H1 showed recovery from infection with the H1N1 virus at statistically significant 75% survival rate (Fig. 1B).

Based on obtained seroconversion and protection data,

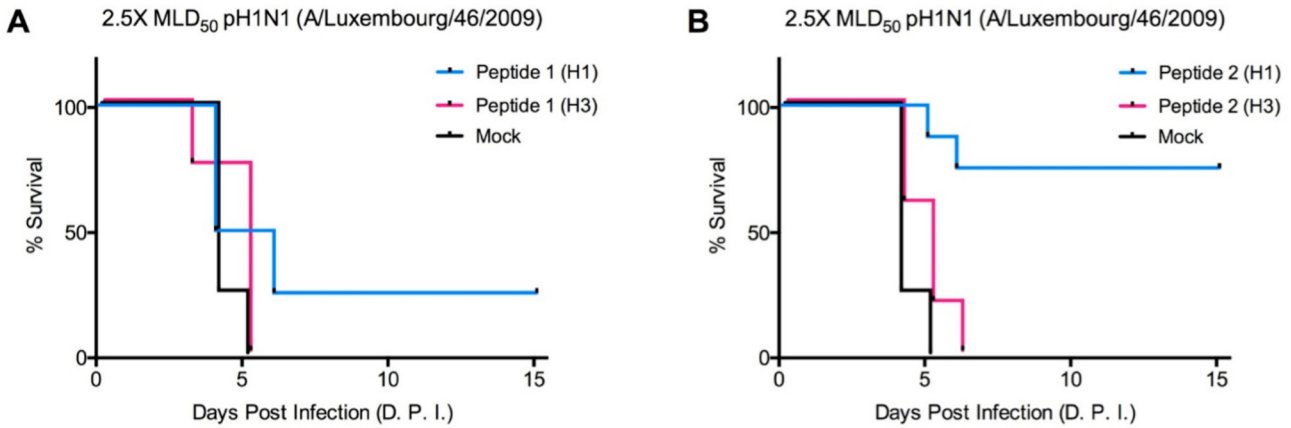


**Fig. 1.** Immunoprotective potential of HA peptides. (A) Vaccination with HA peptide 1 (H1 or H3 subtype) does not elicit sufficient protection against influenza virus challenge in mice. (B) Vaccination with HA peptide 2 (H1 subtype) partially protects from homologous virus challenge in mice.

we conclude that HA stalk peptides from H1 subtype should be considered as better vaccine candidates than subtype H3 derived peptides. In addition, presence of an unstructured loop region at the N-terminus could play a key role in peptide 2 (H1) induced protection. However, challenge-protection using heterologous strain H3N2 is still needed to complete the experiment. In our further research, we will concentrate on increasing the immunogenicity of the stalk peptides by their correct presentation to the immune system. Yet, the present data strongly indicate that the HA stalk based immunization strategy is promising in the development of novel influenza vaccine candidates.

**Acknowledgements**

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# Structural changes of macrozoobenthoss communities after Venta river rapid stage "Ventas rumba" restoration

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**Key words:** macrozoobenthoss, river restoration, Venta River.

Redublicated assessment of macrozoobenthoss with the aim to detect potential changes in macrozoobenthoss communities to compare with results of river restoration in 2011 was performed in Setember 2016. The term "restoration" has been used to describe various activities meant to restore ecological processes or to improve aquatic habitats (Roni, Beechie 2013). Sampling was performed at the same six sampling sites as in 2011. In addition, assessment of macrozoobenthoss in sampling sites where a new river rehabilitation works were performed were carried out.

Due to the river stage restoration, ecosystem of Venta River rapid stage was changed: previously dominated potamal type biotope formed by *Phragmites* and *Scirpus* was replaced by rhithral type biotope formed by sparse macrophytes on dolomite rocks. As a result, physical changes caused by spring floods and ice drift affected outcome of river stage restoration.

Species of macrozoobenthos characteristic for potamal type biotope such as *Radix ovata* (Draparnaud, 1805), *Radix peregra* (O.F. Muller, 1774), *Lymnaea stagnalis* Linneus (L.), *Asellus aquaticus* (Linnaeus, 1758), *Caenis horaria* (Linnaeus 1758) and *Helobdella stagnalis* (Linnaeus, 1758), which dominated in river stages covered by *Phragmites* and *Scirpus*, after river restoration were replaced by reophylic species *Theodoxys fluviatilis* (Linnaeus C., 1758), *Heptagenia sulphurea* Müller, 1776, *Taeniopteryx nebulosa* (Linnaeus, 1758), *Baetis vernus* Curtis 1834, *Baetis niger* (Linnaeus 1761) and *Gammarus lacustris* G.O. Sars, 1864 characteristic for rhithral type biotope. Rare and protected species *Ancylus fluviatilis* O.F. Müller, 1774, *Unio crassus* Philipsson, 1788, *Agrion virgo* (Linnaeus, 1758), *Ophiogomphus cecilia* (Fourcroy, 1785), *Libellula fulva* Müller, 1764 were found in Venta River rapid stage "Ventas rumba".

It was established that in 2016 biomass of macrozoobenthoss (g per $m^2$) and abundance of individuals (individuals per $m^2$) were essentially expanded (total biomass up to 189.4, and the number of individuals up to 68 000).

It appears that restoration of Venta river rapid stretch "Ventas rumba" positive impact on the structure and biological diversity of macrozoobenthoss communities as well as on increase of macrozoobenthoss biomass, that further promote and provide increase of food resources for salmonide fish.

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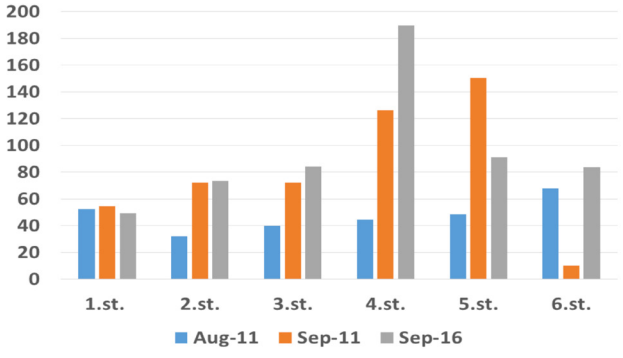


**Fig. 1.** Changes of macrozoobenthoss biomass (mg per m2) in period of five years (2011 to 2016) in six sampling stations in August 2011, September 2011 and September 2016.

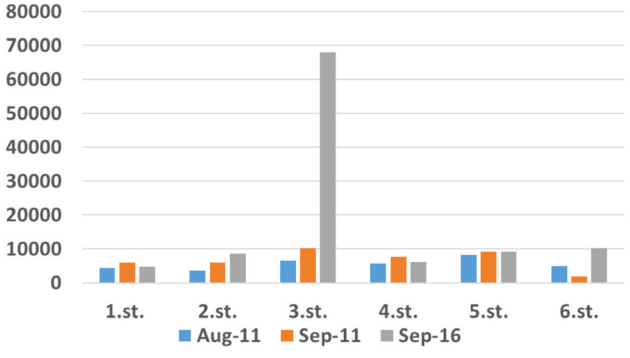


**Fig. 2.** Changes of macrozoobenthoss individuals (individuals per m2) in period of five years (2011 to 2016) in six sampling stations in August 2011, September 2011 and September 2016.

# Dynamics of the quantitative photosynthesis indicators in field beans (*Vicia faba*) induced by zinc accumulation

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**Key words:** chlorophyll concnetration, chlorophyll *a* fluorescence, field beans, hyperaccumulation, photosynthesis, phytoremediation, *Vicia faba*, zinc.

Historically zinc (Zn) pollution has been associated with the development of metallurgy, but nowadays along with the rapid development of the intensive agriculture and increasing use of pesticides more cases of elevated Zn pollution are reported worldwide. In their natural conditions plants are exposed to many factors interfering with the photosynthetic process, leading to decline in growth, development and yield, making photosynthetic measurements an important component of the plant stress studies. In soils, the most common type of Zn is $ZnSO_4$ (Scott 2008), which also is one of the available Zn forms for uptake and accumulation in plants (Prasad 2004). Zn is not only known for participating in the chlorophyll and auxin synthesis (Scott 2008), but also it is an important component in dehydrogenases and thus participates in redox processes (Ali et al. 2013). Zn is also an important component in Cu-Zn-superoxide dismutases, involved in protection against endogenous oxidative stress. As a result of the Zn contamination plants develop leaf chlorosis or necrosis near the main vein (Marmiroli et al. 2013).

The aim of this study was to investigate impact of Zn on leaf chlorophyll concentration, chlorophyll *a* fluorescence and photosynthesis intensity in field beans (*Vicia faba*). The study was carried out from October 2016 till December 2016 in the Department of Plant Physiology, Faculty of Biology, University of Latvia. In the study control, Zn 20 mg $kg^{-1}$, Zn 50 mg $kg^{-1}$ and Zn 70 mg $kg^{-1}$ treatments were used. Plants were grown in quartz sand supplemented with optimum mineral nutrients and cultivated in a semi-controlled greenhouse with additional lighting. Photosynthesis intensity was measured with the LI-6400XT Portable Photosynthesis System (LiCor), Handy PEA (Hansatech) was used for the chlorophyll *a* fluorescence measurements, and the chlorophyll concentration was measured by CCM-300 chlorophyll meter (OptiScience). After a two week growing period measurements were taken once in a week for the next four weeks.

In comparison to control, a significantly lower chlorophyll concentration was observed in Zn70 during the whole experiment. Also a slightly increased chlorophyll concentration was observed in Zn20 treatment. As chlorophyll *a* fluorescence measurements revealed in longer time period, the amount of the inactive chlorophyll (as indicated by RC/ABS) was gradually increasing in treatments with Zn contamination. At the end of the experiment also significantly lower maximum quantum yield of the photosystem II ($F_v/F_m$) and performance index (PI) were observed in plants treated with Zn50 and Zn70 in comparison to these in control plants. This indicates on negative influence of the zinc on plant growth, physiological processes and vitality. Photosynthesis measurements showed that in Zn20 treatment photosynthesis was more intense than in control, but in Zn50 and Zn70 photosynthesis intensity had decreased. Morphologically, formation of bacterial nodules on roots of all Zn-treated plants was not noticed.

The present data suggest that in small amount Zn might be stimulating for the photosynthetic processes in field beans, while in larger amounts Zn inhibits these processes. A further anatomical study of plant roots and stems as well as chemical element analysis is planned.

**Acknowledgements**
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# Seed dormancy in different wild oat populations in Latgale and Vidzeme

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**Key words:** *Avena fatua*, populations, seed dormancy, weeds, wild oat.

Wild oat (*Avena fatua* L.) has been recorded as a weed species in cereal crops in Latvia since the beginning of the XX century, in the 1940-ies its occurrence was characterized as local in certain areas of Latvia (Lapins, Kotovics 1998). However, in terms of the surveys conducted in 2015 and 2016 wild oat was registered in 97 out of 515 surveyed parish territories. Seed dormancy is an important trait in wild oat because it increases the number of seeds persisting in the soil seed bank and makes it harder to control this weed. Duration of primary seed dormancy is highly variable among wild oat populations and depends both on inherited characteristics and environmental influences during seed maturation (Adkins et al. 1987). The aim of this study was to characterize seed dormancy in eight wild oat populations in Latgale (South-Eastern) and Vidzeme (Northern) regions of Latvia and to relate it to morphological traits of the seeds.

Seed samples were collected in cereal fields in late July-early August in four populations in Latgale (Blontu, Rundēnu, Pušmucovas and Asūnes parishes) and four populations in Vidzeme (Blomes Krimuldas, Trapenes and Trikātas parishes), repeatedly in 2015 and 2016. Seeds were stored at room temperature for two weeks to dry and initial germination was tested at 22 °C. Mass of 100 seeds, average seed length and kernel hull percentage were determined for each sample. Seeds were further stored dry at 5 °C, for afterripening seeds collected in 2015 were stored dry at 20 °C for seven months. All germination tests were carried out in four replications of 25 seeds, seeds were germinated in plastic Petri dishes between filter paper (three layers) moistened with deionized water or 1.0 mM solution of GA3 at 22 or 10 °C, tests lasted 49 days.

Comparing morphological seed traits in samples collected in 2015 and 2016 in the same locations, strong and significant positive correlation of 100 seed mass was found between the years ($r^S = 0.796$, $p = 0.032$) after removing one

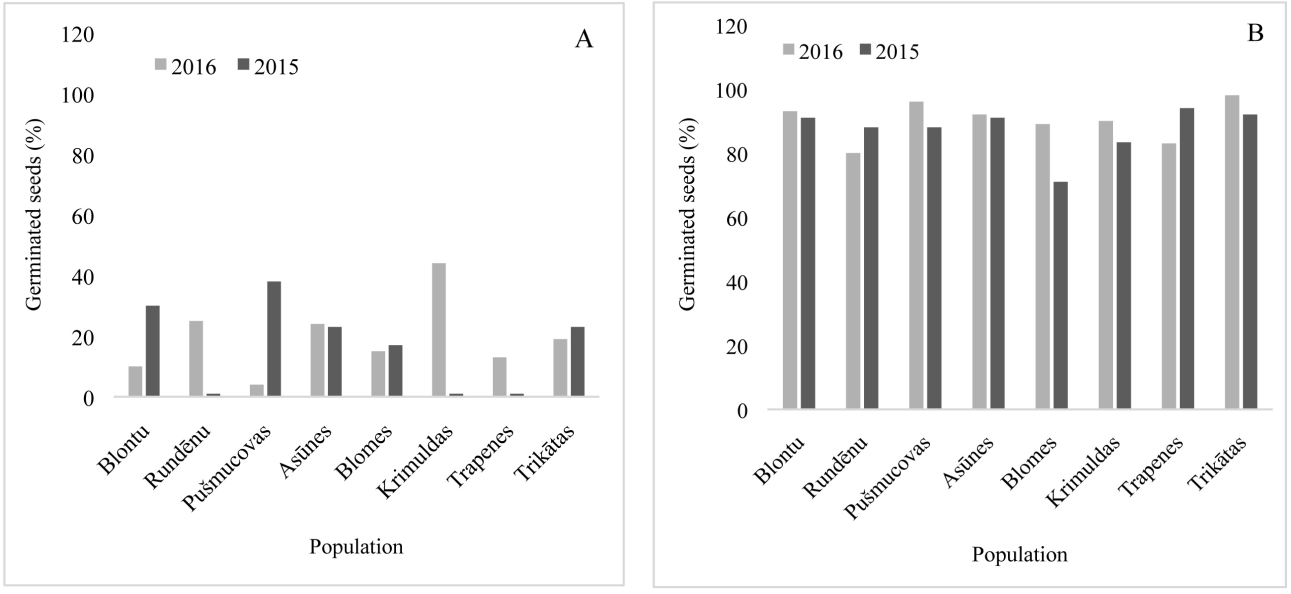


**Fig. 1.** Maximum germination (in 49 days) of wild oat seeds from eight different populations collected in 2015 and 2016, germinated at 10 °C without (A) and with $GA_3$ (B)..

of the samples, but there was no significant correlation of seed length and kernel hull percentage. This means that while 100 seed mass may be a stable trait in the wild oat populations, kernel hull percentage varies between the years and can depend on environmental conditions. Initial germination of all populations in both years did not exceed 30%. Germination was enhanced by $GA_3$ but not equally in all populations, germination ranged from 71 to 98% (Fig. 1). At 10 °C without $GA_3$ slightly higher germination percentage was reached compared to initial germination, indicating that lower temperature is more favourable for germination of dormant seeds. Afterripening did not result in full dormancy release, 35 to 62% germination was achieved in populations from Latgale and 1 to 49% in populations from Vidzeme. There was moderate, significant positive correlation between germination percentage of afterripened seeds and seeds treated with $GA_3$ ($r^S$ = 0.529, $p$ = 0.002). This means that treatment with $GA_3$ imitates prolonged afterripening, but a longer period of afterripening is required for full dormancy release in these populations, especially in Vidzeme where dormancy is more pronounced. In seed samples collected in 2016 there was a moderate, significant negative correlation between kernel hull percentage and germination at 10 °C without $GA_3$ ($r^S$ = –0.443, $p$ = 0.01). In previous experiments piercing the hull enhanced germination and thicker hull can inhibit germination. However, no correlation was found in samples collected in 2015. This result can be explained by the difference in germination percentage between samples collected in the same locations in 2015 and 2016, while hull development could have been influenced by meteorological and growth conditions in each year. The results of this study have practical significance because application of glyphosate-containing herbicides in autumn is a widely used method intended to control wild oats, but taking into account the biology of Latvian wild oat populations that are dormant in autumn, it is mostly ineffective. Further experiments on seed germination are necessary to determine the variability of morphological and physiological traits related to dormancy in these and other wild oat populations in Latvia.

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# Seal influence on costal fishery in Latvia: a case study

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**Key words:** Baltic sea, coastal zone, conflict, fisherman's questionnaires, fishery, seals.

Approximately 10 years ago, seals became the main problem facing Latvian costal fisheries due to the damage caused to fishing gear resulting in reduced catches. The coastal fishery that is most affected by seals is a mixed fishery mainly with stationary gear. It operates in the coastal waters up to 20 m depth or not more than 2 nautical miles from shore. Seal depredation create the substantial economic consequences for fishery. As a result, a gap in understandings of situation have increased between stakeholders, e.g. fishermen and nature conservation specialists. Presently, appropriate solutions to the conflict are limited by insufficient national legislation, e.g. removal of seals is prohibited and fishery loss compensation mechanisms are under-developed.

Two seal species are distributed in the central part of the Baltic Sea: grey seal (*Halichoerus grypus*) and ringed seal (*Pusa hispida*). In Latvian coast, there are no seal haul-outs or breeding areas and coastal waters and central part of Gulf of Riga is used for foraging. The grey seal population has almost tripled in the past 10 years (Härkönen et al. 2013), but ringed seal in the Gulf of Riga have a slight tendency to decrease or have stabilized (Härkönen 2015). Besides, telemetry studies of the ringed seal migration patterns reveal that foraging trips of this species mainly cover the central part of the Gulf of Riga and rarely occur in coastal areas where fishing takes place (Jüssi personal communication). The total abundance of grey seal in the Gulf of Riga is estimated around 2500 individuals and ringed seal 1000 to 1500 individuals (Jüssi personal communication

There are no direct studies of seal population in the Latvia. However, the seal monitoring was conducted during 2001 – 2002 and was based on fishermen inquiries. Fishermen surveys by questionnaires is a method used worldwide to obtain information on stock status, ecosystem changes and economic activities where other research survey possibilities are limited e.g. in case of recreational fishing (ICES 2009). The fishermen interviews were used in Northern Baltic to evaluate the by-catch of seals in fishery. In order to evaluate the possible effects of seal caused damages to fisheries in the coastal zone, a pilot study involving fisherman's questionnaires was conducted in 2016. Questionnaires were distributed to all 141 commercial fishermen units operating in the costal fishery. The first results suggest: (1) Replies were received from 26 fishermen units, mainly in the 1st quarter. Number of replies decreased by quarters in given year and only 3 units provided replies for 3 quarters continuously; (2) From fisheries conducted in the coastal zone the main damages related to gillnets and trap-nets; (3) Representativeness of replies was low: about 5% of gillnet fishing and 14% of trap-net fishing; (4) Seal damaged gears and catch losses varied by local counties and by fishing seasons; (5) The overall preliminary estimate of gillnet damage was around 63 200 EUR (Fig. 1). This was obtained by extrapolating from reported fishing with gear damages to total number of fishing actions from national logbooks and assuming that ~25% of fishing the catches and gears are damaged; (6) Catch losses mainly constituted the commercially important local fish species: salmon, sea trout, herring, smelt, cod, perch, pike perch and vimba bream; (7) The bycatch of seals reported by questionnaires was 55. That is significantly below the number of counted dead seals washed ashore. There were 240 counted in 2016 which constituted around 10% of the grey seal population in the Gulf of Riga (Information from the Nature Conservation Agency, Latvia). In 2015 estimate was 208 indicating the number of dead seals on beaches has increased. This indicate that information obtained from

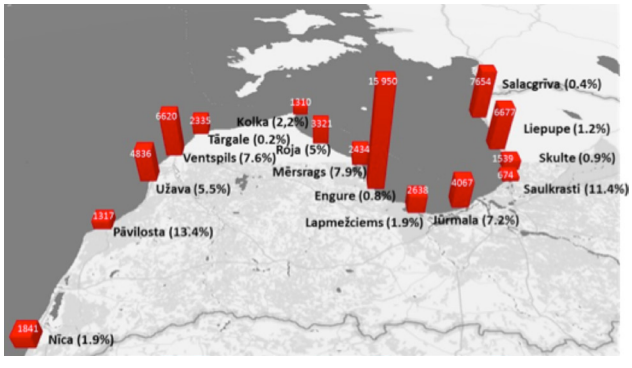


**Fig. 1.** Provisionally estimated losses (in EUR) of gillnet damages by seals. Percentage in brackets after local county name represents the share of reported cases of gear damage from questionnaires to the total number of records of gillnet fishery from national logbooks.

questionnaires is incomplete.

There are several technical measures that restrict the seal damage to fishing gears. First is the electronic seal scarer (Gotz, Janik 2013). Relatively good results show the equipment used for sea fish farming cages. However, it is technical complicated, expensive and require everyday maintenance. In Latvia, such device for first time was implemented in 2016 in fishery operating in River Daugava near outlet (K. Rausis personal communication).

Second, seal safe fishing gears. In the Baltic, has been proposed seal safe gear so called pontoon or push-up trap-net (Varjopuro, Salmi 2006). Several demonstration studies have been carried out in Latvia during 2012 – 2914. However, results revealed that due to geomorphology of Latvian coastal areas (open wind exposed coastline) gear were destroyed and additional construction upgrades are required.

Third, seal abundance regulation and hunting. According to Latvian legislation the seals are protected animals that hunting is prohibited. However, there is several exemptions in Nordic countries, for example in Denmark it is allowed to kill seals if they are in distance of 100 m (Bornholm 500 m) from fishing gear. Studies in northern Sweden shows that generally adult male gray seals have specialized in raiding fishing gear These specialist seals have developed a characteristic behavior pattern and have persisted with it over a long period of time (Königson et al. 2013). Elimination of these "specialists" may be beneficial in certain area. However, it requires special study. As the hunting is assumed as one of reasons of seal abundance decline during previous century, hunting should be treated with great caution. Additionally, it is need to evaluate how much stranding seals already can be assumes as the seal abundance regulation measure.

Fourth, compensation mechanisms of seal damages. Although the direct application of norther Baltic country compensation mechanism to Latvian situation is not applicable. Other, possibly nationally specific, options should be elaborated.

Conclusions: (1) The information obtained so far does not allow a complete evaluation of losses to the fishery but reveal the priorities to be considered. (2) Based on this pilot study, the new questionnaire was developed for 2017 that will be targeted to a subsample of fisherman (agreement with Institute of Food Safety, Animal Health and Environment "BIOR"), allowing the collected seal damage information to be related to logbook statistics. (3) We consider that in Latvia situation none of above mentioned technical regulation mechanisms alone not be able to mitigate the seal depredation. It may be necessary to apply more comprehensive measures including the enhance of the fishery's adaptability and new management approaches for specific coastal fishery conditions.(4) In our vision the mitigation of seal depredation in Latvia maybe by achieved by more closer interactions between the involved institutions where the essential part is development of Seal management/protection plan (Fig. 2).

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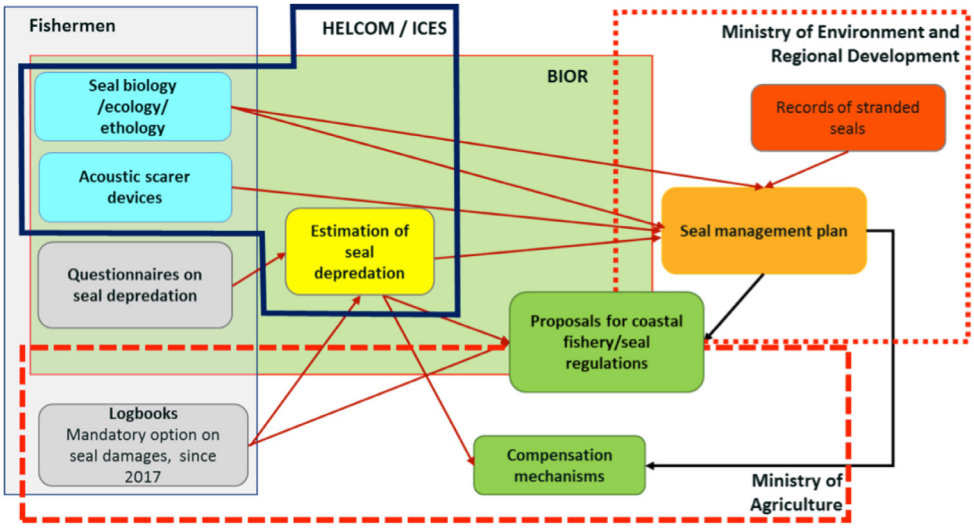


**Fig. 2.** Possible institutional interactions to address the seal fishermen conflict and mitigate the social stress in Latvia.

# Macroscopic green algae from genus *Cladophora* in freshwaters of Latvia

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**Key words:** green algae, *Cladophora*, freshwater, Latvia.

Genus Cladophora and other filamentous green algae are very common in streams and in the shorelines of lakes in Latvia. In the twenties of the last century well-known Latvian phycologist Heinrich Skuja wrote that among green filamentous algae most known in Latvia are *Cladophora glomerata* (Linnaeus) Kützing (Fig. 1, 2) and *Cladophora crispata* (Roth) Kützing, achieving length up to 10 m in rapid waters, but in shallow freshwaters free-floting *Cladophora fracta* (O.F. Müller ex Vahl) Kützing is very common (Skuja 1936).

In total six species of genus *Cladophora* are recognised in Latvia (Rudzroga 1995). Latvian phycologist and limnologist Antonija Kumsare in the monography "Hydrobiology of Daugava River" called the river as 'Cladophora river' due to very high abundance of this filamentous green algae growing on submerged rocks of Daugava River (Kumsare 1967).

Another species from the genus *Cladophora*, *Cladophora aegagropila* (Linnaeus) Trevisan, also known for aquarium hobbyists as Marimo Balls or Moss Balls, forms soft, velvet-like, green algae balls and are typical for deep clean lakes. Nowadays these algae are common only in some lakes in Latvia, for example, in Lake Augstrozes in Northern part of Latvia (https://www.ezeri.lv/). In Latvia this 'Cladophora ball' can reach diameter from 3 to 5 cm (Fig. 3, 4), but according to the literature data it would reach even 20 cm in diameter (Rudzroga 1995).

Assessing cover of *Cladophora glomerata*, mats looks like long, green hair waving in the water. It is a good indicator of eutrophication and shows ecological statuss of freshwaters. Identification of these macroalgae, combined with assessment of cover, permits identification of sites at risk of eutrophication (Martyn et. al. 2016).

As a result of eutrophication, these filamentous green algae (not only *Cladophora*) are very common in biggest rivers in Latvia: River Daugava, River Venta, River Lielupe, River Gauja as well as in salmon River Salaca, where mats of these algae due to action of waves and currents are carried as the dead algae ashore that washes up on beaches often contains dead invertebrates, crustaceans and small fish. Large mass of these decaying mats of *Cladophora* and another filamentous green algae may reduce ecological status of waters, cause odor problems and degrade aesthetic status of shoreline.

**Fig. 1.** *Cladophora glomerata* in Salaca River (photo I. Druvietis).

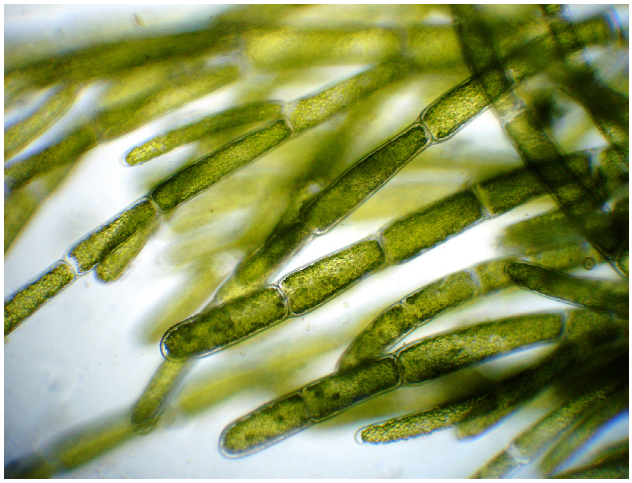

**Fig. 2.** *Cladophora glomerata* (200 ×) in Salaca River (photo I. Druvietis).

**Fig. 3.** *Cladophora aegagropila* ball from Lake Augstrozes (photo I. Druvietis).

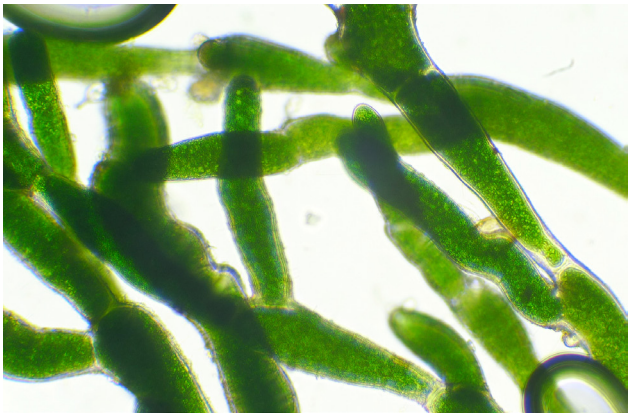

**Fig. 4.** *Cladophora aegagropila* (200 ×) from Lake Augstrozes (photo I. Druvietis).

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# Impact of the growing population of great cormorant *Phalacrocorax carbo* on the trophic status of Lake Engure

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**Key words:** *Chara*, eutrophication, great cormorant, Lake Engure, *Phalacrocorax carbo*, *Ulva intestinalis*.

In 2013, the colony of great cormorants (*Phalacrocorax carbo*) settled down in the central part of lake Engure approximately 500 m NE from the Island Apaļrova (Fig. 1). In 2014, 830 nesting pairs of cormorants were counted there. During the last two years a number of nesting pairs has increased rapidly and already 1500 pairs were nested there in 2016. In order to estimate the impact of the colony on water quality and submerged macrophyte cover, field surveys were carried out from May 2015 until December 2016. Aim of reseach was to start a long-term monitoring of cormorant colony and its impact on the ecosystem of lake Engure. Since it is known that cormorants are feeding on a fish outside the lake in the Riga Gulf, birds can be considered as an important source of nutrients to the lake Engure. It is difficult to measure influence of the cormorants colony to the *Chara* sp., other submerged vegetation and eutrophication of whole lake ecosystem, but it is clear that eutrophicaton caused by cormorants colony strongly influences vegetation in wide territory around the colony.

It was confirmed by chemical analyses which were sampled once a month opposite to the colony as well as visual monitoring of macrophytes an macroscopic algae. It was stated that Charophytes and another submerged macrophytes practically disappiered and now were replaced by macroscopic algae *Ulva intestinalis, Cladophora glomerata, Ulothrix* spp. as well as upper layer of bottom was covered by blue green carpet of filamentous cyanobacteria, that demonstrate results of eutrophication. Macroscopic algae *Ulva intestinalis* formed cormorants colony surrounding green belt (Fig. 2).

Before the appearance of cormorant colony the macroscopic green algae *Ulva intestinalis* was not found in Lake Engure that testify about changes in lake ecosystem. Till now these algae are very common in Riga bay, river estuaries and in lagoon type lakes connected with sea (Rudzroga 1995).

During summer season, lake Engure typically has very low concentrations of nitrogen and phosphorus

**Fig. 1.** Cormorant colony in Lake Engure. Photo A. Skuja.

**Fig. 2.** *Ulva intestinalis* formed belt surrounding cormorant colony. Photo A. Skuja.

compounds, because all available nutrients have already been taken up by macrophytes. However, unusually high concentrations of nutrients indicating on a bad ecological quality were detected in a close proximity to the cormorant colony. For example, concentrations of $P\text{-}PO_4^{3-}$ were 0.114 and 0.092 mg $L^{-1}$ in summer of 2014 and 2016, respectively. Concentrations of $N\text{-}NH_4^{+}$ detected near the colony were twice as high (> 0.40 mg $L^{-1}$) as in other parts of the lake.

Within the EU-funded LIFE project COASTLAKE, monthly in-situ measurements of physico-chemical parameters were carried out in 2015 by using a portable probe Hanna Instruments HI9829. The results show a substantially increased turbidity in a measurement spot near the cormorant colony. Suspended organic matter coming from the cormorant colony or increased phytoplankton growth could be possible reasons for high turbidity. High concentrations of dissolved oxygen (10 to 22 mg $L^{-1}$ or 80 to 250% saturation) are also an indirect indicator for increased primary production in this spot.

**Acknowledgements**

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# Peculiarities of phytoplankton communities in urban lakes of Riga City and its vicinity

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**Key words:** algal blooms, phytoplankton, urban lakes.

The aim of the study was to detect dominating phytoplankton communities forming algal blooms in eight eutrophic urban lakes situated at the vicinity or in the municipal territory of the Riga City. Most of these lakes are used as swimming places for inhabitants of the Riga City in the summer period. Therefore it is important to know presence of potentially toxic algae that would cause low ecological status of waters in these lakes. As a result, the presence of pelagic algae and cyanobacteria affect the function of the food chain and any changes in phytoplankton assemblage influence the entire aquatic ecosystem (Pasztaleniec 2016).

Phytoplankton sampling was performed in the same day (August 20, 2016) in the Lake Ķīšezers, Lake Juglas, Lake Lielais Baltezers, Lake Mazais Baltezers, Lake Bābelītis, Lake Linezers, Lake Velnezers and Lake Gaiļezers. With the help of multi-parameter water quality sonde "YSI 6600 V2" essential water parameters were measured (Table 1).

Conspicuous distinctions in conductivity and salinity were found in waters of lakes that are connected with canal system with Daugava River mouth and Riga bay (Lake Ķīšezers, Lake Lielais Baltezers and Lake Mazais Baltezers). The highest values in biogenic ions were established in small lakes Linezers and Velnezers, whereas the lowest values of biogenic ions were observed in the bigest lakes Lielais Baltezers, Mazais Baltezers and Ķīšezers characterised by reed-formed wide littoral zone and blooms of potentially toxic cyanobacteria *Microcystis* spp. (Fig. 1).

In all the investigated lakes except Lake Gaiļezers potentially toxic cyanobacteria in phytoplankton were found. Highest values of phytoplankton biomass (10.4 mg $L^{-1}$) formed by Cyanobacteria, Cryptophytes and green algae *Tetraedron minimum* was observed in Lake Bābelītis, which is used as one of the more popular swimming places in Riga City (Fig. 2).

The worst situation was observed Lakes Lielais Baltezers and Mazais Baltezers embraced in private properties where the smallest concentration of dissolved

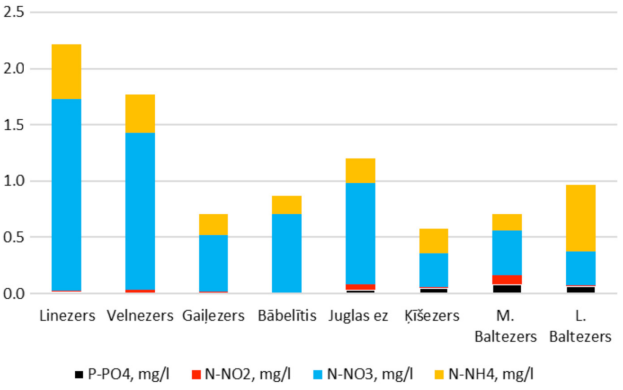


**Fig. 1.** Concentration of biogenic ions in water of urban lakes of Riga City.

**Table 1.** Water parameters of urban lakes of Riga City

| Lake | Temperature (°C) | Conductivity (µS $cm^{-1}$) | Salinity (‰) | pH | $O_2$ (mg $L^{-1}$) |
|---|---|---|---|---|---|
| Ķīšezers | 20.16 | 1744 | 0.98 | 8.16 | 12.21 |
| Juglas ezers | 18.67 | 412 | 0.23 | 7.71 | 7.36 |
| Lielais Baltezers | 22.17 | 1230 | 0.65 | 8.40 | 5.03 |
| Mazais Baltezers | 20.81 | 1154 | 0.63 | 7.61 | 9.17 |
| Bābelītis | 20.70 | 271 | 0.14 | 7.96 | 9.26 |
| Linezers | 21.77 | 117 | 0.06 | 7.92 | 8.96 |
| Velnezers | 20.46 | 320 | 0.17 | 7.38 | 7.47 |
| Gaiļezers | 19.02 | 570 | 0.31 | 8.53 | 9.81 |

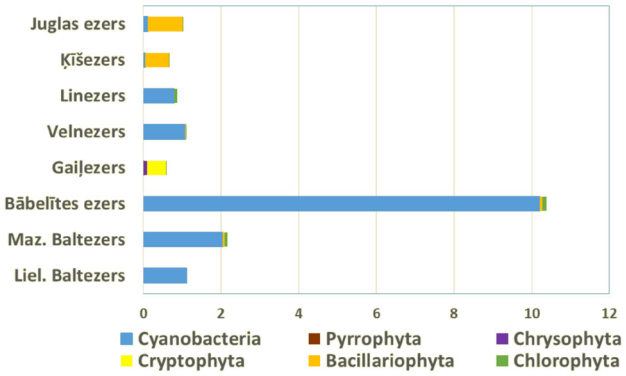


**Fig. 2.** Phytoplankton biomass (mg $L^{-1}$) of Riga City urban lakes in August 20, 2016.

oxygen in water and decay of potentially toxic cyanobacteria *Microcystis aeruginosa* colonies was detected. Two big lakes, Ķīšezers and Juglas ezers, were characterized by a small total phytoplankton biomass (0.64 to 1.02 mg $L^{-1}$) and composition of phytoplankton not typical for summer maximum period, formed mainly by diatoms (Bacillariophyta) *Aulacoseira* spp, *Asterionella formosa, Nitzschia* sp., and *Melosira varians*, with small amounts of Cryptophytes and Cyanobacteria. Dinophyte algae *Ceratium cornutum*, comparatively rare for Latvia, together with Cryptophytes formed practically all the phytoplankton biomass in a macrophyte-type Lake Gaiļezers. Phytoplankton communities of two smallest lakes, Lake Linezers and Lake Velnezers, were dominated by small amounts of cyanobacteria *Planktothrix* sp., *Anabaena* spp. and Cryptophytes (0.87 to 1.09 mg $L^{-1}$).

It can be concluded that at sampling time taxonomic structure of phytoplankton and value of phytoplankton biomass characterized Riga City lakes as very heterogeneous.

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# Fast cefotaxime resistance diagnosis in Enterobacteriaceae using MALDI-TOF

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**Key words:** antibiotic hydrolysis, cefotaxime resistance, Enterobacteriaceae, MALDI-TOF.

Nowadays antibiotic resistant microorganism numbers are expanding and increasing health care problems, especially extended-spectrum β-lactamases of Enterobacteriaceae family. A rapid and trusted detection is required to ensure proper primary treatment therapy. Antibiotic resistance is usually checked on the phenotypic level by the Etest, MIC or disc diffusion that requires another day before reporting results. Lately MALDI-TOF is widely used for fast bacteria identification and now for analyses of β-lactamase activity (Bradford 2012; Sparbier et al. 2012).

The aim of this study was to develop a pattern of fast cefotaxime (CTX) resistance diagnosis in common bacteria of Enterobacteriaceae family using MALDI-TOF Autoflex Speed and compare with clinical approvals.

The present study used clinical isolates of six *Escherichia coli* (3 CTX resistant strains, 2 sensitive, 1 intermediate), one resistant and sensitive *Klebsiella pneumoniae* and resistant *Enterobacter cloacae*. Approximate CFU of bacteria was determined with McFarland. Concentrations of 0.5, 0.25 and 0.1 mg $mL^{-1}$ CTX powder (MIP Pharma GmbH, Germany) diluted in LC-MS Chromasolv water (Sigma Aldrich, Germany). In the first case 50 µL of CTX solution was inoculated with 1 µL loop of bacteria ($4.5 \times 10^8$ CFU), in other 1 µL loop with stack of bacteria ($2.7 \times 10^9$ CFU) and vortexed. Samples were incubated 1, 2 and 3 h at 37 °C under agitation. They were further centrifuged for 2 min at 13 000 rpm and 1 µL of supernatant placed onto polished steel MALDI target plate and covered with α-cyano-4-hydroxy-cinnamic acid (50% acetonitrile, 1% trifluoroacetic acid; Bruker Daltonik, Germany), done twice with two replicates. Spectra were obtained in mass range from 100 to 1000 Da with MALDI-TOF and processed with FlexAnalyses program (Sparbier et al. 2012). Bacteria CTX resistance was verified with disc diffusion method.

CTX peak in mass spectra appeared in 456 Da region where lower signal intensity was evident in the presence of resistant bacteria in comparison to control version and sensitive bacteria presence. Sparbier et al. (2012) separated appeared peaks belonging to sensitive or resistant bacteria. Mentioned peaks in her work were also observed, but they could not be strictly classified for resistant or sensitive strains only, they appeared in both type of strains that could be explained with different MALDI-TOF instrument usage. Further analyses were based on β-lactam ring hydrolysis

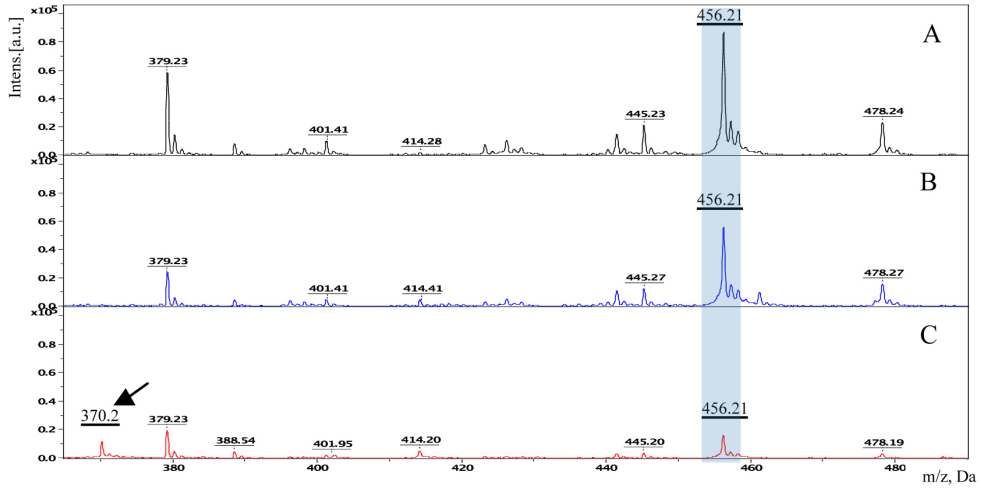


**Fig. 1.** MALDI-TOF spectra of cefotaxime (A to C) after 1 h incubation with Escherichia coli cefotaxime sensitive (B) and resistant strain (C). Highlighted peaks are cefotaxime nonhydrolyzed form. Peak corresponding to hydrolyzed form of cefotaxime and bacteria resistance shown with an arrow.

and decarboxylation peak 370 Da appearance in CTX resistant strains (Fig 1). Overall, this experiment showed that extending incubation time the peak 370 Da intensity increased and 456 Da decreased in presence of resistant bacteria. Amount of inoculated bacteria in CTX solution influenced antibiotic hydrolysis. Higher bacteria CFU resulted in faster and more effective CTX hydrolysis process. It was possible to create a formula for distinguishing CTX resistant from sensitive bacteria according to 370 Da peak magnification: (CTX + bacteria) / CTXcontrol, where 0 to 2 peak increase pointed to sensitive strains but ≥ 2 increase were resistant. One exception was the CTX intermediate *E. coli* strain, which was grouped with resistant strains since the hydrolysis and peak 370 Da was observed.

Data showed that it is possible to distinguish CTX sensitive from resistant Enterobacteriaceae family strains with MALDI-TOF Autoflex Speed. The optimal and fastest way to diagnose CTX resistant bacteria is in 0.5 mg $mL^{-1}$ CTX concentration inoculated with 1 $\mu$L loop of bacteria stack after 1 h incubation. Created formula of peak 370 Da magnification results were in accordance with disc diffusion results.

**Acknowledgements**

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# Opportunity of using mobile genetic elements for the study of genetic diversity of natural populations

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**Key words:** iPBS, LTR-retrotransposons, mobile elements.

Genetic markers have been successfully used in studies of genetic diversity for a long time. The founder of genetics, Gregor Mendel, had used "phenotype-based" markers for analyzing pea plant characteristics already in the 19th century (Agarwal et al. 2008). Nowadays morphological and also biochemical markers are replaced with the different types of molecular markers. Some of them are based on discovery of "jumping genes" by the American scientist Barbara McClintock (Nobel Prize in Physiology or Medicine in 1983) done in the 1950s (Biémont, Vieira 2006; Pray, Zhaurova 2008). Modern knowledge about these ubiquitous and mobile (transposable) elements allows using them as molecular markers for analyses of genetic diversity of many species of animal and plant kingdoms.

LTR-retrotransposons are one of the main groups of all retrotransposons named as Class I transposable elements that occupy significant part of DNA of many eukaryotes (Havecker et al. 2004). These elements consist of two constant Long Terminal Repeats from each side and two GAG and POL coding regions in the middle. The principle of this structure and produced proteins, such as Gag capsid-like proteins, reverse transcriptase, protease and integrase, allow integrating their own copies into host DNA using retrovirus-like "copy-and-paste" mechanism (Elliott, Gregory 2015; Pachulska-Wieczorek et al. 2016). Each place of integration can be used to calculate the number of polymorphic loci and unique alleles within populations and genetic distances between them.

iPBS technique, developed by Kalendar et al. (2010), is applicable to analyze genetic diversity of all eukaryotic organisms using PBS region to design the retrotransposons-specific primers. The aim of this study was to test the possibility of using known primers for analysis of genetic diversity of several animal and plant species. Different types of material were used to extract DNA: blood of European herring gull (*Larus argentatus*), muscle tissue of European perch (*Perca fluviatilis*), foot tissue of Baltic clams (*Macoma balthica*) and leaves/flowers of orchids (*Cypripedium calceolus*). Published information (Kalendar et al. 2010) about sequences and optimal annealing and melting temperature of retrotransposons-based specific primers was used in the research. Twenty-six sequences were applied for primer screening to find the most applicable primer for each species used in this study. PCR products

**Table 1.** Data about specific primers and genetic diversity of chosen populations

| Species | Number of analyzed samples | Primer | Length | Sequence | Total number of all loci | Number of polymorphic loci | Percentage of polymorphic loci | PIC |
|---|---|---|---|---|---|---|---|---|
| Perch *Perca fluviatilis* | 180 | 2080 | 12-mer | CAGACGGCGCCA | 50 | 48 | 96.00 | 0.4663 |
| | | 2081 | 12-mer | GCAACGGCGCCA | 43 | 41 | 95.35 | 0.4739 |
| | | 2239 | 18-mer | ACCTAGGCTCGGATGCCA | 35 | 32 | 91.43 | 0.4989 |
| Gull *Larus argentatus* | 58 | 2239 | 18-mer | ACCTAGGCTCGGATGCCA | 18 | 9 | 50.00 | 0.3225 |
| | | 2378 | 12-mer | GGTCCTCATCCA | 20 | 14 | 70.00 | 0.3876 |
| Clams *Macoma balthica* | 90 | 2232 | 18-mer | AGAGAGGCTCGGATACCA | 17 | 17 | 100.00 | 0.4391 |
| | | 2394 | 12-mer | GAGCCTAGGCCA | 23 | 23 | 100.00 | 0.3805 |
| Orchid *Cypripedium calceolus* | 31 | 2079 | 12-mer | AGGTGGGCGCCA | 29 | 25 | 86.21 | 0.4074 |
| | | 2415 | 18-mer | CATCGTAGGTGGGCGCCA | 38 | 30 | 78.95 | 0.2852 |

were electrophoresed on agarose gel and visualized by ethidium bromide.

To verify the quality and usefulness of the used primers Polymorphic Information Content (PIC) was calculated based on results of polymorphic loci with each primer (Table 1). For dominant markers, such as retrotransposon-based markers, PIC can vary in the range from 0 to 0.5. The PIC value varied in range 0.2852 to 0.4074 among two orchid populations and 0.4663 to 0.4989 among nine populations of perch from Latvian and Lithuanian reservoirs. In all analyzed species each primer revealed high number of loci; among them number of polymorphic loci exceeded 50% even if the amount of collected material was not enough high. We can conclude that used primers designed for PBS region are very effective for the studies of genetic diversity, because of its versatility, availability and ease of use.

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# Investigation of virulence genes, serogroups and antibiotic resistance of *Escherichia coli* isolated from food and livestock

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**Key words:** antimicrobial resistance, *Escherichia coli*, serogrouping, verotoxin.

There are hundreds of different types of *Escherichia coli*. Some subtypes have obtained certain virulence factors that allow them to adapt to new niches and cause wide spectrum of infections (Rasko et al. 2008). Verocytotoxin-producing *E. coli* (VTEC) is one of pathotypes of *E. coli* and it is able to produce one or both verotoxins (VT1, VT2) and it causes a wide spectrum of disease in humans with symptoms varying from mild diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome (Catford et al. 2014). Most often outbreaks are caused by *E. coli* O157:H7 serotype (40 to 50%), although lately records of other serotypes (O26, O111, O103, O145, O121, O104) causing major outbreaks are increasing, reaching 50 to 60% of all cases (EFSA 2013). Antibiotic resistance is increasing concern due to excessive use of antibiotics in agriculture, animal production and transfer of resistant microorganisms in food chain. Studies have shown that antibiotic resistance in VTEC has increased lately (Threlfall et al. 2000). The lack of new antibiotics against gram-negative bacteria, has resulted in revival of using polymyxins. However, genes that encode resistance against colistin (polymixin E) have been detected in both chromosome and plasmids (Liu et al. 2015).

The aim of this study was to describe food and livestock isolates of *E. coli*, their virulence potential, evaluate serogroup composition, determine antibiotic resistance (food isolates) and to monitor the occurrence of *mcr-1* gene in Latvia.

Present study used culture resuscitation, DNA extraction (Birnboim, Doly 1979), polymerase chain reaction, serogroup determination by O antigen. Antibiotic resistance determination and characterization was carried out using Minimal Inhibitory Concentration plates. Plasmids containing resistance genes were transformed to *E. coli* K802 cells. Bacteriophage extraction of colistin-resistant *E. coli* cultures were carried out and electron microscopy was used to prrove the presence of bacteriophages.

From 72 isolates from food samples, tested for the presence of *vtx1*, *vtx2*, *eaeA* genes, 91.7% did not contain any of virulence genes, 8.3% contained intimin gene (*eaeA*), from those 2.8% contained additionally *vtx-1* gene. From 42 isolates from domestic animals 4.8% contained *vtx-1*, 4.8% contained *eaeA* gene, 2.4% contained both *vtx-1* and *eaeA* genes.

As a result of serogroup determination, 29.2% from isolates from food did not belong to verotoxin producing serogroups, 44.4% belonged to O121 serogroup, 9.7% to O103, 12.5% were self-agglutinating, 1.4% belonged to serogroups O26, O145 and O142.

In total, 38.1% of isolates from domestic animals belonged to O:103 serogroup, 26.2% to O:121, 21.4% to O:55 serogroup, 11.9% to O:26 serogroup and 2.4% was O:128 serotype.

From 72 isolates from food, 17 (23.6%) were resistant against at least one antibiotic, all of which were isolated from meat. The majority of samples were resistant against tetracycline (10 samples), ampicillin (eight samples), and sulfamethoxazole (seven samples). Two of the samples were multiresistant, they had resistance against four antibiotic classes. They contained *mcr-1* gene that determines resistance against colistin.

Within "AgroBioRes" project from 313 domestic animals (pigs, broilers and calves) five *mcr-1* gene positive samples were obtained (from swine isolates of *E. coli*). Out of them, two different *E. coli*-specific bacteriophages were obtained.

When detecting verotoxin producing genes with PCR it is possible that their presence is detected from non-viable cells, furthermore due to the unsuited storage conditions bacterial culture could have lost verotoxin genes after first passage (Joris et al. 2011).

Belonging of particular *E. coli* to potentially pathogenic serogroup does not necessarily mean that it is pathogenic and vice versa, therefore several methods should be combined in diagnostics.

There is not one single combination of virulence factors that define pathogenicity of VTEC. However there are speculations that certain combinations of virulence factors

have higher risk to cause severe infections in humans, in comparison with other combinations (EFSA 2013).

The antibiotic resistance depends on the source of isolation. Strains, which have been isolated from meat products, most often are resistant to tetracycline that can be explained by its excessive use in animal production (Schroeder et al. 2002). Two of the samples that were isolated from meat were multi-resistant, they also contained colistin resistance-encoding gene *mcr-1*.

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# Large river phytoplankton index and its relationships with environmental factors

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**Key words:** large rivers, phytoplankton, water quality.

Phytoplankton is one of the elements of biological quality that should be used to assess ecological quality of very large rivers. Although traditionally more used in lakes, phytoplankton can be used as ecological quality indicator of slow-flowing lowland rivers with large water resistance time. In smaller, fast flowing rivers phytoplankton shows trophic level of catchment, and cannot be used for river assessment (Druvietis 1997). According to Nixdorf et al. (2002) rivers with chlorophyll *a* concentration > 18 μg $L^{-1}$ can be described as phytoplankton-rich, in other rivers phytoplankton assessment is not suitable. Until recently there was no accepted assessment method for phytoplankton in Latvia. The new method LatRPI was developed in 2014. It was partly adapted from Hungarian Large River Potamoplankton Index (Borics et al. 2007). LatRPI consists of species composition index Q (based on functional groups) and concentration of chlorophyll *a*.

Our objective was to test suitability of LatRPI for flowing surface waters and its relationship with selected environmental factors.

For this study hydrochemical and phytoplankton monitoring data provided by Latvian Environment, Geology and Meteorology Centre were used. In total, 144 phytoplankton samples from rivers Daugava, Gauja, Lielupe and Venta collected in 2000 to 2013 were used. Data analysis was peformed using PAST statistics.

Largest number of samples corresponding to high quality was observed within Venta River where 88% of samples belonged to high or good quality. Worst quality was observed for Gauja River (67% of samples indicated at least good quality), probably because of hydrological factors that disturbs development of phytoplankton communities. Relatively large number of samples corresponding to high and good quality (71%) within Lielupe River can be linked with poor state of monitoring system because even in summer chlorophyll *a* concentration was unusually low.

We found significant negative relationship between LatRPI and Ptot ($R = -0.557$, $p < 0.05$) as well as LatRIP and BOD5 ($R = -0.728$, $p < 0.5$). Pearson correlation coefficients revealed significant negative correlation between cyanobacteria and total LatRPI index ($R = -0.512$, $p < 0.05$) and national ecological quality index ($R = -0.328$, $p < 0.05$). This indicates that species composition index Q can be successfully used to indirectly assess algal blooms. We did not find any significant relationship between LatRPI and land-use in catchment or hydromorphological modifications. In total, LatRPI can be used to assess eutrophication and organic pollution, but it is not suitable to assess morphological or hydrological modifications of very large rivers.

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# Effects of AV-153-Ca on expression of genes related to DNA repair and nitric oxide metabolism in kidneys of rats with alloxan induced *diabetes mellitus*

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**Key words:** 1,4-dihydropyridines, diabetes mellitus, DNA repair, nitric oxide metabolism.

*Diabetes mellitus* (DM) and its complications cause numerous health and social problems throughout the world. Diabetic nephropathy is one of the most common vascular complications of the disease. The complication is associated with changes in nitric oxide metabolism and impaired DNA repair and also ubiquitine-proteasome system. For instance, some genes of proteasome subunits have shown association with type 1 and type 2 DM.

New drugs with antioxidant and DNA-repair stimulating activity could help to progress in finding a treatment for complications of DM. Some 1,4-dihydropyridines (DHP) might possess these properties. They act as free radical scavengers, normalize production of NO and reduce formation of DNA breaks.

The aim of the research was to analyze influence of 1,4-DHP derivative AV-153-Ca on expression of genes related to DNA repair, nitric oxide metabolism and proteasomes in kidneys of rats with induced DM.

Type 1 DM in Wistar rats was induced by alloxan injection (110 mg $kg^{-1}$). Nine days after DM approval both control and diabetic rats were treated with AV-153-Ca at two doses (0.05 or 0.5 mg $kg^{-1}$) for three days. Expression level of *iNos, eNos, Parp1, Xdh, Psma6* genes in rat kidneys was determined by qPCR. The level of DNA breaks in nucleated blood cells was determined by single cell electrophoresis.

Induction of diabetes significantly increased expression of *iNos, Parp1, Xdh* and *Psma6* genes compared to control rats ($p < 0.05$). Treatment with AV-153-Ca did not influence the expression of genes in control rats. However, in model animals, both doses of AV-153-Ca significantly increased the expression of *eNos* ($p < 0.0001$), *Parp1* ($p < 0.05$), *Xdh* ($p < 0.01$) and *Psma6* ($p < 0.01$) genes compared to diabetic rats. Induction of diabetes did not change the level of DNA breaks in blood cells. Also treatment with AV-153-Ca had no effect both on control rats and diabetic animals.

To conclude, AV-153-Ca can increase the expression of genes related to DNA repair and nitric oxide metabolism, as well as proteasomal genes. This might indicate stimulation of DNA repair.

# Endopolyploidy of *Ligularia sibirica* leaves at different development stages

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**Key words:** endangered species, flow cytometry, *Ligularia sibirica*.

*Ligularia sibirica* (L.) Cass. is herbaceous relict plant species endangered throughout Europe. *L. sibirica* is included in the Annex II of Habitats Directive 92/43/EEK of the Council of European Communities (Council of European Communities 1992). The appropriate protection measures for rare and endangered species should be based on understanding of ongoing processes in populations. Changes on chromosome level, including endopolyploidy, can reflect adaptation under pressure of different stress conditions. Endopolyploidy is result of the exponential replication of nuclear DNA in the absence of mitosis mainly due the endoreduplication which occur in 90% of all angiosperms (Scholes, Paige 2015). Plant endopolypoidization is associated with cell differentiation and metabolic activity and is important for normal organ and tissues growth and development (Barow, Meister 2003; Maluszynska et al. 2013). Endopolypoidization is a consequence of repeated DNA reduplication cycles without occurrence of mitosis and chromosomes segregation. Endopolypoidization leads to presence of various ploidy levels (2C, 4C, 8C etc.) in different cells of the same individual (Maluszynska et al. 2013). Endopolyploidy can be modulated by environmental factors such as light, temperature, nutrient availability, heavy metal pollution, drought and cold stress through activity changes of different molecular processes (Biskup, Izmailow 2004; Betrin 2005; Barow 2006; Jovtchev et al. 2007; Maluszynska et al. 2013; Scholes, Paige 2015). The goal of this study was to determine endopolyploidy occurrence in young and mature leaves of *L. sibirica*.

Leaves of *L. sibirica* were collected in the Krustkalni Nature reserve from 22 specimens with young leaves and 25 from mature leaves (one leaf from each individual). Leaves were dried and kept till analysis in silica gel. Samples for flow cytometry were prepared with the DNA staining kit (Sysmex Partec, PI Absolute, GmbH, Germany), cells

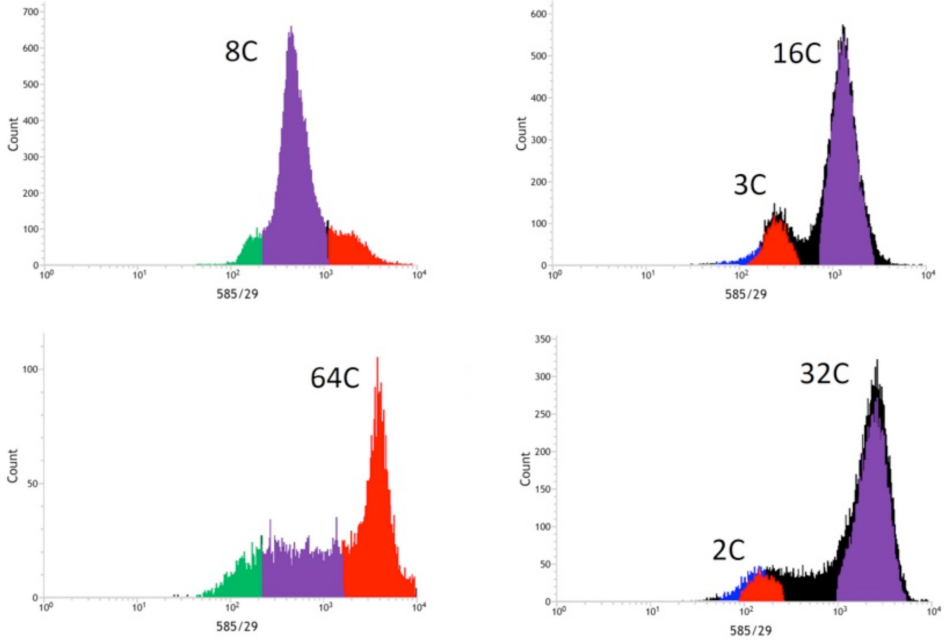


**Fig. 1.** Density plot showing the relative fluorescence of *Ligularia sibirica* nuclei. Abscissa – relative fluorescence units (RFU) in logarithmic scale at 585 nm; ordinate – number of nuclei.

nuclei were stained with 10 μL propidium iodide. BD FACSJazz® cell sorter (BD Biosciences, USA) with flow cytometer function was used to detect DNA content (C-value) of individual cells. Cell counting events were triggered by forward-scattered signal. The excitation of the cell fluorescence was made by 488 nm Coherent Sapphire Solid State (blue) laser. The calibration was considered as successful if the coefficient of variance of the calibration particles relative fluorescence did not exceed 3%. Flow cytometry analysis of DNA content in *L. sibirica* young and mature leaves revealed presence of nine relative fluorescence peaks from 2C up to 64 C (Fig. 1). Most (82%) samples from young leaves had endopolyploidy, only four samples had cells with the same ploidy level. Among them 18% were found to have dominant 2C DNA content, 14% had 4C, 14% 8C and 27% 16C. In contrast, in mature leaves 2C content was the only observed dominant DNA peak in all samples. Percentage of 64C DNA nucleus among all young leaf samples was very low, represented only by 4% of cells. It is known, that high levels of endopolyploidy occur in cells with increased secretory function (Scholes, Paige 2015). So, exhibited endopolyploidy in young leaves of *L. sibirica* individuals reflected growing processes during plant development.

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# Heavy metals and oxidative stress levels in macrophytes in different regions of the Baltic Sea

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**Key words:** Baltic Sea, heavy metals, macrophytes, oxidative stress.

The Baltic Sea as semi-enclosed brackish water ecosystem and, especially, its gulf areas are under remarkable anthropogenic impact. Heavy metals like other contaminants enter in the system and accumulated in aquatic biota. Information on concentrations of contaminants in marine organisms does not indicate the biological effect of them. In contrast to the simple measurements of contaminants accumulating in organisms, biomarkers can offer a more complete an biologically more relevant information on the potential impact of toxic pollutants on the health of organisms, and can be used as early warning signals for general or particular stress.

Macrophyte samples were collected in summer from coastal stations of the Gulf of Riga (Mersrags and Saulkrasti) by scuba diver, according to the HELCOM recommendations (1999). The following species for heavy metal accumulation analyses were macrophytes brown algae *Fucus vesiculosus*, green algae *Enteromorpha intestinalis*, red algae *Furcellaria lumbricalis, Ceramium tenuicorne* and *Polysiphonia fucoides*.

The accumulation of metals (Cd, Pb, Cu, Zn) was determined by AAS method using a flame Varian AA Spectrophotometer (model Spektra A880), but Hg by a Flow Injection Mercury System (Perkin Elmer). The internationally accepted standard samples of plants ($BCR^R$ – 060) were used for quality control.

In long-term studies of metal accumulation in macrophytes it was revealed that *E. intestinalis* had a greater ability to accumulate Hg, Zn, and *F. vesiculosus* to accumulate Cd. In red algae samples most of metals were in much higher concentration than in other macrophytes (Kuļikova, Seisuma 2006; Seisuma et al. 2011).

**Table 2.** Mean glutathione-S-transferase (GST) and glutathione reductase (GR) activities (nmoli $min^{-1}$ $mg^{-1}$ protein) of *Fucus vesiculosus* collected from seven sites with different levels of contamination of the Baltic Sea

| | GST | GR |
|---|---|---|
| Gulf of Riga | 361.25 | 350.77 |
| Gulf of Finland | 314.97 | 323.21 |
| Gulf of Bothnia | 200.01 | 501.95 |

In a frame of international BONUS programme BEAST project, to determine oxidative stress level by enzyme activitity, glutathione-S-transferase (GST) and glutathione reductase (GR) in brown algae *F. vesiculosus* was chosen. *F. vesiculosus* belongs to the dominating macroalgae of the Gulf of Riga as well as of the whole Baltic Sea. Sampling sites were located on the Gulf of Riga (Mersrags, Saulkrasti), Koigustu (Saarema), the Gulf of Finland (Klamilla, Hanko, Tvaerminne), and the Gulf of Bothnia (Rauma) at 3 m depth (2009 – 2010). Activity of enzymes was measured by Microplate Reader Multiscan ASCENT, Thermo Scientific.

*F. vesiculosus* from two sites Mersrags and Saulkrasti (Gulf of Riga) during the last years (2009 to 2016) showed mean activity of GST 279.9 and 261.0, and mean activity of GR 307.1 and 333.1 nmol $min^{-1}$ $mg^{-1}$ protein, respectively.

Statistical analysis (Hammer et al. 2001) applied to the data from sub-regions revealed that enzyme activities and heavy metal concentration showed higher GR activity and Zn concentration in contaminated site of the Gulf of Bothnia (Table 1, 2). This indicate that *F. vesiculosus* communities are suitable for use as biomarkers of environmental contamination in ca oastal zone.

**Table 1.** Mean concentration of heavy metals (mg $kg^{-1}$ DM) in *Fucus vesiculosus* collected from seven sites with different levels of contamination of the Baltic Sea

| | Hg | Cd | Pb | Cu | Zn |
|---|---|---|---|---|---|
| Gulf of Riga | 0.037 | 1.500 | 0.536 | 2.475 | 28.25 |
| Gulf of Finland | 0.049 | 1.049 | 0.355 | 2.421 | 38.60 |
| Gulf of Bothnia | 0.050 | 1.990 | 0.200 | 2.510 | 100.92 |

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# Ecological aspect of the investigations of genetic diversity of species

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**Key words:** genetic diversity.

Nowadays approximately a half of all of the world's human population is concentrated in towns and cities. The rapid development of urban environment poses serious environmental problems: climate change, environmental pollution, noise, waste, landscape fragmentation, a reduction in biodiversity, ecosystem degradation, invasive species, etc. (Niemela 2004). Currently, ecological studies of sustainable urban environment become more and more topical and are one of the most popular and fast growing natural research field in the world with the aim of ensuring the quality of living and working environment for people in the cities and to provide basis of knowledge for balanced development. On the base on the principles of the Copenhagen resolution (2008) of sustainable development of the urban environment in Europe intensive, complex urban studies are initiated. Urban ecology is a cross-disciplinary scientific sector, that research field is the interaction between natural and technogenic environment and development of new methods for investigation of urban environment. Genetic composition and variability of populations might define the relative differences in environmental response of species.

Several plant and animal species: white clover (*Trifolium repens*), lime trees (*Tilia* sp.), a terrestrial pulmonate gastropod mollusk (*Arianta arbustorum*), mute swan (*Cygnus olor*) and herring gull (*Larus argentatus*) were chosen as model species for evaluation of different aspects of adaptation to urban environment. The goal of the presented research was to compare genetic diversity of populations of mentioned species in Riga with diversity of wild Latvian populations, and in a case of white clover also with populations from different urban and non-urban environments of several EU countries. For population's genetic investigations it is important to use a rather simple method, which gives a possibility to analyze a sufficiently large number of samples (Otto 2007). Therefore the universal iPBS (inter primer binding sites) method (Kalendar et al. 2010) was used. The nature of transposable elements and their role in species adaptivity suggested that retrotransposon-based markers would be a relevant choice for studying genetic diversity exposed to challenge of adaptation. The method allows revealing high level of genetic diversity and is cost and labour effective, it was successfully aplied for investigation of genetic diversity for several species (Kalendar et al. 2010). For mute swans and herring gull DNA was extracted from blood samples which were collected in Riga over year (Grauda et al. 2015). The gastropod mollusk DNA was extracted from soft tissue, mollusks were collected from different sites of Riga. The material of plant species was collected in different regions of Riga and in several country side areas of Latvia and for white clover also in different habitats of several EU countries. DNA was extracted from leaves dried in silica gel using standard NucleoSpin® Plant II protocol. DNA was amplified by PCR with specifically selected iPBS primers for each species. High number of polymorphic loci was revealed in all analysed species (Grauda et al. 2015, Kolodinska-Brantestam et al. 2015). Additionally, the BD FACSJazz® cell sorter was used for ploidy level detection for plants. In presentation differences of genetic variation between urban and country side populations were presented.

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# Autoclaving as an effective pre-treatment for enzymatic hydrolysis of wood

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**Key words:** autoclaving, enzymatic hydrolysis, wood.

Recently primary waste treatment technologies for anaerobic recycling have become more actual. Anaerobic process significantly reduces $CO_2$ emissions as compared to other technologies in waste processing. Currently the offer for green waste pre-treatment technologies allowing to increase biogas yield and efficiency is limited. Green waste from forests, parks and gardens is a very complex substrate for efficient biogas production due to lignocellulosic composition. These complications are the reason why a pretreatment step is necessary for obtaining fermentable sugars during the hydrolysis step (Asada et al. 2012).

The aim of this study was to evaluate the efficiency of autoclaving for increasing concentration of derived sugars from coniferous and deciduous wood samples. Shredded branches of spruce (*Picea abies*), pine (*Pinus sylvestris*), maple (*Acer platanoides*), birch (*Betula pendula*), and oak (*Quercus robur*) were autoclaved at 1 atm for 15 min and reducing sugar concentration in extract buffer was determined to evaluate hydrolysis. An increase in sugar concentration was seen in spruce, pine and maple, which had relatively low amount of reducing sugars in the extract buffer before treatment (Fig. 1). It is generally considered that softwoods are difficult lignocellulosic raw materials to hydrolyze to sugars for fermentation, because of the nature and the amount of lignin, 25 to 30% in softwood and 20 to 25% in hardwood (Asada et al. 2012). Autoclaving of birch and oak did not result in statistically significant increase in concentration of reducing sugars in the reaction mixture.

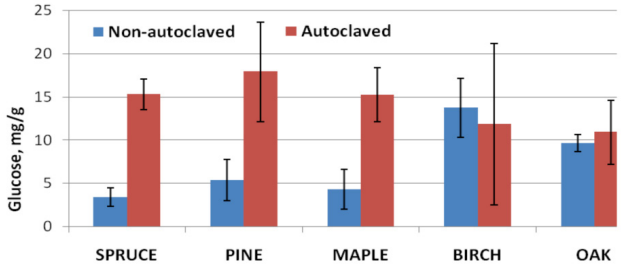


**Fig. 1.** Concentration of reducing sugars in autoclaved wood.

Autoclaved sample supernatants, containing small particles of wood, were analyzed by Fourier transform infrared (FT-IR) spectroscopy. FT-IR spectra were recorded on a HTS-XT microplate reader (Bruker, Germany). Absorption spectra were collected over the range of 4000 to 600 $cm^{-1}$, with 64 scans, and a resolution of 4 $cm^{-1}$. All spectra of wood samples showed absorption bands of carbohydrates, including polysaccharides and hemicellulose at 1000–1150 $cm^{-1}$, and strong bands around 1603 $cm^{-1}$ and 1716 $cm^{-1}$ assigned to aromatic skeletal vibration of lignin and C=C stretching of the aromatic ring. As the intensity of a peak at 1422 $cm^{-1}$ in all samples was the same, it was used as an internal standard for semi quantitative comparison of total carbohydrates by the ratio A1081/A1412. The amount of total carbohydrates in a diminishing row was maple, oak, pine, birch and spruce. The ratio A1716/A1412 shows the relative content of lignin and C=C groups, ranging woods in a diminishing row: pine, birch, spruce, oak and maple. The grouping of woods by content of carbohydrates and lignin are not the same as determined by the reducing sugar content because (1) FT-IR spectral bands are assigned to functional group vibrations, where reducing sugars represent one of components and (2) spectra recorded were sample supernatants composed of liquid and small wood particles. Thus, the effect of autoclaving on wood hydrolysis is species specific. Further experiments will be focused on a comparison of different pre-treatment options for individual tree species and mixtures.

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# Effect of a maple leaf additive on growth of *Lentinula edodes*

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**Key words:** *Acer platanoides*, biological efficiency, *Lentinula edodes*, sawdust, seasonal waste utilization.

Shiitake mushroom *Lentinula edodes* (Berk.) Pegler is edible white-rot fungus that can be used in utilization of lignocellulosic biomass, including seasonal and agricultural waste (Elisashvili et al. 2015). Many seasonal wastes, for example, fallen tree leaves, could be applied as nutrient-rich (Wilkaniec et al. 2012) organic material in mushroom cultivation.

In attempt to develop a more efficient method for the production of shiitake mushroom, we investigated the effect of maple leaves as medium-enriching additive on *L. edodes* mycelium diametric growth, mycelium substrate colonization rate and determined the biological efficiency (fresh fruiting body weight/dry substrate weight) of sawdust-bran substrates.

Mycelium diametric growth was determined on synthetic medium agar plates with or without addition (2.5%) of ground maple (*Acer platanoides*) leaves. Mycelium substrate colonization rate was evaluated by measuring mycelium penetration depth of sawdust-wheat bran (4:1, moisture content 70%) or sawdust-wheat bran-leaves substrates in glass jars. Fruiting period with the highest fruiting body production rate was assessed by weighing fruiting bodies and determining biological efficiency of sawdust-bran substrates in polyethylene bags.

Addition of maple leaves (2.5%) increased *L. edodes* mycelium diametric growth on average by 22.08±2.47% (Table 1). It also increased average substrate colonization rate on sawdust-bran substrates by 37.98±4.79% (Fig. 1), reducing full substrate colonization time by 2 days. The biological efficiency (BE) of sawdust-bran substrates was determined to be 84.30% (for 173 days after inoculation). The most productive fruiting period was for 2 weeks with BE 24.25±7.11% per week after 5 month cultivation.

Our current results suggest maple leaves to be a suitable medium-enriching supplement, therefore we expect an increase in biological efficiency of sawdust-bran substrates upon addition of maple leaves in the future.

**Table 1.** Relative increase (%) of mycelium mean diametric growth upon addition of maple leaves (2.5%). Data are expressed as mean ± SD

| Strain | Days after inoculation | |
|---|---|---|
| | 7 | 14 |
| 361 | 25.81 ± 2.74 | 32.35 ± 0.00 |
| 395 | 20.48 ± 1.70 | 21.62 ± 0.00 |
| 1899 | 14.78 ± 2.46 | 24.60 ± 2.85 |
| 2989 | 13.51 ± 5.10 | 18.42 ± 2.48 |
| 3343 | 33.08 ± 3.19 | 35.61 ± 1.07 |
| 3565 | 11.38 ± 3.39 | 28.67 ± 0.51 |
| 4B2S | 19.05 ± 2.89 | 17.44 ± 3.02 |
| 6B2S | 12.03 ± 11.64 | 18.21 ± 0.47 |
| RA1 | 21.86 ± 0.77 | 15.76 ± 0.00 |
| St-225-99 | 25.33 ± 5.66 | 32.47 ± 0.52 |

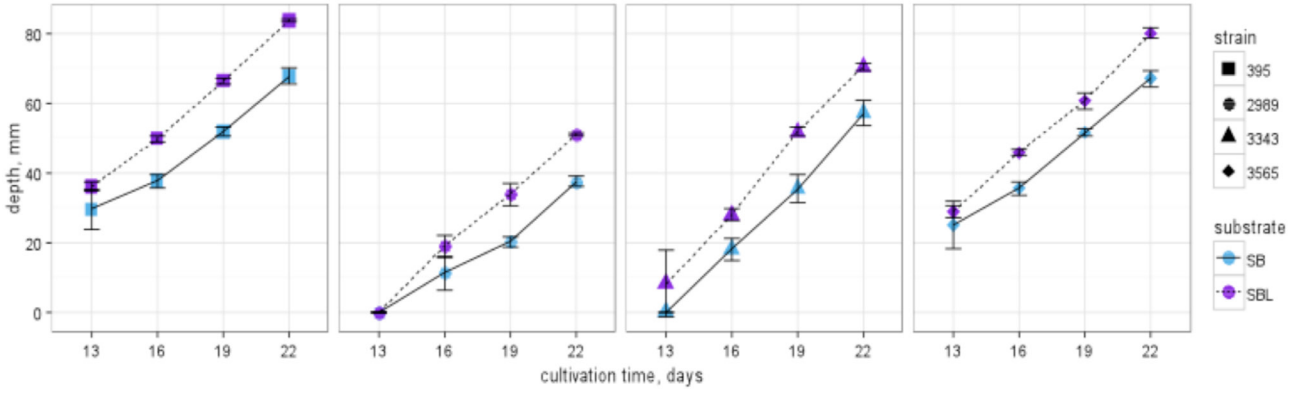


**Fig. 1.** Mycelium substrate colonization depth (mm) of sawdust-bran (SB) and sawdust-bran-leaves (SBL) media. Data are expressed as mean of three biological replicates ± SD.

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# Purification of *Penicillium lanoso-viride* AMP deaminase by column chromatography

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**Key words:** 5'-AMP deaminase, column chromatography, *Penicillium lanoso-viride*.

AMP deaminase (AMP aminohydrolase, adenosine 5'-monophosphate deaminase, EC 3.5.4.6), widely distributed in eukaryotes, catalyzes hydrolytic deamination of AMP to IMP. This deaminase plays a role as a regulatory enzyme for both adenylate energy charge and total adenylate pool size through its participation in the purine nucleotide cycle (Ito et al. 1988; Minami et al. 2011).

Non-specific adenylate deaminase (AMPD) is a glycoprotein (molecular weight 210 kD) produced by microscopic fungus *Penicillium lanoso-viride* during a particular phase of growth, i.e., conidiospore formation. In vivo studies on experimental animals have demonstrated that purified AMPD has diverse immunomodulating properties that influence both cell-mediated and humoral immunity reactions (Nikolajeva et al. 2009).

A method for extraction and purification of AMP deaminase from *P. lanoso-viride* was developed by V. Nikolajeva in 1982 at the Departament of Plant Physiology and Microbiology, Faculty of Biology, University of Latvia. Two techniques of protein purification are employed - precipitation of proteins from crude extract by ammonium sulfate, followed by Sephadex G-200 column chromatography. After these steps purity of AMPD is increased up to 400-fold, yet eluates are not homogenous and contain several other proteins which appear on SDS-PAGE gel.

For comprehensive studies of properties of the enzyme, it is crucial to obtain a homogenous protein sample, therefore the aim of this study was to extend the aforementioned AMPD purification method. Enzyme was purified from protein mixtures with various purification folds and specific activities obtained from Sephadex G-200 column. DEAE-Cellulose, Concanavalin A agarose and 5'-AMP sepharose chromatography columns were used. A spectrophotometric methods to measure enzymatic activity and protein concentration in samples were employed.

Proteins from DEAE-Cellulose column eluted as two peaks. Purification fold of 3.7, and nearly 50% of total AMPD were observed in the first peak (compared to loaded sample).

Concanavalin A is a lectin derived from *Canavalia ensiformis* (Jack bean) seeds and is widely used for the purification of glycoproteins (Rao et al. 2011). Glycoprotein Isolation Kit, ConA (Thermo Fisher Scientific, USA) was employed for AMPD purification. Nearly 100% of total enzyme was detected in the eluate but no increase in purification fold and specific activity was observed. SDS-PAGE showed no differences in protein compositions between loaded and eluted samples which leads to conclude that protein mixtures prior to loading contained mainly glycoproteins.

Deglycosylation of two different protein mixtures (obtained from Sephadex G-200 column) using glycosidases PNGase F (N-glycosidase F, New England BioLabs Inc., USA) and Endo H (endoglycosidase H, New England

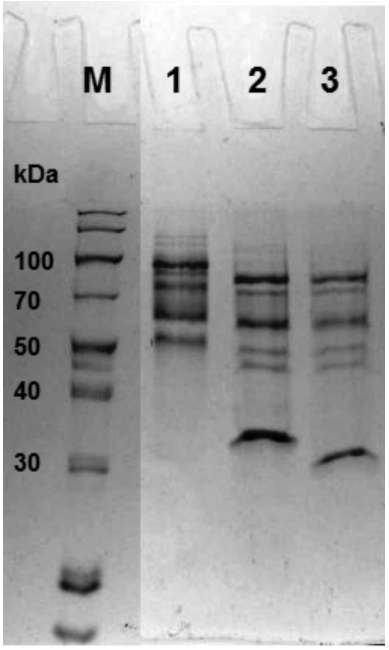


**Fig. 1.** Analysis of changes in protein migration using 8% SDS-PAGE after enzymatic deglycosylation. M-marker (Page RulerTM, Thermo Scientific, USA.). 1, protein mixture prior to deglycosylation; 2, protein mixture after treatment with N-Glycosidase F; 3, protein mixture after treatment with endoglycosidase H.

BioLabs Inc., USA) was performed. Different protein migration patterns were detected on SDS-PAGE gel (Fig. 1) after deglycosylation affirming glycoprotein presence in protein mixtures.

Several authors have reported purification of AMP deaminase to homogenity after application of 5'-AMP sepharose affinity chromatography (Thakkar et al. 1993; Marquez-Rios et al. 2008). AMP-sepharopore 4B-CL (BioWorld, USA) was used in this study to purify AMPD. Number of properties of chromatography column, such as buffer composition, buffer pH, flow rate and ionic strength were altered, yet in all cases nearly all of the total enzyme activity was observed in the first few fractions eluted from 5'-AMP sepharose, pointing to lack of adsorbtion of AMPD to the column. Negligible increase in purification fold was observed in a few eluates, still not due to the enzyme adsorbing to the column matrix as mentioned above, rather other column properties were involved.

In conclusion, the most effective method for AMPD purification was DEAE-Cellulose column chromatography. ConA agarose and deglycosylation results indicate that protein mixtures obtained from Sephadex G-200 contained primarily glycoproteins. AMP deaminase, under conditions tested, did not bind to 5'-AMP sepharose.

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# Prophylactic use of predator and parasitoid complex to control pest populations in polycarbonate greenhouses in Latvia

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**Key words:** biological control, entomophagous, greenhouse pests, natural enemies, parasitoids.

Plant pests in greenhouses cause significant losses of yield. According to the European directives on plant protection, it is necessary to introduce the use of biological control techniques such as application of natural enemies of insect pests (predators and parasitoids). There are many successful examples of the use of beneficial insects and mites in greenhouses (Hoddle et al. 1998; Rabasse, Van Steenis 1999; Wiethof et al. 2004). Long studies on aplications of acarophagous and entomophagous mites in the plant protection are performed at the Institute of Biology, University of Latvia (Petrova et al. 1991). Different biological control agent cultures suitable in Latvian climatic conditions are selected and tested.

The aim of the reecent study was to evaluate success of application of beneficial insect and mites complex in polycarbonate greenhouses according to the dosage and application frequency. Study was conducted in 100 $m^2$ polycarbonate greenhouses located in Jelgava and Salaspils. Greenhouse crops like tomatoes, sweet peppers, cucumbers were inspected. Population density of fungus gnuts were counted on yellow glue traps before beneficial insect and mite release. Used beneficials, their target pests and used doses are described in Table 1. Cultures of *Amblyseius cucumeris, Phytoseiulus persimilis, Aphidius colemani* and *Aphidoletes aphidimyza* reproduced in the Institute of Biology and two comercial Biobest products were used. During the season we counted weekly all pests and beneficials (all stages) on all leaves of selected 20 plants of each crop.

Significant reducion of the average amount of pests per plant was possible when predator / parasitoid combinations before the pest population multiplication were used. We found that the amount of aphids decreased 25 times and the amount of whiteflies 16 times during the season. At the end of the season we observed reduction of all pest populations (less than 10 inviduals per plant). After the biological control agents have been used for two years, we have seen that predatory mites and parasitic wasp *Encarsia formosa* successfully overwinter in the greenhouse. Therefore, in 2015 and 2016 *Amblyseius cucumeris* and *Phytoseiulus persimilis* were applied just once in the beginnig of the seson.

The most effective combination for control of pests: spider mites, trips, aphids and whiteflies, was predatory mites *Ambliseius cucumeris* and *Hypoaspis miles* together with parasitoids *Encarsia formosa* and *Aphidius colemani*.

**Table 1.** Predator/parasitoid complex, target pests, used doses and aplications frequency. *Once in beginning of sesson

| Predator/ parasitoid (comercial product name, producer) | Target pest | Dose per 1 $m^2$ | Application frequency per month | | | |
|---|---|---|---|---|---|---|
| | | | 2013 | 2014 | 2015 | 2016 |
| *Amblyseius cucumeris* | trips, spider mites | 250 mites | 1 | 1 | * | * |
| *Phytoseiulus persimilis* | spider mites | 5 mites | 1 | 1 | * | * |
| *Stratiolaelaps scimitus* = *Hypoaspis miles* (Hypoaspis-System, Biobest) | fungus gnats, trips | 100 mites | 2 | 2 | 1 | 1 |
| *Encarsia formosa* (Encarsia-System, Biobest) | whiteflies | 3 pupae | 2 | 2 | 1 | 1 |
| *Aphidius colemani* | aphids | 0.5 mummies | 2 | 2 | 1 | 1 |
| *Aphidoletes aphidimyza* | aphids | 0.2 cocons | 2 | 2 | 0 | 0 |

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# Evaluation of potential risks of genetically modified seeds and plant propagating material to enter the territory of Latvia

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**Key words:** genetically modified organisms, maize, oilseed rape, propagating material, seeds, soybean.

European Union (EU) is the largest seed exporter in the world providing more than 60% ofthe whole seed market. The largest amounts of seed production are carried out in France, Germany and Italy.

Genetically modified organisms (GMO) in EU are regulated with Directive 2001/18/EC and Regulations (EC) No. 1829/2003 and (EC) No. 1830/2003. Seeds of genetically modified (GM) varieties are included in seed certification framework with Directive 98/95/EC.

Seeds of genetically modified plants can be produced within EU and outside EU. Unintended presence of GM seeds in conventional seed lots cannot be excluded. EU recommendations 2010/c 200/01 recommends 0.9% GM content as the labeling threshold, but the final decision can be made by each member state. In almost all EU countries monitoring plans exist for seed monitoring.

Latvia has not developed any seed monitoring plan yet. Therefore the scientific project by Ministry of Agriculture of Latvia entitled "Assessment of possible risks of genetically modified seeds and propagating material in the territory of Latvia and development of risk management recommendations in accordance with Latvian agro-economic conditions" is initiated to address this issue. It is performed by the Institute of Food Safety, Animal Health and Environment "BIOR" in collaboration with the State Plant Protection Service of Latvia and Ministry of Agriculture from 2016 till 2018. The aim of the project is assessment of potential risk of GM seeds and plant propagating material to enter the Latvian market and development of risk management recommendations. The main outcome of the project will be risk management recommendations according to agro-economical conditions in Latvia ensuring that relevant agricultural sectors are in the line with EU legislation – Directive 2001/18/EC and regulations (EC) No 1829/2003 and 1830/2003.

During the first year of the project a comprehensive literature search about the experience of other countries was carried out. Data about registered GMO contamination incidents were analyzed using EU Rapid Alert System for Food and Feed and GM Contamination Register (maintained by GeneWatch UK un Greenpeace International). The determined possible routs of GM seeds and plant propagating material to enter Latvian market were identified as following: intentional or unintentional import of unlabeled GM seed for cultivation; GM contamination in conventional seed lots; imported GM seed for food and feed; other unintentional import (for example, import of GM seedlings). Crops with increased risk for presence of GM varieties were identified as maize, oilseed rape, soybean, especially if they are imported from the third countries in which GM varieties are authorized for cultivation, e.g., Argentina, United States of America, Brazil or Canada. International Seed Testing Association and International Organization for Standartization standard methods were studied and compared for sampling and testing of presence of GM seeds.