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ORIGINAL PAPERS

SHORT REVIEW

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SUCCINATE, SILICON DIOXIDE, ALUMINIUM OXIDE, AND SILVER NANO- AND MICROPARTICLE INFLUENCE ON THE MODEL ORGANISM *DROSOPHILA MELANOGASTER*

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Abstract: In the study we subjected for biological testing succinate (5–3000 nm), silica (200–300 nm), aluminium (2500 nm), and silver (500–1000 nm) particles potentially applicable in the production of novel 3D biotextile. The effects of selected particles were evaluated in drosophila model by measuring the parameters potentially to be affected during the development. At the concentrations tested (0.01% and 0.1%), no clear adverse effects on egg to imago viability and locomotor activity, size, and phenotype of enclosed flies were found.

Key words: microparticles, biotextile, drosophila, development

Introduction

Inorganic and organic nano- and microparticles have been studied and applied in various industrial areas (Joudeh and Linke, 2022) – food technology (Younes et al., 2018), agriculture (Aqeel et al., 2022), environmental remediation (Liwarska-Bizukojć and Olejnik, 2020), pharmaceutical biotechnology (da Silva et al., 2023), biomedical application (Abbasi et al., 2023), textile manufacturing (Ļašenko et al., 2022), industrial biotechnology (Laible et al., 2021), and other industrial and biotechnological processes.

Nanoparticles are defined as particles with external dimensions between 1–100 nm (Joudeh and Linke, 2022, Engin et al., 2017), and microparticles are considered as particles in the size range from 100 nm up to 1000 μm (McClements, 2020).

Due to the widespread use of nano- and microparticles and their potential release into the environment, it is important to assess the potentially harmful effects of these particles on human health, as well as the impact on living organisms (Kumah et al., 2023).

The effect of particles on living organisms depends on their composition, size, shape, hydrophobic/hydrophilic properties, charge, stiffness, and presence of functional groups (Sabourian et al 2020, Augustine et al., 2020). Within tissues nanoparticles interact with the extracellular matrix (Engin et al., 2017). Upon contact with the cell surface

nanoparticles, depending on their physicochemical properties, can be internalized through passive transport (diffusion) or active cell transport (endocytosis). It is considered that nanoparticle size 10–60 nm is optimal for internalization process (Sabourian et al., 2020). The main nanoparticle internalization process is clathrin-mediated endocytosis which takes part in all eukaryotic cells. In this process 100–150 nm vesicles are formed. Larger particles 0,5–10 μm are obtained within the cell by phagocytosis, the process performed by professional phagocytic cells (Awashra and Mlynarz, 2023). In animals, phagocytosis is carried out by specific immune cells, and in some invertebrates also by enteric phagocytes. The intestinal phagocytes have not yet been described in the model organism *Drosophila melanogaster* (Hartenstein and Martinez, 2019).

Intracellularly nanoparticles interact with cytoplasmic proteins, nucleus, and other cellular organelles, and cause subsequent cellular responses (Augustine et al., 2020).

In the nucleus, particles, up to 10 nm, enter the passive transport path, while larger particles, up to 50 nm, are internalized by active transport (Sabourian et al., 2020). It is considered that smaller-sized nanoparticles have higher cellular uptake and more pronounced toxic effects (Awashra and Mlynarz, 2023).

Nanoparticles cause adverse effects in biological systems, mainly through the formation of reactive oxygen species (ROS) and the resulting oxidative stress (Augustine et al., 2020, Awashra and Mlynarz, 2023). The effects of nanoparticles on living organisms are being studied both in plants (Gao et al., 2023, Grauda et al., 2015) and animals (Lama et al., 2020) using *in vitro* and *in vivo* systems. Nanoparticle effects depends on whether cells are studied *in vitro* or at organism level (Karkossa et al., 2021). It is convenient to use invertebrate models to assess the impact on the whole organism, as they are subjected to less ethical requirements. (Lama et al., 2020). The fruit fly *Drosophila melanogaster* is a commonly used non-mammalian model organism in biological and medical research, including toxicology. It has well-studied biology and evolutionary conserved basic biochemical and genetic processes are shared with higher eukaryotes (Chifiriuc et al., 2016).

The current study was designed to find out if nano- and microparticles subjected for biological testing could be potentially applicable in the production of novel 3D biotextile with enhanced protecting properties. The effects of selected succinate, silica, aluminium, and silver particles (0.1% and 0.01%) were evaluated by measuring drosophila eclosion rate, and other parameters potentially to be affected during the development.

Materials and methods

Chemicals

Four different types of spherical particles (Ps) were tested. Silver particles (Ag) 500–1000 nm (Enola, Latvia), aluminium oxide particles (Al_2O_3) 2500 nm (Alfa Aesar, USA), silicon dioxide particles (SiO_2) 200–300 nm (Sigma-Aldrich, USA), and amber (succinate) composite particles 5–3000 nm (JLU Technologies, Latvia).

The particles were suspended in an aqueous solution of 0.8% Tween® 80 (polysorbate) with a bath sonicator at 40 kHz for 40 minutes, at a final concentration of 0.4%, and used in the test.

Treatment of the flies

Drosophila melanogaster Canton-S strain was used in all tests. The flies were raised on banana medium (Demerc and Kaufmann, 1996) 8 ml in standard 35 ml plastic vials at 25 ± 1 °C, under 12 : 12 h light : dark cycle (9:00 a.m. to 9:00 p.m.) and 60% relative humidity. If necessary for manipulation, CO_2 anaesthesia was applied for adult flies. Testing suspensions were added to banana medium to final Ps concentrations 0.01% and 0.1% in total setting eight experimental groups containing particles (Ps). To ensure appropriate conditions for development, yeast granules were added by spreading to media surface (Becher et al., 2012).

Viability test

Flies were exposed to Ps throughout the life cycle (Rand et al., 2014). Fertilized eggs were obtained by placing reproductively mature drosophila males and females in egg collection cage with standard medium abundantly covered by yeast. Within eighteen hours eggs at the same pose were washed by saline solution using paint brush and collected in sieve and immediately used in experiments. Fifty eggs per vial were placed in Ps containing media and the control media without Ps. Larvae were allowed to hatch, feed, and undergo a metamorphosis. F1 flies were counted after their eclosion to calculate the egg to imago viability. Data were pooled from two experiments and in total 550 eggs per group were used.

Fertilized eggs produced by up two weeks old F1 flies over eighteen hours period were randomly selected and placed in a Ps-containing media. After the completion of metamorphosis, F2 flies were obtained.

Locomotor activity

Locomotor activity was evaluated by the negative geotaxis – climbing test (Nichlos et al., 2012). Two weeks old F1 males and females were separated by CO_2 anaesthesia one day prior the test and maintained in media without Ps.

Flies were moved into glass cylinder, tapped to the bottom of the cylinder, and allowed to climb. After 30 seconds photo was taken, and climbed hight per each fly was measured with ImageJ software. Test were performed within two experiments, 50–71 males and 40–58 females were tested per group. To avoid the influence of circadian rhythm between experiments, the test was carried out at the same time during the light period.

External morphology

F1 and F2 imagoes were examined under stereomicroscope for external morphological deviations in head, thorax, abdomen, wings, and legs. In total 119–162 F1 flies and 68–168 F2 flies per group were analysed. The presence of visual mutant phenotypes was considered as indicator for a genotoxicity of Ps.

Body size

Body size was rated for F1 male and F1 female flies by thorax length (Lafuente et al., 2018). The measurements were made in two experiments, the mean calculated from 57–89 individuals in each group.

Statistical analysis

All data were analysed using Microsoft Office Excel 365 and SPSS 22 for Windows software. The significance of the differences between control and experimental groups were estimated by one-factor ANOVA followed by the Tukey test for normally distributed data sample sets. For other distribution Mann Whitney U-test was applied.

Results and Discussion

A series of different developmental assays are applied to evaluate the overall effects of toxic substances in drosophila model. The toxic effects can be tested within all stages of drosophila development – the egg, larva, pupa, and imago (Rand et al., 2014).

The approach chosen in the study is to expose drosophila to toxic substance throughout all developmental stages. It is a basic method applied in research on the potential deteriorating effects of nanoparticles, microparticles and other compounds.

Drosophila larval period proceeds up to five days at 25 °C, during the stage extensive feeding and ingestion of testing substances occurs. It is followed by pupal period during which complete metamorphosis occurs. Consequently, toxic substance affects not only survival up to the imago stage, but also anatomical, physiological, biochemical parameters of eclosed adult individuals (Rand et al., 2023).

In the current study flies were exposed to Ps within larval stage and consecutive parameters characterizing the drosophila overall physiological state were examined – egg to imago viability, locomotor activity, body size and external morphology.

The F1 egg to pupa viability of Canton-S in control group was on average 55%. In the aluminium oxide and silver Ps 0.1% groups were observed slight reduction of viability up to 15% (Table 1). Sexual dimorphism of locomotor activity (Figure 1) and body size (Figure 2) was observed in all groups. Male flies have faster movements and smaller body size. No clear reduction of the given parameters was observed in the Ps groups. In all groups of F1 and F2 generation fly external morphology matched the wild type.

Table 1. F1 generation egg to imago viability in Ps containing media

Group	*N*	egg to imago viability %		
		Mean	±	*SD*
Control	11	54.9	±	5.54
Succinate 0.01%	11	46.5	±	10.12
Succinate 0.1%	11	55.5	±	8.54
SiO_2 0.01%	11	51.1	±	9.61
SiO_2 0.1%	11	48.7	±	7.55
Al_2O_3 0.01%	11	52.4	±	5.85
Al_2O_3 0.1%	10	42.0	±	12.65*
Ag 0.01%	11	49.8	±	9.05
Ag 0.1%	11	44.0	±	10.39

The data are expressed as mean ± standard deviation. The significance of the differences between means of *Ps* exposed groups and control was determined by the ANOVA Tukey test, $*P < 0.05$ (SPSS 22).

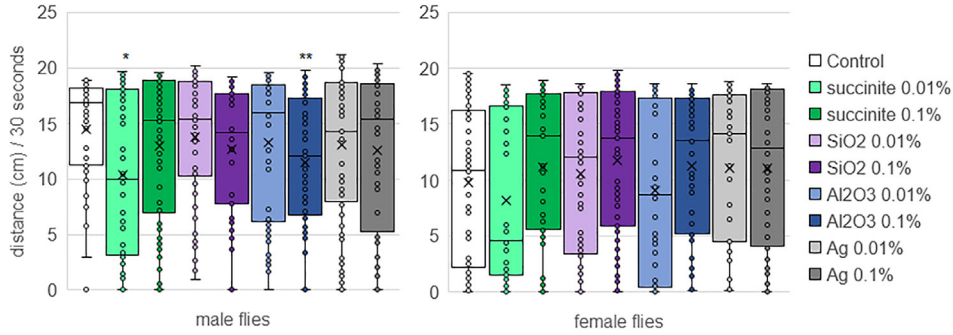

Figure 1. Locomotor activity of F1 imagoes developed in Ps-containing media. The data are expressed as boxplots. The significance of the differences between Ps exposed groups and control was determined by the Mann-Whitney U-test (SPSS 22), **P* ‹ 0.05; ***P* ‹ 0.001.

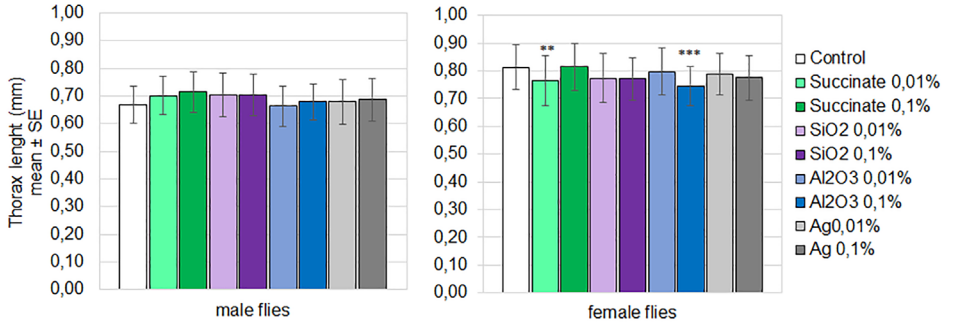

Figure 2. Body size of F1 imagoes developed in Ps-containing media. The data are expressed as mean ± standard deviation. The significance of the differences between means of Ps-exposed groups and control was determined by the ANOVA Tukey test (SPSS 22), *P ‹ 0.05; ***P* ‹ 0.001.

Unlike succinate, Al_2O_3, SiO_2 and Ag nano- and microparticles have been widely applied in different industries, and therefore research data on their toxicity are available. In the drosophila model toxic effects of aluminium oxide, and silver nanoparticles in diameter up to 100 nm have been detected. Al_2O_3 particles (< 50 nm), along phenotypic alterations, induced decrease of locomotor activity of eclosed flies (Anand et al., 2019), similarly, Ag particles (20–100 nm) caused developmental delay, reduced fly size and impaired locomotion (Singh et al., 2021).

In our research Al_2O_3 and Ag Ps in dimeter above 500 nm were tested, and no significant alterations in development, locomotor activity, and phenotype were observed. Non-compliance between our findings and previously published data could be due to the size of tested particles. Larger particles are considered less toxic to the cells (Awashra and Mlynarz, 2023).

The silicon dioxide Ps (> 100 nm) did not exert deteriorating effects on examined parameters, and it is in accordance with previous published data by Peropadre and coauthors in 2023.

Since the ability to induce oxidative stress has been demonstrated for given composition nanoparticles (Mirshafa et al., 2018, Singh et al., 2021, Peropadre et al., 2023), further characterization of Ps included in 3D biotextiles is required, paying attention to biochemical and molecular markers that participate in oxidative stress regulation processes.

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MACROPHYTE *FUCUS VESICULOSUS* AS HABITAT FORMING SPECIES IN THE GULF OF RIGA, BALTIC SEA

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Abstract. In the Gulf of Riga the long-term studies of macrophyte communities and related environmental parameters started at 1999 till 2021. This is first research monitoring combining both species identification, productivity, by SCUBA diving and ecological mapping of two different subregions in the Gulf of Riga. The *Fucus vesiculosus* as habitat forming key species in relation to environment factors described.

Key words: the Gulf of Riga, macrophyte communities, *Fucus vesiculosus*

Introduction

The macrophyte studies in the Gulf of Riga have been performed fragmentary by scientists already from the middle of 19th century (Eichwald, 1852, Skuja, 1924, Kumsare et al., 1974, Kirejeva, 1960). During this period different methods and approaches have been used by researchers to find out the species composition of the underwater vegetation of the Latvian part of the Gulf of Riga. In the 1999 in a frame of Nordic Council Programme "The Gulf of Riga" for the first time phytobenthic communities were sampled using SCUBA techniques on transects from shoreline to macrophyte depth limits. (Kautsky et al., 1999).

From 1999, after first Guidelines for monitoring of phytobenthic plant and animal communities in the Baltic Sea (Annex for HELCOM COMBINE programme, 26 March 1999) appeared, the laboratory of Marine Ecology started long-term research monitoring and ecological studies of macrophyte communities at different eutrophication impact.

The aim of this study was to quantify the macroalgal assemblages on a Gulf of Riga scale on a long-term base.

Material and methods

Two core transects, with two additional (for species diversity) for every transect, were chosen in the Gulf of Riga with different eutrophication impact. One transect was located

on the west coast of the Gulf "Mērsrags", the second – at the eastern part of the Gulf – "Saulkrasti" with three river (Lielupe, Daugava, Gauja) inflow impact. According to guidelines for macrophytes following core variables were measured by SCUBA diver: site position; transect depth profile; substrate; depth distribution of important plant species; composition of plant species; coverage of plant species; temperature; water transparency; salinity. As main parameters were nutrients in the water, algae belt distribution, macrophyte biomass (g dry weight/m^2). The productivity of every species in macrophyte communities are measured as biomass (g dry weight/m^2) by drying in the thermostat at 60° C temperature till the weight of the species is invariable (ICES, 2008) The samples on transects were sampled every second year (1999–2009), later on every year (2009–2021). The Mērsrags site transect belongs to the ILTER (International Long-Term Ecological Research) site, registered as Engure-marine site. The expeditions were organized always in August/early September, when macrophytes reach their physiological/growth maximum. The distance from the shore line to the max depth of Mērsrags transect was 3000 m, for the Saulkrasti transect – 2000 m.

Results and discussion

The Mērsrags core transect (Fig. 1) according to ecological mapping survey is characterized by coverage of plant species. The shallow coastal area (0.1–4 m) more than 75% of the hard substrate (boulders, large stones) is covered by benthic vegetation. The vegetation of hard substrates of upper vegetation zone (0.1–1 m) almost 100% consist of *Cladophora glomerata*. Another green algae *Enteromorpha intestinalis* was abundant only on few bigger boulders at depth of 1.5–2 m. Partial absence of vegetation on small stones is typical for large areas of the coastal zone of the Gulf of Riga. Even bigger stones (40 cm diameter) might be bare in more shallow parts of the coastal zone. It mostly depends on sediment flow and waves in the shallowest part of the coastal waters. The hard bottoms of the shallower part are covered by green algae *Cladophora glomerata*, which dominates above the depth of 2.5 m.

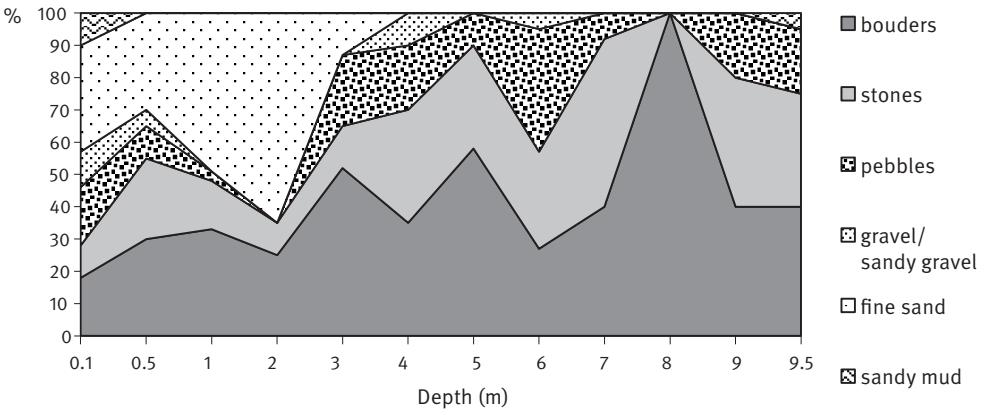


Figure 1. The composition of the substrate on the transect Mērsrags

At depth of 2.5 to 4 m brown algae *Fucus vesiculosus* reaches its maximum that equals to 30–40% of the total plant coverage. In some years its coverage reaches up to 90% of the total vegetation.

At depth of 4 m the abundance of red algae *Ceramium tenuircornis* increases up to 25–30%, down to 5 m *Ceramium* species become in dominance and cover about 60% of the suitable substrate and *Fucus vesiculosus* coverage still remains 5–10% of total plant coverage at this depth.

At the depth of 8.2 m the last shot of red algae *Furcelaria lumbricalis* was observed and brown algae *Sphacelaria arctica* observed on transect already from 1.5 m stays as the only algae reaching lowest limit of the photic zone at depth of 10 m.

The Saulkrasti core transect (Fig. 2) according to ecological mapping survey on a long-term aspect is characterized by following features: the shallow coastal area (0.1–3 m) all of the hard substrate is completely covered by vegetation. The difference in plant coverage on boulders and stones is much less pronounced as it was observed in Mērsrags. The influence of the long-shore sediment flow on the vegetation is less effective in Saulkrasti. However, the causes of the sand scraping were clearly seen in the shallower parts of the monitored area. The green algae *Cladophora glomerata* is dominating species above the depth of 3 m: 75% of the suitable substrate, respectively. *Fucus vesiculosus* occurs on 20% of available bottom at this depth. The coverage of other species living on the hard substrate (*Ceramium* sp., *Sphacelaria artica*, *Furcelaria lumbricalis*) is not more than 5%.

At depth of 3–4 m the coverage of *Cladophora glomerata* has decreased to 30% of the suitable substrate and *Fucus vesiculosus* has declined down to 10%. In contrary, *Sphacelaria artica* covers 35% of the available bottom and becomes a dominating species at this depth. Also red algae *Ceramium* sp. has its increase in coverage up to 20% and below 4 m depth *Sphacelaria artica* becomes an only dominating species. At the depth of 5.2 m the lower limit of distribution of macroalgae was reached. It is likely limited by low light intensity, which may be caused by high degree of dissolved material in water column or high density of the phytoplankton values. Most of the pebbles in Saulkrasti are concentrated at 1–3 m depth (10–20% of the hard bottom).

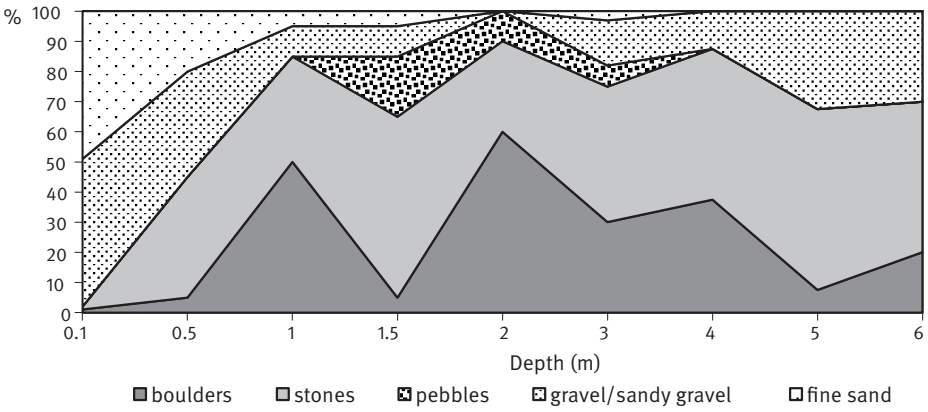


Figure 2. The composition of the substrate on the transect Saulkrasti

The macrophyte communities in the Gulf of Riga represent three main groups close connected with their location along the transects. As dominant species in shallow waters (littoral zone 1–2 m depth) are green algae Chlorophyta, in sublittoral zone (2.5–4 m) brown algae Phaeophyta are prevailing, but in deeper parts red algae Rhodophyta biomass appears more remarkable (Fig. 3).

The dominant species of Chlorophyta *are Cladophora glomerata, Cladophora rupestris, Enteromorpha intestinalis,* of Phaeophyta*: Fucus vesiculosus, Sphacellaria arctica, Pilayella litoralis,* of Rhodophyta – *Ceramium tenuicorne, Polysiphonia fucoides, Polysiphonia fibrillosa* (Boikova et al., 2003, Dekere et al., 2008).

The strong difference between biomass values on both transects for all three macrophyte groups relates with Secchi depth, salinity, nutrient levels. Secchi depth is related to water clarity and is a measure of how deep the light can penetrate into the water. At Mērsrags transect at 3 m depth in the average it was from 2.7 to close 3 m depth, at Saulkrasti transect – 2.1 m. Also, salinity differs at both transects: 5.20 and 4.80 PSU respectively. The average values of total nitrogen (NH_4, NO_2, NO_3) for Mērsrags transect after 2012 slighty lowered in parallel with Cladophora biomass. On Saulkrasti transect this was not observed.

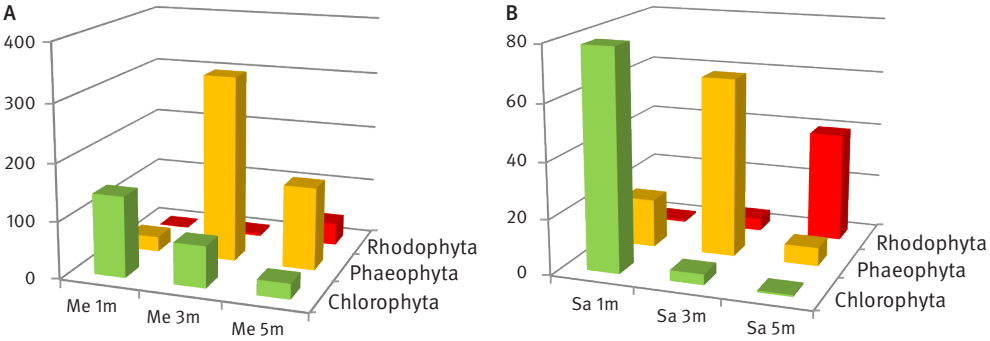


Figure 3. The average biomass (g dry weight/m^2) of macrophyte communities at Mērsrags transect (A), Saulkrasti transect (B) 1999–2021

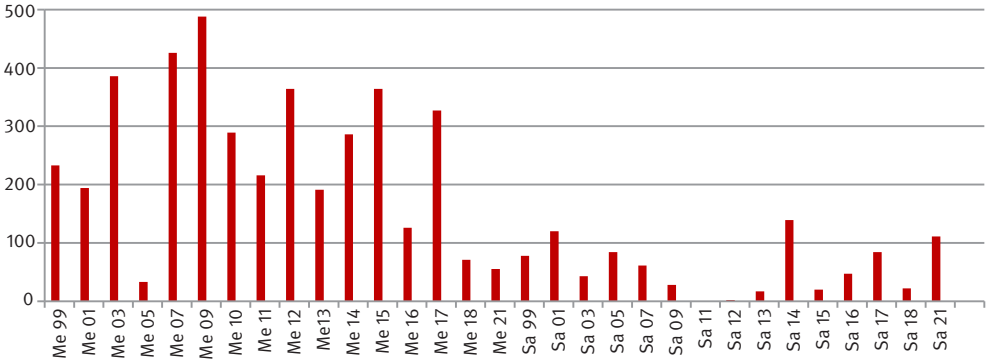
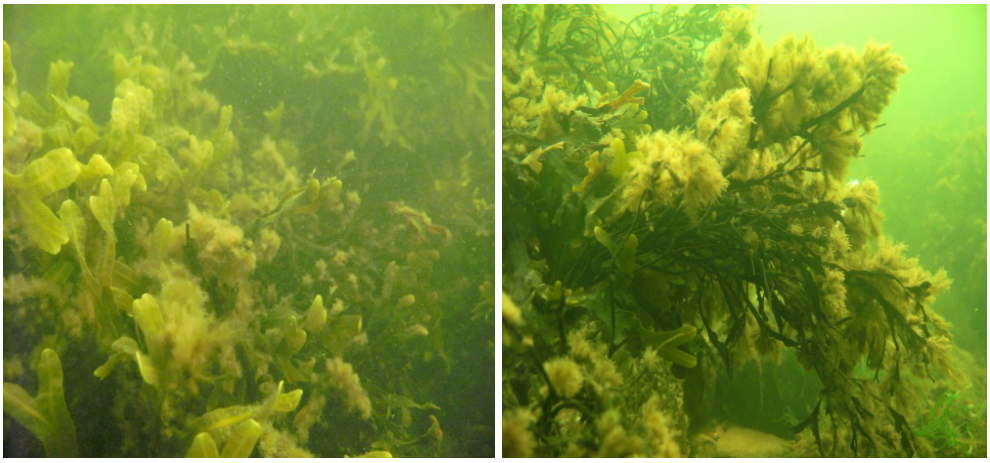


Figure 4. The long-term biomass (g dry weight/m^2) of *Fucus vesiculosus, Mersrags* (west coast) and Saulkrasti (east coast) transects (1999–2021)



A Mērsrags habitat **B** Saulkrasti habitat

Figure 5. Fucus vesiculosus at Mērsrat Mērsrags (A) and Saulkrasti habitats (B) at 3 m depth

The coverage of brown algae *Fucus vesiculosus* reaches the maximum in the depth interval from 2.5 to 4 m and long-term biomass data illustrates this species as a key species in the Gulf of Riga sublittoral zone. Nethertheless, there is remarkable difference in productivity between studied transects: at the Mērsrags transect with the average biomass 253 g dry weight/m^2 and at the Saulkrasti transect only 53 g dry weight/m^2. There was not observed any positive trend in key species *Fucus vesiculosus* and other macrophyte depth distribution as it was described by Kirejeva (1960), where macrophytes reaches 15 m vertical distribution.

Although the Gulf of Riga belongs to eutrophic area in comparison with some other Baltic Sea subregions there are local distribution patterns in relation to environmental variables (Fig. 4).

The *Fucus vesiculosus* habitats are different at both transects not only by substrate type, salinity, Secchi depth, partly by nutrients, but also there is a strong impact of epiphyts on *Fucus* at Saulkrasti transect (Fig. 5).

Still no positive distribution of macrophyte habitats, especially at Saulkrasti coast could be partly explained by possible new pollution elements/climate change.

Acknowledgements

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MIXOTROPHIC AND HETEROTROPHIC CULTIVATION OF DIFFERENT MICROALGAE SPECIES ON DAIRY BY-PRODUCTS FOR FURTHER SUPPLEMENTATION OF POULTRY DIET

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Abstract: Use of alternative cultivation substrates, for instance dairy industry by-products, can significantly reduce the production costs of microalgal biomass. However, dairy by-products are found to be problematic co-substrate for many microalgae, due to inability to hydrolyse lactose. The focus of this study was to assess the ability of microalgal strains to produce biomass in media supplemented with lactose, glucose and galactose. Among them only microalgae *Chromochloris zofingiensis* was capable to assimilate effectively all three sugars.

Key words: microalgae, dairy industry by-products, *Chromochloris zofingiensis*, β-galactosidase

Introduction

Currently the growing consumption of poultry production, including eggs, requires use of a sustainable nutrient sources for satisfying respectively growing feed demand, and to improve production's quality and animal wellbeing. Recent studies indicate that inclusion of microalgae biomass into laying hen diet can significantly improve the fatty acids profile in eggs and meat, i.e., increasing the content of such polyunsaturated fatty acids (PUFA) as docosahexaenoic acid (DHA) and eicosapentaenoic (EPA). Omega-3 are widely recognized as valuable components of a healthy human diet, as well as can improve laying hen immunity (Saadaoui et al., 2021). For instance, supplementation of hen diet with microalgal biomass, containing only 1% of *Trachydiscus minutus* or 1% of *Japonochytrium marinum* in the feed used, resulted in a significant increase in long-chain PUFA in eggs by 26–66%. Moreover, the addition of 1% *Scenedesmus obliquus* to hen feed resulted in an increased content of carotenoids, i.e., lutein and cantaxanthin, in eggs by 48% and 18%, respectively (Jiru et al., 2021).

Despite of reported improvement in product quality, use of microalgal biomass for poultry diet supplementation remains significantly limited. Currently commercial production of many microalgal species is considered economically unfeasible (Benemann et al., 2018).

In order to facilitate commercial application of certain biotechnologically promising microalgal species, use of different agricultural and food processing by-products, e.g., dairy industry by-products (whey, permeate) can be considered as more cost-effective cultivation media (Chakraborty et al., 2023; Kolesovs & Semjonovs, 2023). Additionally, utilisation of such substrates as whey, can decrease the negative environmental impact caused by dairy industry by-products when they are inappropriately disposed into the environment (Koutinas et al., 2009). Furthermore, studies show that some microalgae species can synthesize biomass and bioactive components (lipids, proteins, vitamins, pigments etc.) mixotrophically and heterotrophically – exceeding their productivity by autotrophic growth (Zhan et al., 2021). Nevertheless, there is still little knowledge on the ability of microalgae to efficiently utilise lactose, which is the main carbon (C) source in dairy industry by-products (Kolesovs & Semjonovs, 2023). It should be taken into account however, that lactose is considered to be a problematic substrate due to inability of many microalgal species to hydrolyse this disaccharide due to lack of β-galactosidase enzyme (Brasil et al., 2017) and a selection of suitable species is required.

This study focuses on screening of microalgal strains obtained from microalgal culture collections or environmental isolates for their ability to grow mixotrophically and heterotrophically on lactose, glucose and galactose as main C sources, in a semisynthetic growth media.

Methods

Selection of cultures

Microalgal strains *Chlorella vulgaris* CCAP 211/111, *Chromochloris zofingiensis* CCAP 211/14, *Haematococcus lacustris* CCAP 34/6 and *Scenedesmus quadricauda* CCAP 276/16 were obtained from the Culture Collection of Algae and Protozoa (CCAP, United Kingdom). Additionally, three microalgae freshwater isolates indicated as M1 M2 and M3 were isolated from local freshwater ponds (Riga, Latvia). Bold's basal medium with triple nitrogen and vitamins (3N-BBM-V) (Yee et al., 2019) was used for cultivation of *C. vulgaris, C. zofingiensis S. quadricauda* and for M1 M2 and M3 isolates. Combined *Euglena gracilis* and Jaworski's medium (EG:JM) was used for *H. lacustris* CCAP 34/6 cultivation (Butler et al., 2018).

Experimental design

In this study microalgae were cultivated in test tubes for 21-day long period. Media supplemented with sugar (glucose, galactose and lactose as C source at 5 g/L) were used in two experimental groups, i.e. mixotrophic and heterotrophic cultivation. Medium without added C source was used as a negative control group for both upper mentioned cultivation types (photoautotrophic cultivation for mixotrophic group). The mixotrophic cultivation has been carried out in a presence of LED light source (3000 lux, day : night cycle – 16:8 h), and heterotrophic cultivation has been carried out in darkness, both performed statically in incubators at 25° C.

Changes in biomass

All experimental groups had four replicates (n = 4). After cultivation, samples of cultural liquids were collected, centrifuged at 6000 rpm for 5 minutes, supernatants discarded, and biomass resuspended with distilled water to remove media residues. Afterwards biomass was repeatedly centrifuged and transferred into pre-weighted weighting bottles. Additionally, optical density (OD) of biomass samples was measured at 540 nm in order to estimate growth of microalgae culture.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) to compare means at significance level p = 0.05.

Results and discussion

This study has demonstrated the ability of certain microalgae to utilize sugars from dairy by-products and convert it into additional biomass by mixotrophic growth. Changes in microalgae biomass synthesis are summarised in Table 1. Obtained results are presented as an initial stage evaluation for further in-depth study of lactose metabolism, biomass and its constituents synthesis.

As shown in Table 1, only *C. zofingiensis* was capable of producing significantly higher amounts of biomass in all types of media supplanted with carbohydrates, including lactose (up to 1.03 ± 0.03 g/L, dry weight) supplemented groups (Figure 1). This obviously can be associated with *C. zofingiensis* ability to produce β-galactosidase, i.e., enzyme required for lactose hydrolysis. Further studies would allow to assess *C. zofingiensis* β-galactosidase enzyme activity, as well as changes in C sources during the cultivation. As shown in Figure 1, biomass production under heterotrophic cultivation conditions was significantly lower (>50%) compared to mixotrophic growth.

Table 1. Changes in microalgal biomass compared to negative control ("+" in case of significant increase) after 21 days of mixotrophic and heterotrophic media supplemented with glucose, galactose, and lactose

Microalgal culture	Cultivation type					
	Mixotrophic			Heterotrophic		
	Glucose	Galactose	Lactose	Glucose	Galactose	Lactose
C. vulgaris	+	+	–	+	+	–
C. zofingiensis	+	+	+	+	+	+
H. lacustris	+	–	–	+	–	–
S. quadricauda	+	–	–	+	–	–
M1	+	–	–	+	–	–
M2	+	+	–	+	+	–
M3	–	–	–	–	–	–

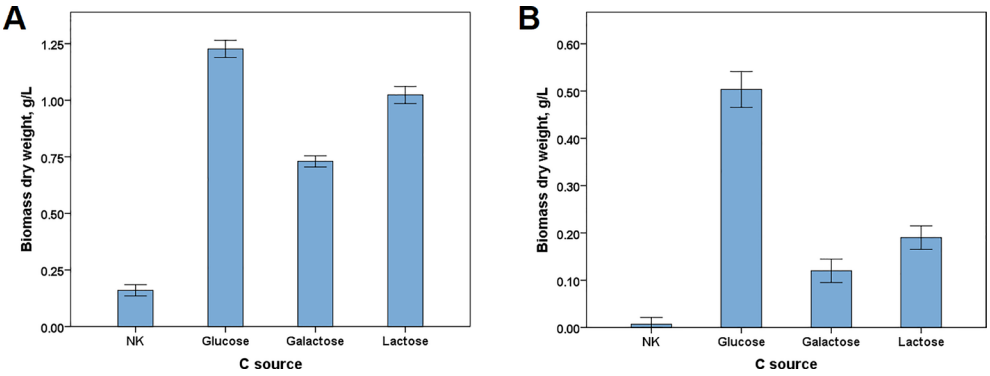

Figure 1. Changes in *C. zofingiensis* CCAP 211/14 biomass synthesis after 21 days of cultivation under mixotrophic (A) and heterotrophic (B) growth conditions with glucose, galactose and lactose as C source or without addition of C (negative control – NK).

Obtained results are in line with data reported previously. For example, it has been shown, that during the evaluation of eight microalgal strains, only three were able to grow on lactose-containing media, and *Dunaliella tertiolecta* (code 117) among them reached highest dry biomass productivity of 0.22 g/L/d on synthetic medium supplemented with lactose as the C source during mixotrophic cultivation. (Zanette et al., 2019). In contrary, another study has demonstrated that addition of lactose can inhibit microalgal growth. Therefore, a selection of suitable strains is necessary in order to achieve higher process feasibility.

Optimisation of *C. zofingiensis* growth conditions, e.g., agitated cultivation, use of more suitable equipment (flasks, bioreactors), pH control and media adjustments (C concentrations, addition of certain growth factors), would allow to achieve significantly higher biomass production rates in further experiments. Additionally, cultivation on lactose-containing substrates, can stimulate the production of valuable bio-active substances such as lipids (including PUFA), pigments (astaxanthin, lutein, β-carotene), proteins, vitamins (Kolesovs & Semjonovs, 2023).

Noteworthy that *C. vulgaris* CCAP 211/111 and M2 isolate showed an enhanced ability to utilize glucose and galactose as main C sources, although, there was no changes in biomass production observed in lactose supplemented media – for both mixotrophic and heterotrophic groups. However, the ability of this strains to utilise glucose and galactose can be considered as beneficial for further formation of microalgal consortia. Use of consortia can stimulate the assimilation of nutrients including high amounts of C present in dairy by-products (Chawla et al., 2020). Further microalgal isolates will be tested using upper mentioned methodology.

Conclusions

Lipid- and pigment-enriched microalgae biomass can potentially be used as a supplement in poultry feed in order to improve both hen immunity as well as production

quality. Currently, main limiting factor for wider use of microalgal biomass in agricultural manufacturing still remains its production costs caused by unsatisfying productivity of the process. Therefore, after extensive research and process optimisation efforts, inclusion of *C. zofingiensis biomass* in poultry feeds can be prospective solution to provide such valuable nutrients as PUFA and astaxanthin. Further improvement of microalgae biomass production process, e.g., mixotrophic cultivation on dairy by-products, can significantly improve process`s efficiency and decrease the respective production costs.

Among tested microalgal strains only *C. zofingiensis* CCAP 211/14 was able to produce significant amounts of biomass using all three C sources including lactose. This shows the suitability of *C. zofingiensis* for growth in dairy by-products for biomass production. Further studies will be focused on the assessment of *C. zofingiensis* ability to produce β-galactosidase under mixotrophic and heterotrophic cultivation conditions, as well as changes in biomass composition. Moreover, further evaluation of microalgae biomass effects on poultry health and production quality should be performed to assess the potential of upper mentioned microalgae cultivation`s approach.

Acknowledgement

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USE OF MICROALGAE *SCENEDESMUS QUADRICAUDA* AND *CHLORELLA VULGARIS* LIVING-CELLS SUSPENSIONS FOR PLANT BIOSTIMULATION

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Abstract: Up to date research on microalgae as plant biostimulants remains fragmented and use of living microalgal cell suspensions is understudied. This research focuses on use of *Scenedesmus quadricauda* and *Chlorella vulgaris* living-cells suspensions as growth stimulants for garden cress (*Lepidium sativum*). Different concentrations of microalgal biomass suspensions were tested. It was found that plant treatment with *S. quadricauda* biomass 0.8 g/L (dry weight) suspension resulted in a significant improvement in *L. sativum* seeds germination and growth rate.

Key words: microalgae, plant biostimulants, plant growth, microalgal biomass, *Lepidium sativum*

Introduction

Currently chemical plant stimulants and synthetic fertilisers remain widespread and cause environmental damage. Microalgae is a valuable but at the same still understudied group of microorganisms, which is attracting an attention as a prospective mean for use as sustainable plant biostimulants (Colla and Rouphael, 2020). This is associated with microalgae ability to produce complex compounds, e.g., lipids, proteins, antioxidants, antimicrobial compounds, polysaccharides, phytohormones. However, up to date the use of microalgae as a sustainable plant biostimulant remains limited (Chiaiese et al., 2018).

Current research shows that microalgal biostimulants trigger natural processes in crops improving nutrient uptake, as well as promoting tolerance to abiotic stress (Colla and Rouphael, 2020). These microalgal properties can promote the replacement of chemical biostimulants or biofertilizers with nature-friendly and sustainable alternatives. Mainly use of microalgal biomass in a form of extracts has been reported up to date, i.e., cell wall is initially disrupted or extraction of bioactive compounds from the cell has been performed by mean of varied solvents and techniques (Colla and Rouphael, 2020; Bella et al., 2021). For instance, in a study conducted by Bella et al. (2021) *C. vulgaris* extract positively influenced the growth of lettuce seedlings, by increasing shoots growth and plant biomass production, chlorophyll and carotenoids concentration in leaves, as well as protein content. However, it can be assumed that use of living cells as foliar sprays

can decrease the costs associated with biomass post-processing and potentially stimulate the synthesis of phytohormones and anti-microbial compounds.

This study focuses on evaluation of effects of living-cells biomass of *Scenedesmus quadricauda* and *Chlorella vulgaris* for treatment of garden cress (*Lepidium sativum*) seeds during germination and further growth in nutrient depleted soil substrate.

Methods

Microalgal strains

Two freshwater microalgae *Scenedesmus quadricauda* CCAP 276/16 and *Chlorella vulgaris* CCAP 211/111 were obtained from Culture Collection of Algae and Protozoa (CCAP, United Kingdom). Both strains were maintained and cultivated in Bold's basal medium with triple nitrogen and vitamins (3N-BBM-V) (Yee et al., 2019).

Preparation of microalgal biomass suspensions

S. quadricauda an *C. vulgaris* were cultivated statically for 21 days in order to achieve high biomass density. Photoautotrophic cultivation has been performed in presence of LED light source (3000 lux), day : night cycle 16 : 8 h, at 25 °C. After the cultivation microalgal was harvested by centrifugation at 6000 rpm for 5 minutes. Supernatant was decanted and the biomass resuspended in distilled water which followed by centrifugation. This procedure was repeated twice to remove medium residues. Subsequently, obtained microalgal biomass was resuspended in distilled water at three concentrations (0.4, 0.8 and 1.2 g/L) and used as foliar spray.

Assessment of plant germination

In order to assess the germination rate, *L. sativum* seeds were placed into containers with 50 g of dry nutrient depleted soil substrate. The soil substrate was watered every two days with tap water. Additionally, 5 mL of microalgae biomass spray (cell suspension) at 0.4 g/L concentration (water as control) was added to respective containers at 1st and 3rd day. After 5 days seed germination rate was calculated.

Plant growth experiment

For assessment of changes in plant shoot system length and plant biomass production, 10 *L. sativum* seeds were placed in pods with 50 g of dry nutrient depleted soil substrate. Each experimental group was grown in triplicates. Approximately 5 mL of microalgal biomass suspension at 0.4, 0.8 and 1.2 g/L concentration was sprayed at plants once a day every two days. For the control group 5 mL of tap water was used instead. Additionally, each container was watered with 10 mL of tap water once a day every two days. After 8 days the length and dry weight of plant shoot system was measured and compared to control. Experiments were performed in controlled environment chambers with 16 : 8 day : light cycle and 22 °C temperature.

Data analysis

SPSS (BM SPSS Statistics for Windows, Version 21.0; IBM Corp, Armonk, USA) was used in order to compare means (analysis of variance) at significance level $p = 0.05$.

Results and discussion

Our research shows, that use of live microalgal cells suspension can promote the germination of *L. sativum seeds* in nutrient depleted soil compared to control (Table 1). After five days of treatment with *S. quadricauda* CCAP 276/16 suspension 60% seed germination rate has been found. Additionally, *C. vulgaris* CCAP 211/11 also promoted the germination of 20% of seeds. Noteworthy that there was no seed germination observed in the control group with no treatment with microalgal suspensions applied. This can be is associated with increase of bioavailability of minerals, and presence of growth promoting factors, i.e., phytohormones, provided by microalgae. Additionally, a significant increase in water holding capacity was found in microalgae supplemented soil. It seems, that living microalgal cells can promote the soil moisture for longer time compared to control group which required additional watering.

Analysis of shoot system`s length showed, that use of live microalgae cells suspension can promote the length of shoot system (Figure 1). It was demonstrated, that *S. quadricauda* promoted a significantly greater increase in *L. sativum* length compared to *C. vulgaris*. Therefore *S. quadricauda* suspension was used in further studies in order to assess the optimal concentration of microalgal cell suspension. Additionally, the untreated control group showed a significantly lower growth rate compared to microalgal supplemented cell groups.

Table 1. Germination of *L. sativum* seeds after 5 days in the nutrient depleted substrate with negative control (water) and *S. quadricauda* or *C. vulgaris* suspensions (0.4 g/L) moisturised containers

Germination rate			
	Control	***S. quadricauda***	***C. vulgaris***
Germinated seeds, %	0	60	20

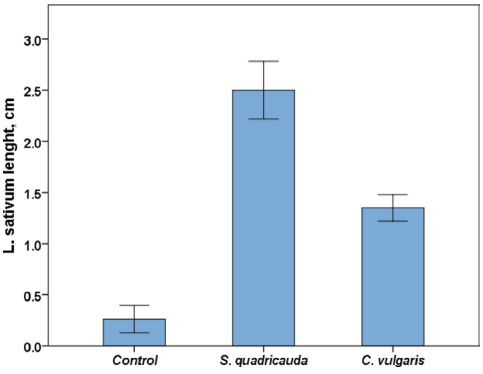

Figure 1. The length of shoot system of *L. sativum* after 5 days of growth in the nutrient depleted substrate with negative control (water) and *S. quadricauda* or *Chlorella vulgaris* suspension at 0.4 g/L treated containers

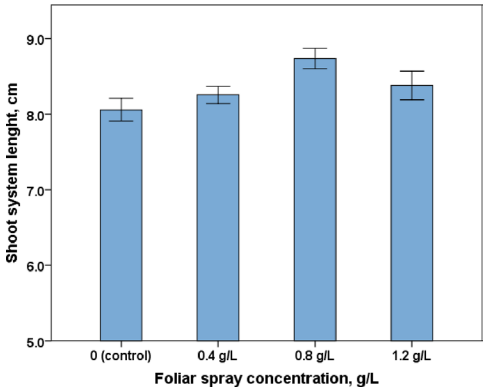

Figure 2. The length of shoot system of *L. sativum* after 8 days of growth in the nutrient depleted substrate with negative control (water) and *S. quadricauda* suspension (0.4, 0.8, 1.2 g/L) treated containers

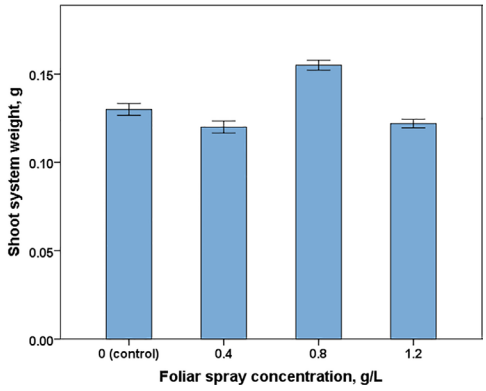

Figure 3. The dry weight of shoot system of *L. sativum* after 8 days of growth in nutrient depleted substrate with negative control (water) and *S. quadricauda* suspension (0.4, 0.8, 1.2 g/L) treated containers

Subsequently, different concentrations of selected microalgae (*S. quadricauda*) cell suspension sprays were tested (Figure 2). A statistically significant ($p < 0.05$) difference between experimental groups was found for microalgal suspension with 0.8 g/L of biomass. As shown in Figure 2, use of cell suspension with higher biomass concentration (1.2 g/L) showed a slight inhibition of plant shoot system growth. This can be associated with excess presence of compounds responsible for growth rate, for example phytohormones (abscisic acid) or relevant compounds (Wang et al., 2022). Further studies should focus on detection of specific stimulants and growth effecting factors present in *S. quadricauda* biomass.

Additionally, plant group treated with 0.8 g/L suspension showed highest increase in dry weight of its shoot system. Such improvement can be associated with increase in uptake of nutrients that microalgal biostimulants can promote (Colla and Rouphael, 2020).

Further research should be caried out to verify whether the improvement was connected to the phytohormonal activity of microalgae or with the release of additional nutrients from the microalgal biomass. Overall, higher germination rate and faster growth of *L. sativum* when microalgal biomass applied on the seeds or plant shoot system were observed.

Similar improvements in plant growth were observed after use of *Desmodesmus subspicatus* microalgal extracts at 0.5, 1.0, 1.5, and 2.0 g/L biomass concentrations. Additionally, a 0.4 g/L extract significantly improved leaf areas and development compared to lower as well as higher extract concentrations (Mazepa et al., 2021).

Conclusion

Results indicate that use of *S. quadricauda* and *C. vulgaris* biomass resulted in a significant improvement of seed germination and plant growth compared to negative control groups. Among tested microalgae the treatment with *S. quadricauda* biomass had a higher positive effect on seed germination and shoot system length compared to control with no microalgal biomass applied or *C. vulgaris suspensions*. Assessment of different concentrations of *S. quadricauda* biomass suspension showed, that at 0.8 g/L the most pronounced impact on *L. sativum* growth can be achieved. Further studies are required in order to select suitable microalgal strains for plant biostimulation, assess optimal biomass concentrations in suspensions and to evaluate the effect of microalgae on specific plant developmental stages.

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ECOLOGICAL QUALITY OF LATVIAN–LITHUANIAN TRANSBOUNDARY LAKES BASED ON BENTHIC MACROINVERTEBRATES AND MACROPHYTES

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Abstract: Macroinvertebrates and macrophytes were used as indicators to assess the ecological quality of five Latvian-Lithuanian transboundary lakes. The aim of the study is to ensure joint quality assessment of trans-boundary lake water bodies, because studied lakes pose a risk for not meeting good status according to the requirements of the EU Water Framework Directive. The fieldwork was performed in May and October 2021. Macroinvertebrates demonstarted good ecological quality at all studied lakes using both Latvian and Lithuanian multimetric macroinvertebrate indices. The non-biting midges Chironomidae, as well as the mayfly species *Caenis horaria* and *Cloeon dipterum*, were dominant in macroinvertebrate communities of the studied lakes. The invasive species zebra mussel *Dreissena polymorpha* was present in four lakes while the spiny-cheek crayfish Orconectes *limosus* was observed in two out of five lakes. In addition, the medical leech *Hirudo medicinalis* was found in Lake Lielais Kumpinišķu; it is a species protected under the Habitats directive (Annex V) and under the Latvian Regulation of the Cabinet of Ministers No 396.

Key words: lentic habitats, aquatic vegetation, benthic invertebrates, ecological status

Introduction

River basins are not restricted to administrative or political boundaries; therefore, some countries can affect the water quality of their neighbours. A variety of threats, e.g., invasive species, diffuse and point source pollution, water abstraction, etc., can affect waterbodies. Trans-boundary cooperation in managing and maintaining a healthy aquatic environment is required to ensure sustainable water resources (Correia and da Silva, 1999).

In the European Union, the Water framework directive (WFD) is the most powerful tool of environmental policy to ensure good ecological status of all waterbodies. The WFD determines the approach of waterbody ecological quality assessment for EU member states, including which biological quality elements to use and the usage of appropriate assessment methods (Directive 2000/60/EC). Despite the WFD being in effect for more than two decades, there are still too many uncertainties concerning it.

The first-time goal for EU member states to ensure good status of their waterbodies was 2015, however, the deadline was extended to 2027 due to the slow process of WFD implementation. Still, for some countries, assessment methods are not yet intercalibrated and new countries might join the EU without intercalibrated methods. Also, transboundary pollution is a serious issue to deal with in terms of river basin management.

In 2021, macrophyte surveys and macroinvertebrate sampling was carried out in five Latvian-Lithuanian transboundary lakes – Lake Ilzu/Garais, Lake Kumpinišķu, Lake Galiņu, Lake Skirnas and Lake Laucesas. The lakes were chosen as posing a risk for not meeting good status according to requirements of the EU Water Framework Directive, and the aim was to ensure joint quality assessment of these trans-boundary waterbodies.

Material and methods

Macrophytes

Latvian macrophyte assessment method (Daugavas upju baseinu ..., 2015) for lakes is primarily based on dominating indicator taxa and the addition of two more parameters: species composition and the depth limit of submerged plants. Passing the littoral of the whole lake by boat, relative abundance of the macrophyte species of all belts (emergent, floating-leaved etc.) and all taxonomic groups were estimated for the lake on the 7-point scale. Using the plant hook with a marked rope (or stock), the zonation and depth limits of macrophytes were determined on transects. The frequency of transects depends on the character of the lake; they have been made after every 100–500 m. In Lithuania, the modified German Reference Index is used for macrophyte-based assessment of the ecological status of lakes (Valstybės žinios, 2013).

Macroinvertebrates

For the sampling, a 50 m-long representative lake littoral zone stretch was chosen at each lake. Sampling was done in proportion to the coverage of dominant habitat types. Kick and sweep approaches were used with 5 replicates collected at each site. All replicates are merged in to one sample and also analyzed as one sample. Further samples were processed at the laboratory – taxa identified, and indices calculated using ASTERICS 4.04 software. For each lake, two multi-metric indices were calculated – the Latvian Lake Macroinvertebrate Multimetric Index (Skuja and Ozoliņš, 2016) and the Lithuanian Lake Macroinvertebrate Index (Šidagytė et al., 2013).

Results and discussion

Macrophytes

In Lake Ilzu (Garais)/Ilge, growth of macrophytes is limited by low water transparency caused by algae blooming or other suspended material. Species diversity is low – only 18 species were found. The dominating macrophyte species in the lake are *Phragmites australis, Nuphar lutea* and *Ceratophyllum demersum*.

In Lake Kumpinišķu, the diversity and abundance of macrophyte species differ in the northern and southern parts. The northern part is shallow, and its whole area is overgrown with macrophytes; species diversity in this part of the lake is high, with 32 species found in total. The southern part of the lake is deeper and the colonization depth of submerged macrophytes is 4 m. *Myriophyllum verticillatum*, charophyte and Potamogetonacea species are most frequent in the lake. Dense halophyte stands grow all along the shore.

In Lake Galiņu, the composition of macrophyte species is typical for slightly eutrophic lakes. The colonization depth of macrophytes is high (3.9 m), and species diversity is also high, however, it is without any charophyte species, thereby indicating a good ecological status. Species typical for eutrophic lakes, e.g. *Ceratophyllum demersum, Sagittaria sagittifolia,* and *Myriophyllum verticillatum*, occur frequently. Overgrowth with macrophytes is characteristic for bays where water exchange rate is lower, sediments are deeper, and macrophyte stands are dense.

In Lake Skirnas the ecological quality is high and species richness is also high (34 macrophyte species). Water transparency is high, therefore the colonization depth of submerged macrophytes is also high – 5 m. Macrophyte development is limited by the steep bottom, but in the shallow parts of the lake, species composition is characteristic of low-impacted lakes. The dominating macrophyte species in Lake Skirnas are *Fontinalis antipyretica*, *Phragmites australis*, *Nuphar lutea, Potamogeton lucens, Scirpus lacustris* and charophyte species.

In Lake Laucesas, macrophyte species composition is consistent for eutrophic lakes. The diversity of macrophyte species in most parts of the lake is moderate, and submerged species occur rarerl due to low water transparency. The species *Phragmites australis, Nuphar lutea, Ceratophyllum demersum,* as well as *Typha latifolia*, dominate in the whole lake. The last two species are typical for eutrophic and polluted waters.

Macroinvertebrates

In Lake Ilzu/Garais, the overall benthic invertebrate taxonomic composition in the littoral zone is characteristic of a eutrophic lake. In spring, altogether 55 macroinvertebrate taxa were identified. Larvae of Chironomidae were the most abundant taxa at all sampling sites. Mayfly nymphs *Caenis horaria* and water mites Hydrachnidia were also common. Having the highest taxonomic diversity was characteristic for caddisfly Trichoptera larvae (14 taxa).

In Lake Kumpinišku, the invasive species zebra mussel *Dreissena polymorpha* and spiny-cheeck crayfish *Orconectes limosus* were observed. Also, a legally protected medical leech *Hirudo medicinalis* was observed in the shallow part of the lake (Council Directive 92/43/EEC Annex V, Regulations of the Cabinet of Ministers No. 396). The overgrown northern part of the lake is more eutrophic and the number of macroinvertebrate taxa and their abundance there is lower than in the deeper southern part. Gastropoda was the species richest in taxa in both seasons.

Table 1. Ecological status of five Latvian-Lithuanian transboundary lakes according to the macrophyte and macroinvertebrate assessment methods of both countries

Lake	Macrophytes		Macroinvertebrates	
	Latvia	Lithuania	Latvia	Lithuania
Lake Ilzu/Garais	0.4 (poor)	0.25 (moderate)	0.67 (good)	0.58 (good)
Lake Kumpinišķu	0.8 (good)	0.51 (good)	0.71 (good)	0.71 (good)
Lake Galiņu	0.6 (good)	0.55 (good)	0.67 (good)	0.53 (good)
Lake Skirnas	1 (high)	0.52 (good)	0.82 (good)	0.69 (good)
Lake Laucesas	0.6 (moderate)	0.18 (poor)	0.88 (good)	0.59 (good)

In Lake Galiņu, the water louse *Asellus aquaticus*, mayfly nymphs from the family Leptophlebiidae, and the species *Caenis horaria* dominate the littoral macroinvertebrate communities. Their abundance varied from 156 to 387 specimens per sample, while the number of taxa was 22 in spring and 34 in autumn. The invasive mussel species *Dreissena polymorpha* and crayfish *Orconectes limosus* were found in the lake.

In Lake Skirnas, the abundance of macroinvertebrates varied from 189 to 509 specimens per sample. The most abundant taxa were Chironomidae larvae, pea clams *Pisidium* sp., and the invasive zebra mussel *Dreissena polymorpha*; the mayfly species *Cloeon dipterum* was also common. In spring, altogether 55 taxa of macroinvertebrates were identified from the littoral samples, while in autumn only 40 taxa were recorded. The highest number of species were represented by aquatic snails Gastropoda and caddisflies Trichoptera.

In Lake Laucesas, the abundance of macroinvertebrates varied from 561 to 2369 specimens. The most abundant taxa were Chironomidae larvae, aquatic Oligochaeta worms, and mayflies *Caenis horaria*. The invasive zebra mussel *Dreissena polymorpha* is also common in Lake Laucesas. A nationally protected species in Latvia – a river nerite, *Theodoxus fluviatilis*, is also common in the lake (Regulations of the Cabinet of Ministers No. 396). In spring, altogether 48 benthic invertebrate taxa were found, while in autumn, 60 taxa were found in the littoral samples.

The results of ecological quality corresponding to macrophyte and macroinvertebrate abundance, according to the assessment methods of Latvia and Lithuania, are comprised in Table 1.

All five studied transboundary lakes were assessed at good ecological status according to the benthic macroinvertebrate methods of both countries, though differences of macrophyte quality classes between both countries were observed. The main differences in the macrophyte methods of both countries are found in the poor/moderate and good/high class boundaries.

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PRELIMINARY STUDIES ON NATURAL ENEMIES OF HARD TICKS AND METHODS OF THEIR POPULATION CONTROL IN LATVIA

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Abstract: Hard ticks Ixodidae (Acari) transmit pathogens causing dangerous diseases such as tick-borne encephalitis and Lyme disease. These tick species in Latvia are dog tick *Ixodes ricinus* and taiga tick *Ixodes persulcatus*. The use of acaricides to control tick populations raises concerns over environmental pollution. Environmentally friendly way to control ticks is necessary to develop. Parasitoid tick wasp *Ixodiphagus hookeri* (Hymenoptera, Encyrtidae) are natural enemies of Ixodidae ticks. The aim of the current research was to study natural enemies of *Ixodes* spp. and determine causes of tick mortality, and development of methods for tick rearing in laboratory conditions. In order to carry out the artificial feeding of ticks, a prototype of a tick feeding device using skin-imitating membrane was created.

Key words: parasitic wasp, parasite, Ixodidae, *Ixodiphagus hookeri*, entomopathogenic fungi

Introduction

Hard ticks *Ixodes ricinus* (Fig. 1) and *Ixodes persulcatus* are found across Europe. Ticks are ectoparasites feeding with animal and human blood. Since hard ticks often carry bacterial or / and viral pathogens, they are vectors for potentially lethal diseases, such as tick-borne encephalitis (TBE) and Lyme disease (Hillyard, 1996).

The number of Lyme disease cases in Latvia has dropped considerably in the last 5 years (from 612 cases in 2017 down to 272 in 2022) Latvian Center of Disease Prevention and Control data (CDPC, 2023). The number of reported tick-borne encephalitis (TBE) cases remain stable at around 200 every year, as shown by the recent data collected by the CDPC. According to the WHO (2020), TBE is the most prevalent arthropod-borne disease in Europe.

Vaccination against tick-borne encephalitis is an effective tool for disease control, still more measures are needed to actively control tick populations. Synthetic acaricides can be used as a short-term solution, there are valid concerns over long-term pollution caused by man-made "forever-chemicals" as well as growing resistance towards commonly used acaricides. A recent study showed that ticks over time become resistant towards common man-made acaricides (Obaid et al., 2022).

Figure 1. Female of dog tick *Ixodes ricinus* (Photo: I. Salmane)

Use of plant-derived alternatives (natural oils), vaccines and introduction of tick control agents, pathogens (fungi, bacteria, viruses) and parasitoids are essential (Jamil et al., 2022; Obaid et al., 2022). Studies have found at least 58 pathogenic fungal species that kill Astigmata, Oribatida, Prostigmata, Mesostigmata and Ixodida mites (Chandler et al., 2000; Ravensberg, 2010). *Beauveria bassiana, Metarhizium anisopliae, Isaria farinosa*, *I. fumosorosea* and *Lecanicillium lecanii* infect Ixodida ticks under natural conditions (Perinotto et al., 2012). Many mycosecticides have been developed with the fungi *B. bassiana* and *M. anisopliae* (Copping, 2009; Fernandes et al., 2012).

At the beginning of the 20th century, endoparasitic wasp of the genus *Ixodiphagus* (Hymenoptera, Encyrtidae) was discovered to parasitize *Ixodes spp.* ticks (Hu et al., 1998; Bohacsova et al., 2016; Sormunen et al., 2019; Gaye et al., 2020). *Ixodiphagus* wasps lay eggs in tick larvae and nymphs, devour their internal organs during the larval development and then pierce the back of a tick with their mouthparts. Female wasps lay significantly fewer eggs in blood-sucking larvae than in nymphs. Eggs develop only in nymphal ticks which are fed by blood. (Hu et al., 1998; Takasu & Nakamura, 2008). An adult wasps emerge from the tick about 20–57 days after the tick falls off the host (Hu et al., 1998; Bohacsova et al., 2016). One female *Ixodiphagus hookeri* can parasitize two blood-sucking tick nymphs (42–50 eggs in each nymph) or three non-blood-sucking nymphs during her lifetime (Takasu & Nakamura, 2008). In Europe, *I. hookeri* has a wide variety of tick hosts: *Ixodes ricinus*, *I. persulcatus*, *Dermacentor reticulatus*, *D. pictus*, *D. marginatus*, *Haemaphysalis concinna*, and *Rhipicephalus sanguineus* (Buczek et al., 2021). Parasitoid wasp *I. hookeri* (Hymenoptera, Encyrtidae) might offer a new environmentally friendly way of controlling tick populations (Hu et al., 1998). Method has limitations, such as the necessity to constantly reproduce and reintroduce the parasitoids, as the parasitoid coexist naturally with the pest (ticks) and cannot completely eradicate the latter. Feeding

of ticks using artificial membranes is becoming more common, as modern materials make it possible to emulate animal skin, with appropriate thickness and texture.

Institute of Biology, University of Latvia in cooperation with LTD "Kukaiņu garāža" started a study of biological control of hard ticks *Ixodes spp.* in Latvia. The objectives of research were: 1) the study of causes of mortality and natural enemies of *Ixodes spp.* in climatic conditions of Latvia; 2) the development of methods for *Ixodes* tick control using their natural enemies; 3) the development of a prototype for the feeding and reproduction of *Ixodes spp.* ticks as host of parasitic wasps.

Materials and methods

Collection of Ixodes spp. and determination of mortality causes of ticks

During the 2021 season ticks of the genus *Ixodes* were collected at Ziemeļblāzma (Skulte parish) by the flag dragging method. Fed ticks were collected from migratory birds in Pape ornithological station (Latvia) and from cats in Salaspils and Ziemeļblāzma. The presence of parasitoids and pathogens was observed immediately after collection. Repeated inspection of tick individuals was carried out after storage in an incubator at +23 ± 2 °C temperature for 60 days. The mortality factor was referred to one of the categories: parasitoids, bacteria, nematodes, pathogenic fungi, mechanical or another cause. Specimens with symptoms of infection (cuticle covered with fungal mycelia or conidia) were used for entomopathogenic fungi isolation.

Isolation and determination of entomopathogenic fungi

Collected dead ticks were surface sterilized in 1% sodium hypochlorite for 30 sec., rinsed three times in sterile distilled water and placed in humid conditions to stimulate fungal growth and sporulation. Preliminary identification of fungi was confirmed by slide preparation. For specimens with conidial cushions preparations of conidia were obtained by film method. The shape and size of conidia were examined using a microscope fitted with a micrometer scale. Squash preparations of various infected tissues were viewed in light microscope Olympus CX41 with magnification of 400× (Lacey & Brooks, 1997). Agar-coated slide technique and staining with Lactophenol Cotton Blue were used for observation of sporulating structures and spores. Fungi were isolated and maintained on Malt extract agar. Keys for the identification were used (Lacey & Brooks, 1997, Samson et al., 1988).

Tick storage

Collected alive *Ixodes ricinus* ticks were transferred to vials with nylon fabric end caps (Fig. 2 A) and stored in jars halfway filled with wet sand for transfer to the laboratory. Afterwords vials were put into desiccators with saturated magnesium sulfate solution to ensure 98% humidity. The desiccators were placed into incubators set to +22 °C with a 16 : 8 day–night light cycle for long-term storage.

Feeding unit construction

The feeding units were built according to Oliver et al. (2016) with some minor alterations. Polycarbonate pipe (diameter 14 mm) was cut into equal pieces (6 cm in length). Two-component silicone rubber (Smooth-On with Shore hardness of 00–10 and 00–50 for nymphs and adult ticks, respectively) was mixed with petroleum ether (PE 40 °C) in the ratio of 1 : 2 (by volume). Thin pieces of rayon paper (12 g/m^2) were impregnated with the silicone-PE mixture, which was then dispersed using a soft silicone wiper until no more silicone collected on the brush. The sheets were left to air dry for 24 hours. After drying, the silicone paper was hand-pressed to improve tackiness. Greenhouse silicone (A-10 Shore hardness, with no added preservatives) was applied to the edge of the tubes, which were then attached to the silicone paper and slightly twisted to ensure a complete seal. The feeding units were left to dry for another 24 hours. Then a scalpel was used to detach the feeding units from the sheet by carefully cutting around the tube. The membrane was tested for leaks using 70% ethanol for 10 minutes. Faulty units were discarded. The assembled membranes had a thickness of 14–20 µm for 00–10 silicone and 18–28 µm for 00–50 silicone, respectively, which corresponds well to the results obtained by Oliver et al. (2016).

Preparation of tick attracting extract

The hair extract was prepared according to Krull et al. (2017). Dog hair was collected from the dog that at least one year was not treated with tick/flea repellents. 10 g of hair was cut into small pieces and suspended in 50 mL of dichloromethane (DCM, analytical grade). The mixture was stirred with mild heating (+40 °C) for 20 minutes. DCM was separated and a new portion of the solvent (25 ml) was added and stirred as before. Extraction was repeated two more times. All organic extracts were combined and evaporated down to 7 mg of dog hair per 1 ml of solvent. The extract was stored in the freezer (–20 °C) and used within the same week.

Tick feeding

Tick feeding was attempted using the procedure developed by Oliver et al. (2016) with alterations. 160 µl of dog hair extract was poured into the feeding unit and left to dry for at least 2 hours. 4,5 ml of thawed out bovine blood was used for each feeding unit. Each blood portion was poured into feeding plate (or beaker) and supplemented with 15 µl of aqueous glucose solution (1 g/l), 45 µl of the 100x antibiotic-antimycotic solution (BioReagent, 10,000 units penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml), 4 µl of aqueous 0,1 M ATP (disodium salt) solution. The blood meal was warmed up to +37 °C. Twelve adult ticks were placed into the feeding units (Fig. 2 B), which were inserted into the feeding plate in direct contact with the blood. The top of the feeding unit was covered with Parafilm. The assembled units were placed in a water bath set to +37 °C. The blood was changed every 12 hours, and the feeding units were carefully rinsed with sterile buffered saline to remove old blood prior to adding new blood.

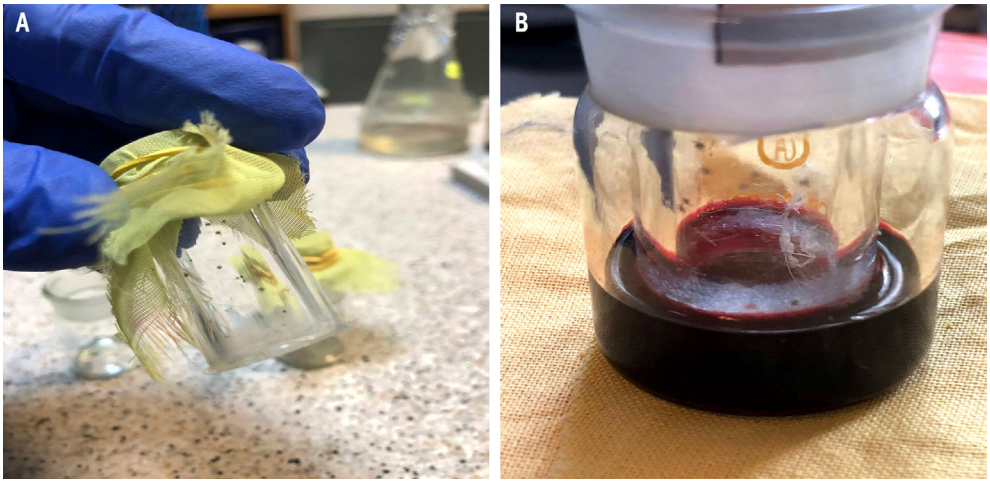

Figure 2. A – tick storage vials and B – tick feeding unit

Results and discussion

The examination of 89 fed ticks from animals was performed immediately after collection. The presence of parasitoids and pathogens was not observed immediately after collection. After storage in a desiccator at +22...+25 °C for 60 days, a repeated inspection of tick individuals was carried out. It was established that 16 ticks or 18%, were dead. In two cases (2%), a hole in the tick's abdomen was found, indicating hatching of a parasitic wasp. The wasps themselves were withered and it was not possible to determine the species.

500 ticks collected by the flag dragging method were examined after 60 days, as according to the literature, parasitic wasps develop for 30–70 days (Collatz et al., 2011). In the nymph stage were 263 ticks and 237 were imagoes. Males were 1.6 times more in number than females. It was concluded that after 50–60 days 122 ticks or 24% were dead. Causes of mortality for both groups of ticks are shown in Fig 3. A hole in the abdomen of three ticks was observed, indicating hatching of parasitic wasps. In several cases, ticks drowned in droplets of condensate (mechanical cause of death). In other cases, the cause of death has not been determined.

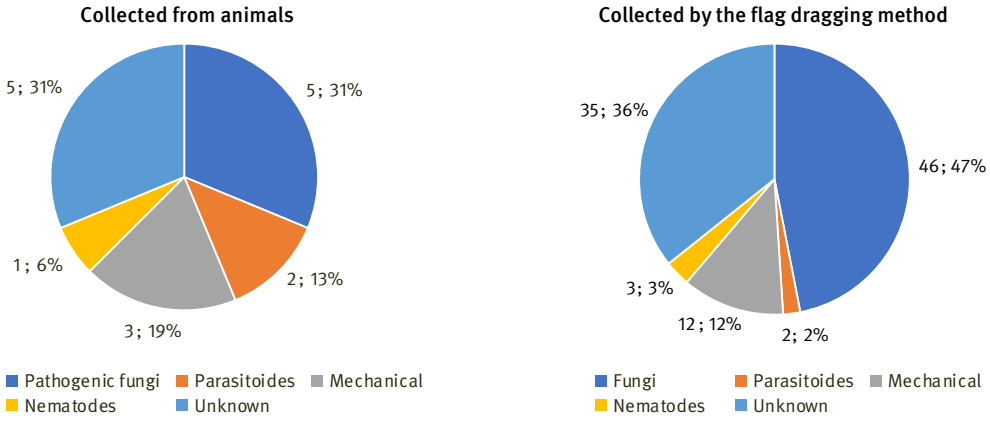

Figure 3. Mortality causes of ticks that died after 60-day incubation. In total dead 16 ticks collected from animals and 122 collected by flag dragging method were investigated

Infection with entomopathogenic fungi was found in 5.6% of all ticks collected from animals and 9.2% of all ticks collected by the flag dragging method. 36 isolates were isolated in pure cultures. Among them *Beauveria bassiana* (8 isolates), *Lecanicillium lecanii* (6 isolates), *Metarhizium anisopliae* (4 isolates), *Beauveria sp.* (6 isolates) and 10 isolates were not identified. Our study showed that tick parasitoid wasps are also found in Latvia, as 2% of the ticks collected from animals and 0.6% of the tested ticks collected by the flag dragging method had a hole in abdomen.

Tick survivability

High humidity conditions eventually result in a rapid fungal growth within the storage unit and causes death of ticks, making it difficult to preserve ticks in laboratory till next summer. High mortality during feeding for both nymphs and adult ticks after 2 months of uninterrupted storage over saturated magnesium sulfate solution was observed. It is possible that repellent residue used in previous season from collected dog hair used in preparation of attracting extract, might negatively affect ticks in the feeding chamber. Shampoo residue from occasional grooming also might be a source of potentially harmful chemicals. Collecting hair samples only from farm animals (sheep, goat and cow hair) with no history of acaricide/repellent use might be a viable solution. Moreover, tick excrements, a phagostimulants used by Oliver et al. (2016) was not available during tick feeding experiments. Additional use of tick excrements as attractor may improve the feeding process.

In the future to improve the membrane feeding prototype, different phagostimulants should be used (or a combination), such as tick excrements, extract of tick excrements as well as hair collected from wild or free-range animals. Different temperature regimes should be tested as well (for example, room temperature during tick feeding).

Conclusions

Populations of the *Ixodes* ticks in Latvia are not regulated by parasitoids (level of mortality < 2.3%).

The impact of pathogens on hard ticks was low – mortality rate of all inspected ticks was 5.6–9.2%. 36 isolates of pathogenic fungi, associated with *Ixodes spp.,* were isolated. Isolates of determined pathogenic fungi were representatives of genera *Beauveria, Metarhizium* and *Lecanicillium*. As next step, it is necessary to carry out molecular identification of fungal isolates. In future it is necessary to improve tick feeding technics in laboratory conditions.

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TYPICAL BERRIES OF THE HEMIBOREAL ZONE: TRADITIONAL USE AND THE POTENTIAL FOR CULTIVATION – A SHORT REVIEW

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Abstract: Wild berries – bilberry, lingonberry, cranberry, and cloudberry are typical boreal forest species and have been a source of food and traditional medicine since ancient times. Based on the SCOPUS database, this review evaluates the popularity of wild berries in Latvia and the world, comparing the latest scientific research on each species. The literature highlights the global popularity of bilberry and a notable research interest on cloudberry in Latvia. The large proportion of articles in health-related fields emphasizes the importance of using wild berries for health benefits.

Keywords: boreal forests, V*accinium myrtillus, Oxycoccus palustris, Vaccinium vitis-idaea*, *Rubus chamaemorus*

Wild berries of Latvia – worldwide distribution and growth environment

Wild berry species such as wild bilberry *Vaccinium myrtillus, bog cranberry Oxycoccus palustris, lingonberry Vaccinium vitis-idaea*, and cloudberry *Rubus chamaemorus* are typical boreal forest species in the subarctic-temperate zone, however, their distribution and use differ throughout the regions of the northern hemisphere. Wild berries have been used as a source of food and medicine for thousands of years, including both berry fruits and leaves, finding use for most, if not all, parts of the plant (Vanhanen, Pesonen, 2016).

In Latvia, the most popular are wild blueberries and cranberries, although other berry species of the hemiboreal zone can also sustain good harvests. Bilberry is widely distributed throughout the northern hemisphere, the subarctic and temperate zone. France is the southern limit, where it mostly grows in the colder mountain areas. Most important factors determining distribution is mean annual temperature, soil pH and C : N ratio (Coudun, Gégout, 2007), colder regions with poor, peaty soils with pH of 4–5 are especially favorable. Cranberries are distributed mainly in the subarctic and temperate zones, particularly in bogs, tundra and boreal forests (Adamczak et al., 2009). Lingonberry distribution is similar to wild bilberries (Lee, Finn, 2012; Hirabayashi et al., 2022).

Cloudberry is naturally distributed in the arctic-subarctic regions, sustaining most abundant harvests in the Scandinavian countries, but still can be found in the northern parts of Poland (Thiem, 2003; Koczur, 2011). Overall, a similar characteristic for these wild berry species is the requirement for wet, acidic habitats that are poor in nutrients.

Yield variations of wild berry species

Current scientific literature reports on annual yield variations for all berry species included in the review (Wallenius, 1999). In general, bilberry and lingonberry yields are similar, literature reports on yield range 12–38 kg ha^{-1} for bilberry, 12–35 kg ha^{-1} for lingonberry (Turtiainen et al., 2011). Thus, average yields *can variate by more than three times from year to year (Turtiainen et al., 2011, Nestby et al., 2011). Bilberry blooms in spring, so in most cases low yields have occurred in years with spring frosts during flowering (*Nestby et al., 2011). As lingonberry usually blooms in late spring or early summer, risk for frosts is lower. However, concerns regarding climate change predict on earlier blooming for all vegetation, which can result in higher risk for frost during blooming for all berry species (Hirabayashi et al., 2022). Bog cranberry can be considered as the highest yielding berry shrub between mentioned species, with average yields reportedly ranging 92–2420 kg ha^{-1}, which can be attributed to the species sustaining large stands throughout the bog landscape (Adamczak et al., 2009). Meanwhile, the yields of *cloudberry* in the northern countries are highly variable with reported results ranging from 2 to 300 kg ha^{-1} (Kortesharju, 1984; Thiem, 2003, Li et al., 2015). Although yields in Latvia are likely not comparable to those reported in Finland or Canada (Thiem, 2003; Li et al., 2015), local businesses get by to make cloudberry liquor and wine (Ligatne winery, S. a.). It should be noted that so far data on yearly yields in Latvia is limited for all wild berry species (Bārdule et al., 2020).

Research on health benefits of various wild berry species

Current research suggests health benefits of using both leaves and fruits of mentioned berries, as well as ongoing demand for wild berry products (Ferlemi, Lamari, 2016; Vaara et al., 2013). Fruits of each species has a unique set of compounds associated with specific taste and health benefits. Leaves are gathered and even sold in pharmacies as teas or transdermal agents for a wide range of health problems (Ferlemi, Lamari, 2016; Rubine et al., 1977). Therefore, although yield variability and distribution affect availability, all wild berry species are still widely sought after on the world market.

Research on health benefits is abundant for all four berry species, however, only cranberries, bilberries and lingonberries are mentioned in Latvian traditional plant medicine books (Rubine et al., 1977; Groms, Hammermane, 1971). Scientific literature describes cloudberries as an important vitamin C source in the northern regions, using berries and leaf tea (Thiem, 2003; Nilsen, 2005). However, the lack of mentions in Latvian popular scientific literature indicate that the cloudberry has been overlooked in spite of being relatively abundant in the wild. In general, scientific research is most scarce regarding cloudberries, but in Latvia, as of August 2023, both cranberries and cloudberries have

been mentioned in 4 SCOPUS indexed articles. This contradicts the assumption that annual yield highly determines the popularity and level of knowledge about berries, regarding the high popularity of cranberry daily use.

Teas or infusions prepared from berry leaves have several common effects e.g., as diuretics, antipyretics, diaphoretics, and scurvy remedies. Bilberry leaves are also known to reduce gastrointestinal pain and are traditionally widely used against diabetes, although there is little research on this (Rubine et al., 1977; Ferlemi, Lamari, 2016). Cranberry leaves are commonly used to treat urinary tract infections and discomfort (Rubine et al., 1977). Lingonberry has similar properties regarding treating fever and urinary tract issues (Ferlemi, Lamari, 2016). Cloudberries have been shown to contain the highest amounts of ellagic acid – an antioxidant associated with anti-carcinogenic properties (Landete, 2011; Ferlemi, Lamari, 2016). Ellagic acid has also been found in cloudberry leaves (Thiem, 2003).

Cultural and scientific popularity

Although studies show great potential for wild berries to improve human health, there is evidence of decreasing demand and knowledge of non-wood forest product picking. This is due to both socio-economic and ecological reasons, as nowadays visiting forest sites is regarded mostly for recreational rather than harvesting purposes (Anderson et al., 2018; DiCori et al., 2021; Lindhagen, Hörnsten, 2000). It should be noted that fresh fruit is preferable due to evidence regarding fruit losing bioactive compounds during processing and freezing (Howard et al., 2012). Therefore, the trend of declining berry picking in the Northern Hemisphere is not encouraging. Especially since the demand for fresh berry supply had increased at least up until 2022 (Rijswick, 2022). High demand has also remained for processed berry products like liquors, wine, jam, frozen fruit, sweets and canned products. This highlights the gap between knowledge on traditional product use and the modern lifestyle. However, an interest in methods and technologies of cultivating various species has remained in the scientific community, providing theoretical and practical knowledge to the fields of food science and biochemistry.

In this literature review, the popularity of various berries was analyzed by comparing scientific research conducted on each species, as well as including current knowledge on their uses in traditional medicine in Latvia.

The SCOPUS database provides a search function for the comprehensive analysis of research documents within its repository. We performed a comparative analysis of research articles mentioning four wild berry species, conducting search for their respective Latin taxonomic names either in the article title, abstract or keywords. The inclusion of Latin taxonomic names in scientific literature is a common practice, ensuring accessibility and clarity for researchers, regardless of the diverse common names assigned to these species.

For instance, "bilberry" is frequently used interchangeably with "blueberry," even though *V. myrtillus* is the Latin nomenclature for bilberry, while "blueberry" primarily denotes the cultivated highbush blueberry *Vaccinium corymbosum* and other varieties.

Furthermore, *Vaccinium uliginosum* is also referred to as bilberry, though its distinct English common name is "bog bilberry".

Our search methodology involved specifying the inclusion of both the genus name and specific epithet within the article title, abstract, or keywords, spanning the time frame from 2000 to August 2023. The search terms for each berry species were defined as follows: "vaccinium myrtillus" for bilberry, "vaccinium vitis idaea" for lingonberry, and "rubus chamaemorus" for cloudberry. However, for cranberry, both "oxycoccus palustris" and "vaccinium oxycoccus" were specified, with the results combined, given their interchangeability in wild bog cranberry research.

Analysis revealed bilberry as the most frequently referenced berry species among the four, with mentions in 1793 SCOPUS-indexed articles (Figure 1). This aligns with its distribution throughout temperate and subarctic zone and its widespread use as a dietary supplement or additive in everyday culinary practices. Lingonberry was the second most cited species, also widely distributed in the temperate zone and containing various bioactive compounds known for their health benefits.

Interestingly, the analysis revealed unexpected differences in research popularity between cloudberry and cranberry. Despite the significantly narrower geographic distribution as compared to cranberries, we found a higher number of indexed articles on cloudberries, indicating a notable research interest in this species. This also applies to the ongoing interest, both scientific and practical, in the commercial cultivation of cloudberry (Boulanger-Pelletier, Lapointe, 2017; Rapp, Martinussen, 2002). Furthermore, even though cloudberry in the wild covers significantly less area than cloudberries, these species have the same number of indexed articles from Latvian authors.

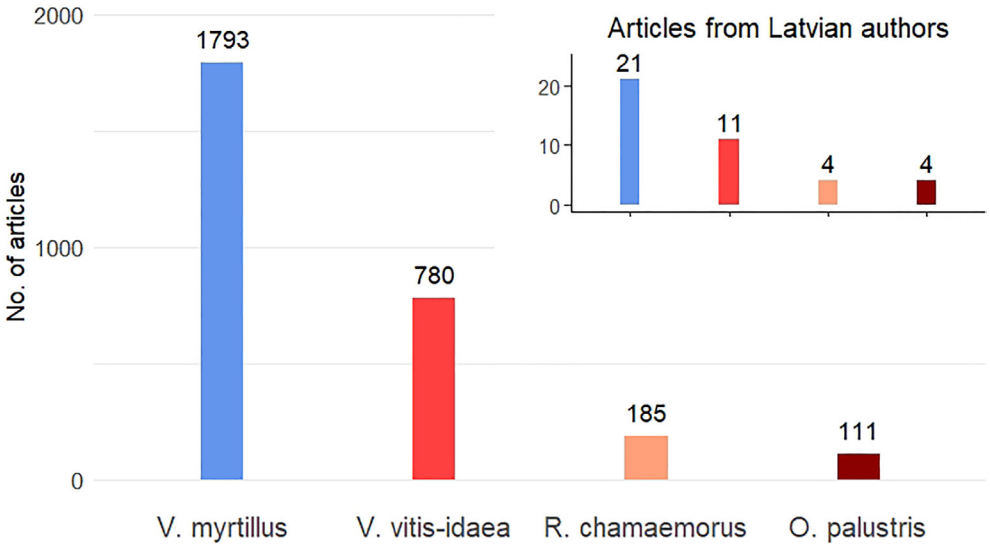

Figure 1. Comparative analysis of species name occurrence in titles, abstracts, and keywords within the SCOPUS database

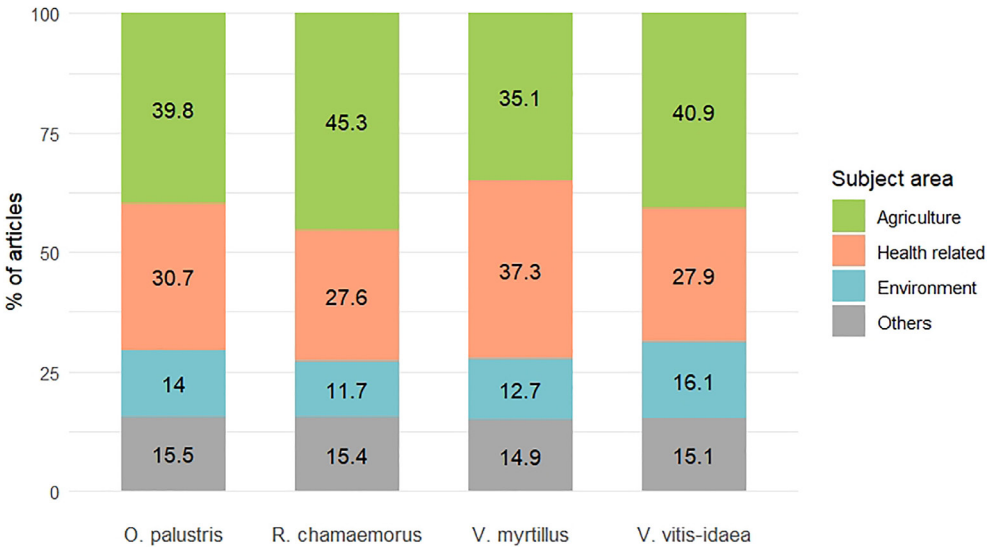

Figure 2. Comparative analysis of articles by subject area for wild berry species

The document analysis reveals that research on all species has been done primarily within the domains of agricultural and biological sciences (Figure 2). This highlights the significance of investigating wild species as a means to develop cultivars for commercial production. They are followed by research in health-related fields, including biochemistry, medicine, pharmacology, immunology, and chemistry. The third most notable subject area is environmental sciences.

In the case of cloudberries, the current focus remains on the selection of varieties for commercial cultivation purposes. Currently commercially grown cultivars include female cultivars like "Fjordgull" and "Fjellgull," male cultivars like "Apollen" and "Apolto," and the hermaphrodite variety "Nyby" (Rapp, Martinussen 2002; Uosukainen, 2010).

Conclusions

Demand for fresh produce has increased over the last decades, however, the popularity of picking wild produce has decreased. This highlights the gap between knowledge on traditional product use and the modern lifestyle. However, an interest in cultivation of species like bilberries and cranberries has remained in the scientific community, providing theoretical and practical knowledge for growers and consumers alike. Research on cloudberry cultivation is also promising, currently focusing on finding the optimal conditions for successful propagation and growing in field. Only wild bilberries, bog cranberries and lingonberries have been mentioned in Latvian traditional plant medicine books. This indicates that the cloudberry has been overlooked, considering that it is relatively abundant in Latvian bogs and forests on peatlands. Our comparative analysis of research articles mentioning wild berry species revealed bilberry as the most popular

among the scientific community globally, including Latvian authors. Interestingly, the analysis also revealed a higher number of articles mentioning cloudberries as compared to cranberries, indicating a notable research interest in the species. The high percentage of articles conducted within the subjects of health-related areas also underlines the importance and potential of wild berry use for their health promoting properties.

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THE UPPER DAUGAVA MEANDERS ON THE WAY TO THE WORLD HERITAGE NOMINATION

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Abstract In 2011 the UNESCO World Heritage Committee approved the Upper Daugava landscape area with nine impressive river meanders on the UNESCO Tentative List. This means that this area belongs to the UNESCO National Heritage. Although the protected landscape area "Augšdaugava" was established in 1991 and belongs to the NATURA 2000 network from 2004, the nature protection plan was designed only for the nature park "Daugavas loki" (2010–2022). The new protected Landscape nature protection plan (2023–2035) covers 52 098 ha, including the nature park "Daugavas loki". The new protection plan presents the research results of EU habitat types, their quality, flora and fauna species lists, as well as the level of threats and challenges. Landscape planning, functional zonation and large programme of management activities are offered for Augšdaugava and Krāslava municipalities for the first time to promote tourism industry in future and protect the nature values. This area under UNESCO umbrella was nominated as mixed because of unique combination of nature, geology, long time history of different nations, their religion, culture. The nomination criteria are enriched both for nature and culture in a protection plan process, meaning that the scientific background is high and it is time to start the process to the nomination of the Upper Daugava and meanders to the UNESCO World Heritage list.

Key words: Upper Daugava and meanders, Nature protection plan, UNESCO criteria

Introduction

The Upper Daugava valley with nine unique meanders (98 km from Piedruja to Daugavpils) is a depositary of outstanding values of nature, biodiversity and landscapes reflecting also historical and cultural significance. This object represents a mixed – natural and cultural – world heritage value.

Nomination of the Upper Daugava and meanders as world heritage object according to the UNESCO methods consists of several stages. At first the nomination place should be recognized as UNESCO National nomination category with 5-year regularity assessment of the nomination place. The Tentative List is a tool and a pre-condition of the nomination of the site to the World Heritage List, only sites included on a State Party's Tentative List can be nominated for inscription on the World Heritage List. Following a positive assessment by an international panel of experts, a World Heritage nomination is possible.

The Nature Management Plan for the Nature Park "Daugavas loki" 2010–2020, as well as information on the NATURA 2000 network in the region since 2004 were used as background material for the preparation of the national nomination. In 2018, according to the Nature Census project, financed by the EU Cohesions Fund, the first nature protection plan for the Landscape area"Upper Daugava" ("Augšdaugava") and the nature park "Daugava meanders" ("Daugavas loki") was elaborated as an integrated unit with different levels of protection (htttps://www.daba.gov.lv). Following EU guidelines (European Union Protected Habitats in Latvia, 2013), the experts prepared assessments on habitat types and quality, biodiversity of flora and fauna, quality of river Daugava, small streams, lakes and the geological character of the Daugava valley. Based on this information a very detailed territory management plan was created with the aim to develop the well-being of the population of local peoples, tourists and balanced socio-economic goals in harmony with nature values for the next 12 years (2023–2035). UNESCO's nomination regulations provide specific criteria to be used for the evaluation of nominations at both national and global levels. Justification of outstanding universal values should be in compliance with UNESCO corresponding criteria (https://whc.unesco.org/en/criteria/), (Guidance on Developing and Revising World Heritage Tentative Lists, 2020). In this process, evidence of authenticity and/or integrity, as well as comparison with other similar properties, plays an important role. There are 10 accepted criteria and three of them were appropriately for the nomination of Upper Daugava and meanders in the World Tentative List (https://whc.unesco.org/en/tentativelists/5610/).

Results and discussion

Tentative List selection criteria (V): to be an outstanding example of a traditional human settlement, land–use, which is representative of a culture (or cultures), or human interaction with the environment especially when it has become vulnerable under the impact of irreversible change. The Daugava as a natural watershed, transport and trade route has been beneficial and meaningful in cultural history. People in this region began to settle a long time ago – around 11 thousand years ago, creating rich cultural impact on the landscape. Numerous archaeological, architectural, and historical monuments indicate intense human activity since the archaic period, as shown by the remained ancient burial grounds, hillforts, castle ruins, palaces, churches, and settlements. Therefore, the territory is an excellent example of multicultural living from ancient Balts, Vikings, and Crusaders. The name Daugava (Dyna) is mentioned in the 10th–11th century in written Scandinavian Gutasaga and the waterway Daugava was the road from Varangians to Greeks. Later this waterway was taken over by Russians, Poles and Swedes. Daugava was a permanent trade route between Western and Eastern Europe until the North War (1700–1721). All these historical events are reflected in nowadays. The Krāslava city has a logo of boat with five oars presenting five nationalities living together for many years – Latvians, Poles, Russians, Belarussians, Jews. The nomination area contains about 60 archaeological, historical, and cultural monuments. Recently, four ancient mounds have been found using new technologies (Lidar).

Tentative List selection criteria (VIII): to be outstanding examples representing major stages of earth history, including the record of life, significant on-going geological processes in the development of landforms, or significant geomorphological or physiographical features. The river Daugava valley with its characteristic arches and typical upper flood terraces formed before 13–15 thousand years, after the Last Glacial Period. During that time the river stream was so strong that it carried great masses of sand, gravel, large stones and even cliffs, creating picturesque hills, deep ravines with springs, rivers, and large river Daugava riffles. In 45.4 km stretch from Daugavpils and Krāslava, the river is composed of nine outstanding meanders that represent unique and nowadays exceptionally rare features (Fig. 1). These unchanged geomorphologic formations belong to the context of large rivers of the world's Northern hemisphere. The river Daugava is a key element in forming the landscape. Its course is full of riffles, banks with flat and steep slopes and nine very diverse meanders forming dynamic changing and highly three-dimensional landscape that has high aesthetic value. Especially high value of landscape is formed by ratio of woods, meadows, agricultural areas, and relief. This boreal region with its different landscape types changes with the seasons – from white winter, green and sparkling spring, rich green summer forests and flowering meadows to a brilliant palette of colours in autumn. In the territory of meanders is a very dense network of ravines with unique ecological niches for flora and fauna.

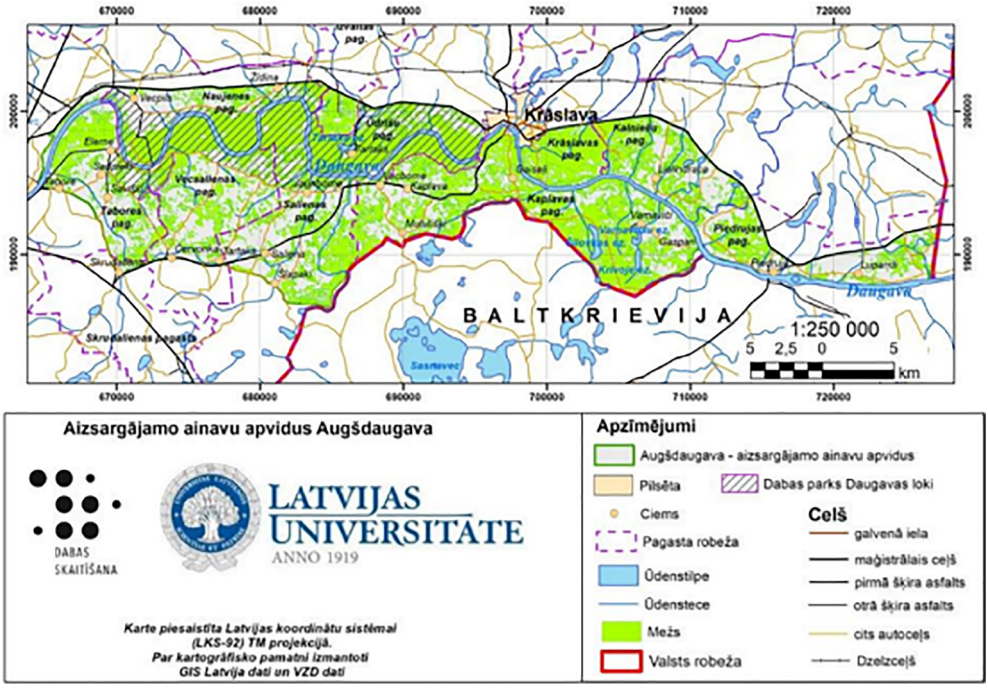

Figure 1. The UNESCO Tentative List nomination area "Upper Daugava and meanders"

Tentative List selection criteria (X): to contain the most important and significant natural habitats for in-situ conservation of biological diversity, including those containing threatened species of outstanding universal value from the point of view of science or conservation: The nominated area habitats host very rich and rare fauna and flora species belonging to boreal and nemoral regions. The Daugava serves as an important ecological corridor for species migration, and from this point of view its value is also high in the context of transnational species migration. The microclimate of the river valley, the highest positive summer temperatures in eastern areal of Europe and the calcareous soil types create unique plant and animal communities, and some species are at the edge of their ranges in these areas and require special attention for their protection. Since 2004 this territory is included in NATURA 2000 network. In the nomination territory there are 28 EU level protected habitat types. The most important by coverage area are: with the code 9010* Western taiga (*veci un dabiski boreāli meži*), 9050 Fennoscandian herb-rich forests with Picea abies (*lakstaugiem bagāti egļu meži),* 6430 Hydrophilous tall herb fringe communities of plain and of the montane to alpine levels (*eitrofas augsto lakstaugu audzes*), 6450 Norden boreal alluvial meadows (*eitrofas augsto lakstaugu audzes)*, 7140 Transition mires and quaking bogs (*pārejas purvi un slīkšņas)*, 3150 Natural eutrophic lakes with *Magnopotamium or Hydriharition* – type vegetation (*eitrofi ezeri ar iegrimušo ūdensaugu un peldaugu augāju)*, 3130 Oligotrophic to mesotrophica waters (*ezeri ar oligotrofām līdz mezotrofām augu sabiedrībām).* The area is one of the richest in species in Eastern Europe. In a comparatively small area near the town Krāslava on a meander "Priedaine", there is located the nature monument "Sproģu grava" with 28 ravins, covering four EU importance habitats with rich list of protected species.

In the nomination territory, according to the botany experts, were found ca. 900 vascular plants species, 71 protected species. In the territory ca. 130 bird species are living, 39 of them are included in the EU Bird directive 1 attachment. There is a rich fauna of insects, about 70 species, 12 of them Annex II and Annex IV species. It is very rich in raspy/amphibians fauna – altogether 11 protected species. The site is particularly important for the conservation of all bat species. According to data, their main feeding ground is the Daugava River.

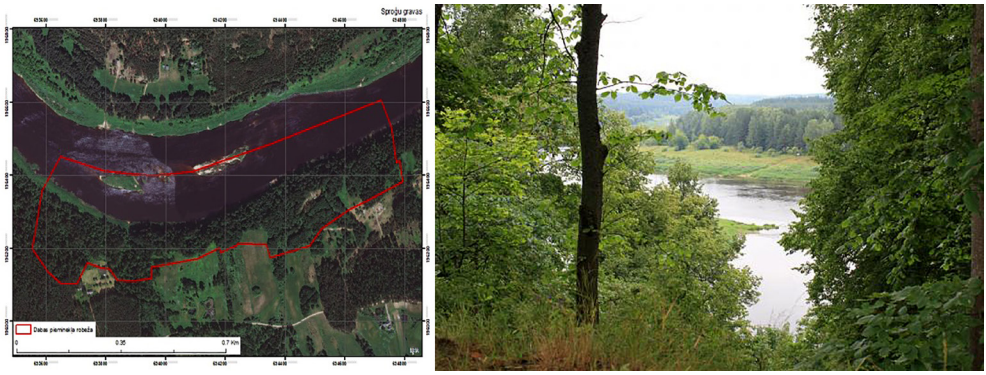

Figure 2. The nature monument "Sproģu grava"

To protect this unique area, a comprehensive management plan has been developed and is on its way to the Ministry of Environment and Regional Development for implementation. A total of 75 measures are proposed for nature conservation, society information and education, tourism/recreation activities, protection of landscapes and cultural sites, science and monitoring of management activities.

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