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Content
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Natura 2000 excellence values and management challenges in the protected landscape area "Augšdaugava"

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Abstract: The ideal or long-term goal of the management plan is to preserve the natural and cultural-historical values of the "Augšdaugava". It means to preserve the unchanged section of the Daugava River and the adjacent territory, its landscape structure, habitat and species diversity, in the same time promoting sustainable values and socio-economic interests. This complicated goal is demonstrated in the new functional zonation and management proposals.

Key words: EU habitats, rare and threatened species, risk factors, functional zonation, management plan

Introduction

The goal of biodiversity according to the European Environment Agency – good status or increasing trend still is not reached: 1/3 of species & habitats still is not in a good condition and further deterioration is in progress (H. Bruyninckx, 2021). There are 27 850 Natura 2000 sites in Europe. In Latvia, the Natura 2000 network includes 333 territories. The protected landscape area "Augšdaugava" belongs to the Natura 2000 site with an area of 52 078 ha and represents the largest site among the landscape-protected areas in Latvia. The characterization of this unique territory is based on three pillars: the upper Daugava region is formed by geomorphologically unchanged nine river meanders forming the landscape of the ancient valley from the Latvian state border to the Daugavpils City by 98 km long. At the country border, Daugava River flows as a lowland (potomal) river. Near the town Krāslava the valley becomes deeper (40 m) and broader (2–4.5 km) starting to flow via meanders. The protected landscape area represents 27 European Union-protected habitats with flora and fauna species. belonging to both Latvia`s and EU protection.

A peculiar cultural environment has historically developed by merging the way of life of several nations already from Vikings. Today it manifests itself in a rich and unique architectural, cultural, and historical heritage.

Results and discussion

The most significant part of the protected habitats and species are concentrated in the territory of the nature park "Daugavas loki" forming the key zone in the protected landscape area. The microclimate of the Daugava valley especially the very dense net of ravines and the calcareous soils hosts unique vegetation. Some plants can be considered elements of the steppes in the Latvian flora. Due to the warm summers some plant species whose main distribution area is in central Europe. are found there. For a lot of species this region belongs as border zone of their distribution from north and east. The Daugava River valley is an important migration path for new species. According to the data obtained historically as well as during elaboration of the management plan for the protected landscape area "Augšdaugava", more than 900 species of vascular plants, 71 of which are specially protected, are found there. In this Natura 2000 site, 38 bird species are nesting which are included in Annex I of the Birds Directive. And in addition to them, 16 specially protected bird species have been identified. In the "Augšdaugava" experts have found 26 specially protected insect species in Latvia and 12 of them are included in the annexes of the Habitats Directive. Four protected species of fish and one lamprey species, as well as 20 rare and protected species of mollusks, four of which are included in the annexes of the Habitats Directive. The "Augšdaugava" area is of immense importance for the conservation of bat species: nine species have been identified all of which are specially protected in Latvia and are included in Annex IV of the Habitats Directive. "Augšdaugava" is rich in reptile and amphibian fauna. There are 11 amphibian species (or 85% of Latvian amphibian species) and five reptile species (or 71% of Latvian reptile species) are included in the annexes of the Habitats Directive.

Table 1. Protected habitats in the landscape area "Augšdaugava"

EU habitat code. name	Habitat area (ha) in the protected landscape area	Ratio of protected landscape area habitat to the total Latvia habitat area (%)	Assessment of the situation in Latvia
1	2	3	4
Freshwater habitats			
3130 Oligotrophic to mesotrophic standing waters with vegetation of the *Littorelletea uniflorae and/or of the Isoëto-Nanojuncetea*	59.80	1.11	U2d
3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* — type vegetation	259.3	0.43–0.65	U1s
3260 Water courses of plain to montane levels with the *Ranunculion fluitantis* and *Callitricho-Batrachion*	1532.06	8.96–13.43	U1s
3270 Rivers with muddy banks with *Chenopodion rubri p.p.* and *Bidention p.p.* vegetation	19.96	159.28–238.92 ha	XX
Natural and semi-natural grassland habitats			
6120 Xeric sand calcareous grasslands	18.56	2.4–3.19	U2x
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (* important orchid sites)	383.7	6.62–8.61	U2d
6230 Species-rich *Nardus* grasslands. on silicious substrates in mountain areas	1.36	0.22–0.28	U2d
6270 Fennoscandian lowland species-rich dry to mesic grasslands	252.33	1.25–1.63	U2d
6410 *Molinia* meadows on calcareous. peaty or clayey-silt-laden soils *(Molinion caeruleae)*	5.17	0.12–0.16	U2x
6430 Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels	119.59	17.83–23.21	U1s
6450 Northen boreal alluvial meadows	122.38	0.68–0.88	U2d
6510 Lowland hay meadows (*Alopecurus pratensis. Sanguisorba officinalis)*	28.04	0.52–0.68	U2d
Mire habitats			
7110 Active raised bogs	4.67	0.042–0.005	U1s
7120 Degraded raised bogs still capable of natural regeneration	0.96	0.006–0.008	U2x

7140 Transition mires and quaking bogs	49.25	0.58–0.74	U1s
7160 Fennoscandian mineral-rich springs and springfens	16.2	2.26–2.87	U1x
7220 Petrifying springs with tufa formation (*Cratoneurion)*	0.56	1.12–1.83	U1s
Forest habitats			
9010 Western Taïga	1073.76	1.47–2.21	U2x
9020 Fennoscandian hemiboreal natural old broad-leaved deciduous forests (*Quercus. Tilia. Acer. Fraxinus or Ulmus*) rich in epiphytes	32.1	0.23–0.29	U2s
9050 Fennoscandian herb-rich forests with Picea abies	110.81	1.01–1.03	U2x
9060 Coniferous forests on. or connected to. glaciofluvial eskers	3.16	0.19	U2x
9080 Fennoscandian deciduous swamp woods	59.81	0.26–0.29	U2d
9160 Sub-Atlantic and medio-European oak or oak-hornbeam forests of the *Carpinion betuli*	10.31	0.03–0.06	U1x
9180 Tilio-Acerion forests of slopes. screes and ravines	227.18	3.66–4.25	U1x
91D0 Bog woodland	123.7	0.11–0.21	U1s
91E0 Alluvial forests with Alnus glutinosa and Fraxinus excelsior (*Alno-Padion. Alnion incanae. Salicion albae)*	32.81	0.29–0.4	U1x
91T0 Central European lichen Scots pine forests	5.15	0.20–0.21	U1x

(green)	FV: The state of protection is favourable;
(yellow)	U1: The state of protection is unfavourable-Inadequat;
(red)	U2: The state of protection is unfavourable-bad;
(grey)	XX: The state of protection is unknown;
Trend in the conservation status of the species I – positive. D – negative. S – stable or unknown. Source: Latvia report to the EU, 2019.	

According to the assessment, there are 27 EU habitat types in the protected nature area, but with low habitat quality (U1, U2). There are many reasons for this situation in the territory. Although this is Natura 2000 site and covered 51% by forests, their management by Joint Stock Company "Latvia's State Forests" (LVM) by 65% is industrially motivated. Increasing trend in clearcutting in the last three years, new technical roads (data from the State Forest Agency. VMD, 2020) illustrates the negative impact on forests, especially fragmentation of high conservation value habitats. Also, private owners are far away from the will to introduce good practice in forest management. Agriculture is responsible for pesticides, phosphorus, and nitrate leakage to the environment, especially in water ecosystems. Therefore, in this management plan for the first time, a new approach is recommended to reduce the eutrophication effect on several lakes (Boikova et al., 2021). A long-lasting problem is not recultivated sand and gravel quarries. These territories are suitable for the establishment of invasive plant species. There is a strong necessity to improve the knowledge of nature's values and the possibility to manage the area in a more sustainable way.

The first functional zonation of this large area was created. Five functional zone, each of which with different management, were proposed. The largest one is the landscape protection zone with 65% of the total area, and the second is the nature park zone "Daugavas loki" ("Meanders of Daugava") – 25.5%. nature reserve zone – 5.8%. neutral zone – 2.9% and strictly protected zone – 0.3%. Recommended new management activities for the period between 2022 and 2034 are presented:

- Administrative and organizational arrangements – six activities,
- Protection and management of nature values – 23 activities,
- Organization and harmonization of tourism and recreation – 32 activities,
- Education and information activities – 20 activities,
- Management of cultural and historical values – six activities,
- Research and monitoring – five activities.

Acknowledgements

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Diatom-based assessment of the ecological status of the Venta River, Kuldīga

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Abstract: Pressure from land use patterns, climate change, and urbanisation on rivers is an important socio-ecological issue that requires management and biomonitoring. The ecological status of the Venta River at Kuldīga was assessed using bioindicators, specifically diatoms, which are widely used in monitoring of streams and rivers worldwide, especially in European countries. Ecological status was defined by calculating Specific Pollution Sensitivity Index (IPS). In addition, diatom diversity was determined in the studied part of the river. A total of six sampling sites were selected along a 10 km stretch of the river in the territory of Kuldīga. The average distance between sampling sites was 1.5 km.

A total of 118 taxa were identified in all samples. The highest species diversity (67 taxa) was observed in sample 2. Individual rarefaction was calculated if exactly 500 diatom valves were counted in all samples. The lowest species diversity was observed in samples 6 (45 taxa) and 6A (17 taxa). Samples 1 and 5 were the most similar in terms of species composition. This is probably because both sites have similar physical characteristics that include sandy beaches with a slow current.

The most abundant diatoms were *Cocconeis placentula* Ehrenberg, *Amphora pediculus* (Kützing) Grunow, *Nitzschia fossilis* (Grunow) Grunow, *Navicula capitatoradiata* Germain ex Gasse, *Navicula antonii* Lange-Bertalot, *Amphora libyca* Ehrenberg, and *Sellaphora nigri* (De Notaris) Wetzel & Ector, which are commonly found in eutrophic waters. Diatom analysis suggests that the Venta might be at risk for eutrophication. For the most abundant diatom taxa, the susceptibility to pollution was assessed as III (medium), but there were also diatoms with IV and V (very sensitive to pollution). This shows that the Venta River in Kuldīga overall has low levels of pollution. All study sites were rated as having 'good' ecological status according to the IPS index (12.4-14.1 IPS).

Key words: aquatic pollution, bioindicators, eutrophication, Specific Pollution Sensitivity Index (IPS), diatoms, Latvia

Introduction

According to the Europe 2020 strategy, one of seven societal challenges Europe must face is the adaptation to climate change and its influence on the aquatic environment. Freshwater resources are essential for human well-being. However, over the last decades Europe's freshwater resources have faced increasing pressure (EEA, 2018). The main drivers of deterioration in water quality are urbanisation, industrialisation, intensive agriculture, and climate change (Alan, 2004; Jury & Vaux, 2005). A cornerstone of EU environmental policy is protecting water resources and ensuring their ecological quality with an aim of ensuring access to good or high quality water for all EU citizens. One of the Water Framework Directive (WFD) targets was to create uniform standards for water policy within the EU and to aim for at least 'good status' for all water bodies in the EU by 2015. In 2018, the European Environment Agency (EEA) reported that only 40% of monitored European water bodies met water quality standards (EEA, 2018). This means that stakeholders (e.g., water resource management organisations and policymakers, nature protection organizations) and researchers must work closely together to reach the WFD aims for 'good status' for all water bodies.

In general, the term "ecological status" is used to describe the combination of biological quality, physicochemical and hydromorphological quality. This combination is generally expressed as an ecological quality ratio (observed/reference), which is into five scale-status classes (high, good, moderate, poor, and bad) (EC, 2003; Masouras et al., 2021). In lotic ecosystems, the most used bioindicators are phytobenthos, benthic invertebrates, fish, macrophytes, as well as phytoplankton (Masouras et al., 2021). Diatoms are the dominant component of phytobenthos and have a fundamental ecological role in aquatic ecosystems. During photosynthesis, inorganic carbon (CO_2) is taken up and converted into organic compounds, as well as diatoms participate in phosphorus, nitrogen and silica cycles (Julius and Theriot, 2010; Mann, 1999). Due to their short lifecycle, diatoms respond fast to any natural and anthropogenic disturbance, making them more sensitive to changes in environmental parameters (e.g., inorganic nutrients, organic pollutants, pH, temperature, salinity, and heavy metals) than other biotic groups (Julius and Theriot, 2010; Kelly and Whitton, 1995; Laine et al., 2014; Wu et al., 2016). Therefore, they are good indicators of water quality and land use change (Stevenson, 2014; Stevenson et al., 2010), and as such are the most common indicator organisms in biological assessment in the WFD (Almeida et al., 2014).

Several diatom indices are regularly implemented to evaluate the ecological status of water bodies, these include the Specific Pollution Sensitivity Index (IPS; Coste, 1982), Trophic Diatom Index (TDI; Kelly and Whitton, 1995), Biological Diatom Index (BDI; Coste et al., 2009) and others (Masouras et al., 2021; Stevenson et al., 2010; Tan et al., 2017). For this study, the IPS was chosen to determine the water quality in a river, as it correlates with water quality parameters such as nutrient enrichment, organic pollution, and conductivity (Descy and Coste, 1991). The IPS is one of the most precise diatom-based indices (Tan et al., 2017), as it incorporates the largest data set among all diatom indices, more than 2000 taxa (Descy and Coste, 1991).

The ecological quality of different aquatic ecosystems in the territory of Latvia has been monitored by Latvian Environment, Geology and Meteorology Centre (LEGMC), but benthic diatoms have not been regularly and extensively used as bioindicators. Therefore, this study focuses on a particular part of the Venta River that flows through Kuldīga. The catchment includes residential areas with allotments, industrial areas and agricultural lands. Urbanization and intensive agriculture often have an observable impact on the river's ecology, as increased pollution tends to interfere with physiological processes in living organisms. This often leads to pathologies, which in turn can further reduce quality and expectancy of life at a societal cost. The monitoring of pollution in the Venta is crucial for establishing a baseline and timing of informed and appropriate municipal-level interventions for prevention and mitigation.

The aim of this study was to assess the ecological status of the Venta at Kuldīga using diatoms as bioindicators and to determine diatom diversity in the studied part of the river.

Study site

The Venta River begins in Užventis, Šiauliai County, Lithuania (168 km), runs through western part of Latvia (178 km) and flows into the Baltic Sea at Ventspils, Latvia. The basin system is ca. 11 800 km^2 large, of which ca. 50% is forested. The upper part of the river has a faster current, while downstream of Kuldīga, the river meanders along a calm course. The shores are mostly overgrown and have a few bathing areas. In some places, there are exposed outcrops of reddish sandstone. The largest tributary Abava (Latvia) is 134 km long. The river usually freezes at the end of December, and the ice starts to melt at the beginning of March. The largest settlements along the Venta River are Mažeikiai (population size: 38 819 in 2018) in Lithuania and Kuldīga and Ventspils (population sizes: 10 109 and 33 372 in 2021, respectively) in Latvia. The Venta River flows from a dolomite bed into sandstone, forming the largest waterfall in Latvia – the Venta waterfall (in Latvian: Ventas Rumba) in the town centre of Kuldīga.

The study was carried out along a 10 km stretch of the Venta River in the territory of Kuldīga town (Fig. 1). The average distance between sampling sites was 1.5 km. In total, six sampling sites were selected (Table 1). Fig. 2 illustrates sampling sites along the Venta River.

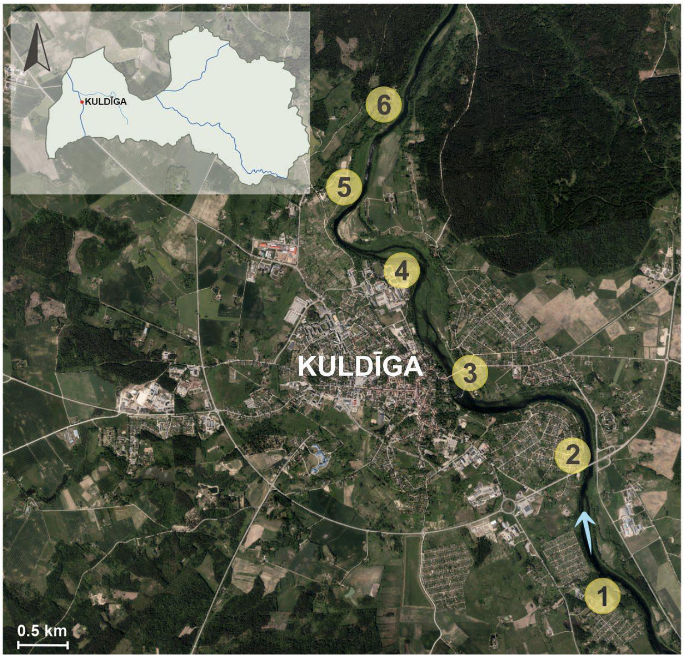

Figure 1. Study area of Venta in Kuldīga, Latvia. Sampling sites are marked with yellow circles. The light blue arrow indicates the direction of flow of the Venta River (LGIA, 2019)

Table 1. Description of sampling sites

Nr.	Water depth	Coordi-nates	Description	Potential sources of pollution
1.	0.5 m	56°56′55″ 22°0′40″	Swimming area on the left bank of the Venta in Ābeļciems, near private houses	Adjacent nearby allotments, agricultural land
2.	0.5 m	56°57′42″ 22°0′05″	Left bank of the Venta near the bridge, construction works on the opposite bank	Construction works, pollution from road traffic, using sand and salt on the road
3.	0.2 m	56°58′09″ 21°58′47″	Venta waterfall. The right bank of the Venta	Popular tourist destination, located in the city centre, 400–600 m from construction debris disposal site on the left bank of the Venta
4.	0.5 m	56°58′49″ 21°58′15″	A swimming area on the left bank of the Venta, with automobile repair shops nearby	Automobile repair shops, industrial area

5.	0.7 m	56°59′17″ 21°57′35″	Swimming area on the left bank of the Venta River. Opposite is the water treatment plant	Agricultural land, incompletely treated sewage, residents using the bathing area
6.	0.5 m	56°59′40″ 21°57′57″	Left bank of the river Venta, near the castle mound	Water from the treatment plant flows into the opposite bank. Here, the tributary Krāčupīte flows past the factory and the allotment gardens

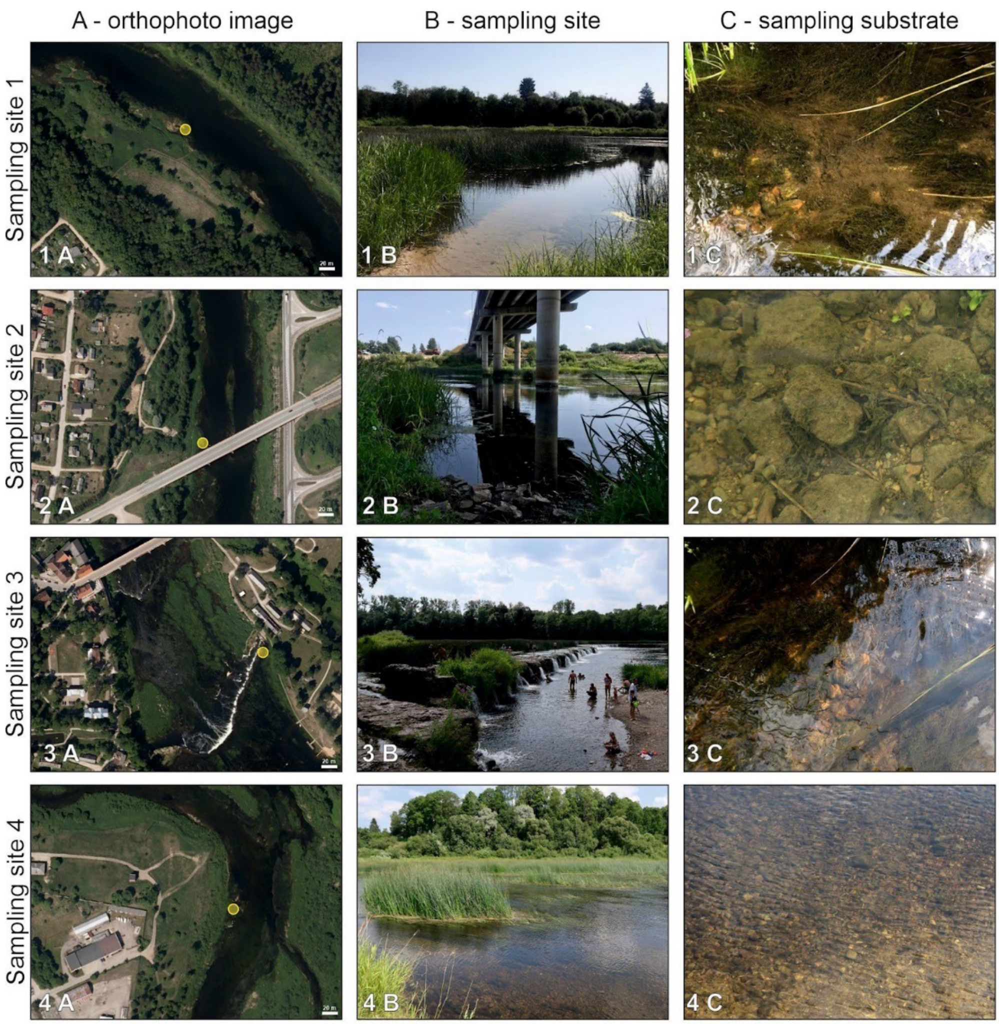

Figure 2a. Sampling sites along the Venta River. A – orthophoto image (LGIA, 2019), sampling site indicated with a yellow circle, B – sampling site, C – sampling substrate

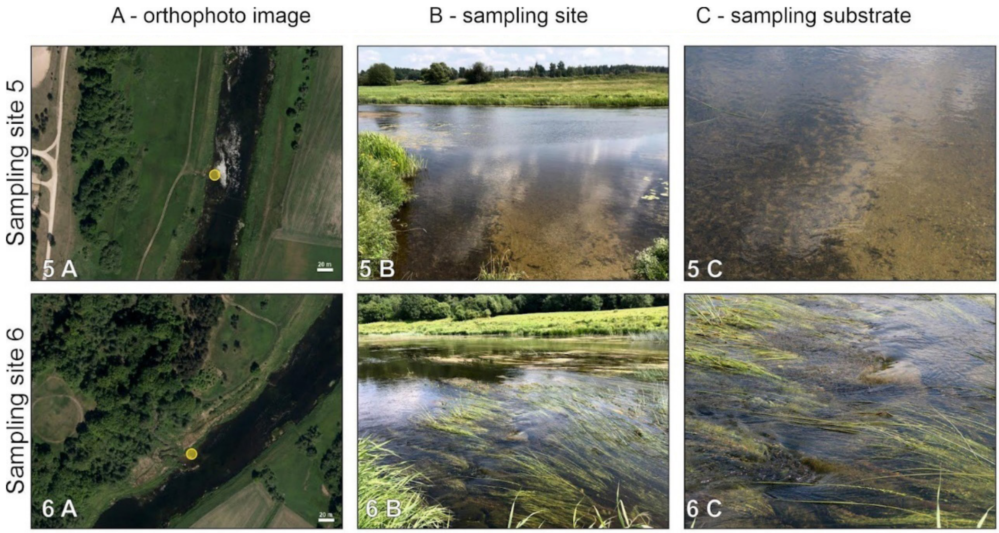

Figure 2b. Sampling sites along the Venta River. A – orthophoto image (LGIA, 2019), sampling site indicated with a yellow circle, B – sampling site, C – sampling substrate

Material and methods

Sampling

In total, six sites were selected in the Venta River in Kuldīga, Latvia. The study sites were selected by assessing the potential sources of pollution in the river. The samples were collected on 26th July 2021. Before sampling, the area was visually documented by photo camera, and exact coordinates were marked on the map.

Benthic diatoms were sampled on rocks, which were chosen as targeted natural substrata, as periphyton samples on aquatic plants can be highly variable (Stevenson et al., 2010). The sampling procedure was carried out in accordance with the guidelines for sampling diatoms on rocks described in Kelly et al., (1998), recommendations in the European Union monitoring programs (WFD: EC, 2000) and the EU Standard for sampling (EN 13946; EU, 2014). In the river, five rocks (samples 1–6) of similar size with an obvious diatom film were chosen. In addition, periphyton from aquatic plants (samples 2A, 3A and 6A) in three sites were sampled in order to compare diatom composition on different substrata. The water depth, at which the stones were found, was measured and marked in a field journal. The rocks or aquatic plants were placed in a bowl and softly brushed with a toothbrush. Each sample was placed in a clean 50 ml centrifuge tube labelled with the name and number of the sampling site and date of the fieldwork campaign.

Microscope slide preparation

In the laboratory, the samples were centrifuged to remove excess water. The remaining residue was treated following the diatom sample preparation protocol (Battarbee et al., 2001). At first, samples were treated with 10% hydrochloric acid (HCl) to remove $CaCO_3$. After carbonates have reacted in the samples, they were washed three times with distilled water. Then, 35% hydrogen peroxide (H_2O_2) was added to remove organic matter. Samples were placed in a water bath where the temperature gradually increased from 50 °C to 85 °C. During the gradual temperature rise, a few drops of H_2O_2 were added to the sample until the reaction was complete. The samples were again rinsed three times with distilled water. A drop of the remaining material was put on a cover slip and left to dry overnight at room temperature. The microscope slides were properly labelled and placed on a hot plate. A drop of *Naphrax* was applied on the warm microscope slide, on which the dried cover slip with the sample on it was carefully placed. Since *Naphrax* contains the organic solvent toluene, the microscope slides were placed on the stove in a fume hood and remained there until the toluene evaporated (i.e., the sample stopped bubbling).

Diatom identification and analysis

The European Standard (EN 14407; EU, 2014) for the WFD was followed for diatom analysis. Diatoms were identified and enumerated to species level (if possible) under the microscopes AmScope SME-F8BH and Leica DM2500 LED by using oil immersion at 1000× magnification. A minimum of 500 diatom valves was counted per slide to estimate relative abundance of taxa. The diatom taxonomy was based on diatom floras (Krammer and Lange-Bertalot, 1986, 1988, 1991a, 1991b; Lange-Bertalot et al., 2017), as well as on the internet sources Diatoms of North America (diatoms.org).

Samples were grouped by applying incremental sum of squares cluster analysis (CONISS) performed on the full percentage sum (transformed by square root function) diatom data with Tilia (Grimm, 2011). The diatom species richness was assessed using rarefaction analysis (Birks and Line, 1992) in the software PAST 4.03 (Hammer et al., 2001). Rarefaction analysis was chosen as it fits well for data in which count sizes vary and, thus, must be normalised (n = 500).

Calculation of Specific Pollution Sensitivity Index

The Specific Pollution Sensitivity Index (*IPS*) is one of the most commonly used diatom indicators, which uses taxonomic composition of assemblages, the relative abundance of taxa in the sample (taxonomic composition) and the environmental preferences and tolerances of taxa (autecological attributes of taxa or taxa traits) (Stevenson et al., 2010; Masouras et al., 2021). Calculations of *IPS* are usually done at the species level. The OMNIDIA software (Lecointe et al., 1993) was used to calculate the IPS indices.

Calculation formula:

$IPS_{(1-5)} = \Sigma\, S \times V \times A \Sigma V \times A$ (Coste, 1982)

where: A – Abundance of the alga
V – species ecological sensitivity index (Versatility)
S – Sensitivity to pollution

Algal abundance (A) is calculated as: the number of individuals of a species to the total number of diatoms in the sample. The ecological sensitivity index (V) of a species is a number between 1 and 3, where 1 stands for the widest ecological niche while 3 indicates diatoms with the narrowest ecological niche. Sensitivity to pollution (S) is characterised by a number between 1 and 5, where 5 denotes the taxa most sensitive to pollution.

The $IPS_{(1-5)}$ index calculated above is used to calculate the final *IPS*. This is used to determine the ecological status of a water body.

$IPS_{(final)} = (IPS_{(1-5)} \times 4.75) - 3.75$

Table 2. IPS ecological quality indicators (Kokorite et al., 2018)

IPS Specific Pollution Sensitivity Index				
High	Good	Moderate	Poor	Bad
› 15.5	15.5–12.0	12.0–9.5	9.5–6.9	‹ 6.9

Results and discussion

Benthic diatom assemblages in Venta

A total of 118 diatom taxa belonging to 47 genera were found in nine samples (six from rocks, Samples 1–6, and three, Samples 2A, 3A and 6A, from macrophytes) from the Venta River. Only diatom taxa accounting for ≥ 2% of the diatom abundances are presented in the diatom diagram of Venta (Fig. 3).

The most abundant diatoms were *Cocconeis placentula* Ehrenberg (mean = 18.9%; max = 52.8%), *Amphora pediculus* (Kützing) Grunow (mean = 15.9%; max = 30%), *Nitzschia fossilis* (Grunow) Grunow (mean = 4.5%; max = 13%), *Navicula capitatoradiata* Germain ex Gasse (mean = 3.9%; max = 8.1%), *Navicula antonii* Lange-Bertalot (mean = 2.6%; max = 6.9%), *Amphora libyca* Ehrenberg (mean = 3%; max = 6.4%), and *Sellaphora nigri* (De Notaris) Wetzel & Ector (mean = 2.5%, max = 5.1%), which are commonly found in eutrophic waters. In significant quantities indifferent taxa, such as *Achnanthidium minutissimum* (Kützing) Czarnecki (mean = 2.5%, max = 6.1%), *Navicula cryptotenella* Lange-Bertalot (mean = 6%; max = 10.3%) in all samples and *Ulnaria ulna* (Nitzsch) Compère (max = 8.6%) in sample 4. Diatom data suggests that the Venta River might be at risk of eutrophication.

The majority are eutrophic diatoms 49–72% (median 60%), the lowest number of eutrophic diatoms is in sample 2 (49%), but the highest (72%) in sample 3, while in samples 6 and 6A were observed the highest abundance of hypereutrophic taxa (4–5%). The highest species diversity (rarefaction n = 500) was observed in sample 2 (67 taxa), while the lowest species diversity was observed in samples 6 (45 species) and 6A (17 taxa). The most similar samples in terms of species are samples 1 and 5 (Fig. 3). This is probably because both sites have similar physical characteristics that include sandy beaches with a slow current (Fig. 2 – 1B and 5B).

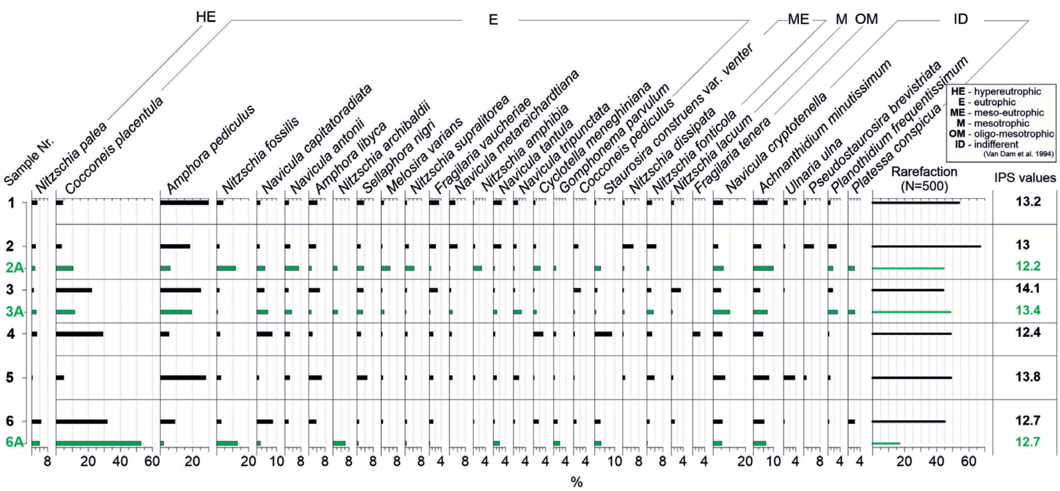

Figure 3. Diagram of selected diatoms. Only diatoms › 2% at least in one sample are presented. Diatoms are grouped according to the trophic state where they are found most often (Van Dam et al., 1994). 'A' indicates samples taken from macrophytes

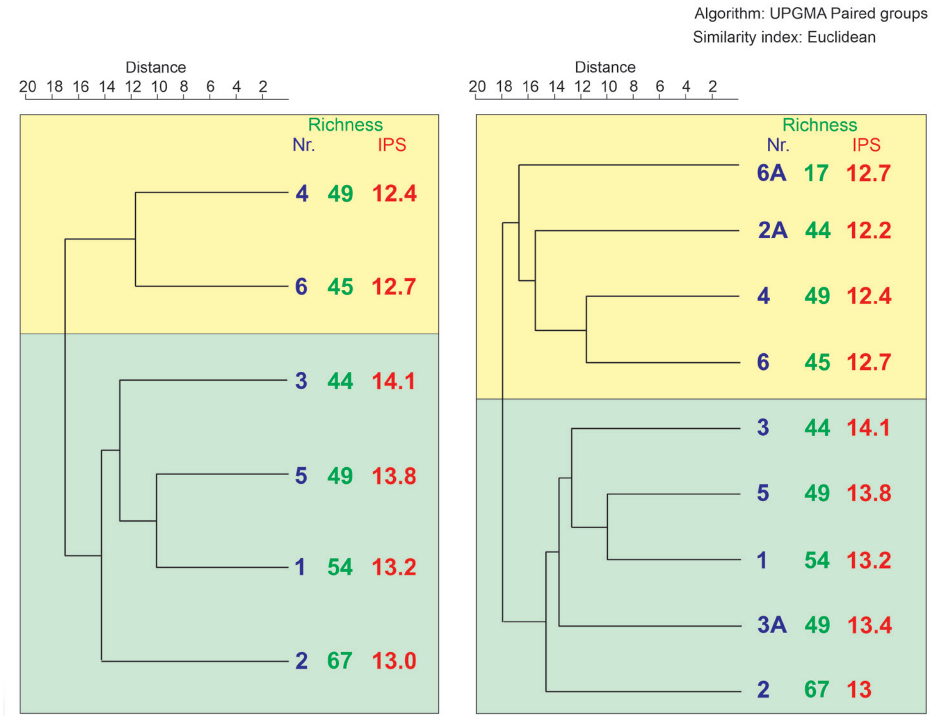

Figure 4. Cluster plots from clustering analysis (CONISS). On the left side, diagram shows clusters of samples scraped from rocks, and the diagram on the right side shows the clusters of the most similar samples taken from different substrates (rocks and macrophytes)

Water quality assessment in the Venta River

The precision and accuracy of performance of the IPS partly depends on degree of overlap between the taxa list integrated in the IPS and those that occur in the analysed samples (Tan et al., 2017). More than 95% of all diatom taxa identified in the Venta River samples were found in the IPS list.

Overall, all samples met the 'good' ecological status according to the IPS values (Fig. 3 and 4, Table 2) in the part of Venta River flowing through Kuldīga town. The lowest value from samples scraped from rocks was calculated for sample 4 (12.4 IPS), while the highest IPS value had sample 3 (14.1 IPS). If samples brushed from macrophytes are considered, then the lowest IPS value was calculated to sample 2A (12.2 IPS). IPS values tend to be lower for samples 2A and 3A, which biofilms were taken from submerged macrophytes, it might be explained by epiphytic diatom ability to uptake additional nutrients through the plant. Therefore, it is important to choose a targeted substrate (if possible) at the beginning of field work (Stevenson et al., 2010). As mentioned before, in this study the targeted substrate is submerged medium size rocks.

Water quality and ecological status determined by IPS indices of diatom samples collected in 2021 indicate good water quality and correspond to data collected by the Latvian Environment, Geology and Meteorology Centre (LEGMC) in 2018 and 2019 (Table 3). The latter data provide a total biological assessment of two closest monitoring stations to the study site and show 'good' ecological quality. While total physicochemical assessment indicates 'poor' water quality in two upstream monitoring stations in 2019 due to elevated total nitrogen (TN; 4.3–4.5 mg/l), other chemical indicators show 'high' or 'good' water quality. Observed elevated TN might be caused by increased runoff from agriculture lands (Kaliff, 2002). Data from 2020 (LEGMC, 2021) from two other monitoring stations along the Venta River upstream from Kuldīga also show elevated TN (3.45–3.72 mg/l), indicating 'moderate' ecological status. In addition, biological assessment from one of the stations (representing 2020) also shows 'good' ecological status in the Venta River, as the ones close to Kuldīga (representing 2018 and 2019).

Table 3. Monitoring results (LEGMC, 2020 and 2019): the numbers for biological quality elements, hydromorphological and total ecological quality indicate ecological quality class (1 – high, 2 – good, 3 – moderate, 4 – poor, 5 – bad), and numbers for chemical parameters represent yearly average concentrations

Monitoring station	Venta		
	0.5 km upstream of Kuldīga	**1.0 km downstream of Kuldīga**	**Upstream of Ēdas**
Year	**2019**	**2018**	**2019**
Benthic invertebrates	2	1	1
Macrophytes	2	N	3
Phytobenthos		2	
Total biological assessment	2	2	3
O_2, mg/l	11.4	11.4	11.2
BOD_5, mg O_2/l	1.4	1.07	1.4
N-NH_4, mg/l	0.03	0.06	0.05
Total N, mg/l	4.5	1.9	4.3
Total P, mg/l	0.055	0.051	0.058
Cu, µg/l	1.6	1.1	1.5
Zn, µg/l	1.1	1.1	1.0
Total physicochemical assessment	4	2	4
Summary ecological quality	3	2	3
Hydromorphological modifications	2	2	3

Colour chart of quality criteria:

High	Good	Moderate	Poor	Bad

Rivers usually experience high variability in discharge, water chemistry, temperature, and light availability (Stevenson et al., 2010) throughout the vegetative period. Therefore, one-time measurements of environmental parameters might not provide a precise characterisation of physical and chemical conditions of the river or stream (Stevenson et al., 2010). Thus, phytobenthos (e.g., diatom-based) autecological indices are particularly valuable in water quality assessments, as a one-time assay of diatom taxa composition and abundance in lotic ecosystems provides a better characterisation of environmental conditions (Stevenson et al., 2010, Stevenson, 2014).

Conclusions

Calculating autecological indices based on species composition and abundance of benthic diatoms is an effective approach to determine ecological status of aquatic ecosystems including rivers and streams.

Diatom analysis suggests that the Venta River might be at risk of eutrophication. This could be mitigated by controlling the use of fertilizers on the surrounding agricultural lands. Pollution might also enter the river from neighbouring allotments. To reduce the risk of pollution, it should be checked whether all allotments with summer houses are connected to the urban sewerage network.

Calculated IPS index values show that the Venta River in Kuldīga overall has low levels of pollution. All study sites were rated as having 'good' ecological status on the IPS index. The highest diatom diversity was observed in Sample 2 (67 taxa), which was taken from the rocks, and the lowest diversity was in the sample taken from the water plants (17 taxa) – sample 6A.

Acknowledgements

We thank Ilga Kokorīte for providing us with monitoring data from the Venta River and Iveta Šteinberga for technical support. We very much appreciate the involvement of our family members (Armands Elsbergs, Inta Vaselāne and Andris Vaselāns) in the development of this project, especially in the field work campaign.

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Investigation of occurrence of specially protected whorl snails *Vertigo* spp. in the habitats of EU importance

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Abstract: In Latvia the conservation status is described as unfavourable bad for three whorl snail species: *Vertigo angustior*, *V. geyeri* and *V. genesii*. Therefore we studied the malacofauna and assessed the species' occurrence in specially protected habitats of EU importance with different management practices.

Key words: Vertigo *angustior*, *V. geyeri* and *V. genesii, V. moulinsiana*

Introduction

In Latvia, the following four specially protected *Vertigo* species from Annex II of the Habitats Directive occur: *Vertigo angustior*, *V. genesii, V. geyeri* and *V. moulinsiana*. According to the report of the European Commission on the conservation status of specially protected species of EU importance for 2013 and 2018, in Latvia the conservation status is described as unfavourable bad for three species: *V. angustior*, *V. geyeri* and *V. genesii* (Rudzīte et al., 2010). The aim of this study was investigation of the malacofauna and the assessment of species occurrence in specially protected habitats of EU importance with different management practices.

Materials and Methods

Sampling was performed in accordance with the methods of the Latvian Natura 2000 sites invertebrate monitoring (compiled in 2013), method *BEZ2*: examination of whorl snail habitats. Survey sites were selected from maps, based on presence of potentially suitable habitats and previously known species localities. For the selection of potentially suitable habitats, available habitat survey protocols from 2017–2019 from the project "Nature Census" ("Dabas skaitīšana") were used. The selection of the sampling sites and number of samples was left to an expert. The investigation was carried out at ten specially protected nature territories and eight habitats of EU importance from 2019 to 2021 (Table 1).

Table 1. Habitats of EU importance, number of collected samples and number of samples, where especially protected whorl snail species were found (*Vertigo angustior* – *V. ang.*, *V. geyeri* – *V. gey.*, *V. genesii* – *V. gen.*)

Habitat of EU importance	**Number of collected samples**	***V. ang.***	***V. gey.***	***V. gen.***
2190 Humid dune slacks	9	0	0	0
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils (Molinion caeruleae)	13	9	8	1
6450 Northern boreal alluvial meadows	7	2	0	0
7140 Transition mires and quaking bogs	3	2	2	0
7160 Fennoscandian mineral-rich springs and springfens	6	2	1	0
7210 Calcareous fens with Cladium mariscus and species of the Caricion davallianae	2	0	1	0
7220 Petrifying springs with tufa formation (Cratoneurion)	5	1	1	0
7230 Alkaline fens	8	8	7	0
Total	53	24	20	1

Results and discussion

In the investigated habitats, three specially protected whorl snail species were found: *V. angustior*, *V. genesii* and *V. geyeri*. *V. genesii* is considered very rare in Latvia and during our study was found associated with only one habitat type – 6410 *Molinia meadows on calcareous, peaty or clayey-silt-laden soils* (*Molinion caeruleae*). This species *V. g*enesii usually in EU is related with habitat type 7230 *Alkaline fens* un 7220 *Petrifying springs with tufa formation* (*Cratoneurion*). *V. angustior* and *V. geyeri* were more common and found in almost all studied habitats, but more often in 6410 *Molinia meadows on calcareous, peaty or clayey-silt-laden soils (Molinion caeruleae)* and 7230 *Alkaline fens*, as well as in 7140 *Transition mires and quaking bogs*. *Vertigo* was absent in habitat 2190 *Humid dune slacks*. This habitat is apparently the least suitable for *Vertigo* species in Latvia, although the association of this habitat with *V. angustior* has been reported in the literature (Cameron et al., 2003). Studies on the occurrence of protected whorl snail species in this habitat have yet to be continued.

Several factors influencing the quality of *Vertigo* habitats and the occurrence were identified during the investigation: 1) overgrowth of the habitat with shrubs/trees because of non-management or natural succession; 2) changes in the hydrological regime due to melioration; 3) regular mowing or intensive grazing. Good populations were found in habitats with no management activities for the last ten years despite the overgrowth. However, removal of overgrowth could improve the population status of the species. It is recommended to plan mowing adaptively: to divide the managed area into several patches and to mow each of them every few years. In addition, grazing must be extensive.

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Impact of light source on distribution of greenhouse whiteflies *Trialeurodes* spp. (Hemiptera: Aleyrodidae) on tomatoes in greenhouses

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Abstract: Nowadays to ensure better plant production various artificial light sources are used in greenhouses. Light in terms of photoperiod length, wavelength and temperatures is one of the most important factors in the insect life. Whiteflies *Trialeurodes* spp. are known to feed on many plant species, including agriculturally important ones, and in favourable conditions can rapidly reproduce and cause vast plant damage. During our monitoring the significant increase of whitefly numbers was observed under high-pressure sodium vapor lamps Helle Magna compared to LED and induction lamps.

Key words: pests, control, monitoring, yellow sticky traps, artificial light

Introduction

Nowadays, to provide fresh vegetables, all year-round growing in greenhouses during the cold and dark autumn-spring seasons is a fundamental part of agriculture. Various artificial light sources are used to ensure better plant growth, development and flowering during the short-day periods. Plant growth and reproduction are influenced by light intensity and photoperiod. The light in greenhouses may be that of natural sunlight, artificially provided, or both. Lately, beside the other light sources, light emitting diodes are introduced in greenhouse systems, as they are safer, easier to control digitally and are cooler than high-pressure sodium vapor lamps.

In greenhouses, not only plants can flourish under favourable living conditions, but various pests also thrive. As for plants light, and especially ultraviolet light, is one of the most important factors in insect life. Light can stimulate or supress insect development, their feeding habits and life cycles. Insects can suffer mortality from collisions with hot lamps, exhaustion, or increased predation due to the attraction of predators and/or increased visibility (Donners et al., 2018). Light may influence insect activity, orientation, and dispersal. On the other hand, the degree to which insects are attracted to light is influenced by its intensity, polarisation and the spectral composition of the light.

Whiteflies *Trialeurodes* spp. (Insecta: Hemiptera: Aleyrodidae) are known to feed on many plant species, including agriculturally important ones. In favourable conditions, whiteflies can rapidly reproduce as their life cycle takes about three weeks to develop and cause vast plant damage (White, 2013). They do not have dormant stages and in greenhouse conditions develop year-round and are year-round pests (van der Ent et al., 2017). *Trialeurodes* spp. feed on plant fluids and sap and infested leaves may lose vigour, become yellow and may drop prematurely. In case of severe infestation, plants can be destroyed. Whiteflies must eat large quantities of dilute sap to obtain the necessary nutrients. The liquid and excess sugar ends up being excreted as shiny, sticky, sugary honeydew on a plant's surface that may lead to black sooty mould starting to appear on the foliage. Still, what harms plants the most is a whitefly's ability to transfer plant viruses. They can transmit over a hundred different plant viruses, like begomoviruses (Geminiviridae), criniviruses (Closteroviridae), and torradoviruses (Secoviridae). The viruses are taken by whiteflies during the feeding on an infected plant. Then whiteflies move to new plants and start feeding, and viral particles enter new plants. Whitefly transmitted viruses are more frequently associated with vegetables than ornamental crops in greenhouses.

Materials and methods

The study was performed in the polycarbonate greenhouse of Faculty of Agriculture of the University of Life Sciences and Technologies of Latvia in Jelgava. The investigation was performed during the winter season 2020/2021. Three additional light sources were used: LED COB Helle Top LED 280 luminary, induction lamps and high-pressure sodium vapor lamps Helle Magna (HPS) (Fig. 1).

Figure 1. LED COB Helle Top LED 280 luminary, induction lamps and high-pressure sodium vapor lamps Helle Magna (from left to right)

These three light sources differ by light wavelength (Fig. 2). Tomatoes were grown under a 16-hour photoperiod.

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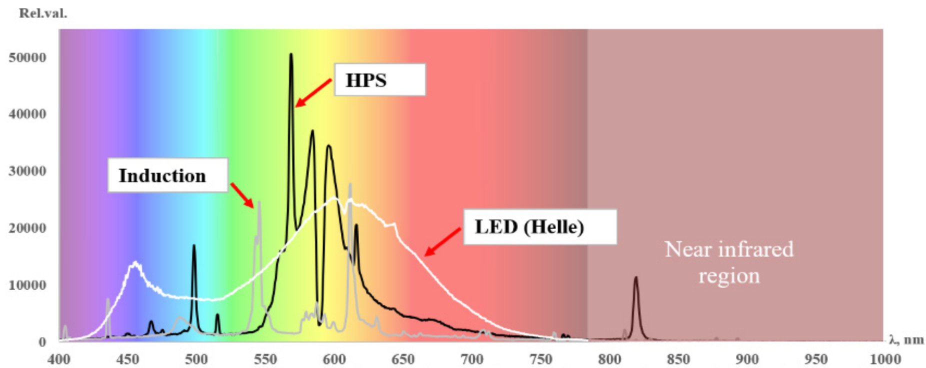

Figure 2. Spectral distribution of investigated light sources

Adult whiteflies were collected by means of yellow sticky traps placed about 50 cm below the light source (Fig. 3). One sticky trap was placed for each tomato plant. These traps were monitored every 2 weeks during the ten-week period. Traps were changed and whiteflies were counted by means of reflected light microscope. The air temperature was measured hourly 50, 100 and 150 cm below the light source. Afterwards average day temperatures were calculated.

Figure 3. Yellow sticky traps exposed for whiteflies control

Results and discussion

During the whitefly monitoring, we observed temperature differences under the investigated light sources (Fig. 4). Temperature was measured 50, 100 and 150 cm under the lamps. The highest temperature was observed under HPS lamps, and this difference remained even 150 cm down from the lamps.

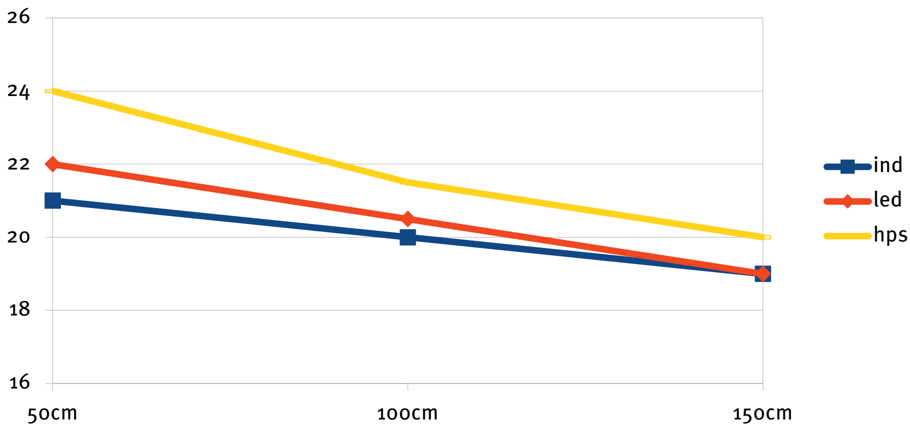

Figure 4. Temperature (°C) changes with distance from light source (blue-induction, green-LED, yellow-HPS lamps)

Obviously, adult whitefly distribution was affected by two factors: environmental temperature in greenhouse and spectral properties of lamps. HPS lamps emitted higher temperatures in comparison to the other light sources throughout the investigation period. This difference is retained also with the increase of distance from the lamps (Fig. 4). Gammara's (et al., 2020) investigation demonstrated that optimal temperature for whitefly development is 22–24 °C. Typical wavelength of HPS lamps (Fig. 2) was observed to be as the most attractive to whiteflies since some authors (Zhang et al., 2020) found the peak sensitivity of greenhouse whitefly optical receptors occurring at 525 nm wavelength which corresponds with green light.

During the ten weeks period, numbers of whiteflies increased, and their distribution was observed variable under the investigated light sources. The least pronounced increase was recorded under induction lamps (Fig. 5). Increase of whitefly numbers under LED lamps was also similar. The comparatively dramatic increase of adult whiteflies was observed under high-pressure sodium vapor lamps (Fig. 5). There were recorded 300 000 individuals/m^2 during the last observation, e.g., about six times the number under LED and induction lamps at the same time.

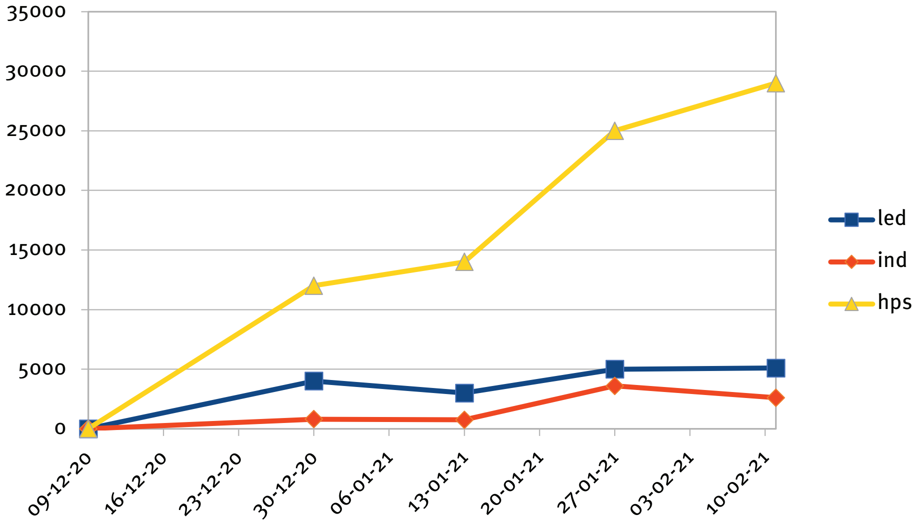

Figure 5. Increase of greenhouse whitefly numbers during the ten-weeks period under LED COB Helle Top LED 280 luminary (LED), induction (IND) and high-pressure sodium vapor lamps Helle Magna (NA)

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Evaluation of the biological activity of sugar-free fractionated red beetroot juice

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Abstract: In the case of type II diabetes, the most important preventive and therapeutic effect gives a diet with a minimal amount of easily digestible carbohydrates. Vegetable juices are positioned as healthy food, because of the high content of phenolic and other biologically active compounds. However, due to the high glycemic index, juices are contraindicated in obesity, and diabetes, while juices with a reduced glycemic index, are not available on the market. We have developed a technology for the fractionation of red beetroot juice based on molecular mass using ultrafiltration. The resulting fraction stimulates the absorption of iron, increases blood hemoglobin level, and enhances capillary blood flow more effectively than native juice does. Both effects are important for patients with diabetes because the impaired blood supply to tissues and organs is an important pathogenetic factor in the development of diabetic renal failure, blindness, and gangrene. The sugar content in fractionated beetroot juice is 5–7%, which makes its use in diabetes problematic. The purpose of the study was to develop a technology for removing sugar from fractionated red beetroot juice and assessing the safety of its functional properties. The fractionated native red beetroot juice and fractionated fermented juice were studied. Fermentation was carried out using pre-activated yeast *Saccharomyces cerevisiae*. It was found that after 5-day fermentation, the sugar content in the fermented fractionated juice fell to 0.5–0.7%, while maintaining functional activity.

Key words: rat, chicken, ultrafiltration, sugar removal, fermentation, absorption of iron

Introduction

Diabetes mellitus can be called a disease of civilization. This pathology is becoming more and more global. In 2019, the number of patients in the world reached 463 million, of which 58 million were in Europe (Fuentes-Merlos et al., 2021) In the case of type II diabetes, the most important preventive and therapeutic value is the modification of the chemical composition of the diet, which should contain a minimum amount of easily digestible carbohydrates. Vegetable juices are an essential component of the modern man's diet and, usually, they are positioned as healthy food products. Due to the high content of phenolic and other biologically active compounds, juices contribute to maintaining health. However, due to the high glycemic index, juices are contraindicated in diabetes. The glycemic index, a measure that ranks carbohydrates by their effect on the body's postprandial glycemic response, was introduced at the beginning of 20th century to facilitate glycemic control in diabetic patients (Jenkins et al., 1981). Unfortunately, juices with a reduced glycemic index are not yet available on the market.

A relatively low glycemic index is usual for juices derived from celery, parsley, and spinach. The sugar content of these juices is 0.9–2.0%. In our previous research, it was found, that the sugar content in red beets depends both on growing conditions and on the variety; in beetroot juice it ranges from 3 to 7.5% (Babarykin et al., 2018). We have developed a technology for the fractionation of red beetroot juice based on molecular mass using ultrafiltration. The resulting fraction can stimulate duodenal absorption of iron and increase hemoglobin levels in blood of the animals with iron deficiency anemia as well as to enhance capillary blood flow more effectively than native juice. Both effects are important for patients with diabetes because the impaired blood supply to tissues and organs is an important pathogenetic factor in the development of diabetic renal failure, blindness, and gangrene. The sugar content in fractionated beet juice is 5–7%, which makes its use in diabetes problematic.

As it is known, the most common way to reduce the sugar content in a food product is fermentation. The production of fermented juices is traditional in the countries of Southeast Asia and the Caucasus (Shet, 2017). Fermentation, used as a method of preserving vegetable raw materials in the production of semi-finished and finished food products, has become popular in Europe today. There is a lot of interest in the sector of non-dairy probiotics based on soy, juices, and cereals. Fermentation has been shown to increase the antioxidant activity of apple juice but cause a decrease in total phenols and flavonoids (Li et al., 2019). Similar changes were recorded by the authors during the fermentation of black carrot juice (Toktaş et al., 2018).

The purpose of the study was to develop a technology for removing sugar from fractionated red beetroot juice by fermentation and assessing it's the safety and its functional properties.

Materials and methods

Production of fractionated and fermented red beetroot juices

By household juicer red beetroot juice was squeezed, and centrifugated for 15 min at 5000 g. The obtained juice was tested on the content of iron, sucrose and reducing sugars. Then fresh juice was diluted with distilled water (added 10% of juice volume), fermented for 48 h (at 25 °C) using dried and previously activated culture of *Saccharomyces cerevisiae* (2 g·l^{-1}), filtrated via paper and centrifugated for 20 min at 5000 g. The centrifugate was evaporated to the initial juice volume in a vacuum at 40 °C to remove ethanol. Sucrose and reducing sugar concentration dynamics were checked. The next step was the fractionation of the resulting fermented juice according to the previously described method (Babarykin et al., 2018).

The content of total sugar and reducing sugar were determined according to the classical Nelson-Somogyi method (Nelson, 1944). Ascorbic acid concentration in beet juice fraction was determined by 2,6-dichlorindophenol titrimetric AOAC Official Method 967.21 (1968, 2006). Atomic absorption spectrometry *(Perkin-Elmer, Analyst 700)* was used to analyse iron (Fe),calcium (Ca) and zinc (Zn) concentration in samples (Jorhem et al., 2000).

Animals

Chickens. 30-day old Lohmann Brown cockerels have been used in the study. Chickens were housed in cage units with free access to food and water. The animals were fed a full-fed diet containing 208 mg of iron per 1 kg. In an *in vivo* experiment, native red beet juice, fractionated (FRBJ) and fermented fractionated (FFRBJ) ones were studied. The chickens received 0.35 mg of iron (as sulfate water solution) *per os* once, alone, and in combination with 1 ml of the test juice. After 100 minutes, the iron content was determined in blood, duodenal mucosa, liver, and spleen.

The influence of the obtained fractions on the mineral balance of Fe^{2+}, Zn^{2+} and Ca^{2+} in the body of chickens was evaluated. Chickens received orally 0.35 mg of iron and 1 ml of a fraction of native (FRBJ) or fermented beetroot juice (FFRBJ) for 7 days. The balance of trace elements in the body of chickens was assessed by the amount received with feed and excreted with dung during the last 3 days. At the end of experiment cockerels were decapitated in accordance with the recommendations for the euthanasia of experimental animals of the European Convention (Close et al., 1997).

Rats. A similar experiment was carried out using laboratory rats with experimental iron deficiency anemia. The rats of the control group consumed standard laboratory animal food in the form of biscuits, consisting of proteins, carbohydrates, fats, minerals, and vitamins. The content of iron in diet was 270 mg/kg. Other groups received a semisynthetic diet containing 16.3 mg Fe/kg. Test solution, containing 0.35 mg of iron (iron sulfate dissolved in water) alone or in combination with experimental juices (FRBJ) or (FFRBJ) was administered *per os* for 3 days. Rats were euthanized by transcervical dislocation after liver, spleen and blood sampling (Close et al., 1996). The experiments were approved by the local Animal Ethics Committee.

All statistics were performed using the software Statistica 7. Results of body weight and visceral fat mass of rats and biochemical parameters are presented as means ± SE. Multiple group comparison was done using one-way ANOVA and Post-hoc Tukey HSD test. Statistical significance was attributed to $P < 0.05$.

Results and discussion

Table 1 presents the comparative results of the composition of fractionated and fractionated fermented red beetroot juice. Fermentation significantly has reduced the carbohydrate content of red beetroot juice. The fraction obtained from such juice contained 10 times less sucrose than the fraction obtained from natural beet juice.

Table 1. Chemical composition of fractioned and fractioned fermented red beetroot juices

Parameter	Fractionated red beetroot juice (FRBJ)	Fractionated fermented red beetroot juice (FFRBJ)
Sucrose, %	5.00 ± 1.20	0.51 ± 0.04
Reducing sugars, %	2.02 ± 0.51	0.06 ± 0.02
Ascorbic acid, mg/L	0.09 ± 0.15	0.03 ± 0.19
Fe, mg/ml	0.96 ± 0.24	0.77 ± 0.19
Ca, mg/ml	26.2 ± 2.80	20.0 ± 3.10
Zn, mg/ml	1.10 ± 0.27	0.67 ± 0.21

It was observed that the decrease of reducing sugars content by more than 30 times and ascorbic acid – almost three times. The concentration of the studied minerals in the juice after fermentation practically did not change.

It was found that because of 5-day fermentation, the sugar content in the native and fractionated juice fell to 0.5–0.7%. Fractionation as well as fermentation impacted the ability of red beetroot juice to stimulate intestinal absorption of iron and its concentration in the blood. Moreover, the fermented product showed a tendence to increase the level of iron in the blood by reducing the accumulation of the element in the liver (Table 2).

Table 2. The content of iron (Fe) in blood serum and organs of chickens administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated red beetroot juice (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Fe content			
	Liver, mg/g wet w	Spleen, mg/g wet w	Duodenum, mg/g wet w	Blood serum, mg /ml
1. Control	121.3 ± 4.1[a*]	103.4 ± 2.5[a]	17.8 ± 1.2[a]	2.10 ± 0.06[a]
2. + Fe (Fe sulphate buffer solution)	123.3 ± 5.1[a]	102.9 ± 3.5[a]	18.6 ± 2.1[a]	2.29 ± 0.05[b]
3. + Fe +FRBR	126.5 ± 6.3[a]	103.8 ± 2.8[a]	19.6 ± 1.6[a]	2.32 ± 0.07[b]
4. + Fe + FFRBR	116.00 ± 7.1[a]	103.0 ± 3.1[a]	18.5 ± 1.8[a]	2.44 ± 0.11[b]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)				

Judging by the content of the studied elements in the dung, *per os* ingested fractionated, and especially fractionated fermented beet juice, stimulated of iron assimilation in the body, without affecting the balance (the ratio between the amount of consumed and excreted with dung) of zinc and calcium (Table 3).

Table 3. Content of mineral elements in dry dung of chickens administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Fe, mg/g	Zn, mg/g	Ca, mg/g
1. Control	833.3 ± 15.3[a, b*]	458.3 ± 11.4[a]	22.03 ± 1.22[a]
2. + Fe (Fe sulphate buffer solution)	866.7 ± 14.5[a]	383.3 ± 15.2[b]	23.75 ± 2.31 [a]
3. + Fe +FRBR	800.0 ± 15.8[b]	375.0 ± 20.1[b]	21.17 ± 1.56 [a]
4. + Fe + FFRBR	780.0 ± 16.6[b]	366.7 ± 14.9[b]	21.08 ± 1.92 [a]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)			

In iron-deficient rats, both studied types of beetroot juice contributed to increased assimilation of Fe in the body (Table 4). However, the fermented fractionated juice contributed to a more intensive (22.6%) accumulation of Fe in the liver and an increase of the element concentration in blood.

Table 4. The iron content in blood serum and organs of iron-deficient rats administered *per os* by Fe (0.35 mg) only and simultaneously with fractionated (FRBR) or fractionated fermented red beetroot (FFRBR) juice

Group	Iron content		
	Liver, mg/g	Spleen, mg/g	Blood serum, mg/ml
1. Control (healthy rats)	467.3 ± 19.2[a*]	708.5 ± 25.9[a]	10.5 ± 2.1[a]
2. Iron-deficient rats	61.8 ± 2.20[d]	153.7 ± 4.7[b]	7.7 ± 0.9[b]
3. Iron-deficient rats (+ Fe)	81.6 ± 3.10[c]	153.3 ± 4.5[b]	10.2 ± 1.5[a]
4. Iron-deficient rats (+ Fe + FBRJ)	86.0 ± 4.10[c, b]	158.8 ± 6.9[b]	10.4 ± 0.8[a]
5. Iron-deficient rats (+ Fe + FFBRJ)	105.5 ± 3.51[b]	153.3 ± 5.3[b]	10.8 ± 1.0[a]
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($p < 0.05$)			

Based on the data obtained in the study, it is difficult to explain opposite direction of the vectors of changes in the iron content in the liver between chickens and rats under the influence of both types of juice. The issue requires further study. Nevertheless, from the point of view of the possibility to achieve the required outcomes (maintaining the level of iron in the blood in the norm and in conditions of alimentary iron deficiency), the use of fermented fractionated beetroot juice seems justified. This is especially important for those at risk of obesity and diabetes.

The obtained results testify to the prospects of using the fermentation of beetroot and other sugar-containing juices. Fractionation based on molecular weight and the removal of easily digestible sugars significantly expands the target audience of juice consumers and provides new opportunities for health promotion through functional food.

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The role of natural dietary antioxidants in animals under oxidative stress

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Abstract: Among the main environmental stressors the most toxic are heavy metals including cadmium (Cd). Stress conditions caused by Cd are associated with overproduction of free radicals following a disturbance in prooxidant-antioxidant balance and tissue damage. The protective action of nutrients with natural antioxidative activities against Cd-induced oxidative stress in chickens was investigated. The experimental analyses demonstrated enhanced production of reactive oxidation species and immunosuppressive effect in Cd-exposed chickens. As antioxidant dietary supplements the salts of zinc ($ZnCl_2$) and selenium (Na_2SeO_2), and vitamin C (ascorbic acid) were used in the experiments with chickens administered orally 100 mcg of Cd (water solution of $CdCl_2$). The antioxidative effect of developed new natural innovative product from red beetroot (fractionated juice) was studied in additional experiment, when chickens exposed to Cd with diet (50 mg/kg). Experimental results demonstrated the prospect of preventive role of Zn, Se, ascorbic acid, and beetroot fractionated juice in the improvement of Cd-induced disorders in the body in case of environmental pollution with heavy metals.

Key words: cadmium, zinc, selenium, ascorbic acid, red beetroot juice, pro/antioxidative action

Introduction

The harmful effect of the most dangerous environmental heavy metal cadmium (Cd) is accompanied by an antioxidant-prooxidant imbalance in animal organs and tissues. Cd catalyzes the formation of reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide in cell membranes following the oxidative stress and the risk of developing metabolic disorders and diseases (Bull, 2010). Oxidative processes are believed to be leading causes of the detrimental consequences of stress in biological systems and resulted in disturbance of antioxidant defense system (Surai, 2003). Natural antioxidants including minerals and vitamins play a vital role in the maintenance of antioxidant status in biological systems

(Surai, 2019). The protective effect of dietary zinc (Zn) and selenium (Se) against Cd toxicity was studied in broilers (Zoidis et al, 2020). Zn is one of the most important essential trace elements and forms an integral component of several cellular enzymes. Moreover, the structural similarities of the Zn and Cd atoms ensure their competitive interaction and leads to their physiological antagonism. The protective effect of Se against Cd-induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage was revealed in rats (El-Boshy et al., 2015). Vitamin C or ascorbic acid (AA) belongs to antioxidant vitamins and is capable of scavenging both superoxide and hydrogen peroxide (Elias A. & Deo, 2013). Dietary natural antioxidant supplements are used to protect the body from the oxidative stress consequences. A special interest for these purposes is the use of the local natural sources – such as beetroot juice and its innovative products. The technology of fractionated red beetroot juice was developed and obtained in the Institute of Innovative Biomedical Technology (Latvia).

The aim of the present study was to investigate the protective action of natural antioxidants as additional dietary support for the antioxidant defence system in Cd-exposed chickens.

Materials and methods

Ethics Statement

All experimental procedures were approved by the animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia, authorization reference number 53 (April 10, 2012).

Material

For the experimental dietary additions, used for two different experiments, the following supplements were used:

1. Chemicals: Cd (cadmium chloride, $CdCl_2$,), Zn (zinc chloride, $ZnCl_2$), Se (sodium selenite, Na_2SeO_2), AA (ascorbic acid) obtained from Sigma Chemical Co, EU.
2. Fermented fractionated (FF) beet root juice obtained from squeezed and centrifuged red beet root juice by the fermentation during 48 h (at 25 °C) using activated culture of *Saccharomyces cerevisiae* (2 $g \cdot L^{-1}$). (Babarykin et al., 2018)

Experimental design and animals

Two different experiments on Cd-exposed male Lohmann brown chickens were undertaken.

The first treatment was performed on 1 to 34 days old cockerels. For the first 20 days, all chickens were fed a standard starter fool-fed basal diet (BD-1). Then the birds were divided into 5 groups of 25 heads each. Group 1 (Control-1) continued to consume BD-1 without any supplements. Chickens of the other 4 groups during next 14 days daily were administered *per os* 100 mg of cadmium (water solution of $CdCl_2$) per 100 g of body mass and also provided the same diet but differed by dietary supplements: Group 2 (+Cd) – fed the BD-1 without any supplements. Group 3 (+Cd+Zn) received BD-1, calculated to contain zinc 500 mg kg^{-1} (as $ZnCl_2$). Group 4 (+Cd+Se) consumed BD-1, calculated to contain 1 mg selenium kg^{-1} food (as Na_2SeO_2). The same BD-1 of Group 5 (+Cd+AA) was supplemented by 100 mg AA kg^{-1}.

For the second treatment 35-day-old male Lohman Brown chickens were obtained and used for 10 days of investigation. The chickens were divided into 4 groups of 7 heads each. Group 1 (Control-2) fed corresponding for the birds of this age standard grower fool-fed basal diet (BD-2) without any supplements. Group 2 (+Cd) was given the same BD-2 but supplemented by Cd 50 mg kg^{-1} ($CdCl_2$). Group 3 (+FF) consumed the BD-2 and each chicken was administered by 1 ml of FF *per os* daily. Chickens of Group 4 (+Cd+FF) were fed the same diet as Group 2 but administered *per os* by 1 ml of FF daily.

At the end of experiments, chickens were weighed and sacrificed by decapitation in accordance with the Recommendation for Experimental Animals of the European Convention (Close et al., 1997). Whole blood, blood serum and liver of chickens were collected and used for analyses.

Biochemical and immunological assays

The content of cadmium and zinc in blood serum and liver was determined by atomic absorption spectrophotometer Perkin-Elmer (model AAnalyst 700) according to the procedures of the AOAC (1999). The content of selenium in whole blood and liver was estimated by fluorometry applying 2,3-diaminonaphthalene reagent (AOAC, 1997). The antioxidant status was evaluated by measuring the level of lipid peroxidation product malodialdehyde (MDA) in liver homogenate by the thiobarbituric acid reaction (Surai et al., 1996). Activity of glutathionperoxidase (GSH-P_x) in liver homogenate was measured based by modified the method described by Pinto and Bartley (1969).

Immunological analyses

The parameters of humoral immunity were investigated using the complex of methods presented in Vasilyeva et al., (2001). Serum lysozyme content was evaluated by a modified nephelometric assay by determining the decrease in turbidity of a suspension of *Micrococcus lysodeicticus*. Nonspecific circulating immune complexes (CIC) in blood serum were estimated spectrophotometrically using precipitation with polyethylene glycol.

Statistical analyses

All statistics were performed using the software *Statistica* 7. Results of Cd, Zn, Se content and biochemical parameters are presented as mean ± SE. Multiple group comparison was done using *one-way* ANOVA and *Post-hoc* Tukey HSD test.

Results and discussion

Cd-exposed chickens during the experimental periods of both presented treatments showed no evidence of clinical toxicity. However, a slight tendency of chicken growth suppression was observed in both Cd exposure experiments.

Administration of 100 mg of cadmium for 14 days *per os* caused a non-significant decrease of chicken body mass by 3.0% ($P > 0.05$) compared to the Control-1: 421.6 ± 20.4 g *vs* 434.8 ± 22.2 g. After consumption of the diet supplemented by Cd 50 mg kg^{-1} for 10 days chicken body mass was very close to the Control-2: 473.4 ± 19.8 g *vs* 480,8 ± 23.6 g and tended to decrease only by 1.6% ($P > 0.05$).

Amelioration of the adverse effect of Cd had observed when antioxidants Zn, Se and AA were added to the diet and when beet juice FF was given *per os* to Cd-treated chickens.

At the first treatment body weight of Cd-co-administered chickens with Zn, Se and AA on average approached the Control-1, and by 3–6% ($P > 0.05$) non-significant exceeded +Cd group. At the second experiment administration of FF to the Cd-treated birds resulted in a non-significant (about 2%, $P > 0.05$) increase in body mass.

It is possible that the small negative effect of Cd on chicken growth was due to the short period of Cd exposure experiments.

A more noticeable effect of Cd was manifested in the blood serum of chickens. Both when cadmium was administered to chickens *per os,* and when it supplemented in the diet, the concentration of Cd in the blood serum significantly increased by 40% and 134% correspondingly (Table 1 and Table 3). Analysis of biochemical parameters after Cd exposure at the first treatment showed a tendency to decrease blood Zn and Se levels in chickens of +Cd group.

Table 1. Effect of antioxidative dietary supplements on the concentration of trace elements in blood of Cd-exposed chickens

Group	Cd in blood serum, $\mu g \cdot dL^{-1}$	Zn in blood serum, $\mu g \cdot dL^{-1}$	Se in whole blood, $\mu g \cdot dL^{-1}$
Control-1	$5.0 \pm 0.6^{a*}$	$300.0 \pm 10.3^{a\,b}$	14.3 ± 1.5^{a}
+Cd	7.0 ± 0.5^{b}	276.0 ± 17.9^{a}	12.2 ± 2.0^{a}
+Cd+Zn	4.3 ± 0.4^{a}	353.0 ± 30.0^{b}	11.6 ± 2.1^{a}
+Cd+Se	4.7 ± 0.5^{a}	$293.0 \pm 24.0^{a\,b}$	25.3 ± 2.2^{b}
+Cd+AA	4.3 ± 0.3^{a}	$310.0 \pm 16.9^{a\,b}$	–

* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$)
Cd exposure: 100 mg of cadmium administered per one chicken *per os* daily

The observed trace element imbalance accompanied by enhanced production of reactive oxidation species (Table 2). Very often overproduction of free radicals, a major cellular source of oxidative stress in biological systems, compromise antioxidant defense in the cell/whole body (Surai et al., 2019).

The results represented in Table 2 Cd-induced decrease of antioxidant enzyme GSH-P_x activity in blood and growing extent of lipid peroxidation production (increase of MDA level in liver) indicated the development of oxidative stress in chickens.

Table 2. Parameters of lipid peroxidation in liver and blood in chickens induced by Cd and effect of antioxidative dietary supplements

Group	Malondialdehyde, $\mu mol \cdot g^{-1}$ liver	Glutathionperoxidase, μmol GSH$\cdot min^{-1} \cdot ml^{-1}$ blood
Control-1	16.70 ± 0.55[a*]	2.75 ± 0.07 [b]
+Cd	18.10 ± 0.30[b]	2.33 ± 0.03[a]
+Cd+Zn	15.10 ± 0.51[a]	2.95 ± 0.11 [b]
+Cd+Se	15.57 ± 0.64 [a]	3.97 ± 0.12 [c]
+Cd+AA	15.83 ± 0.48 [a]	2.69 ± 0.09 [b]

* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$)
Cd exposure: 100 mcg of cadmium administered per one chicken *per os* daily

All the experimental antioxidative nutrients demonstrated preventive effect against the harmful impact of Cd. In the first treatment, the most pronounced protective result manifested in the case of Zn supplement. This effect may be due to synergistic and antagonistic interactions between Cd and Zn at the molecular level (Markovs et al., 1997). The physiological role of Se in the body relates to activity of glutathionperoxidase (GSH-Px). Se is incorporated in the active site of this enzyme which acts as a free radical scavenger in the body protecting cellular membranes against oxidative stress products (Zoidis et al., 2020). The data of Table 2 showed that intake of diet supplemented with Se prevented Cd-induced disturbance of antioxidant-prooxidant balance in chickens. Consumption of the diet supplemented with AA also provided an improvement of Cd-induced damage.

An increased Cd accumulation in chicken blood after Cd exposure in the second experiment is accompanied by enhance of oxidative processes activity in organs (Table 3).

The results of this treatment showed that administration of FF to Cd-exposed chickens prevented prooxidative impact of this heavy metal. The data of MDA level and GSH activity in chickens after FF administration approached to the Control-2 group.

Table 3. The effect of red beetroot juice fermented fraction (FF) on cadmium (Cd) concentration in blood serum and indices of oxidative stress in liver of Cd-exposed chickens

Group	Cd, $\mu g \cdot dL^{-1}$ blood serum	Malondialdehyde, $\mu mol \cdot g^{-1}$ liver	Glutathionperoxidase, mM GSH·min^{-1} g^{-1} liver
Control-2	$4.4 \pm 0.2^{a*}$	16.90 ± 0.76^{a}	9.60 ± 0.23^{b}
+Cd	10.3 ± 0.7^{c}	18.30 ± 0.37^{b}	8.20 ± 0.28^{a}
+FF	4.6 ± 0.7^{a}	16.60 ± 0.52^{a}	9.51 ± 0.19^{b}
+Cd+FF	8.0 ± 0.3^{b}	17.10 ± 0.40^{a}	$8.90 \pm 0.27^{a, b}$
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$). Cd exposure: diet supplemented by Cd 50 mg kg^{-1}			

In addition to Cd accumulation in blood and oxidative imbalance in liver a suppression of humoral immunity parameters was observed (Table 4). The index of nonspecific humoral resistance is characterized by a decrease of lysozyme enzymatic activity in blood serum of Cd-exposed chickens. This enzyme plays an important role in natural defense reactions, participating in regulation of immune responses by creating the host antibacterial barrier (Ragland & Criss, 2010). A suppressive effect of low concentration of cadmium on serum lysozyme content was observed in common carp serum after 30 days (Ghiasi et al., 2010). The noticed increase of antigen – antibody nonspecific CIC level in Cd-administered chickens' serum also indicated a disturbance of immune response (Table 4). Normally, CIC formed in the bloodstream, phagocytized, and destroyed. Pathological reactions to CIC may be due to an increase in the rate of their formation over the rate of elimination (Cacheiro-Haguno et al., 2021). FF administration in experimental Cd-exposed birds provided anti-stress defense at the level of nonspecific humoral immunity.

The preventive action of FF may relate to its components – pigments of betalain group. Betalains act as a free radical scavenger and an inductor of defense mechanism in cells (Esatbeyoglu et al., 2014). It determines their antioxidative capacities.

Table 4. The influence of red beetroot juice fermented fraction (FF) on humoral immune parameters in blood serum of Cd-exposed chickens

Group	Lysozyme, $\mu g \cdot mL^{-1}$	Circulating immune complexes (CIC), extinction x 100
Control -2	$6.9 \pm 1.10^{b*}$	2.9 ± 0.11^{a}
+Cd	4.4 ± 0.91^{a}	3.4 ± 0.08^{b}
+FF	$6.6 \pm 0.90^{a, b}$	$3.0 \pm 0.12^{a, b}$
+Cd+FF	$5.0 \pm 0.88^{a, b}$	3.3 ± 0.09^{b}
* Statistically different or similar within column according to Post-hoc Tukey HSD test ($P < 0.05$). Cd exposure: diet supplemented by Cd 50 mg kg^{-1}		

The preventive action of FF may relate to its components – pigments of betalain group. Betalains act as a free radical scavenger and an inductor of defense mechanism in cells (Esatbeyoglu et al., 2014). It determines their antioxidative capacities.

The present investigations had demonstrated that Cd is an environmental stressor for chickens. It may cause an imbalance of mineral nutrients and oxidative stress development. The adverse effect of Cd is manifested regardless of the method of this heavy metal administration to the chickens. It resulted in the development of oxidative stress and suppressed parameters of innate humoral immunity in Cd-exposed chickens. Supplementation of natural antioxidants Zn, Se and ascorbic acid to the diet prevented Cd-induced oxidative damage. The protective role of red beetroot juice fermented fraction against Cd danger was to support the antioxidant and humoral immunity defence systems.

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Effects of Low Frequency Electromagnetic Radiation on *Lemna minor* growth parameters and generation of point mutations at GPx, CAT and APx genes

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Abstract: Development of new technologies distributing electric power from power stations to our homes through a *network* of cables and wires, including numerous electric devices at working places and home environment become a source of electromagnetic radiation (EMR) much stronger than EMR of natural origin. To provide a better understanding of the impact of the EMR of anthropogenic origin on living organisms, we investigated the long-term effects of EMR on *Lemna minor*. In this study, plants of the *L. minor* laboratory clone were exposed to low frequency (50 Hz) electromagnetic radiation (LF EMR) growing clones in Petri dishes placed on the coils initially generating magnetic flux (MF) of 1 μT for first three weeks, 2 μT until 12th week and after 12th week of the experiment MF was enhanced up to 300 μT. We examined the response of the plants by sequencing DNA fragments that included promoter, intron, and exon regions of ascorbate peroxidase (APx), glutathione peroxidase (GPx), and catalase (Cat) genes) as well measured growth parameters growth intensity, frond area, and number of fronds.

Comparison of growth parameters of *L. minor* clones exposed to 2 μT and 300 μT magnetic flux revealed positive effect stimulating growth of experimentally affected plants at 2 μT After the first 14 weeks of treatment, the growth parameters were lower in the directly exposed by LF EMR group than in the group grown distantly from the source of EMR. However, after 18 weeks from the beginning of the experiment no significant difference was observed between two groups of *L. minor* including directly and indirectly affected by LF EMR plants. Moreover, the signals of the impact of LF EMR on the plants as rising of new point mutations were detected. The significantly enhanced number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones were revealed at the introns of APx ($P = 0.011$), GPx ($P = 0.009$), and Cat ($P = 0.044$) genes starting from the 10th week of the experiment

In conclusion, the comparison of DNA sequencing data together with measurements of growth parameters revealed differences in response at molecular and physiological levels of directly and indirectly affected *L. minor* clones providing evidence that response to the impact of LF EMR depended on the prolongation of the impact and the magnetic flux density.

Key words: Low frequency electromagnetic radiation, duckweed, point mutations, catalase, glutathione peroxidase, ascorbate peroxidase

Introduction

Lemna minor is an aquatic plant with all parts of the plant floating in water. This plant belongs to the Lemnaceae family, which consists of five genera: *Landoltia, Lemna, Spirodela, Wolffia and Wolffiella*. Currently, 37 species belonging to this family have been identified (Les et al., 2002). The small float has one to four leaves that attach to a root that floats in the water. Their roots are 1–2 cm long and their floating leaves are oval shaped, 1–8 mm long and 0.6–5 mm wide (Wolverton and McDonald, 1980). *L. minor* is often used as a model organism in various studies (Hoeck et al., 2015). For example, due to its ability to absorb heavy metals from water and adapt to different environmental conditions, *L. minor* is an excellent organism for studying water pollution levels (An et al., 2018).

Although the variability of glutathione peroxidase (GPx) genes of *L. minor* has not been investigated so far, several studies related to stress and changes in GPx enzyme activity have been conducted. For example, Tjidjen and colleagues studied the effect of oxidative stress on *L. minor* and used the GPx enzyme as a biomolecular marker. During the study, *L. minor* was exposed to the herbicide diclofop-methyl at different concentrations of 17.5 µg/L, 35 µg/L, 70 µg/L. After 21 days, the obtained results showed that GPx activity is directly dependent on the duration of cultivation with herbicide and its concentration (Tlidjen et al., 2012). Another group of researchers cultivated *L. minor* for 21 days exposed to biopesticides at different concentrations (4 µL/L, 80 µL/L, 120 µL/L). Compared to the control, GPx activity also increased significantly with biopesticide concentration and cultivation time (Atamanalp et al., 2019). A considerable amount of research has been done with enzymatic activity of catalase (Cat) using *L. minor* as a model organism. Unfortunately, investigations of *L. minor* DNA sequences coding Cat gene starting from construction of primers for amplification of DNA fragments that could be suitable genetic marker to investigate impact of various factors on stability of DNA strand including generation of new point mutations have not been developed yet. Available publications mostly represent results related to measurements of changes of Cat enzyme activity under stress conditions. For example, in one study, smallmouth bass fishes were exposed to mercury, cadmium, and chromium at concentrations ranging from 0.02 to 20 mg/L. The obtained results showed that Cat activity began to increase significantly in the case of mercury, cadmium, and chromium when their concentration reached 0.2 mg/L (Varga et al., 2013). Ascorbate peroxidase (APx, EC 1.11.1.11) is one of the key enzymes that detoxifies H_2O_2 in plants. This APx gene belongs to a family of multigene coding peroxidases that have a heme group and can catalyse the H_2O_2-dependent oxidation of various organic molecules (Lazzarotto et al., 2011).

Oxidative stress to the body can also be caused by an electromagnetic field (EMF), the background level of which is low in the natural environment, and the effect is almost imperceptible. EMFs of various frequencies are caused by human activities and are increasing every year due to the use of wireless technologies, including mobile phones, Wi-Fi, and other related devices. Since the numbers and variety of devices emitting non-ionizing radiation in the living environment is increasing significantly more and more attention is paid to their effects depending on the EMF amplitude, frequency, wavelength, tissue distance from the source of EMF or other parameters (Vian et al., 2016). To provide a better understanding of the impact of the EMR of anthropogenic origin on living organisms, we investigated the long-term effects of EMR on *Lemna minor* clones based on changing growth parameters as well screening of appearance of new point mutations in DNA sequences of candidate genes involved into regulation of oxidative stress in cells.

Materials and methods

Plant material

L. minor indicated as S2 clone was collected from the Neris River above city of Vilnius (54° 45‘48.25“, 25° 21‘14.53“) and was chosen for testing of LF EMF in the laboratory conditions. The sterilized plants were transferred to Petri dishes with Steinberg medium (ISO 20079). The Steinberg medium contains 350 mg KNO_3, 295 mg Ca $(NO_3)_2 \cdot 4H_2O$, 90 mg KH_2PO_4, 12,6 mg K_2HPO_4, 100 mg $MgSO_4 \cdot 7H_2O$, 120 µg H_3BO_3, 180 µg $ZnSO_4 \cdot 7H_2O$, 44 µg $Na_2MoO_4 \cdot 2H_2O$, 180 µg $MnCl_2 \cdot 4H_2O$, 760 µg $FeCl_3 \cdot 6H_2O$, 1500 µg EDTA Disodium-dihydrate per litter of distilled water. The culture of *L. minor* was maintained under continuous light (OSRAM L 36/77), photoperiod of 16 h/8 h day/night of fluorescent light of 90–100 µE m^{-2} s^{-1} intensity at 25 ± 1 °C.

EMF treatment

For the test, representatives of one colony (3 clones) of *L. minor* S2 were grown in 15 ml of Steinberg’s medium in a Petri dishes. Every seven days, the plants were transplanted into a new medium. After 2 weeks, the material was fixed, leaving one clump from each variant plate for continuation of the test by transferring to a new medium. Lighting conditions ~70–80 µmol. Temperature 25 °C ± 2 °C. For each test option, 3 Petri dishes were placed on the coils representing plants indicated as directly exposed to EMF, these samples were coded as group EMF, and the clones experiencing remote EMF exposure (plants grown in Petri dishes at 1.5 m distance from the EMF generating coils) were coded as group C. To create EMF conditions for the cultivation of *L. minor*, a generator with a frequency of 50 Hz was used (Fig. 1).

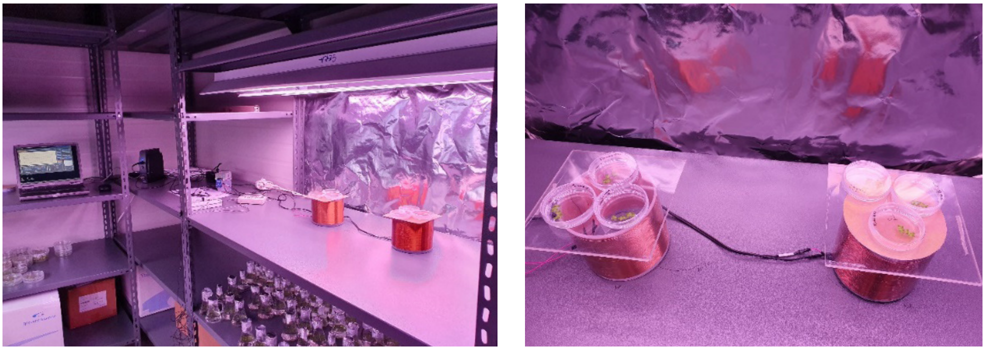

Figure 1. Cultivation of ***L. minor*** on EMF generating equipment. ***L. minor*** samples are grown on electromagnetic radiation generating coil (10 cm in diameter and 10 cm in height)

The plants were transplanted every week and the experimental conditions changing the effect of EMF on *L. minor* clones are described in Table 1. Growing parameters of experimentally affected plants were analysed after 3, 6, 8, 10, 14 and 18 weeks from the beginning of the experiment. After each week, the green mass of the plant was weighed, and the number and area of nodules were measured by ImageJ software. For DNA analysis, the material was fixed at –20 °C.

Table 1. Electromagnetic radiation parameters and experimental duration

Test duration, week	EMF induction	Strength of current
1–3	1 μT	1.2 mA
4–11	2 μT	2.4 mA
12–18	300 μT	0.4 A

DNA extraction

The whole plants with fronds and roots were grounded in liquid nitrogen and total DNA was extracted using Dneasy Plant Mini Kit method according to manufacturer's protocol (QIAGEN). Aliquots of the extracted DNA were used for measuring DNA quantity with NanoDrop ND1000. DNA extracts were diluted to a final concentration of 10 ng/μL in distilled water. DNA was stored at –20 °C until use.

PCR and sequencing conditions

L. minor sequences of antioxidant genes were used for designation of primers were obtained from CoGe database (https://genomevolution.org/coge/) (Van Heck et al., 2015). Specific primers for amplification of different parts of gene including promoter, introns and exons were designed using Primer3Plus program (Table 2).

PCR was performed in 10 μL of final solution volume containing 2 μL of DNA (10 ng/μL), 1 μL of each primer (10 μM), 5 μL DreamTaq PCR Master Mix, 1 μL nuclease-free water. The PCR was performed in Eppendorf Mastercycler thermal cyclers. The thermocycling program started from 5 min at 94 °C; followed by 35 cycles of 94 °C for 45 s, annealing temperature (indicated in Table 1) for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. Amplified products were analyzed by electrophoresis in 1.5% agarose gel in 1X Tris-acetate-EDTA (TAE) buffer using Thermo Scientific Gene-Ruler DNA ladder and visualized by ethidium bromide staining. The PCR products were purified with exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) and then sequenced by 3500 Genetic Analyser.

Table 2. Primer sequences of antioxidant gene markers used to study impact of LF EMF on *L. minor*

Gene	Primer name	Primer sequences (5'–3')	Annealing temperature (°C)	Product Length (bp)	Type
Glutathione peroxidase	GPx6	F: TGTGCAAACACATAATCCCAAT R: TGATCATGACCAATAGATCGTT	49	902	Promoter + Exon 1 + Intron 1
Catalase	Cat7	F: CGCGGTTTGGTTCAATTCGT R: TGGACTTGATCAGCGGTGAC	55	785	Promoter + Exon 1 + Intron 1 + Exon 2 + Intron 2 + Exon 3 + Intron 3 + Exon 4
Ascorbate peroxidase	APx1	F: AAATTCGAGCCGTCAGATTG R: CCGAGATCCGACCTGATAGA	56	772	Promoter 1 + Intron 1 + Promoter 2 + Exon 1 + Intron 2 + Exon 2

Molecular data analysis

The sequenced DNA fragments were aligned using MUSCLE approach (Edgar, 2004) option in MEGA-X (Kumar et al., 2018). The aligned sequences were further analysed indicating rising of point mutations at sequences of affected by EMF colonies of S2 line in comparison to control grown distantly (1.5 m) from the source of EMF colony of the same S2 line. Before the start of the experiment plants representing non-affected by EMF colony were collected and kept frozen and used as a source of reference DNA sequences. The presented data are means ± standard errors of at least three independent measurements for each term. The Student's t-test was used to estimate the statistically differences between the two groups representing directly and indirectly affected by EMF groups. The difference was considered significant at p levels lower than 0.05 ($p < 0.05$).

Results and Discussion

The entire length of the GPx gene is 3167 bp, therefore ~~bso~~ to make easier amplification of shorter sequences of the GPx gene, it must be broken down into smaller fragments that overlap. In this case, we constructed primers for amplification of 7 overlapping fragments: GPx1, GPx2, GPx3, GPx4, GPx5, GPx6, GPx7 (Fig. 2). All generated fragments contain at least one exon and one intron. Of the seven GPx primer pairs generated, one fraction is obtained only with the GPx6 and GPx7 primer pairs. Sequencing of these gene fragments resulted in clean sequences that were used for further analysis.

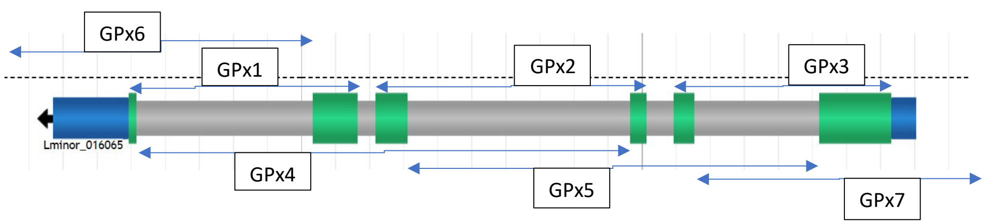

Figure 2. Positions of 7 DNA fragments encompassing whole sequence of glutathione peroxidase gene. Exon region is shown in green, intron in grey, promoter, and terminator regions in blue. Arrows indicate the relative length of the corresponding fragments for which primer pairs were created for amplification

The length of the Cat gene is 3788 bp, therefore, to sequence the entire gene, it must be broken down into smaller fragments that overlap. In this case, 9 fragments were created: Cat1, Cat2, Cat3, Cat4, Cat4a, Cat4b, Cat5, Cat6, Cat7 (Fig. 3). Fragments with Cat4, Cat4b, Cat7 primer pairs were selected for further analysis. Sequencing of fragments of this gene resulted in clean sequences that were used for further analysis.

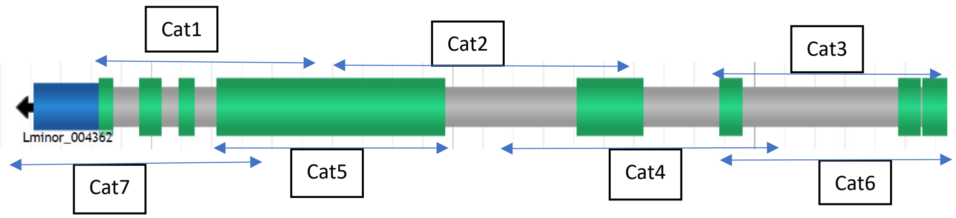

Figure 3. Positions of 9 DNA fragments encompassing whole sequence of catalase gene. The exon region is shown in green, the intron region in grey, and the promoter region in blue. Arrows indicate the relative length of the corresponding fragments for which primer pairs were created for amplification

Since the length of ascorbate peroxidase gene is 2085 bp, to perform sequencing of the entire gene, it must be split into smaller fragments that overlap. In this case, 4 fragments were obtained: APx1, APx2, APx3, APx4 (Fig. 4). Each fragment with designed primer pairs includes at least two exon and two intron regions. Sequencing results in each case showed impure sequences, making such sequences ineligible for further analysis. Primer pairs Apx1 and Apx2 that yield clean sequences are selected for further analysis

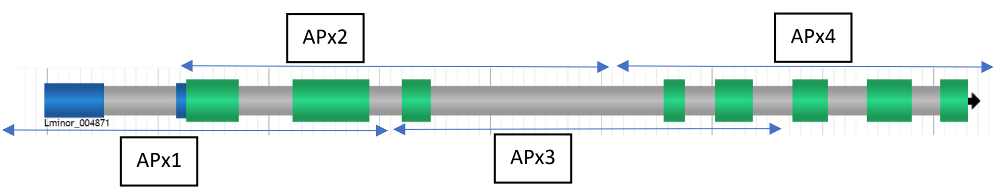

Figure 4. Positions of 4 DNA fragments encompassing whole sequence of ascorbate peroxidase gene. The exon region is shown in green, the intron region in grey, and the promoter region in blue. Gene fragments marked with arrows were amplified with the help of appropriate primers

When analysing gene markers encoding antioxidant enzymes, it was observed that the cleanest sequences were obtained with the Cat7 primer pair for all tested samples of the wild population *L. minor* (Table 3). This marker was also chosen for the study of experimental clones related to electromagnetic radiation. In the fragments amplified with the Cat7 primer pair, most nucleotide substitutions were observed in exon (623, 625, 627, 628, 629, 633, 634) in both treatment groups.

Table 3. Nucleotide substitutions and deletions detected at Cat7 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct electromagnetic radiation, K1, K2, K3 – plants exposed to indirect electromagnetic radiation. The exon region is marked in green; blue – promoter; grey – intron

		19 (9510)	21 (9512)						44 (9535)			71 (9562)	74 (9565)	168 (9659)	170 (9661)	193 (9684)	194 (9685)	197 (9688)	199 (9690)	215 (9706)	216 (9707)	407 (9898)	412 (9903)	451 (9942)	458 (9949)	464 (9955)		466 (9957)	467 (9958)	468 (9959)	488 (9979)	489 (9980)	490 (9981)	497 (9988)	509 (10000)	519 (10010)	527 (10018)	564 (10055)	587 (10078)	589 (10080)	599 (10090)	601 (10092)	606 (10097)	614 (10105)	616 (10107)	621 (10112)	623 (10114)	625 (10116)	627 (10118)	628 (10119)	629 (10120)	633 (10124)	634 (10125)	635 (10126)		642 (10133)
	Cat7 Sta-2	G	G	-	-	-	-	-	C	-	-	T	C	A	C	G	C	C	G	A	C	G	G	A	A	G	-	C	C	A	C	A	C	T	G	G	A	C	G	G	G	G	A	A	A	G	A	A	A	A	C	G	T	C	-	G
10 week	K1	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	G	.	.	.	.	.	-	.
	K2	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
	K3	.	.	A	A	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	-	A
	E1	.	.	A	-	-	-	-	.	-	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	-	A
	E2	A	.	A	-	-	-	-	.	-	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	-	.
	E3	A	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
14 week	K1	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	C	G	.	G	.	-	.
	K2	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	-	.
	K3	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
	E1	.	.	-	-	-	-	-	.	-	-	.	.	-	T	C	.	.	A	-	T	C	.	.	.	T	T	T	T	T	T	C	.	.	.	-	C	.	.	T	T	.	.	T	T	C	.	.	.	.	.	C	.	.	-	.
	E2	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A	.	A	.	-	A
	E3	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	-	A
18 week	K1	.	.	-	-	-	-	-	.	-	-	.	.	-	T	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.	C	.	.	.	.	-	A
	K2	.	.	G	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	.
	K3	.	.	-	-	-	-	-	.	-	-	.	.	.	.	A	T	A	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	C	.	.	.	.	-	.
	E1	.	.	-	-	-	-	-	.	-	-	.	.	-	T	.	.	.	.	.	.	.	T	G	T	.	-	.	T	G	.	.	T	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
	E2	.	.	-	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
	E3	.	.	-	-	-	-	-	.	A	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	-	A
48 wek	E1.1	.	.	A	-	T	T	C	T	-	-	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	A
	E1.2	.	.	A	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
	E2.1	A	.	A	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	G	.	.	.	.	A	.	A	.
	E2.2	.	.	A	-	-	-	-	.	-	-	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	-	.
	E3.1	.	.	A	-	-	-	-	.	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	-	.
	E3.2	.	T	A	-	-	-	-	.	G	-	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	G	-	.

In the fragments amplified with the GPx6 primer pair, nucleotide substitutions were observed at several positions (95, 97, 99, 101, 102, 199, 202 – promoter, 226, 228, 242 – exon) after 14 weeks and no point mutations were detected after 18 weeks in EMF S2 clones exposed independently to electromagnetic radiation (Table 4).

Table 4. Nucleotide substitutions and deletions of GPx6 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct EMR, K1, K2, K3 – plants exposed to indirect EMR. The exon region is marked in green; blue – promoter; grey – intron

Week	Sample	95 / 9244	97 / 9246	99 / 9248		101 / 9250	102 / 9251					199 / 9348	202 / 9351	226 / 9375	228 / 9377	242 / 9391					295 / 9444	345 / 9494	561 / 9710	709 / 9858	759 / 9908			839 / 9988	840 / 9989	848 / 9997		860 / 10009	861 / 10010
	GPx6 Sta-2	A	C	A	-	G	T	-	-	-	-	G	A	G	A	G	-	-	-	-	T	A	A	A	G	-	-	C	A	A	-	A	C
10 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
10 week	K2	.	.	.	C	.	.	A	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
10 week	E1	.	.	.	-	.	.	-	T	T	-	.	.	.	.	.	-	-	-	-	.	.	.	T	-	-	-	.	-	.	-	.	.
10 week	E2	.	.	.	G	T	G	A	-	-	-	.	.	.	.	.	-	-	-	-	.	C	.	.	-	-	-	.	-	.	-	.	.
14 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
14 week	K2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
14 week	K3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
14 week	E1	G	T	C	-	-	-	-	-	-	-	-	-	.	C	-	C	-	C	-	C	.	C	.	-	-	-	.	-	.	-	T	.
14 week	E2	.	.	.	G	T	C	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	A
14 week	E3	-	-	-	-	-	-	-	-	-	-	T	G	A	.	A	-	T	T	T	.	.	.	.	-	-	-	.	-	.	A	.	.
18 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
18 week	K2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
18 week	K3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
18 week	E1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
18 week	E2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
18 week	E3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
48 week	E1.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	G	-	.	.
48 week	E1.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
48 week	E2.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
48 week	E2.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	A	-	.	-	.	-	.	.
48 week	E3.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	A	-	A	.	-	.	-	.	.
48 week	E3.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	A	C	.	-	.	.

In the fragments amplified with the APx1 primer pair, nucleotide substitutions were observed at several positions in promoter, exon, and intron after 10, 14 and 18 weeks in EMF S2 clones exposed independently of electromagnetic radiation exposure distance (Table 5). The new point mutations that do not persisted till the end of the experiment should be considered as repaired by the DNA repair mechanism.

Table 5. Variable positions and nucleotide substitutions of APx1 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct EMR, K1, K2, K3 – plants exposed to indirect EMR. The promoter region is marked in blue, the rest is the intron

Week	Sample	39 (28286)		71 (28318)	72 (28319)	73 (28320)		191 (28438)	199 (28446)	382 (28629)			465 (28712)	482 (28729)			581 (28828)			586 (28833)		607 (28854)			629 (28876)		664 (28911)		668 (28915)	669 (28916)	677 (28924)	678 (28925)			694 (28941)				717 (28964)				730 (28977)
	Apx1 Sta-2	G	-	T	C	T	-	G	A	G	-	-	C	T	-	-	G	-	-	C	-	C	-	-	G	-	T	-	A	T	C	T	-	-	G	-	-	-	C	-	-	-	G
10 week	K1	-	-	.	.	C	T	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	A	.	-	-	-	.	-	-	-	.
	K2	.	-	.	.	.	C	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	K3	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E1	-	-	-	-	-	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	C	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E2	.	-	-	-	-	-	.	.	.	C	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	A	A	.	.	.	.	-	-	.	-	-	-	.	T	-	T	.
	E3	-	-	-	-	-	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
14 week	K1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	A	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	K2	.	-	.	.	C	C	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	K3	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	T	C	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	C	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E3	.	-	.	.	.	-	.	G	.	-	-	.	.	-	-	.	-	-	.	-	.	G	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
18 week	K1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	C	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	G	.	-	-	-	.	-	-	-	A
	K2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	K3	.	A	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	A	.	-	-	-	.	-	-	-	.
	E3	.	-	.	.	C	-	T	.	.	-	-	T	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	T	.	-	-	-	.
48 week	E1.1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E1.2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	A	.	-	-	-	.	-	A	-	.
	E2.1	A	G	.	.	.	-	.	.	.	-	C	.	.	T	-	.	G	C	A	T	.	-	A	A	A	A	A	T	.	.	.	G	G	.	G	T	-	G	C	G	T	A
	E2.2	.	-	.	.	.	-	.	.	.	-	-	.	C	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.
	E3.1	.	-	.	.	.	-	.	.	C	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	A	T	C	T	C	T	-	A	-	-	T	.	-	-	-	A
	E3.2	.	-	C	T	C	T	.	.	.	-	-	.	.	-	-	.	-	-	.	-	.	-	-	.	-	.	-	.	.	.	.	-	-	.	-	-	-	.	-	-	-	.

Comparison of growth parameters of *L. minor* clones exposed to 1 μT, 2 μT, and 300 μT magnetic flux revealed positive effect stimulating growth of experimentally affected plants at 2 μT. After the first 14 weeks of treatment, the growth parameters were lower in the directly exposed to LF EMR group than in the group grown distantly from the source of EMR. However, after 18 weeks from the beginning of the experiment no significant difference was observed between two groups of *L. minor* including directly and indirectly affected by LF EMR plants. Moreover, the signals of the impact of LF EMR on the plants rising point mutations were detected. The significantly enhanced number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones were revealed at the introns of APx ($P = 0.011$), GPx ($P = 0.009$), and Cat ($P = 0.044$) genes starting from the 10th week of the experiment.

In conclusion, the comparison of DNA sequencing data together with measurements of growth parameters revealed differences in response at molecular and physiological levels of directly and indirectly affected *L. minor* clones providing evidence that response to the impact of LF EMR depended on the prolongation of the impact and the magnetic flux density.

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Influence of peat substrate composition on indicators of physiological vitality of cloudberry (*Rubus chamaemorus* L.) during the rooting period

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Abstract: The growing of cloudberry *Rubus chamaemorus* in Latvia and globally is still done in rather small areas and harvests are mainly obtained in wild stands, thus making cloudberry cultivation in Latvia a promising research field. The aim of the study was to determine the most suitable substrate composition for successful rooting of cloudberry in greenhouse conditions. Results indicated that unfertilized peat (K) was the most suitable for cloudberry rooting, as it ensured the highest survival and vitality of plants in the first year.

Key words: cloudberry, rooting, peat, shoot physiological status, chlorophyll fluorescence

Introduction

The growing of cloudberry *Rubus chamaemorus* in Latvia and globally is still done in rather small areas and harvests are mainly obtained in wild stands, thus making cloudberry cultivation in Latvia a promising research field. Planting different berries in cutover peat bogs is a method successfully developed over the last 20–30 years. For example, in Latvia, the highbush blueberry *Vaccinium corymbosum* or the American cranberry *Vaccinium macrocarpon* are both widely commercially grown in extracted peatlands. Berry plantations are known to protect the upper peat layer from wind erosion and water loss, as well as to reduce GHG emissions. Production of high-quality berry yields and their highly demanded processed products also contribute to the local economic development. Determining the conditions for successful propagation of seedlings is an important aspect in the development of cloudberry cultivation technology. Therefore, the aim of the study was to determine the most suitable substrate composition for successful rooting of cloudberry in greenhouse conditions.

Material and methods

In 2020, hermaphroditic cloudberry variety *Nyby* (originating in Finland) was vegetatively propagated by dividing rhizomes and then planting them in 3 variations of peat substrate with different levels of acidity and nutrient supply. Variations were formed by mixing bare peat (K-without added lime or fertilizer), with limed and fertilized peat substrate (M1) in the following proportions: 1) K, 2) 1K:1M1, 3) M1. Rhizomes were divided into 10–15 cm long parts and planted in pots (11 × 11 × 15 cm in size). Only living parts of the rhizomes with a visible bud at the tip were planted (Fig. 1). A total of 300 new cloudberry seedlings were planted, 100 pots in each substrate variation.

The survival and vitality of plants were recorded in a non-destructive way. In 2020, in the middle of the growing season (July), when all the surviving buds had developed leaves, morphological parameters as number of young shoots, leaf amount and size were recorded to determine survival rates among substrates. In the second half of the growing season (August), when most of the leaves had fully developed, leaf plate size was measured for each variant (Fig. 2). In 2021, leaf measurements and survival recordings were carried out in May.

Figure 1. Cloudberry rhizome with a visible bud

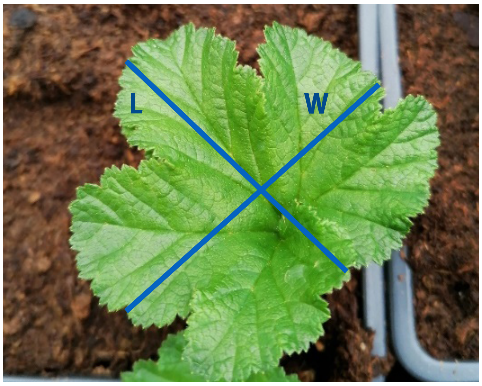

Figure 2. Leaf blade measurements. Leaf size = (L + W)/2

In 2020, chlorophyll fluorescence activity (Fv/Fm) and the complex fluorescence parameter Performance Index (PI) were measured using a chlorophyll fluorimeter *Handy PEA*, which indicate the overall physiological status of plants. The concentration of total *a* and *b* chlorophyll in SPAD units was measured with a chlorophyll meter, by which it is possible to detect changes in leaf chlorophyll as a reaction to various stressors, the parameter also indicates the general condition of the plant (Neufield et al., 2006, Yuan et al., 2016). Measurements were taken 3 times during the growing season (n = 30).

Results and discussion

First year results marked higher rooted cloudberry vitality and photosynthetic productivity in the bare peat variant (K), which held the highest values of all three chlorophyll-related parameters ($p < 0.05$). The optimal value of Fv/Fm is considered to be a result above 0.800, indicating good vitality of the plant (Öquist et al., 1992; Andersone et al., 2011). Only the leaves from cloudberries grown in substrate K reached this value. Meanwhile, the lowest values for these indicators were characteristics for cloudberries in substrate M1. The highest survival rates (number of shoots and leaves) were also observed in variant K – 78 young shoots with a total number of 218 leaves; the lowest rates – in variant M1: 24 young shoots with a total number of 51 leaves.

In the second year of development, describing the productivity of rooted cloudberry rhizomes, the highest results corresponded to M1 – on average 4.1 shoots per pot. It should be noted that in the first year, the rooting of cloudberry in substrate M1 was the least successful. For variant K, an average of 3.4 seedlings per pot were found.

There were no significant differences between the variants regarding the size of leaf blades in the first year: in substrate K the average leaf size was 4.47 cm, in 1K1M1 – 4.56 cm, in M1 – 4.52 cm (Fig. 3).

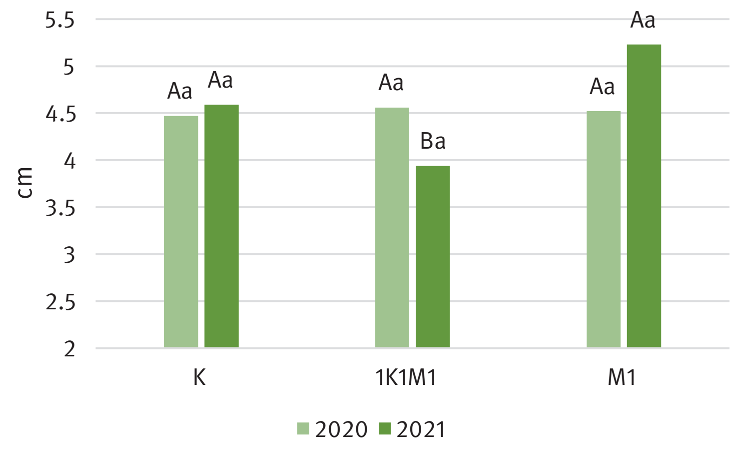

Figure. 3. Average cloudberry leaf blade size by substrate in study years 2020 and 2021. Values with different letters differ significantly (*p < 0.05*), according to Student's t-test

[1] Uppercase letters compare variants within a year.

[2] Lowercase letters compare two study years for each variant.

In the second year, the largest average leaf size was obtained for cloudberry seedlings grown in the nutrient-richest substrate M1 – 5.23 cm. However, differences between K and M1 were not significant (t-test), leaf size in substrate K on average was 4.59 cm. Only the 1K1M1 variant (3.94 cm) differed significantly (t-test, $p < 0.05$). Overall, there were no significant differences in either variant between study years regarding leaf size.

When propagating cloudberry, successful rooting is essential for high survival rates of rhizomes and their ability to form young shoots and vital leaves. According to these criteria, results indicated that unfertilized peat (K) was the most suitable for cloudberry rooting, as it ensured the highest survival and vitality of plants in the first year. Unfertilized or slightly fertilized peat substrate could be considered as most suitable for propagated cloudberry rooting.

In contrast, the heavily fertilized M1 substrate is not considered suitable for rooting, since the high concentrations of most nutrients, as well as too high pH and electric conductivity levels can suppress the development of seedlings in the first year. However, in the second year, higher green mass production in M1 indicated the positive effect of fertilizer on the rooted cloudberry to form shoots and leaves in further development. After rooting, cloudberry seedlings should be fertilized with low doses of fertilizer as it enhances growth of leaves and shoots. Further research including fertilizer and moisture effects on cloudberry production must be done to understand the preconditions of the first steps of cloudberry propagation and commercial growing.

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Study of the antioxidants and nutrients in cloudberry (*Rubus chamaemorus* L.) in Latvia

Short communication

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Abstract: Cloudberry (*Rubus chamaemorus* L.) is an economically important plant that is already cultivated. The aim of the study is to create and analyze a collection of cloudberry samples representing Latvian population.

Key words: antioxidants, phenolic compounds, macro- and microelements

Cloudberry (*Rubus chamaemorus* L.) is a herb species of the boreal zone with a distribution areal in the northern hemisphere. In Latvia cloudberry localities are close to the southern distribution area of the species in Europe (Thiem, 2003; Auniņš, 2013). It is an economically important plant that is already cultivated in Fennoscandia. Fresh cloudberry fruits and compounds derived from fruits and leaves contain several health-promoting substances: vitamins, flavonoids and phenolic acids with antioxidant properties (tannins, quercetin, naringenin). The most valuable components are those with antioxidant properties, such as ascorbic acid, carotenoids, and polyphenolic compounds (Jaakkola et al., 2012; Whaley et al., 2021).

The aim of the study is to create and analyze a collection of cloudberry samples representing Latvian population. Processed cloudberry seeds will be further used as breeding material for establishment of agricultural culture adapted to regional climatic and ground conditions.

Cloudberry samples from eight deposits in Latvia were analyzed. Presence of antioxidants including ascorbic acid, β-carotene, xanthophylls and total phenols were determined in the berries. Macroelements – N, P, K, Ca, Mg, S and microelements – Fe, Mn, Zn, Cu, Mo, B were determined in the leaves.

Level of all detected antioxidants found in berries harvested in bogs Baltais and Zaļezers was higher in comparison to samples representing Nītaure and Lielais un Pemme bog. Highest content of all identified macro- and microelements was found in the leaves harvested in Lauga and Pelečāre bogs.

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