Alterations in the Low Molecular Weight Metabolome of *Potamogeton perfoliatus* L., One of the Key Species in Littoral Habitats of Lake Ladoga, Concerning Phytoplankton Development

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Abstract: Perfoliate pondweed (*Potamogeton perfoliatus* L.) is one of the key species in plant associations in the coastal waters of Lake Ladoga. The chromatograph mass spectrometric study of the low molecular weight metabolome (LMWM) of this species growing in various littoral biotopes of Lake Ladoga, which differ in the degree of anthropogenic load and phytoplankton development, revealed the relationships between the LMWM of *P. perfoliatus* and the degree of anthropogenic disturbance of littoral biotopes, as well as the level of phytoplankton development and its composition. We noted a decrease in the content of low molecular weight organic compounds, as well as the absolute and relative content of fatty acids in the LMWM of *P. perfoliatus*, with increasing anthropogenic impact. The observed changes in LMWM of *P. perfoliatus* are a reflection of physiological processes that ensure the ecological and biochemical adaptation of the plant to changing environmental conditions, the deterioration of which as a result of excessive development of cyanobacteria is often associated with anthropogenic factors. Changes in the LMWM of *P. perfoliatus* can be used as diagnostic signs of deterioration of the lake environment in the coastal zone of Lake Ladoga. The anthropogenic factor decreases the stability and resistance of the aquatic ecosystem, making it more vulnerable to harmful algal blooms due to reduced synthesis of fatty acids by macrophytes.

Keywords: Low-molecular-weight metabolome, Fatty acids, Cyanobacteria, Aquatic macrophytes, *Potamogeton perfoliatus*, Anthropogenic impact, Aquatic ecosystems, Phytoplankton, Lake Ladoga, Indication of ecological state, Eutrophication, Pollution.

INTRODUCTION

The unique ecosystem of Lake Ladoga, the biggest freshwater lake in Europe, has long been a major area of study for limnologists, hydrobiologists, and environmental protectionists (Rumiantsev & Kondratyev, 2013). Significant anthropogenic influences (mostly eutrophication and pollution) have caused unfavorable changes in the littoral biocenoses in some regions of the lake's littoral zone, which is essential to the ecosystem as a whole (Kurashov, 2011). Harmful algal blooms (mostly cyanobacterial blooms) (HAB) are one of the most destructive processes linked to anthropogenic influences; hence, it is imperative to investigate the effects of excessive cyanobacterial development in the lake and look for ways to decrease it. The consequences of HAB development include a variety of threats to aquatic ecosystems as a whole, as well as to aquatic organisms, semi-aquatic animals, and humans (Šulčius *et al*., 2017; Huisman *et al*., 2018). Furthermore, growing anthropogenic impacts and climate change are continuously making these issues worse (Gobler, 2019; Griffith, Gobler, 2019; Brenckman *et al*., 2025).

The findings from the investigation of low-molecular-weight organic compounds (LMWOC) in aquatic ecosystems (Gurevich, 1953, 1973; Fink, 2007; Kurashov *et al*., 2014; Nezbrytska *et al*., 2022) indicate that allelopathy is a natural mechanism upon which contemporary nature-inspired technologies (Kovalchuk & Naraikin, 2017; Zhironkin *et al*., 2019; Nature-like…, 2019) for the management of phytoplankton communities in inland waters can be founded. This natural phenomenon may prove to be very useful for the effective prevention and reduction of HAB manifestations in water bodies (Hu & Hong, 2008; Macías *et al*., 2008; Kurashov *et al*., 2014; Kurashov *et al*., 2021; Reynolds & Aldridge, 2021; Gao *et al*., 2025).

Allelopathy can be found in aquatic and terrestrial environments (Mallik & Inderjit, 2002). It might show up as a plant's stimulatory or inhibitory effects on other plants and microorganisms, including cyanobacteria (Mushtaq *et al*., 2020). As in terrestrial ecosystems, allelopathic interactions linked to aquatic macrophytes appear to be common in the littoral zone of water

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bodies and, generally speaking, in shallow waters (Kurashov *et al*., 2021; Nezbrytska *et al*., 2022). Note that many fish species spawn and nurse in Lake Ladoga's littoral zone, which is particularly significant for young fish. Thus, maintaining the littoral zone's favorable biological state and comprehending and researching the mechanisms that support it are essential.

Key aquatic plants such as *Potamogeton perfoliatus* L. (perfoliate pondweed) play a significant role in shaping the lake environment (Raspopov, 2011). This species also exhibits allelopathic effects on phytoplankton, including cyanobacteria, by the synthesis of allelochemicals—particularly carboxylic acids—that inhibit the growth of cyanoprokaryotes (Kurashov *et al*., 2018).

The substantial potential of fatty acids as allelochemicals that support aquatic ecosystem restoration and protection from HAB has so far been demonstrated by observational and experimental research (Kurashov *et al*., 2021; Nezbrytska *et al*., 2022; Krylova *et al*., 2023). However, there is proof that anthropogenic pressure reduces this potential (Kurashov *et al*., 2018; Kurashov *et al*., 2024).

This study seeks to elucidate the correlation between the low-molecular-weight metabolome (LMWM) of *P. perfoliatus* in various water zones of Lake Ladoga, characterized by differing levels of anthropogenic impact and phytoplankton development, specifically focusing on carboxylic acids generated by perfoliate pondweed.

MATERIALS AND METHODS

The material for this study consisted of *P. perfoliatus* samples collected during the flowering and fruiting phase (late July – early August 2019) in various littoral habitats of Lake Ladoga, differing in the level of anthropogenic impact (Figure **1**).

According to available information on the state of the Ladoga littoral zone (Kurashov, 2011; Mitrukova *et al*., 2020; Krylova *et al*., 2022), sampling was carried out in three types of coastal waters of Lake Ladoga: 1. Conditions for minimal anthropogenic impact: the aquatory near Mantinsaari Island; 2. Conditions of high anthropogenic impact: the water area near Impiilahti Bay with a pronounced HAB; 3. Conditions of moderate, multi-faceted anthropogenic impact: the waters near Koyonsaari Island, the mouth of the Svir River, in Volkhov Bay (Voronovo settlement), and Osinovets Cape.

Macrophyte samples were collected at standard littoral stations (Kurashov, 2011; Figure **1**) during the

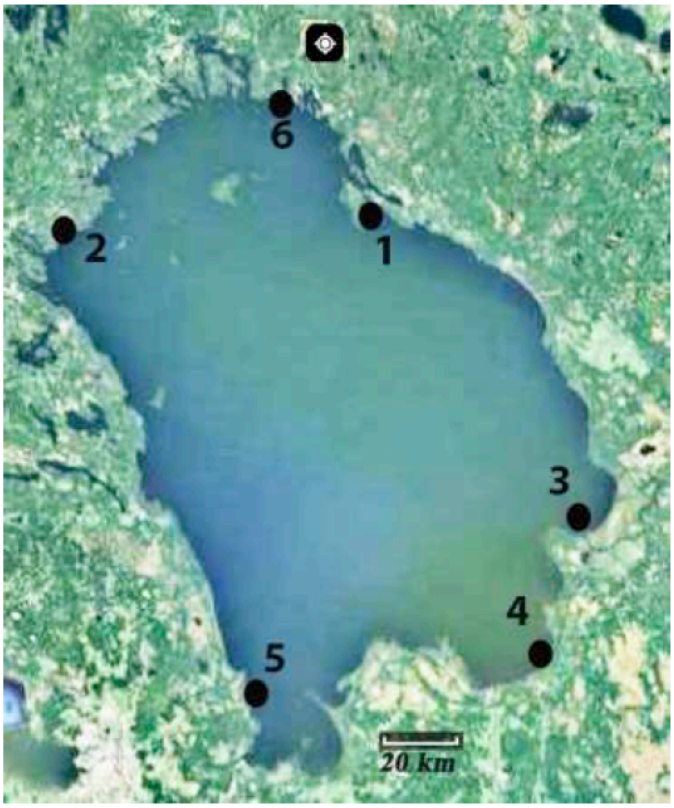

Figure 1: Location of *P. perfoliatus* sampling sites in Lake Ladoga: **1** – Mantinsaari Island; **2** – Koyonsaari Island; **3** – Svirskaya Bay; **4** – Volkhov Bay (Voronovo settlement); **5** – Osinovets Cape; **6** – Impilahti Bay (cartographic materials from the website https://www.google.ru/maps/ were used).

period of maximum plant vegetation. Simultaneously, phytoplankton samples were collected from the same biotopes. The collected plants were carefully washed free of any overgrowth and dried in the shade, away from direct sunlight, until they reached an air-dry state. Essential oil containing LMWOC was obtained from dried plants by hydrodistillation for 6 hours using a Clevenger apparatus (Clevenger, 1928; GOST (State Standard) 1980, 1988). Before distillation, the dry plant material was ground to a powdered state in a Waring BB-25ES blender (Waring, USA). To obtain the essential oil, 9-15 g samples of dry plant material were taken. The resulting distillate was extracted with hexane. The hexane extracts for further chromatograph mass spectrometric analysis was stored in tightly closed vials in a freezer at T = –18°C.

The qualitative and quantitative composition of LMWOC in LMWM of *P. perfoliatus* was identified using gas chromatography-mass spectrometry with a SHIMADZU GCMS-QP2010 Ultra mass spectrometry complex equipped with a non-polar MTX-1 column 30 m × 0.25 mm × 0.25 µm. Helium served as the carrier gas. Mass spectra were recorded in full scan mode (30–1090 m/z) using a temperature program (35°C – 3 min hold, heating at 2°C/min to 60°C – 3 min hold, heating at 2°C/min to 80°C – 3 min hold, heating at 4°C/min to 120°C – 3 min hold, heating at 5°C/min to 150°C – 3 min hold, heating at 15°C/min to 240°C – 10 min hold). Non-automatic step-by-step processing of chromatograms was used to identify LMWOCs. The LMWOCs were identified using the NIST-2014 and Wiley mass spectra libraries (Wiley, 2011; NIST/EPA/NIH…, 2014). For more accurate identification, linear retention indices (Tkachev, 2008) were used, obtained using C7–C30 alkane standards. Quantitative analysis was performed using benzophenone as an internal standard.

Phytoplankton studies were based on methodological protocols described in (Abakumov, 1983; Krylov, 2024). Water samples were collected from the surface layer of the water using a bathometer. The samples were fixed with Lugol's solution (10 g potassium iodide + 50 mL H_2O + 5 g iodine), followed by the addition of formalin to a final concentration of 4%. After settling and concentrating each sample, a portion of it was counted in a Nageotte chamber (V = 0.05 ml) under a light microscope (AxioLab A1 and AxioVert CFL 40 ("Carl Zeiss", Germany) (Guseva, 1959). Species identification was performed using light microscopy with the help of identification guides (Komárek & Fott, 1983; Topachevsky & Masyuk, 1984; Krammer & Lange-Bertalot, 1991; Barinova & Medvedeva, 1996).

RESULTS AND DISCUSSION

The study results demonstrated a clear relationship between the level of LMWOC synthesis by perfoliate pondweed and phytoplankton development in different littoral biotopes. The highest LMWOC content in the LMWM of *P. perfoliatus* was observed in this plant growing in the waters off Mantinsaari Island, the least affected by anthropogenic impact among the studied locations in the lake's intertidal zone (Figure **2**, Table **1**).

The lowest values of LMWOC content were observed in samples of pondweed from the habitat at the exit from Impilahti Bay into the open lake at maximum values of phytoplankton abundance. This habitat is characterized by the greatest development of

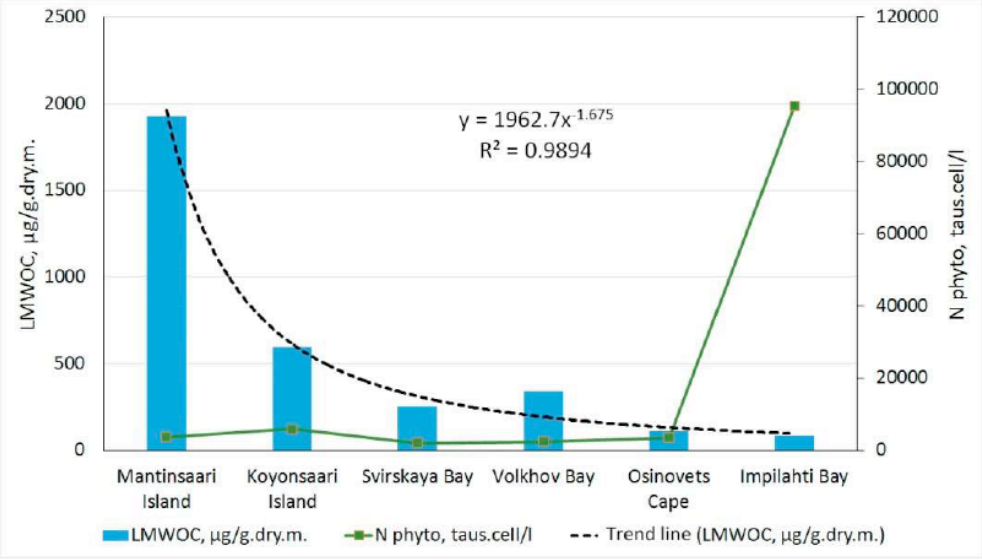

Figure 2: The relationship between the content of low-molecular-weight organic compounds (LMWOC, µg/g.dry.m.) in the NM of *P. perfoliatus* and the number of phytoplankton (N phyto, thousand cells/l) in the littoral biotopes of Lake Ladoga.

Table 1: Total Content of LMWOC (µg/g.dry.m.), Composition and Content (%) of Fatty Acids in LMWM of *P. perfoliatus* from Different Littoral Biotopes in Lake Ladoga

Compounds	Formula	Mantinsaari Island	Koyonsaari Island	Svirskaya Bay	Volkhov Bay	Osinovets Cape	Impilahti Bay
Dodecanoic acid; [Lauric Acid]	$C_{12}H_{24}O_2$	1.42	0.93	–	0.14	1.82	2,14
Tridecanoic acid	$C_{13}H_{26}O_2$	–	0.36	–	–	0.15	–
Tetradecanoic acid; [Myristic acid]	$C_{14}H_{28}O_2$	11.44	20.54	7.05	4.98	3.18	11,96
Pentadecanoic acid	$C_{15}H_{30}O_2$	1.26	4.32	0.91	0.90	0.99	4,32
(Z)-hexadec-9-enoic acid; [Palmitoleic acid]	$C_{16}H_{30}O_2$	6.70	0.37	5.29	3.10	5.35	16,64
Hexadecanoic acid; [Palmitic acid]	$C_{16}H_{32}O_2$	38.09	17.63	35.79	26.37	16.97	7,14
Heptadecanoic acid [Margaric acid]	$C_{17}H_{34}O_2$	0.28	0.31	–	0.11	0.32	–
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid; [alpha-Linolenic acid]	$C_{18}H_{30}O_2$	–	5.33	–	1.08	0.58	–
(9Z,12Z)-octadeca-9,12-dienoic acid; [Linoleic acid]	$C_{18}H_{32}O_2$	–	0.73	–	0.05	0.14	–
(Z)-octadec-9-enoic acid; [Oleic acid]	$C_{18}H_{34}O_2$	–	3.61	2.40	–	–	–
Octadecanoic acid; [Stearic acid]	$C_{18}H_{36}O_2$	0.76	1.44	1.82	0.39	0.57	–
(8E,11E,14E)-icosa-8,11,14-trienoic acid	$C_{20}H_{34}O_2$	0.91		1.36	3.09		–
(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraen oic acid; [Arachidonic acid]	$C_{20}H_{32}O_2$	–	0.01	–	–	–	–
Icosanoic acid; [Arachidic acid]	$C_{20}H_{40}O_2$	–	0.15	0.23	–	–	–
TOTAL, %		60.85	55.72	54.85	40.20	30.07	42.20
Total content of LMWOC, µg/g.dry.m.		1929	596	255	343	112	83

Note: Trivial or most frequently used names of acids are given in square brackets; "–" – component not identified.

cyanobacteria as part of the phytoplankton community (more than 95000 cells/mL; 91% of the total number).

In biotopes with moderate anthropogenic impact, a gradual decrease in the LMWOC content in the pondweed LMWM is observed. This dependence can be described by a power equation with the coefficient of determination R^2=0.9894 (Figure **2**).

As mentioned above, allelochemical substances in the LMWM of aquatic plants are of greatest interest, since they allow macrophytes to reduce the

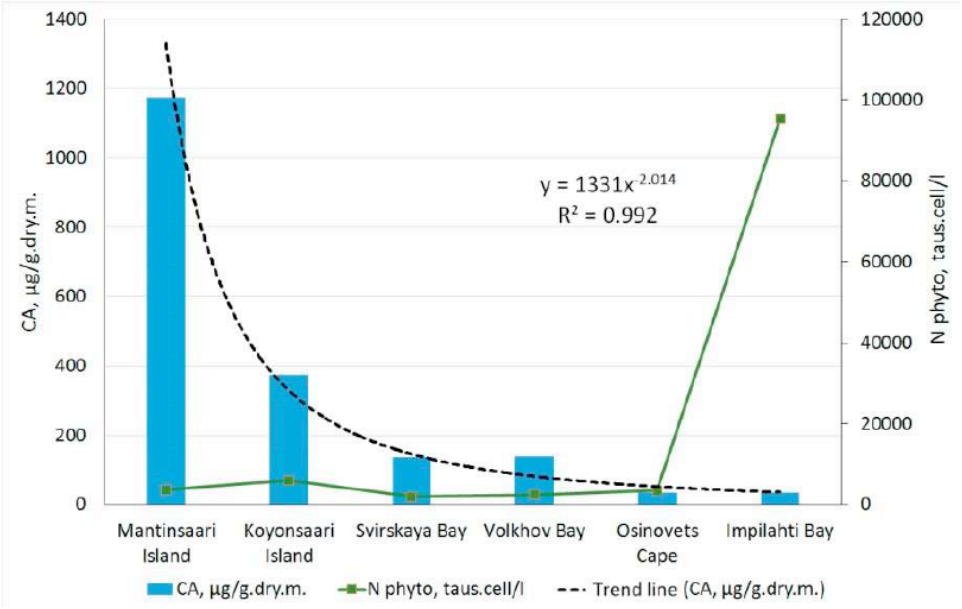

Figure 3: The relationship between the content of carboxylic acids (CA, µg/g dry weight) in LMWM of *P. perfoliatus* and the abundance of phytoplankton (N phyto, thousand cells/L) in the littoral habitats of Lake Ladoga.

development of phytoplankton, including cyanobacteria. Such allelochemicals, well represented in pondweed in Lake Ladoga, include carboxylic (fatty) acids (Krylova *et al*., 2024).

It turned out that, like the total content of LMWOC, the content of carboxylic acids in *P. perfoliatus* in Lake Ladoga is linked to the development of littoral phytoplankton with a very clear dependence (Figure **3**) with a coefficient of determination of R^2=0.992.

The proportion of carboxylic acids in the composition of LMWM of *P. perfoliatus* was also found to be related to phytoplankton abundance (Figure **4**). However, this relationship is less pronounced than in the first two cases (coefficient of determination R^2=0.7161).

A total of 14 fatty acids were identified in the studied samples of *P. perfoliatus* from various habitats in Lake Ladoga (Table **1**). At the same time, 9 of them acted as major components in some cases (content exceeding 1% of the total LMWOC content). Myristic acid, palmitic acid, and oleic acid have always been major components, but only the first two were present in all samples. In addition to myristic acid and palmitic acid, pentadecanoic acid and palmitoleic acid were detected in all samples, but in some cases, they could also be minor components of LMWM.

If we examine the composition and structure of phytoplankton in the most contrasting biotopes in terms of anthropogenic impact (Mantinsaari Island and Impilahti Bay), as well as in biotopes with moderate anthropogenic impact (as an example, Svirskaya Bay) (Figure **5**), we can note a pronounced dominance of cyanobacteria in the biotope with the highest anthropogenic impact (Impilahti Bay) and a more diverse algal community in biotopes with lower anthropogenic impact, where cyanobacteria accounted for only about 50% of the total phytoplankton abundance (Figure **5**).

The following species predominated in terms of abundance at Mantinsaari Island and in Svirskaya Bay: among cyanobacteria - *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek and *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault; among cryptophytes - the small-celled *Plagioselmis lacustris* (Pascher & Ruttner) Javornický; among diatoms - *Skeletonema subsalsum* (A. Cleve) Bethge; among golden algae - *Uroglenopsis americana* (G.N. Calkins) Lemmermann.

In the waters near Impilahti Bay, cyanobacteria were dominant in terms of abundance: *Dolichospermum lemmermannii* (P.G. Richter) P. Wacklin, L. Hoffmann & J. Komárek, *Aph. flos-aquae*, *Aphanizomenon gracile* Lemmermann, *Aphanizomenon klebahnii* (Elenkin) Pechar & Kalina ex Komárek & Komárková, *Limnothrix redekei* (Goor) Meffert, *Woronichinia naegeliana* (Unger) Elenkin.

The results obtained show that anthropogenic impact on the lake environment in such a large body of water as Lake Ladoga leads to a decrease in the biochemical impact of macrophytes on the lake environment, as the overall level of LMWOC synthesis, and particularly the synthesis of carboxylic acids, decreases. This conclusion aligns with the analysis conducted in the studies (Gladyshev *et al*., 2009; Kurashov *et al*., 2024), which showed that the role of

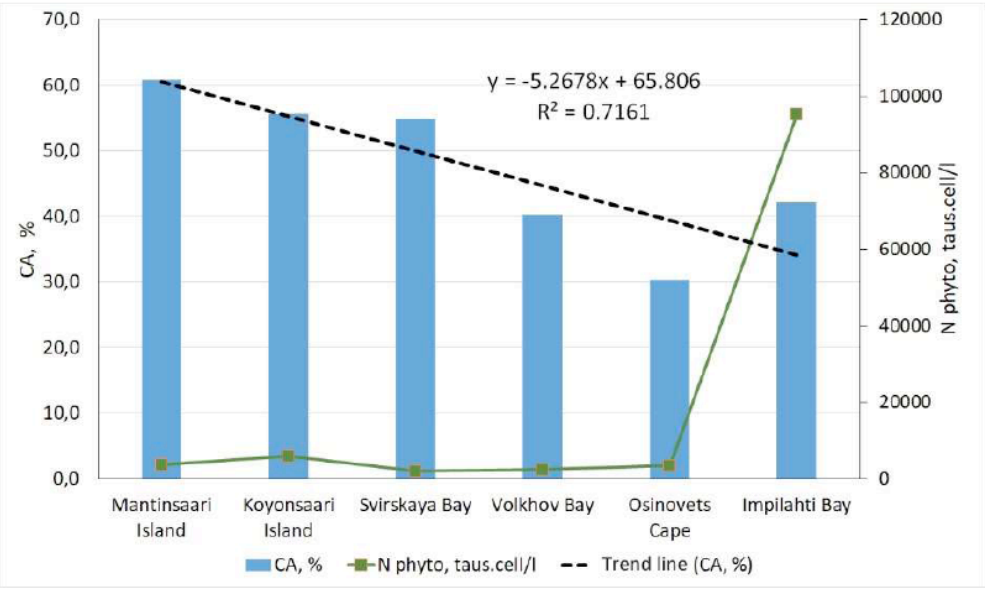

Figure 4: The relationship between the proportion of carbon acids (CA, %) in LMWM of *P. perfoliatus* and the abundance of phytoplankton (N phyto, thousand cells/L) in the littoral habitats of Lake Ladoga.

fatty acids produced by macrophytes and phytoplankton in aquatic ecosystems decreases with anthropogenic eutrophication and the proliferation of cyanobacteria.

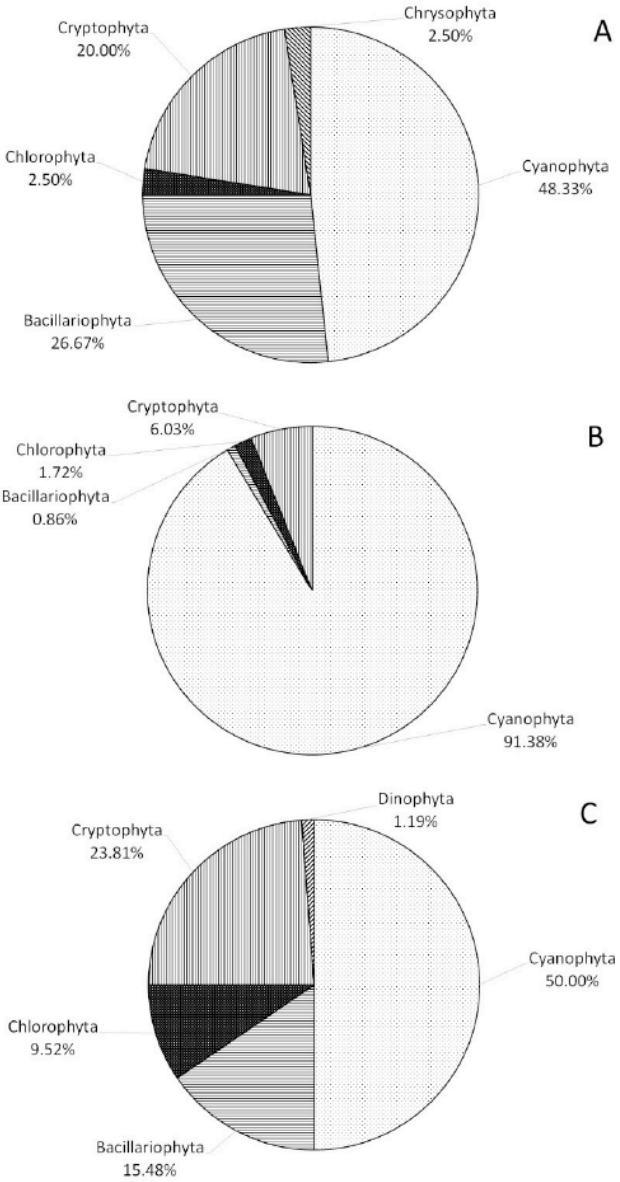

Figure 5: Phytoplankton structure by abundance in the littoral habitats of Lake Ladoga: A – Impilahti Bay; B – Mantinsaari Island; C – Svirskaya Bay.

The decrease in fatty acid synthesis by macrophytes leads to a reduction in the aquatic ecosystem's resistance to HAB development, as many fatty acids (including those identified in *P. perfoliatus* in the present study) are active allelochemicals and suppress the growth of cyanobacteria. Thus, a direct allelopathic effect on phytoplankton is characteristic and shown for lauric acid (Zhang *et al*., 2009a; Hu *et al*., 2010; Wang *et al*., 2015), myristic acid (Nakai *et al*., 2005; Zhang *et al*., 2009a; 2009b), pentadecanoic acid (Clément *et al*., 2007; Quan *et al*., 2022), palmitoleic acid (Wu *et al*., 2006; Krylova *et al*., 2023), palmitic acid (Nakai *et al*., 2012; Hu *et al*., 2025), linoleic acid (Nakai *et al*., 2005; 2012; Wang *et al*., 2015), α-linolenic acid (Gallardo-Williams *et al*., 2002; Mohamed, 2017), oleic acid (Nakai *et al*., 2012; Huang *et al*., 2025), stearic acid (Nakai *et al*., 2005; Wang *et al*., 2017), and arachidonic acid (Alamsjah *et al*., 2005; Sun *et al*., 2021).

Reliable existing literature sources suggest that margaric acid can play an allelopathic role in the aquatic environment through mechanisms common to free fatty acids (Li *et al*., 2014; Li *et al*., 2021; Nezbrytska *et al*., 2022). α-Linolenic acid can also indirectly act as an allelochemical, but not in its original form itself, but through its oxidation products (various oxylipins), which exhibit pronounced allelopathic activity. α-Linolenic acid acts as a precursor to bioactive oxylipins (jasmonates, phytoprostanes) that can mediate stress responses and interspecific chemical interactions (Leung *et al*., 2022).

At the same time, the most significant allelopathic synergistic effect is often observed when a mixture of different fatty acids acts on phytoplankton (Zhang *et al*., 2009a), meaning that macrophytes affect phytoplankton thru a so-called "allelopathic cocktail." It is the mixtures of fatty acids (saturated and unsaturated) with benzoic/lactic acids and polyphenols that are responsible in most cases for the allelopathic suppression of cyanobacteria by macrophytes in real aquatic environments (Nakai *et al*., 2012; Wang *et al*., 2015).

The relationships and results of the LMWOC and carboxylic acid content assessment in *P. perfoliatus*, obtained in this study based on material from 2019, are in complete agreement with similar results for this plant studied in a lake in 2014 (Krylova *et al*., 2024), including samples taken from habitats that do not overlap with those we investigated.

Thus, the results presented in this study, along with the results of research on the LMWOC of pondweed in 2014 (Krylova *et al*., 2024), show that the composition and content of LMWOC in LMWM (especially carboxylic acids) can be a good bioindicator of changes in the lake environment in the coastal zone of Lake Ladoga under the influence of anthropogenic factors.

The possibility of using changes in the chemical composition of pondweed LMWM, particularly in its glycopolymer and free amino acid content, for monitoring and indicating the impact of pollutants was demonstrated in studies of this species in habitats differing in the degree of nitrogen and oil hydrocarbon contamination in the Volga River near Saratov (Sachkova *et al*., 2005). As our data show, carboxylic acids can also serve the same purposes.

The results of this study are consistent with current evidence indicating that the low-molecular-weight metabolome of aquatic macrophytes is highly responsive to changing environmental conditions. Experimental research has shown that submerged plants undergo rapid biochemical adjustments when directly exposed to phytoplankton. In co-culture experiments with the cyanobacterium *Microcystis aeruginosa*, *Myriophyllum spicatum* exhibits the induction of novel low-molecular-weight metabolites and a distinct shift in its metabolic profile relative to control plants, reflecting the activation of specific physiological and biochemical pathways involved in plant–phytoplankton interactions (Nam *et al*., 2008). These findings demonstrate that the metabolome of macrophytes is highly sensitive to the presence and development of phytoplankton.

Field observations further support the link between macrophyte metabolomes, trophic state, and phytoplankton dynamics (Riedl *et al*., 2012). The metabolic fingerprint of *M. spicatum* differs systematically among lakes with contrasting water chemistry and trophic conditions, and also varies over time within the same population. Such spatial and temporal patterns are strongly associated with gradients in nutrient concentrations and other hydrochemical parameters that influence the structure and seasonal development of phytoplankton communities. Together, these results indicate that macrophytes record both short-term fluctuations and long-term ecosystem-level gradients through metabolomic adjustments, which makes their metabolome a sensitive indicator of ecological condition (Riedl *et al*., 2012).

A central role in these metabolomic responses is played by fatty acids, which act not only as structural components of plant cells but also as functional agents mediating biochemical interactions with phytoplankton. Several studies have shown that saturated and unsaturated fatty acids released by *M. spicatum*, including nonanoic, tetradecanoic, hexadecanoic, octadecanoic, and octadecenoic acids, account for a substantial proportion of the plant's inhibitory activity against *M. aeruginosa* (Nakai *et al*., 2005). Moreover, experimental mixtures constructed from measured release rates of polyphenols and fatty acids reproduce more than half of the allelopathic activity observed in intact plants (Nakai *et al*., 2012). These findings indicate that variation in fatty-acid profiles and release intensities is not a passive consequence of plant metabolism, but rather constitutes an active biochemical mechanism of ecological adaptation to HAB.

Finally, changes in fatty-acid composition also reflect macrophyte responses to abiotic and anthropogenic stressors. Exposure to heavy metals, such as cadmium, and to thermal stress causes rapid and highly specific alterations in the fatty-acid profiles of *M. spicatum* and *Elodea canadensis* (Kirichenko & Pobezhimova, 2013). These shifts are interpreted as metabolic markers of adaptive stress responses and have been proposed as indicators of environmental contamination and altered thermal regimes. Thus, the fatty-acid component of the macrophyte metabolome plays an essential role not only in the plant's interactions with phytoplankton but also in its adaptation to anthropogenic influences.

Our findings on *P. perfoliatus* from Lake Ladoga are consistent with the modern understanding that shifts in the LMWM of aquatic macrophytes arises from coordinated biosynthetic and regulatory mechanisms. Lake Ladoga is characterized by pronounced spatial and temporal variability in hydrochemical conditions and phytoplankton dynamics, including periodic cyanobacterial development in littoral zones. Under such conditions, the metabolome of *P. perfoliatus* reflects a suite of adaptive responses involving reconfiguration of metabolic fluxes, changes in fatty-acid composition, and other components of LMWM. It is possible that the central component of these shifts is the oxylipin (jasmonate) pathway, which mediates de novo synthesis of oxylipins from polyunsaturated fatty acids through the action of lipases, lipoxygenases and cytochrome P450 enzymes (Howe & Schilmiller, 2002). Jasmonates function as key signals (JA) that trigger transcriptional cascades and systemic defense responses, linking changes in fatty-acid–derived metabolites to gene-level regulatory processes. In macrophytes such as *P. perfoliatus*, activation of JA signaling is expected to redistribute carbon fluxes among respiration, storage, and defense-related biosynthesis—as shown in metabolomic studies of terrestrial plants (Savchenko *et al*., 2019; Xu & Fu, 2022) — and this framework perhaps explains the observed variability in fatty acids and the entire LMWM of *P. perfoliatus* in different habitats in Lake Ladoga.

These metabolic reconfigurations are frequently accompanied by the upregulation of specialized secondary metabolic pathways (Vogt, 2010). The phenylpropanoid pathway, regulated by transcription factors and hormonal signals, produces phenolic and flavonoid compounds with key photoprotective and antioxidant roles (Vogt, 2010). For *P. perfoliatus* growing in the chemical and trophic gradients characteristic of Lake Ladoga, activation of phenylpropanoid biosynthesis likely also represents an important mechanism of protection against oxidative stress and adverse factors.

Furthermore, studies in *Arabidopsis* and leafy vegetables demonstrate that signaling mechanisms and transcriptional networks act as integrated regulatory modules that coordinate complex metabolome responses (Kusano *et al*., 2011; Kitazaki *et al*., 2018). Given the wide variation in environmental conditions (including anthropogenic pressure) in Lake Ladoga, similar signaling processes are likely to influence the metabolic architecture of *P. perfoliatus*, including its fatty acid and phenolic pools.

CONCLUSION

Thus, the established correlation between the LMWM of *P. perfoliatus* and the extent of anthropogenic disruption in littoral biotopes, alongside the degree of phytoplankton proliferation and composition, indicates that the LMWM of aquatic macrophytes may serve as a metric for assessing the degradation of the aquatic ecosystems (including littoral zone of Lake Ladoga), particularly in relation to the emergence of HAB, as exemplified in Impilahti Bay. At the same time, standard, swiftly fluctuating hydrochemical measures may not consistently represent the comprehensive scenario linked to anthropogenic influence (Krylova *et al*., 2024).

A decrease in the absolute and relative content of fatty acids in the LMWM composition of macrophytes in any body of water during monitoring studies can be an indication that the anthropogenic load on this aquatic ecosystem, such as pollution or eutrophication, is increasing. Since each species of aquatic macrophytes has unique biological and ecological characteristics, particularly in terms of stressor resistance (including anthropogenic ones), it is likely that the observed pattern will not apply to all of them. This emphasizes the necessity of doing additional in-depth studies on the LMWM composition of aquatic macrophytes and its variations, broadening the scope of species examined in diverse ecological settings.

The anthropogenic factor leads to a decrease in the resilience and resistance of the aquatic ecosystem to such a negative factor as HAB thru processes that reduce the synthesis of fatty acids by macrophytes. Additionally, the high content of fatty acid - allelochemicals found in *P. perfoliatus* in Lake Ladoga suggests that this species could be a potential raw material source for obtaining allelochemicals for their further use in creating new-generation algicides (Kurashov *et al*., 2021).

Taken together, the available evidence demonstrates that the LMWM of aquatic macrophytes—particularly its fatty-acid fraction—forms an integrated biochemical system that reflects the combined effects of biotic interactions, ecosystem-level trophic gradients, and anthropogenic stressors. This highlights the value of metabolomic approaches for assessing ecological adaptation and environmental status in aquatic ecosystems.

In conclusion, metabolome shifts in *P. perfoliatus* from Lake Ladoga reflect the activity of integrated hormonal, transcriptional, and enzymatic regulatory systems that enable the plant to adapt to changing phytoplankton dynamics, hydrochemical regime, and trophic gradients. These findings support the use of macrophyte metabolomes as sensitive indicators of ecological conditions in freshwater ecosystems.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

[1] Abakumov VA, editor. Guide to Methods of Hydrobiological Analysis of Surface Waters and Bottom Sediments. L.: Gidrometeoizdat; 1983. (in Russian)

[2] Alamsjah MA, Hirao S, Ishibashi F, Oda T, Fujita Y. Isolation and structure determination of algicidal principles from Ulva fasciata. Biosci Biotechnol Biochem 2005; 69(11): 2186-2192. https://doi.org/10.1271/bbb.69.2186

[3] Barinova SS, Medvedeva LA. Atlas of Algae Indicators of Saprobity (Russian Far East). Vladivostok: Dalnauka; 1996. 364 p. (in Russian)

[4] Brenckman CM, Parameswarappa Jayalakshmamma M, Pennock WH, Ashraf F, Borgaonkar AD. A Review of Harmful Algal Blooms: Causes, Effects, Monitoring, and Prevention Methods. Water 2025; 17: 1980. https://doi.org/10.3390/w17131980

[5] Clément M, Tremblay J, Lange M, Thibodeau J, Belhumeur P. Whey-derived free fatty acids suppress the germination of Candida albicans *in vitro*. FEMS Yeast Res 2007; 7(2): 276-285. https://doi.org/10.1111/j.1567-1364.2006.00166.x

[6] Clevenger JF. Apparatus for the determination of volatile oil. J Am Pharm Assoc 1928; 17(4): 345-349. https://doi.org/10.1002/jps.3080170407

[7] Fink P. Ecological functions of volatile organic compounds in aquatic systems. Mar Freshw Behav Physiol 2007; 40(3): 155-168. https://doi.org/10.1080/10236240701602218

[8] Gallardo-Williams MT, Geiger CL, Pidala JA, Martin DF. Essential fatty acids and phenolic acids from extracts and leachates of southern cattail (Typha domingensis P.).

Phytochemistry 2002; 59(3): 305-308.
https://doi.org/10.1016/S0031-9422(01)00449-6

[9] Gao X, Gao AG, Bai ZX, Yuan JR, Liang J, Wu AP. Submerged plant allelopathic effects on the growth of a harmful bloom-forming cyanobacterium (Microcystis aeruginosa): a diversity gradient experiment. Mar Freshw Res 2025; 76: MF23149.
https://doi.org/10.1071/MF23149

[10] Gladyshev MI, Arts MT, Sushchik NN. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA+DHA) from aquatic to terrestrial ecosystems. In: Lipids in Aquatic Ecosystems. Springer; 2009: 179-210.
https://doi.org/10.1007/978-0-387-89366-2_8

[11] Gobler CJ. Climate change and harmful algal blooms: insights and perspective. Harmful Algae 2019; 91: 101731.
https://doi.org/10.1016/j.hal.2019.101731

[12] GOST 24027.2-80. Medicinal plant raw materials. Methods for determining moisture, ash content, extractive and tannins of essential oil. Moscow: Izd. Standartov; 1980. (in Russian)

[13] GOST 7082.5-88. Fruits of essential oil crops. Industrial raw materials. Methods for determining the mass fraction of essential oil. Moscow: Izd. Standartov; 1989: 13-24. (in Russian)

[14] Griffith AW, Gobler CJ. Harmful algal blooms: a climate change co-stressor in marine and freshwater ecosystems. Harmful Algae 2019; 91: 101590.
https://doi.org/10.1016/j.hal.2019.03.008

[15] Gurevich FA. On the protistocidal properties of aquatic and coastal-aquatic plants. In: Proceedings of Krasnoyarsk State Medical Institute. 1953; 3: 212-214. (in Russian)

[16] Gurevich FA. Phytoncides of aquatic and coastal plants, their role in hydrobiocenoses. Doctoral dissertation abstract. Irkutsk State University named after A.A. Zhdanov, Irkutsk; 1973. (in Russian)

[17] Guseva KA. On the method of accounting for phytoplankton. Proc Inst Biol Reservoirs 1959; 2: 44-51. (in Russian)

[18] Howe GA, Schilmiller AL. Oxylipin metabolism in response to stress. Curr Opin Plant Biol. 2002; 5(3): 230-236.
https://doi.org/10.1016/S1369-5266(02)00250-9

[19] Hu CY, Ge FJ, Zhang SH, Liu BY, Wang J, Gao YN, Wu ZB. Isolation of antialgal compounds from Potamogeton malaianus and algal inhibitory effects of common fatty acids. J Lake Sci 2010; 22(4): 569-576.

[20] Hu H, Hong Y. Algal-bloom control by allelopathy of aquatic macrophytes—a review. Front Environ Sci Eng China 2008; 2(4): 421-438.
https://doi.org/10.1007/s11783-008-0070-4

[21] Hu N, Tan Y, Xiao X, Gao Y, Zheng K, Qian W, Zhang Y, Zhao Y. Antialgal effects of nonanoic and palmitic acids on Microcystis aeruginosa and the underlying mechanisms. Sustainability 2025; 17(3): 1207.
https://doi.org/10.3390/su17031207

[22] Huang X, Hu Y, Cheng X, Pan W. Preliminary study on the inhibitory effect and mechanism of oleic acid in Cylindrospermopsis raciborskii. Water 2025; 17(17): 2561.
https://doi.org/10.3390/w17172561

[23] Huisman J, Codd GA, Paerl HW, Ibelings BW, Verspagen JMH, Visser PM. Cyanobacterial blooms. Nat Rev Microbiol 2018; 16: 471-483.
https://doi.org/10.1038/s41579-018-0040-1

[24] Kirichenko K, Pobezhimova T. The influence of cadmium chloride and hyperthermia on the fatty acid composition of high aquatic plants from Angara River. Biologija. 2013; 59(3).
https://doi.org/10.6001/biologija.v59i3.2787

[25] Kitazaki K, Fukushima A, Nakabayashi R, Okazaki Y, Kobayashi M, Mori T, Nishizawa T, Reyes-Chin-Wo S, Michelmore RW, Saito K, Shoji K, Kusano M. Metabolic reprogramming in leaf lettuce grown under different light quality and intensity conditions using narrow-band LEDs. Sci Rep. 2018; 8(1): 7914.
https://doi.org/10.1038/s41598-018-25686-0

[26] Komárek J, Fott B. Das Phytoplankton des Susswassers: Systematik und Biologie. Binnengewasser. 1983. V. 7.

[27] Kovalchuk MV, Naraikin OS. Nature-like technologies—new capacities and new challenges. Security Index 2017; 22(3-4): 118-119.

[28] Krammer K, Lange-Bertalot H. Bacillariophyceae. Teil 3: Centrales, Fragilariaceae, Eunotiaceae. Susswasser flora von Mitteleuropa. B. 2(3). Stuttgart, Jena; 1991.

[29] Krylov AV, editor. Methods of hydrobiological studies of inland waters. Borok, Yaroslavl region: IBIW RAS; Yaroslavl: Filigree; 2024. (in Russian)

[30] Krylova JV, Kurashov EA, Ponomarenko AM, Svetashova ES, Sinyakova MA, Ekimova SB, Protopopova EV, Kolosovskaya EV, Grebennikov VA. Assessment of the ecological state of the littoral zone of Lake Ladoga based on research results of 2019. Proc Karelian Sci Centre RAS 2022; 6: 102-120. (in Russian)
https://doi.org/10.17076/lim1474

[31] Krylova JV, Kurashov EA, Protopopova EV, Khodonovich VV, Iavid EYa. Saturated and unsaturated fatty acids as potential allelochemicals for aquatic ecosystems rehabilitation. Ecosyst Transform 2023; 6(5): 29-42.
https://doi.org/10.23859/estr-221005

[32] Krylova JV, Kurashov EA, Protopopova EV, Khodonovich VV, Yavid EYa, Kuchareva GI. Composition of the low molecular weight metabolome of Potamogeton perfoliatus (Potamogetonaceae) as an indicator of the transformation of the ecological state of the littoral zone. Inland Water Biol 2024; 17(4): 560-570.
https://doi.org/10.1134/S1995082924700202

[33] Kurashov EA, editor. The Littoral Zone of Lake Ladoga. St. Petersburg: Nestor-Istoriya; 2011. (in Russian)

[34] Kurashov EA, Krylova JV, Chernova AM, Khodonovich VV, Yavid EYa. On the ecosystem and indicator significance of fatty acids in the low molecular weight metabolome of water macrophytes. Inland Water Biol 2024; 17(5): 921-925.
https://doi.org/10.1134/S1995082924700585

[35] Kurashov EA, Krylova JV, Egorova AA, Khamitov AS, Khodonovich VV, Yavid EYa. Prospects for using the low-molecular-weight metabolome of aquatic macrophytes to indicate the ecological state of aquatic ecosystems. Water: Chem Ecol 2018; 1-3: 68-79. (in Russian)

[36] Kurashov EA, Krylova JV, Mitrukova GG, Chernova AM. Low-molecular-weight metabolites of aquatic macrophytes growing on the territory of Russia and their role in hydroecosystems. Contemp Probl Ecol 2014; 7(4): 433-448.
https://doi.org/10.1134/S1995425514040064

[37] Kurashov EA, Krylova JV, Protopopova EV. The use of allelochemicals of aquatic macrophytes to suppress the development of cyanobacterial blooms. In: Pereira L, Gonçalves AM, editors. Plankton Communities. London: IntechOpen; 2021.
https://doi.org/10.5772/intechopen.95609

[38] Kusano M, Tohge T, Fukushima A, Kobayashi M, Hayashi N, Otsuki H, Kondou Y, Goto H, Kawashima M, Matsuda F, Niida R, Matsui M, Saito K, Fernie AR. Metabolomics reveals comprehensive reprogramming involving two independent metabolic responses of Arabidopsis to UV-B light. Plant J. 2011; 67(2): 354-369.
https://doi.org/10.1111/j.1365-313X.2011.04599.x

[39] Leung KS, Oger C, Guy A, Vigor C, Durand T, Lee JCY. Alpha-linolenic acid, phytoprostanes and phytofurans in plant, algae and food. In: Advances in Botanical Research. 2022; 101: 437-468.
https://doi.org/10.1016/bs.abr.2021.09.005

[40] Li B, Yin Y, Kang L, Feng L, Liu Y, Du Z, Tian Y, Zhang L. Application of allelochemicals in water ecological restoration—algal inhibition. Chemosphere 2021; 267: 128869.
https://doi.org/10.1016/j.chemosphere.2020.128869

[41] Li D, Zhang H, Fu L, An X, Zhang B, Li Y, Chen Z, Zheng W, Yi L, Zheng T. A novel algicide: evidence of the effect of a fatty acid compound from the marine bacterium Vibrio sp. BS02 on the harmful dinoflagellate Alexandrium tamarense. PLoS One 2014; 9(3): e91201.
https://doi.org/10.1371/journal.pone.0091201

[42] Macías FA, Galindo JLG, García-Díaz MD, Galindo JCG. Allelopathic agents from aquatic ecosystems: potential biopesticide models. Phytochem Rev 2008; 7: 155-178. https://doi.org/10.1007/s11101-007-9065-1

[43] Mallik AU, Inderjit, editors. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Basel: Springer; 2002. https://doi.org/10.1007/978-3-0348-8109-8

[44] Mitrukova GG, Kapustina LL, Kurashov EA. Ecological assessment of the water quality of the littoral zone of Lake Ladoga based on microbiological studies. Proc Karelian Sci Centre RAS 2020; 9: 88. https: //doi.org/10.17076/lim1277 (in Russian) https://doi.org/10.17076/lim1277

[45] Mohamed ZA. Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management—a review. Limnologica 2017; 63: 122-132. https://doi.org/10.1016/j.limno.2017.02.006

[46] Mushtaq W, Siddiqui MB, Hakeem KR. Allelopathy: Potential for Green. 2020. https://doi.org/10.1007/978-3-030-40807-7

[47] Nakai S, Yamada S, Hosomi M. Anti-cyanobacterial fatty acids released from Myriophyllum spicatum. Hydrobiologia 2005; 543: 71-78. https://doi.org/10.1007/s10750-004-6822-7

[48] Nakai S, Zou G, Okuda T, Nishijima W, Hosomi M, Okada M. Polyphenols and fatty acids responsible for anti-cyanobacterial allelopathic effects of submerged macrophyte Myriophyllum spicatum. Water Sci Technol 2012; 66(5): 993-999. https://doi.org/10.2166/wst.2012.272

[49] Nam S, Joo S, Kim S, Baek N-I, Choi H-K, Park S. Induced metabolite changes in Myriophyllum spicatum during co-existence experiment with the cyanobacterium Microcystis aeruginosa. J Plant Biol. 2008; 51: 373-378. https://doi.org/10.1007/BF03036141

[50] Nature-like and Convergent Technologies Driving the Fourth Industrial Revolution. Vienna: United Nations Industrial Development Organization; 2019.

[51] Nezbrytska I, Usenko O, Konovets I, Leontieva T, Abramiuk I, Goncharova M, Bilous O. Potential use of aquatic vascular plants to control cyanobacterial blooms: a review. Water 2022; 14(11): 1727. https://doi.org/10.3390/w14111727

[52] NIST/EPA/NIH MS/MS Mass Spectral Library. 2014. Available from: https: //www.sisweb.com/software/nist-msms-2014.htm [accessed 2025 October 10].

[53] Quan W, Wang A, Li C, Xie L. Allelopathic potential and allelochemical composition in different soil layers of Rhododendron delavayi forest, southwest China. Front Ecol Evol 2022; 10: 963116. https://doi.org/10.3389/fevo.2022.963116

[54] Raspopov IM. Species and coenotic diversity of higher aquatic and coastal-aquatic plants in the littoral zone of Lake Ladoga. In: Kurashov EA, editor. The Littoral Zone of Lake Ladoga. St. Petersburg: Nestor-Istoriya; 2011. p. 52-64. (in Russian)

[55] Reynolds SA, Aldridge DC. Embracing the allelopathic potential of invasive aquatic plants to manipulate freshwater ecosystems. Front Environ Sci 2021; 8: 551803. https://doi.org/10.3389/fenvs.2020.551803

[56] Riedl J, Kluender C, Sans-Piché F, Heilmeier H, Altenburger R, Schmitt-Jansen M. Spatial and temporal variation in metabolic fingerprints of field-growing Myriophyllum spicatum. Aquat Bot. 2012; 102: 34-43. https://doi.org/10.1016/j.aquabot.2012.03.017

[57] Rumiantsev VA, Kondratyev SA, editors. Ladoga. St. Petersburg: Nestor-Istoriya; 2013. (in Russian)

[58] Sachkova OA, Konnova SA, Ignatov VV, Melnikov GV. Study of carbohydrates and free amino acids of higher aquatic plants. Izv Saratov Univ New Ser Chem Biol Ecol 2005; 5(2): 3-7. (in Russian)

[59] Savchenko TV, Rolletschek H, Dehesh K. Jasmonates-mediated rewiring of central metabolism regulates adaptive responses. Plant Cell Physiol. 2019; 60(12): 2613-2620. https://doi.org/10.1093/pcp/pcz181

[60] Šulčius S, Montvydienė D, Mazur-Marzec H, Kasperovičienė J, Rulevičius R, Cibulskaitė Ž. The profound effect of harmful cyanobacterial blooms: from food-web and management perspectives. Sci Total Environ 2017; 609: 1443-1450. https://doi.org/10.1016/j.scitotenv.2017.07.253

[61] Sun S, Hu S, Zhang B, Sun X, Xu N. Allelopathic effects and potential allelochemical of Sargassum fusiforme on red-tide microalga Heterosigma akashiwo. Mar Pollut Bull 2021; 170: 112673. https://doi.org/10.1016/j.marpolbul.2021.112673

[62] Tkachev AV. Study of Plant Volatiles. Novosibirsk: Ofset; 2008. 969 p. (in Russian)

[63] Topachevsky AV, Masyuk NP. Freshwater Algae of the Ukrainian SSR. In: Makarevich MF, editor. Kyiv: Vyshcha Shkola; 1984. 336 p. (in Russian)

[64] Vogt T. Phenylpropanoid biosynthesis. Mol Plant. 2010; 3(1): 2-20. https://doi.org/10.1093/mp/ssp106

[65] Wang H, Liang F, Zhang L. Composition and anti-cyanobacterial activity of essential oils from six different submerged macrophytes. Pol J Environ Stud 2015; 24(1): 333-338. https://doi.org/10.15244/pjoes/26383

[66] Wang H, Zhang L, Wang Y. Isolating and identifying organic acids from Portulaca oleracea and their allelopathic effects on Microcystis aeruginosa. Pol J Environ Stud 2017; 26(1): 441-445. https://doi.org/10.15244/pjoes/64465

[67] Wiley STM Databases. Mass Spectral and GC Data of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites. 2011.

[68] Wu JT, Chiang IZ, Huang WY. Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. Aquat Toxicol 2006; 80(4): 338-345. https://doi.org/10.1016/j.aquatox.2006.09.011

[69] Xu Y, Fu X. Reprogramming of plant central metabolism in response to abiotic stresses: a metabolomics view. Int J Mol Sci. 2022; 23(10): 5716. https://doi.org/10.3390/ijms23105716

[70] Zhang TT, He M, Wu AP, Nie LW. Allelopathic effects of submerged macrophyte Chara vulgaris on toxic Microcystis aeruginosa. Allelopathy J 2009a; 23(2): 391-402.

[71] Zhang TT, Zheng CY, He M, Wu AP, Nie LW. Inhibition on algae of fatty acids and the structure-effect relationship. China Environ Sci 2009b; 29(3): 274-279.

[72] Zhironkin S, Demchenko S, Kayachev G, Taran E, Zhironkina O. Convergent and nature-like technologies as the basis for sustainable development in the 21st century. E3S Web Conf 2019; 105: 03008. https://doi.org/10.1051/e3sconf/201910503008

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