

ANALYSIS OF THE DEPENDENCE OF THE COMPOSITION OF THE METABOLOME OF HIGHER AQUATIC VEGETATION AND PHYTOPLANKTON ASSOCIATIONS TO ASSESS THE ECOLOGICAL STATE OF THE WATER ECOSYSTEM (LAKE SUURI)

Khodonovich V.V.¹, Iavid E.Ia.^{1,2}, Krylova Y.V.¹, Kurashov E.A.^{1,3}, Bakulina A.E.⁴

¹St. Petersburg Branch of FGBNU "Vniro" ("Gosniorkh" Named After L. Berg), 199053, St. Petersburg, Makarova Emb., 26, Russia

²Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences (SRCES RAS), 197110, St. Petersburg, Korpusnaya St., 18., Russia

³Institute of Limnology of the Russian Academy of Sciences (IL RAS), 196105, St. Petersburg, Sevastianova St., 9, Russia

⁴Ltd «Nord», 192102, St. Petersburg, Buharestskaya St., 8, Russia

Abstract

Aquatic and coastal macrophytes play an important role in the formation of the chemical composition of organic matter in fishery reservoirs. The success of the study of low-molecular-weight organic compounds of aquatic plants shows that they are one of the most important factors in the formation of hydrobiocenoses in the littoral of lakes. Higher aquatic plants are one of the key components of inland water bodies, performing an important ecological function due to biologically active substances. This paper presents the results of the study of the volatile low molecular weight organic compounds of perennial higher aquatic vegetation of the following species: Potamogeton perfoliatus L., Potamogeton natans L., Equisetum fluvatile L. Plant material was collected from Lake Suuri, Leningrad region. Research has shown that these species are capable of synthesizing over 79 secondary metabolites. Secondary metabolites of macrophytes have an allelopathic effect on unicellular algae, which can be divided into suppressive and stimulating. Also in the course of the work, the dominant species of phytoplankton communities in plant associations, total biomass, abundance, biomass and abundance by divisions of algae were identified. The limnological characteristics of the reservoir in the places where higher aquatic plants grow are shown. The composition of low-molecular organic compounds of macrophytes was studied by gas chromatography-mass spectrometry. For a number of algal species, saprobity values have been established. In the investigated reservoir of the Leningrad region, more than 27 indicators of saprobity were revealed. It is shown that the indicator role of phytoplankton is determined not only by the presence or absence of certain species in the reservoir, but also by the degree of their quantitative development in the associations of higher aquatic plants. Specific biologically active volatile low-molecular-weight organic compounds have been identified, which can serve as an indicator of the state of the aquatic ecosystem under study. The regularity between the quantitative and qualitative composition of low-molecular organic substances of aquatic plants and the ecological situation of the habitat is shown.

Keywords: *aquatic macrophytes, low-molecular-weight organic compounds, low-molecular-weight metabolic profiles, gas chromatography-mass spectrometry, anthropogenic impact, ecological state, water ecosystems*

1. INTRODUCTION

The success of studying low molecular weight organic compounds of aquatic plants shows that they are one of the most important factors in the formation of hydrobiocenoses in the littoral zone of lakes [1, 2]. At the same time, it was found that the system of low molecular weight metabolome of aquatic macrophytes is extremely mobile, responsive to changes in the growing conditions of plants and their hydrobiological environment [3].

Allelopathy is one of the mechanisms by which the development of populations of plants and cyanobacteria in aquatic hydrobiocenoses is regulated [1]. Allelopathic interactions are carried out through the synthesis of specific low molecular weight organic compounds (LMWOC) - allelochemicals. Higher aquatic plants are one of the key components of inland water bodies, performing the most important environmental function due to biologically active substances. It is known that secondary metabolites of macrophytes have an allelopathic effect on unicellular algae, which can be divided into suppressive and stimulatory.

At the same time, the situation with the development of this direction in Russia, unfortunately, continues to remain unsatisfactory. The patterns of low molecular weight organic compounds production by aquatic macrophytes have been studied extremely insufficiently. In particular, there is still little information on how the low molecular weight organic compounds of various species of aquatic macrophytes change in habitats subject to varying degrees of anthropogenic impact.

The purpose of this work is to assess the possibility of using the LMWOC of higher aquatic vegetation as an integral indicator of the stability of aquatic ecosystems of fishery reservoirs, as well as to assess the regularities associated with the suppression of phytoplankton development in those ecosystems where macrophytes are actively developing.

2. MATERIALS AND METHODS

2.1. Materials

This paper presents the results of the study of the volatile low molecular weight organic compounds of perennial higher aquatic vegetation of the following species: *Potamogeton perfoliatus* L., *Potamogeton natans* L., *Equisetum fluvatile* L. Plant material was collected from Lake Suuri, Leningrad region. The collection of material was carried out on July 14-15, 2012. Also, in the studied associations, samples of phytoplankton were taken and analyzed. In the course of the work, the dominant species of phytoplankton communities, total biomass, and abundance, biomass, and abundance by algae divisions were determined.

During the work, the following limnological parameters of the surface layer of the water body were determined using the YSI 6600-D multiparameter probe: temperature, pH, concentration of dissolved oxygen, concentration of nitrate and ammonium nitrogen, turbidity, electrical conductivity, salinity, redox potential, chlorophyll concentration, number of blue-green algae.

Lake Suuri is located in the Priozersk District of the Leningrad Region near the Lekhmalahti Bay of Lake Ladoga (61.132291 °, 29.921373 °) (Fig. 1). The lake belongs to the river basin of the Neva, the area is 1.3 km². It is a small weak-flowing lake with depths of up to 5.5 m, located at an altitude of 12.5 m above sea level, 1.5 km from Lake Ladoga in the north-west of the Karelian Isthmus in the area of distribution of the selga relief. A significant amount of precipitation exceeding evaporation, falling unevenly throughout the year, and the predominance of natural moisture input over its actual consumption for evaporation determine the general regularity of the hydrological regime of the area [4].

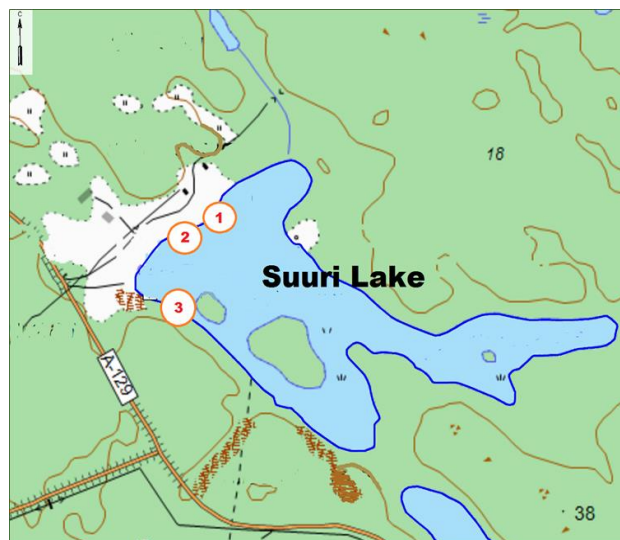


Fig 1. Lake Suuri. Sampling points of associations *Potamogeton perfoliatus* L. (1), *Potamogeton natans* L. (2), *Equisetum fluviatile* L. (3)

2.2. Methods

The shoots of higher aquatic vegetation during the selection were thoroughly washed from possible fouling and then dried in a darkened room without the penetration of direct sunlight to an air-dry state. Essential oils containing volatile low molecular weight organic compounds were obtained from the dried material by the Clevenger method by steam distillation of samples for 6 hours. Before distillation, the dried plant material was ground in a Waring BB-25ES blender to a powder. From the obtained essential oil, volatile low molecular weight organic compounds were extracted into hexane.

This extract was studied by gas chromatography-mass spectrometry on a TRACE DSQ II gas chromatography-mass spectrometric complex (Thermo Electron Corporation) and TRACE ISQ. The columns used, respectively, Thermo TR-5ms SQC 15 m × 0.25 mm with a phase ID of 0.25 µm and Thermo TG-SQC 15 m × 0.25 mm with a phase ID of 0.25 µm. The carrier gas is helium. Before chromatography, the extract was diluted 3 or 5 times (depending on the concentration of the investigated substances). Decafluorobenzophenone and benzophenone were used as internal standards.

Deciphering of chromatography-mass spectrograms was carried out using special software "Xcalibur" and libraries of mass spectra "Nist-2008" and "Wiley".

Phytoplankton was sampled with a bathometer in the surface water horizon and fixed with a Lugol solution with glycerin. Thickening of quantitative phytoplankton samples was carried out by sedimentary method. To count the cells, a 0.05 ml Nageotte counting chamber was used. The calculation of the abundance and biomass of phytoplankton was carried out according to the corresponding algorithms and formulas [9, 10, 11]. The calculation of the biomass of phytoplankton is carried out by the method of summing up the biomasses of individual populations, which is most generally accepted in freshwater hydrobiology, which requires the determination of the average mass (volume) of algal cells that make up the population in the sample [8]. Species identification was carried out under a universal microscope Carl Zeiss Axioplan 2 imaging according to standard methods using keys [7].

Saprobity was assessed in accordance with the Pantle and Bukk method as modified by Sladeczek. The method is based on the ability of organisms to survive in conditions of organic pollution of the environment, called saprobity. For a number of algae species, saprobic values have been established. The degree of saprobity of the reservoir is characterized by the saprobity index, which takes values from 0 (for xenosaprobic reservoirs) to 8.5 (ultra-saprobic reservoirs), and corresponding to six classes

of water purity. The frequency of occurrence of algae is taken into account on a six-point scale (from single to mass) [2, 12].

3. RESULTS AND DISCUSSION

The results obtained show that the formation of LMWOC of aquatic plants occurs as a result of their active interaction with the environment, while the influence of both biotic and abiotic factors, including anthropogenic ones, is important, leading to diverse disturbances of aquatic biotopes and changes in the low molecular weight profile.

A study of the metabolomes *Potamogeton perfoliatus* L., *Potamogeton natans* L., *Equisetum fluviatile* L. in the Suuri reservoir showed that these species are capable of synthesizing more than 130 primary and secondary low molecular weight metabolites. In the sample *Potamogeton perfoliatus* L., 72 LMWOC were found, in *Potamogeton natans* L. - 115 and in *Equisetum fluviatile* L. - 138 LMWOC respectively.

Limnological characteristics inside investigated plant associations are presented in table 1.

Table 1. Average values (median) of temperature (T, °C), electrical conductivity (E, mS cm⁻¹), mineralization (TDS, g l⁻¹), oxygen concentration (O₂, % above the line, mg l⁻¹ below the line), pH, redox potential (Eh, mV), ammonium nitrogen concentration (NH₄⁺, mg l⁻¹), nitrate nitrogen concentration (NO₃⁻, mg l⁻¹), chloride concentration (Cl⁻, mg l⁻¹), chlorophyll concentration (Chl, µg l⁻¹) in the surface water layer in the macrophyte associations of Lake Suuri in 2012.

Association	T	E	TDS	O ₂	pH	Eh	NH ₄ ⁺	NO ₃ ⁻	Cl ⁻	Chl
<i>E. fluviatile</i> L.	21,12	0,07	0,05	84,0/ 7,47	7,5	119	0,03	1,74	31,40	4,6
<i>P. natans</i> L.	21,7	0,07	0,05	89,8/ 7,99	7,38	113	0,03	2,12	44,01	3,8
<i>P. perfoliatus</i> L.	21,08	0,07	0,05	84,7/ 7,5	7,35	124	0,03	3,37	32,65	6,0

Phytoplankton was obtained on July 14-15, 2012, and formed 67 species of algae from 7 divisions: blue-green (Cyanophyta), diatoms (Bacillariophyta), green (Chlorophyta), yellow-green (Xanthophyta), golden (Chrysophyta), euglena (Euglenophyta), and cryptophyta (Cryptophyta). According to the number of species, the leading place was occupied by green algae - 27 species, followed by diatoms - 19 species and blue-green - 12 species. The indicator role of phytoplankton is determined not only by the fact of the presence or absence of certain species in the reservoir but also by the degree of their quantitative development, which can be seen in Table 2.

Table 2. Characteristics of phytoplankton by phylum in macrophyte associations: A) Phytoplankton abundance in macrophyte associations in Lake Suuri (cells / ml); B) Distribution of phytoplankton biomass (mg/ l)

Phytoplankton	<i>E. fluviatile</i>		<i>P. natans</i>		<i>P. perfoliatus</i>	
	B	A	B	A	B	A
Cyanophyta	0,11	223455	0,02	27239	0,07	178550
Bacillariophyta	0,30	108	0,12	257	0,51	145
Chlorophyta	0,08	1482	0,14	2451	0,10	2705
Cryptophyta	-	0	0,02	38	0,01	20
Chrysophyta	0,06	65	0,02	34	0,02	35

Xanthophyta	-	7	-	-	-	10
Euglenophyta	-	-	0,01	17	-	-
Total	0,55	225 117	0,33	30 036	0,71	181 465

As can be seen from the table, at all stations of the lake Suuri in 2012, the representatives of the Cyanophyta division made the greatest contribution to the phytoplankton abundance. Among them, the dominant species can be considered *Aphanothece* sp., which forms numerous colonies of small cells (size about $0.9 \times 0.9 \mu\text{m}$). According to the Table 2, the minimum amount of phytoplankton was observed in the *P. natans* association, while the maximum was shared by the other associations (with a slight excess in the *E. fluviatile* association).

In the course of the work, the biomass of algae was also investigated, the values of which are presented in Table 2. It should be noted that the pattern of the total phytoplankton abundance generally repeated the biomass pattern. Thus, the association of *P. natans* was also characterized by the minimum biomass value and the association with the largest biomass – *P. perfoliatus*.

Analysis of the component composition of metabolites of the studied plants showed the presence of a large number of substances belonging to different chemical classes (Table 3). In general, the plants contained from 79 to 138 biologically active substances, namely: *P. perfoliatus* L. – 79, *P. natans* L. – 115, *E. fluviatile* L. – 138.

E. fluviatile L. contained the largest number of LMWOC in the metabolome - 138 substances. These data can be explained by the fact that plant was selected in the most favorable conditions for its existence, that is, macrophytes were not oppressed by external factors, therefore, they could synthesize a larger number of metabolites than the same plants, but in stressful situations (polluted water bodies or intraspecific and interspecific competition).

On the contrary, the smallest amount of biologically active substances was recorded in *P. perfoliatus* L., which may indicate the suppression of the synthesis of endometabolites due to biological (competition) or anthropogenic loads (the plant grew most close to the place of sewage drainage).

Table 3. Comparative content (% in relation to the whole essential oil) of the main functional groups of volatile low molecular weight organic compounds in the studied samples.

Functional class	<i>P. perfoliatus</i> L.	<i>P. natans</i> L.	<i>E. fluviatile</i> L.
Hydrocarbons	20,87	1,41	6,08
Ketones	3,70	8,52	2,57
Aldehydes	1,41	2,18	18,26
Alcohols	20,90	27,99	13,43
Carboxylic acids	20,30	10,81	12,12
Aromatic Hydrocarbons	0,52	0,08	14,79
Ethers	0,42	7,43	52,5
Nitrogen-containing	0,08	1,4	1,72
Diverse functional groups	21,30	37,72	10,00
Not detected	10,15	0	0

Comparing the obtained data on the characteristics of phytoplankton and metabolites produced by macrophytes, it can be assumed that *P. natans* has the greatest ability to inhibit phytoplankton. This conclusion may be associated with a significant predominance in endometabolites of substances from the class Diverse functional groups, which are characterized by a complex structure and which include some steroids, for example, Pregnenolone (P4) (endogenous steroid).

It is also interesting that the highest phytoplankton biomass was recorded in the association of *E. fluviatile* L. With the condition that Ethers was the prevailing class of substances produced here, it can be assumed that this class of compounds does not affect the suppression of blue-green algae and can be regarded as neutral in relation to phytoplankton.

The assessment of the ecological state of the reservoir was calculated according to the indicators of the saprobity index. In the littoral zone of the lake Suuri revealed 27 indicators of saprobicity, of which 9 are β -mesosaprobic, 5 are oligosaprobic, and 3 are oligo- β -mesosaprobic forms. The saprobic index ranged from 1.68 to 1.86, which corresponds to beta-oligosaprobic and oligo-alphamesosaprobic waters. In particular, *E. fluviatile*, *P. natans* grew in oligo-alphamesosaprobic waters, *P. perfoliatus* - beta-oligosaprobic, which corresponds to class II and III of their purity (moderately and slightly polluted, respectively). The given data are confirmed by the chemical composition of investigated plants.

4. CONCLUSIONS

Studies of the metabolomes of aquatic plants *Potamogeton perfoliatus* L., *Potamogeton natans* L., *Equisetum fluviatile* L. showed that these species are capable of synthesizing more than 79 organic substances. In general, the plants contained from 79 to 138 biologically active substances, namely: *P. perfoliatus* L. – 79, *P. natans* L. – 115, *E. fluviatile* L. – 138. The main groups of compounds in samples were Ethers and Diverse functional groups.

The phytoplankton of Lake Suuri is represented by 67 species, the dominants of which were colonies of blue-green algae of the genus *Aphanothece* and green algae of the genus *Dictiosphaerium*. The representatives of the Cyanophyta division made the greatest contribution to the phytoplankton abundance. The minimum amount of phytoplankton was observed in the *P. natans* association, while the maximum was shared by the other associations (with a slight excess in the *E. fluviatile* association). The association of *P. natans* was also characterized by the minimum biomass value and the association with the largest biomass – *P. perfoliatus*.

Comparing the obtained data on the characteristics of phytoplankton and metabolites produced by macrophytes, it can be assumed that *P. natans* has the greatest ability to inhibit phytoplankton due to the predominance in the composition of endometabolites of compounds belonging to the «Diverse functional groups» class. Whereas substances from class «Ethers» does not affect the suppression of blue-green algae and can be regarded as neutral in relation to phytoplankton.

According to the saprobity index, the water quality of the studied lake corresponds to the II and III purity classes, which characterizes it as slightly and moderately polluted. It can be assumed that the exometabolites. *Equisetum fluviatile* L. stimulated the development of microalgae. In associations of *P. natans*, the minimum values of the abundance and biomass of phytoplankton were observed, which may be the result of the inhibitory effect of exometabolites of macrophytes.

REFERENCES

1. Fink, P 2007, 'Ecological functions of volatile organic compounds in aquatic systems', *Marine and Freshwater Behaviour and Physiology*, vol. 40, pp. 155-168.
2. Kurashov, EA, Krylova, YuV, Mitrukova, GG & Chernova, AM 2014, 'Volatile low molecular weight metabolites of aquatic macrophytes growing on the territory of Russia and their role in hydroecosystems', *Siberian Journal of Ecology*, vol. 4, pp. 573-591.

3. Krylova, YuV & Kurashov, EA & Mitrukova, GG 2016 'Component composition of volatile low molecular weight organic compounds *Ceratophyllum demersum* (Ceratophyllaceae) growing in different climatic conditions' , *Water: chemistry and ecology*, vol. 8, pp. 11-25.
4. Blindow, IA, Hargeby & Andersson, G 2002, 'Seasonal changes of mechanisms maintaining clear water in a shallow lake with abundant *Chara* vegetation', *Aquatic Botany*, vol. 72, pp. 315-334.
5. Hilt, S & Gross, EM 2008, 'Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes?', *Basic and Applied Ecology*, vol. 9, pp. 422-432.
6. Kurashov, EA & Krylova, Yu.V 2013, 'Low molecular weight secondary metabolites of higher aquatic plants and perspectives of autotrophic link management in aquatic ecosystems', *Biology of Inland Waters: Materials of the XV School-Conference of Young Scientists (Borok, October 19–24, 2013)*, Kostroma Printing House LLC, Kostroma, pp. 34.
7. Barinova, SS & Medvedeva, LA 1996, *Atlas of algae-indicators of saprobity (Russian Far East)*, Dalnauka, Vladivostok.
8. Kondakova, LV & Domracheva, LI 2007, *Flora of the Vyatka Territory. Part 2. Algae (Species composition, specificity of water and soil biocenoses)*, Kirov Regional Printing House, Kirov, 192 p.
9. Giants, LL, Garibova, LV, Gorbunova NP, Gorlenko, MV & etc. 1981, *Course of lower plants: A textbook for students of high fur boots*, Higher school, Moskov, 504 p.
10. Rossolimo, LL 1977, *Changes in limnic ecosystems under the influence of anthropogenic factors*, Nauka, Moskov, 144 p.
11. Abakumov, VA 1983, *Guide to methods of hydrobiological analysis of surface waters and bottom sediments*, Gidrometeoizdat, Leningrad, 239 p.
12. Pearsall, WH 1932, 'Phytoplankton in English lakes. II. The composition of the phytoplankton in relation to dissolved substaces', *Journal of Ecology*, vol. 20 (2), pp. 241-262.