Characteristics of the Low Molecular Weight Metabolome of *Potamogeton Natans* L. (Potamogetonaceae) from Lakes of Different Trophic State (Karelian Isthmus, Northwest Russia)

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Abstract: The qualitative and quantitative component composition of low molecular weight volatile organic compounds (VOCs) of the essential oil of the floating-leaf pondweed (*Potamogeton natans* L., Potamogetonaceae family) growing in various lakes of the Karelian Isthmus (North-West of the Russian Federation) in the fruiting stage was investigated in detail for the first time by gas chromatography-mass spectrometry. The low molecular weight metabolome (LMWM) of *P. natans* contained 138 components, 128 of which were identified. VOCs belonging to esters, alcohols, and various functional groups dominated the LMWM of floating leaf pondweed from mesotrophic and eutrophic lakes. A significant similarity was found between the component composition of VOCs of floating leaf pondweed from mesotrophic and eutrophic lakes. Many of the substances found in the LMWM of *P. natans* can be attributed to biologically active compounds. This opens up prospects for the use of this plant (particularly manool and ecdysteroids from its LMWM) for various economic applications as a valuable natural raw material. Due to the characteristic of the floating leaf pondweed's substantial resistance of its LMWM to the factor of the trophic status of the lakes, it is feasible to use it as an ecological indicator of significant disruptions in aquatic environments.

Keywords: *Potamogeton natans* L., floating-leaf pondweed, Low molecular weight organic compounds, Low molecular weight metabolome, Gas chromatography-mass spectrometry, Essential oil, Component composition, Lakes, Karelian Isthmus, Trophic state.

INTRODUCTION

Species of the genus Potamogeton (Family: Potamogetonaceae) are widespread throughout the world [1-5] and play an important role in aquatic ecosystems [6-8]. Potamogeton is one of the most important genera in the aquatic environment, particularly when it comes to providing food or habitat for aquatic creatures [9-12].

Potamogeton species are important for obtaining useful bioadditives for animal feed and/or the pharmaceutical and food industries, and several species can be eaten directly [13-15].

Currently, research on the discovery and application of medications derived from plant components is becoming increasingly significant. These medications have a high chemical variety and the best ADME/TOX ratios (absorption, distribution, metabolism, and excretion/toxicity) due to their biological origin. Back in1999, in particular, these benefits, relevance, efficacy, and possibilities for using herbal products as antibacterial agents and components of complex therapy were extensively detailed [16].

In this regard, it is important to obtain knowledge about the complete composition of the low molecular weight metabolome (LMWM), in particular, representatives of the genus Potamogeton.

Chromato-mass spectrometry has only been used to study a few Potamogetonaceae species. For instance, *Potamogeton crispus* L., *Potamogeton pusillus* L., and *Potamogeton pectinatus* L. were investigated in [15].

In aquatic habitats in the North-West of European Russia, pondweeds continue to be a poorly studied group of macrophytes, especially with regard to the study of their LMWM. We previously published thorough data on the LMWM composition of *Potamogeton perfoliatus* (L.) and *P. pectinatus* from diverse lake habitats [17-19].

Although floating-leaf pondweed (*Potamogeton natans* L., Potamogetonaceae) is abundant in many bodies of water, detailed research on its LMWM is still lacking.

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Thirteen *ent*-labdane diterpenes were isolated from *P. natans* [20]. This study also showed that the abundant bioactive diterpenes in this plant may affect the equilibrium of aquatic systems by interacting with other aquatic animals.

It has been demonstrated that aquatic macrophytes growing in geographically dissimilar regions of the range may substantially change their LMWM. Examples such as *Nuphar lutea* (L.) Sm., *P. pectinatus*, and *Ceratophyllum demersum* L. are used to demonstrate this [8, 19, 21]. Uncertainty exists on how a water body's trophic state influences LMWM variability. When researching the lakes of the Karelian Isthmus (North-West Russia), where lakes of various trophic levels are situated close to one another, the chance to test this emerged. In this situation, the influence of zonal geographical factors may be excluded.

In this regard, the goal of this study was to evaluate how the LMWM of the floating-leaf pondweed *P. natans* varies in lakes that are geographically separated by just 2.7 km but have various trophic statuses.

MATERIALS AND METHODS

Plant Materials, Trial Lakes

The floating-leaf pondweed is widespread in the Northern Hemisphere. It is frequently discovered in freshwater reservoirs with stationary or (less frequently) slowly moving water, such as lakes, ponds, oxbow lakes, and canals. One of the most common aquatic plants in the North-West and Central regions of Russia, it is widespread across the country [22, 23].

P. natans samples were taken at the end of July, during the fruiting stage, in two lakes of the Karelian Isthmus (Northwest Russia), which differed in trophic status (Table **1**) and were situated apart by 2.7 km (Figure **1**).

Lake Suuri is a lake with a silty bottom. The lake basin has an 836768 m^3 volume and a 0.4 km^2 surface area. The average depth of the lake is 2.2 m, and the maximum depth is 5.5 m. The lake's absolute water level is 12.1 m, which is 7.5 m higher than Lake Ladoga's typical water level. In May and June, Lake Suuri reaches its highest water level. It is practically not flowing, the water flow is carried out only through the overgrown lake Mäntylampi. The lake receives the only unnamed permanent stream feeding from the swamp in addition to other minor, temporary streams [24].

Lake Kuznechnoye is an oblong lake with no current and a silty bottom. The area of the lake is 0.21 km^2. It is subject to strong anthropogenic impact, on the shore of the lake there are a sand-granite quarry and the village Kuznechnoye. The absence of runoff, the abundance of suspended small particles, and the huge

Figure 1: Location of sampling of *P. natans* in lakes Suuri and Kuznechnoe. (cartographic materials used from the site: https://yandex.ru/maps/).

Table 1: Average Values (median) of some Basic Indicators of the Aquatic Environment State in Habitats of *P. natans* in Lakes Suuri and Kuznechnoe during the Study Period (July 2015) and the Coordinates of the Sampling Points

Indicators	Lake Suuri	Lake Kuznechnoe
Temperature, °C	20.6	19.8
Conductivity, mS/cm	0.080	0.301
Total Dissolved Solids, g/L	0.057	0.217
pH	7.31	9.91
Redox potential, mV	116	88
Turbidity, NTU	0.0	51.0
Chlorophyll-a concentration, µg/L	4.88	15.0
Cyanobacteria abundance, cells/mL	176	81828
Dissolved oxygen, %	103.6	140.6
Dissolved oxygen, mg/L	9.31	12.91
Trophic type	mesotrophic	eutrophic
Transparency of water according to the Secchi disk, m	2.5	0.3
Coordinates of the sampling points of *P.natans*	61.1318256; 29.9204041	61.1182139; 29.8793020

number of cyanobacteria that form a noticeable "bloom" are the reasons why the indicator of water transparency (according to the Secchi disk) in this lake has a critical mark of 30 cm (Figure **2**).

According to all available information, Lake Suuri has a mesotrophic status, while Lake Kuznechnoye has a eutrophic status [25, 26]. The differing trophic states of the lakes are particularly evident in Figure **1**. Lake Kuznechnoye's surface is so green (owing to an intensely developing cyanobacterial "bloom") that it is difficult even to detect the lake's contours on the map. At the same time, Lake Suuri has a dark color since phytoplankton is not actively growing there.

The floating-leaf pondweed association was well-developed in the mesotrophic Lake Suuri, although signs of its degradation were found in Lake Kuznechnoye (Figure **2**).

The collected plants were manually cleaned to remove contaminants and fouling. The plants were air-dried in a ventilated room without access to direct sunlight (in the shadow). The conventional shade-drying process is the best way to dry herbal raw materials in order to produce the highest yield of essential oil during subsequent hydrodistillation extraction [27, 28]. Until the essential oil was extracted, air-dried plants were kept in the lab at a relative humidity of no more than 75%.

Table **1** provides the primary limnological indicators of the condition of the aquatic environment in *P. natans* habitats that were obtained in situ during sampling using the multi-parameter automatic probe YSI 6600D (YSI Incorporated, USA).

Extraction of the Essential Oil

The dried plant material was ground to a powder in a Waring BB 25ES blender (Waring, United States) prior to distillation. The same Clevenger-type apparatus approach used to acquire essential oils from terrestrial plants [29, 30] was utilized to extract a fraction of low molecular weight volatile organic compounds (VOCs) from dry aquatic plant material. The hydrodistillation process took 8 hours. Hexane (5 mL) was then used to extract the samples. Before GC/MS analysis, the extracts were kept in a freezing cell (-18 °C).

Gas Chromatography / Mass Spectrometry (GC/MS) Analysis

Following hexane extraction, the concentrations of VOCs were measured using a TRACE ISQ gas chromatograph-mass spectrometer (Thermo Electron Corporation) equipped with a quadrupole mass analyzer and a Thermo TG-SQC Column (15 m, inner diameter: 0.25 mm, and 0.25 µm film). The carrier gas was helium, and the ionization voltage was 70 eV.

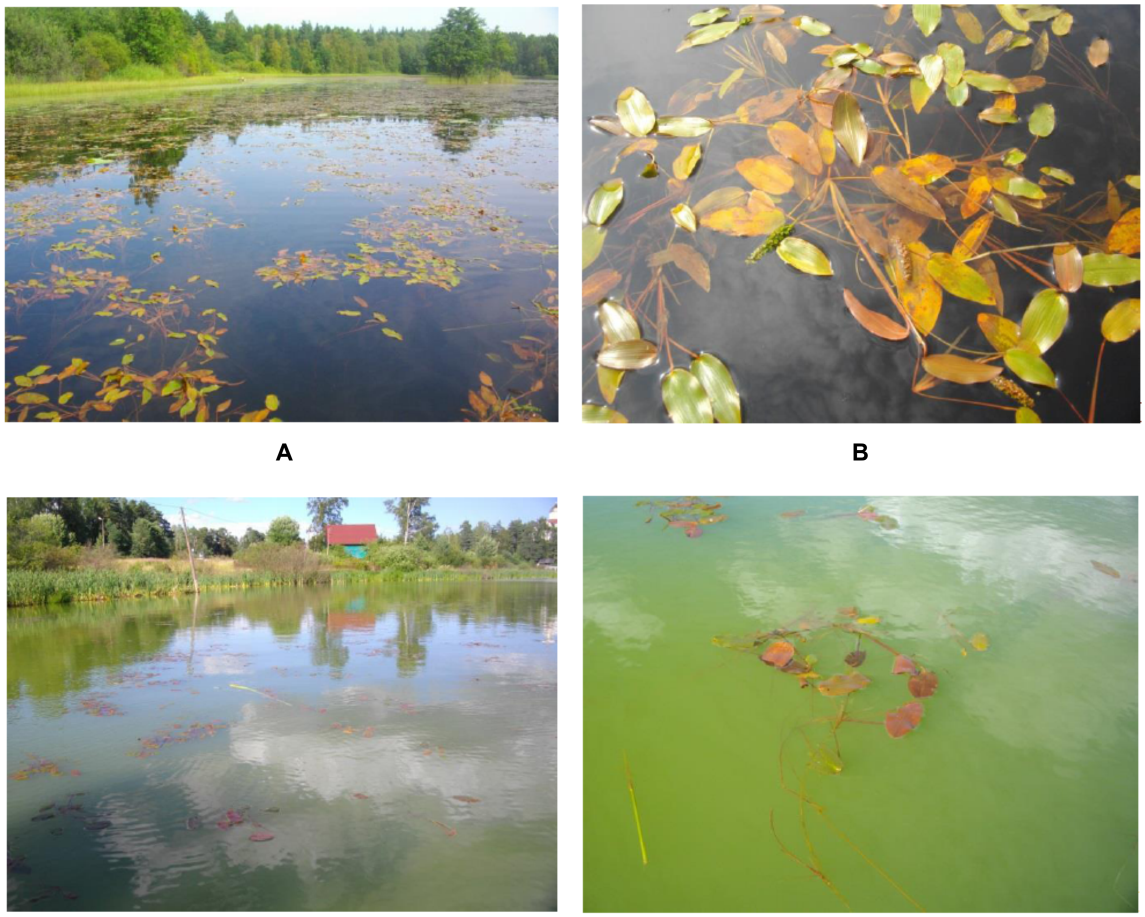

Figure 2: *P. natans* association in Lake Suuri (**A**) and Lake Kuznechnoye (**C**) from a general perspective; **B** and **D**, the state of specific plants in Lake Suuri and Lake Kuznechnoye.

The mass spectra were recorded in scan mode for the entire mass range (30-580 amu) in a programmed temperature regime: the oven temperature was kept at 35 °C for 3 minutes; then it was increased to 60 °C at a rate of 2 °C /minute and kept constant for 3 minutes; then it was increased to 80 °C at a rate of 2 °C /minute and kept constant for 3 minutes; was increased to 120 °C at a rate of 4 °C /minute and kept constant for 3 minutes; was increased to 150 °C at a rate of 5 °C /minute and kept constant for 3 minutes; was increased to 240 °C at a rate of 15 °C /minute. Finally, it was kept at isothermal temperatures for 10 minutes.

The VOCs found in aquatic plant samples were identified by comparing their mass spectra to those found in the NIST_2014 and Wiley mass spectral databases. The identification of the compounds was confirmed by Linear Retention Indices obtained from a series of straight-chain alkanes (C7–C30) [31]. Quantitative analysis was performed using Merck-certified reference materials decafluorobenzophenone and benzophenone (CAS Numbers 119-61-9 and 853-30-4) as internal standards.

Assessment of Metabolome Similarity

The similarity of the component composition of VOCs of *P. natans* essential oil from the studied lakes was estimated using the similarity coefficients of Jaccard (J) [32] and Sørensen-Czekanowski (Qs) [33, 34], calculated using the following formulas:

$$J = \frac{c}{a+b-c},$$

$$Qs = \frac{2c}{a+b},$$

where c is the number of common VOCs found in samples A and B; a - VOCs found in A; b - VOCs found in B.

The similarity of samples in terms of quantitative data (by the content of individual compounds and groups of compounds) was assessed using the Morisita (Morisita-Horn) index [35]:

$$Cmh = \frac{2\sum_i (an_i \cdot bn_i)}{(da + db) \cdot aN \cdot bN},$$

where an_i is the content of the i-th compound (group of compounds) in sample A; bn_i - the same for sample B; aN is the total VOCs content in sample A; bN - the same for sample B; $da = \sum(an_i^2)/aN^2$, $db = \sum(bn_i^2)/bN^2$.

RESULTS

A GC/MS analysis of the component composition of the essential oil of *P. natans* shoots from the investigated lakes revealed the presence of a significant number of VOCs belonging to several chemical compound classes (Tables **2**, **3**). Figure **3** displays an overall view of the chromatograms of the floating-leaf pondweed samples analyzed.

The results of the component composition research of LMWM of floating-leaf pondweed from the examined lakes showed that 138 components, of which 128 VOCs were identified, were present in essential oil samples. The LMWM of pondweed from the mesotrophic Lake Suuri included 135 compounds, whereas a sample from the eutrophic Lake Kuznechnoe contained only 109 compounds. There were 106 common chemicals. Only three minor components were detected in the composition of Lake Kuznechnoye pondweed LMWM that were not found in Lake Suuri pondweed LMWM.

The total concentration of VOCs in Lake Suuri was 437.03 µg/g dry plant weight and 381.03 µg/g dry plant weight in Lake Kuznechnoye (Table **2**). The number of major compounds (greater than 1% of total VOCs content) in both samples was nearly identical (19 and

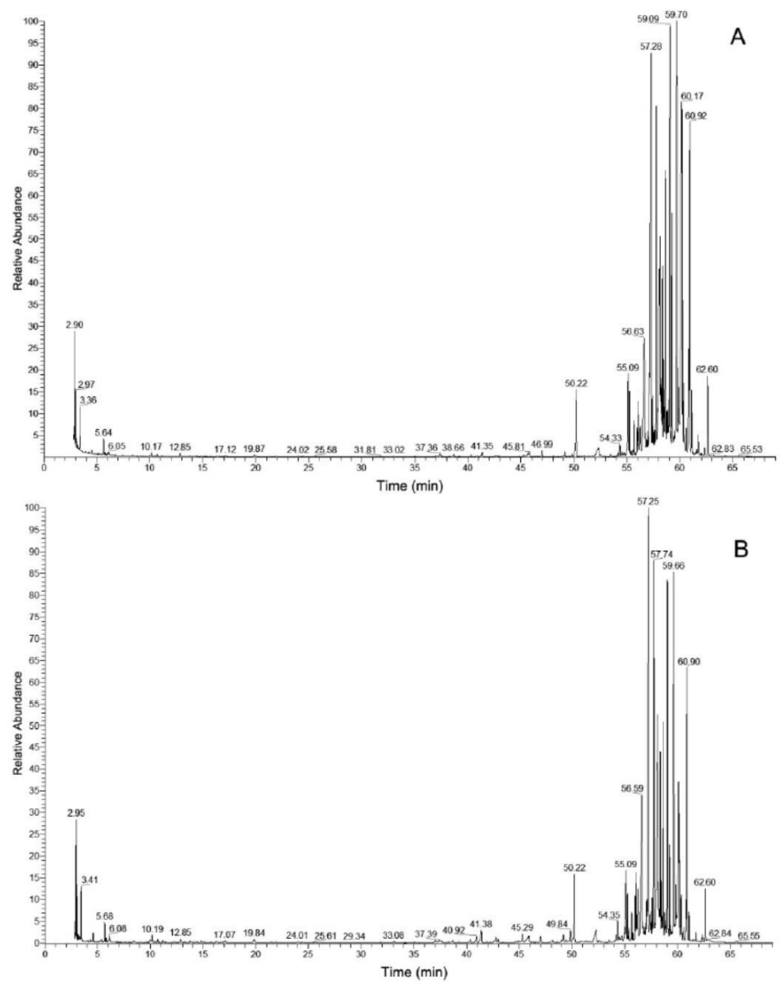

Figure 3: General view of essential oil chromatograms of air-dry samples of *P. natans* from Lake Suuri (**A**) and Lake Kuznechnoe (**B**).

Table 2: Component Composition of the Essential oil of *P. natans* from Investigated Lakes (RI is the Retention Index; % is the Percentage of the Substance from the Sum of All Substances of the Essential Oil; C is the Absolute Content of Compounds, µg/g of Dry Plant Weight)

№	Component	Formula	RI	Lake Suuri		Lake Kuznechnoe	
				%	C	%	C
1	2-ethyl-5,5-dimethylcyclopenta-1,3-diene	C_9H_{14}	843	0.03	0.14	0.07	0.27
2	1,2,3-trimethylcyclohexane	C_9H_{18}	848	0.02	0.08	0.07	0.26
3	(E)-hex-2-enal	$C_6H_{10}O$	853	0.07	0.31	0.24	0.93
4	4-methyloctane	C_9H_{20}	865	0.01	0.05	0.02	0.08
5	ethylbenzene	C_8H_{10}	867	0.01	0.04	–	–
6	1,3-xylene	C_8H_{10}	869	–	–	0.02	0.07
7	3-methyloctane	C_9H_{20}	872	0.01	0.05	0.02	0.08
8	1-ethylcyclohexa-1,4-diene	C_8H_{12}	881	0.02	0.10	–	–
9	(4E)-4-ethylidenecyclohexene	C_8H_{12}	883	–	–	0.06	0.22
10	2,2,4-trimethylheptane	$C_{10}H_{22}$	887	0.01	0.05	0.02	0.07
11	heptan-2-one	$C_7H_{14}O$	898	0.04	0.20	0.12	0.44
12	nonane	C_9H_{20}	900	0.05	0.23	–	–
13	3,3,5-trimethylheptane	$C_{10}H_{22}$	901	0.01	0.05	0.02	0.09
14	(Z)-hept-4-enal	$C_7H_{12}O$	905	0.03	0.12	0.10	0.37
15	2,7-dimethyloxepine	$C_8H_{10}O$	935	0.02	0.07	0.02	0.08
16	ethenyl hexanoate	$C_8H_{14}O_2$	990	0.02	0.11	0.17	0.65
17	2-pentylfuran	$C_9H_{14}O$	994	0.07	0.32	0.28	1.08
18	3-ethyl-1-cyclohexene	$C_7H_{10}O$	1000	0.02	0.09	0.04	0.14
19	2-[(E)-pent-2-enyl]furan	$C_9H_{12}O$	1004	0.04	0.18	0.15	0.58
20	(2E,4E)-hepta-2,4-dienal	$C_7H_{10}O$	1011	0.01	0.05	0.08	0.31
21	2,2,6-trimethylcyclohexan-1-one	$C_9H_{16}O$	1030	0.02	0.07	0.04	0.17
22	2-phenylacetaldehyde	C_8H_8O	1039	0.10	0.44	0.22	0.82
23	(E)-oct-2-enal	$C_8H_{14}O$	1057	0.02	0.07	0.05	0.18
24	3-(chloromethyl)heptane	$C_8H_{17}Cl$	1064	0.02	0.09	0.06	0.22
25	(3E,5E)-octa-3,5-dien-2-one	$C_8H_{12}O$	1071	0.02	0.11	0.06	0.23
26	2,6-dimethylcyclohexan-1-ol	$C_8H_{16}O$	1098	0.01	0.04	0.05	0.19
27	nonanal	$C_9H_{18}O$	1103	0.06	0.25	0.13	0.49
28	2,6,6-trimethylcyclohex-2-ene-1,4-dione	$C_9H_{12}O_2$	1138	0.10	0.43	0.27	1.04
29	(2E,6E)-nona-2,6-dienal	$C_9H_{14}O$	1152	0.01	0.06	0.03	0.13
30	(E)-non-2-enal	$C_9H_{16}O$	1160	0.02	0.07	0.05	0.19
31	1,3,5,5,6,6-hexamethylcyclohexa-1,3-diene	$C_{12}H_{20}$	1164	0.03	0.13	0.06	0.25
32	2,6,6-trimethylcyclohexa-1,3-diene-1-carbaldehyde	$C_{10}H_{14}O$	1192	0.02	0.10	0.08	0.29
33	octanoic acid	$C_8H_{16}O_2$	1197	0.02	0.08	0.03	0.12
34	decanal	$C_{10}H_{20}O$	1207	0.01	0.04	0.03	0.12
35	2,6,6-trimethylcyclohexene-1-carbaldehyde	$C_{10}H_{16}O$	1212	0.03	0.11	0.07	0.28
36	2-(2,6,6-trimethylcyclohexen-1-yl)acetaldehyde	$C_{11}H_{18}O$	1220	0.01	0.05	0.03	0.10
37	2,4,4-trimethylcyclohexane-1,3-dione	$C_9H_{14}O_2$	1259	0.00	0.02	–	–
38	1H-indole	C_8H_7N	1285	0.00	0.01	–	–
39	nonanoic acid	$C_9H_{18}O_2$	1292	0.02	0.09	0.04	0.15

40	4-ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	1310	0.04	0.16	0.10	0.37
41	(2E,4E)-deca-2,4-dienal	$C_{10}H_{16}O$	1317	0.01	0.04	0.02	0.09
42	(6E)-6-methyl-5-propan-2-ylidenenona-6,8-dien-2-one	$C_{13}H_{20}O$	1336	0.01	0.04	–	–
43	1,5,8-trimethyl-1,2-dihydronaphthalene	$C_{13}H_{16}$	1344	0.03	0.15	0.05	0.21
44	4,4,7-trimethyl-2,3-dihydro-1H-naphthalene	$C_{13}H_{18}$	1349	0.02	0.07	0.02	0.09
45	3,4,8-TRIMETHYL-2-NONENAL	$C_{12}H_{22}O$	1379	0.02	0.11	0.13	0.49
46	4-(7,7-dimethyl-3-bicyclo[4.1.0]hept-3-enyl)butan-2-one	$C_{13}H_{20}O$	1386	0.08	0.37	0.05	0.17
47	tetradecane	$C_{14}H_{30}$	1400	0.03	0.11	0.03	0.10
48	4-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)butan-2-one	$C_{13}H_{20}O$	1412	0.12	0.54	0.08	0.29
49	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one; [α-ionone]	$C_{13}H_{20}O$	1424	0.01	0.02	0.01	0.05
50	4-(2,4,4-TRIMETHYL-1,5-CYCLOHEXADIEN-1-YL)-3-BUTEN-2-ONE	$C_{13}H_{18}O$	1426	0.01	0.04	0.03	0.12
51	(5E)-6,10-dimethylundeca-5,9-dien-2-one	$C_{13}H_{22}O$	1454	0.07	0.30	0.12	0.45
52	(1S,4S,4aS,6R,8aR)-1,6-dimethyl-4-propan-2-yl-1,2,3,4,4a,5,6,8a-octahydronaphthalene; [cadinene]	$C_{15}H_{24}$	1469	0.04	0.19	0.20	0.75
53	(E)-4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one	$C_{13}H_{20}O$	1480	0.16	0.70	0.67	2.56
54	pentadecane	$C_{15}H_{32}$	1500	0.01	0.03	0.02	0.09
55	tridecanal	$C_{13}H_{26}O$	1510	0.03	0.11	0.08	0.29
56	4-[(1R,2R)-2-methyl-3-oxocyclohexyl]butanal	$C_{11}H_{18}O_2$	1513	0.02	0.07	0.20	0.75
57	8a-methyl-3,4,4a,5,6,7-hexahydro-2H-naphthalene-1,8-dione	$C_{11}H_{16}O_2$	1518	0.03	0.12	0.11	0.41
58	(6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol	$C_{15}H_{26}O$	1562	0.02	0.10	–	–
59	2,2,7,7-tetramethyltricyclo[6.2.1.01,6]undec-5-en-4-one	$C_{15}H_{22}O$	1571	0.04	0.19	0.33	1.25
60	dodecanoic acid	$C_{12}H_{24}O_2$	1584	0.17	0.74	0.48	1.82
61	2-[(5E)-6,10-dimethylundeca-1,5,9-trien-2-yl]oxirane	$C_{15}H_{24}O$	1598	0.01	0.06	0.02	0.06
62	hexadecane	$C_{16}H_{34}$	1600	0.01	0.05	0.02	0.06
63	tetradecanal	$C_{14}H_{28}O$	1614	0.15	0.65	0.23	0.86
64	methyl 2-[(1R,2R)-3-oxo-2-[(Z)-pent-2-enyl]cyclopentyl]acetate; [methyl jasmonate]	$C_{13}H_{20}O_3$	1649	0.03	0.11	0.13	0.50
65	tributyl phosphate	$C_{12}H_{27}O_4P$	1662	0.01	0.04	0.04	0.16
66	cyclotetradecane	$C_{14}H_{28}$	1681	0.01	0.03	0.23	0.88
67	heptadecane	$C_{17}H_{36}$	1700	0.00	0.01	0.36	1.36
68	***pentadecanal***	$C_{15}H_{30}O$	***1714***	***1.50***	***6.55***	***2.28***	***8.68***
69	6-[1-(HYDROXYMETHYL)VINYL]-4,8A-DIMETHYL-1,2,4A,5,6,7,8,8A-OCTAHYDRO-2-NAPHTHALENOL	$C_{15}H_{24}O_2$	1724	0.02	0.09	–	–
70	***tetradecanoic acid; [myristic acid]***	$C_{14}H_{28}O_2$	***1781***	***0.54***	***2.38***	***1.03***	***3.94***
71	hexadecanal	$C_{16}H_{32}O$	1825	0.00	0.02	0.09	0.35
72	6,10,14-trimethylpentadecan-2-one	$C_{18}H_{36}O$	1859	0.01	0.03	0.44	1.68
73	***nonadec-1-ene***	$C_{19}H_{38}$	***1889***	***0.09***	***0.39***	***1.56***	***5.93***
74	(7Z,10Z,13Z)-hexadeca-7,10,13-trienal	$C_{16}H_{26}O$	1895	0.73	3.20	0.81	3.07
75	(E)-heptadec-9-enal	$C_{16}H_{30}O$	1898	0.04	0.17	–	–
76	nonadecane	$C_{19}H_{40}$	1900	0.00	0.02	0.20	0.74
77	unidentified m/z 270 [M+], 119 (100)	–	1916	0.07	0.30	–	–

78	unidentified m/z ? [M+], 87 (100)	–	1919	0.01	0.05	–	–
79	(5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one	$C_{18}H_{30}O$	1922	0.42	1.85	0.58	2.22
80	(4aS,4bS,7R,10aS)-7-ethenyl-1,1,4a,7-tetramethyl-3,4,4b,5,6,9,10,10a-octahydro-2H-phenanthrene; [Sandaracopimaradiene]	$C_{20}H_{32}$	1931	0.02	0.11	0.14	0.54
81	2,6,6,10,11-pentamethyl-14-oxatetracyclo[9.2.1.01,10.02,7]tetradecane	$C_{18}H_{30}O$	1947	0.26	1.13	0.74	2.81
82	(1E,5E,9E,12R)-1,5,9-trimethyl-12-prop-1-en-2-ylcyclotetradeca-1,5,9-triene; [neocembrene]	$C_{20}H_{32}$	1951	0.15	0.67	0.16	0.60
83	***3,7,11,15-tetramethylhexadec-1-en-3-ol; [isophytol]***	$C_{20}H_{40}O$	***1955***	***0.52***	***2.27***	***1.06***	***4.06***
84	(Z)-hexadec-11-enoic acid	$C_{16}H_{30}O_2$	1970	0.53	2.30	0.82	3.12
85	***(3S,5S,8S,9S,10S,13S,14S)-9,10,13-trimethyl-1,2,3,4,5,6,7,8,11,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-3-ol; [9-methylandrostan-3-ol]***	$C_{20}H_{34}O$	***1996***	***1.35***	***5.89***	–	–
86	***hexadecanoic acid; [palmitic acid]***	$C_{16}H_{32}O_2$	***2000***	***3.53***	***15.43***	***7.37***	***28.07***
87	5,5,9,13-tetramethylpentacyclo[11.2.1.01,10.04,9.012,14]hexadecane ; [trachylobane]	$C_{20}H_{32}$	2007	0.12	0.53	–	–
88	3-(2-((1S,2R,4aR,8aR)-1,2,4a,5-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)ethyl)furan; [annonene]	$C_{20}H_{30}O$	2022	0.12	0.54	0.13	0.49
89	(8S,9S,10R,13S,14S)-10,13-dimethyl-1,2,3,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-4-one; [androst-5-en-4-one]	$C_{19}H_{28}O$	2033	0.13	0.56	0.12	0.47
90	***(6E,10E)-3,7,11,15-tetramethylhexadeca-1,6,10,14-tetraen-3-ol***	$C_{20}H_{34}O$	***2042***	***1.19***	***5.18***	***0.15***	***0.57***
91	***(3R)-5-[(1S,4aS,8aS)-5,5,8a-trimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol; [manool]***	$C_{20}H_{34}O$	***2068***	***16.12***	***70.43***	***19.61***	***74.72***
92	(8S,9S,10R,13S,14S)-4,4,10,13-tetramethyl-1,2,3,5,6,7,8,9,11,12,14,15,16,17-tetradecahydrocyclopenta[a]phenanthren-3-ol ; [4,4-dimethylandrostan-3-ol]	$C_{21}H_{36}O$	2081	0.44	1.92	–	–
93	unidentified m/z 276 [M+], 91 (100)	–	2085	0.02	0.09	–	–
94	octadecan-1-ol	$C_{18}H_{38}O$	2090	0.15	0.67	0.36	1.35
95	heneicosane	$C_{21}H_{44}$	2100	0.12	0.53	0.44	1.66
96	***(E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol; [phytol]***	$C_{20}H_{40}O$	***2122***	***3.98***	***17.41***	***6.72***	***25.62***
97	(E)-5-[(1S,4aR,5S)-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-2-enoic acid; [agatholic acid]	–	2135	0.02	0.08	0.04	0.15
98	2-hydroxy-1-[(3S,5S,8R,9S,10S,13S,14S,17S)-3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl]ethanone; [3β,5α-tetrahydrodeoxycorticosterone]	$C_{21}H_{34}O_3$	2139	0.02	0.07	–	–
99	(9Z,12Z)-octadeca-9,12-dienoic acid; [linoleic acid]	$C_{18}H_{32}O_2$	2149	0.13	0.58	–	–
100	***ethyl octadeca-9,12-dienoate***	$C_{20}H_{36}O_2$	***2168***	***2.50***	***10.91***	–	–
101	***ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate***	$C_{20}H_{34}O_2$	***2172***	***5.19***	***22.69***	***5.03***	***19.17***
102	2-(4-butoxyphenyl)pyrimidin-5-amine	$C_{14}H_{17}N_3O$	2181	0.30	1.32	–	–
103	(2E,4Z)-5-(4,5-dimethyl-7a-prop-1-en-2-yl-	$C_{20}H_{34}O_2$	2185	0.43	1.86	–	–

	2,3,3a,5,6,7-hexahydro-1H-inden-4-yl)-3-methylpenta-2,4-dien-1-ol						
104	nonadeca-1,18-diene-7,10-dione	$C_{19}H_{32}O_2$	2190	0.20	0.86	0.54	2.04
105	***[(3S,8R,9S,10R,13S,14S)-10,13-dimethyl-17-oxo-1,2,3,4,7,8,9,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-yl] acetate***	$C_{19}H_{28}O_2$	***2204***	***1.51***	***6.59***	***2.67***	***10.17***
106	(4Z,8Z,13Z)-1,5,9-trimethyl-12-propan-2-ylcyclotetradeca-4,8,13-triene-1,3-diol	$C_{20}H_{34}O_2$	2219	0.88	3.84	0.66	2.52
107	7-ethenyl-1,1,4a,7-tetramethyl-3,4,4b,5,6,9,10,10a-octahydro-2H-phenanthren-2-ol; [sandaracopimaradien-3 β -ol]	$C_{20}H_{32}O$	2230	0.30	1.30	–	–
108	***(1R,2R,4aS,8aS)-1-[(3R)-3-hydroxy-3-methylpent-4-enyl]-2,5,5,8a-tetramethyl-3,4,4a,6,7,8-hexahydro-1H-naphthalen-2-ol; [sclareol]***	$C_{20}H_{36}O_2$	***2249***	***3.77***	***16.49***	***3.53***	***13.44***
109	Methyl kaur-16-en-18-oate	$C_{21}H_{32}O_2$	2264	0.37	1.63	0.56	2.13
110	(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,14,15,16-tetradecahydrocyclopenta[a]phenanthren-17-one; [androsterone]	$C_{19}H_{30}O_2$	2283	0.67	2.93	–	–
111	***(17-acetyl-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl) acetate***	$C_{23}H_{36}O_3$	***2315***	***10.19***	***44.54***	***8.73***	***33.25***
112	(E)-5-[(1S,4aR,5S,8aR)-5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-2-en-1-ol; [agathadiol]	$C_{20}H_{34}O_2$	2326	0.10	0.42	0.10	0.39
113	***1-Naphthalenecarboxylic acid, 5-[2-(3-furanyl)ethyl]decahydro-1,4a-dimethyl-6-methylene-, methyl ester, [1S-(1α,4aα,5α,8aβ)]***	$C_{21}H_{30}O_3$	***2340***	***2.49***	***10.87***	***1.40***	***5.34***
114	pentadec-2-ynyl furan-2-carboxylate	$C_{20}H_{30}O_3$	2366	0.04	0.15	0.06	0.22
115	5-[5-(hydroxymethyl)-5,8a-dimethyl-2-methylidene-3,4,4a,6,7,8-hexahydro-1H-naphthalen-1-yl]-3-methylpent-1-en-3-ol; [18-Hydroxymanool]	$C_{20}H_{34}O_2$	2379	0.06	0.26	–	–
116	***methyl (1S,4aR,5S,8aR)-5-[(E)-5-methoxy-3-methyl-5-oxopent-3-enyl]-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylate; [methyl athecate]***	$C_{22}H_{34}O_4$	***2407***	***11.21***	***49.01***	***10.03***	***38.21***
117	methyl daniellate	$C_{22}H_{3402}$	2432	0.47	2.03	0.65	2.46
118	***(1S,4aS,5R)-5-[2-(furan-3-yl)ethyl]-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid; [polyalthic acid]***	$C_{20}H_{28}O_3$	***2469***	***6.31***	***27.58***	***4.73***	***18.03***
119	***(1S,4aR,5S)-trimethylsilyl 1,4a-dimethyl-5-(3-methyl-5-oxopentyl)-6-methylenedecahydronaphthalene-1-carboxylate***	$C_{22}H_{34}O_4$	***2474***	***4.58***	***20.00***	***3.02***	***11.49***
120	***[(8R,9S,10R,13S,14S)-4,10,13-trimethyl-3-oxo-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-17-yl] acetate***	$C_{22}H_{32}O_3$	***2487***	***1.38***	***6.02***	***1.07***	***4.10***
121	***Pentacosane***	$C_{25}H_{52}$	***2500***	***0.92***	***4.02***	***1.06***	***4.05***
122	(1R,4aS,5R,8aS)-5-[2-(furan-3-yl)ethyl]-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid; [daniellic acid]	$C_{20}H_{28}O_3$	2531	0.34	1.48	0.28	1.08
123	unidentified m/z 298 [M+], 147 (100)	–	2554	0.30	1.32	0.18	0.67
124	***(3R,5R,7R,8R,9S,10S,13S,14S,17S)-7,10,13,17-tetramethyl-1,2,3,4,5,6,7,8,9,11,12,14,15,16-tetradecahydrocyclopenta[a]phenanthrene-3,17-diol; [7a,17a-Dimethyl-5b-androstane-3a,17b-diol]***	$C_{21}H_{36}O_2$	***2557***	***1.26***	***5.49***	***–***	***–***
125	unidentified m/z ? [M+], 149 (100)	–	2557	–	–	0.70	2.67
126	***methyl (1R,4aR,4bS,7R,10aR)-7-ethenyl-1,4a,7-***	$C_{21}H_{32}O_2$	***2568***	***6.53***	***28.54***	***0.67***	***2.55***

	trimethyl-3,4,4b,5,6,9,10,10a-octahydro-2H-phenanthrene-1-carboxylate; [methyl sandaracopimarate]						
127	unidentified m/z 316 [M+], 121 (100)	–	2588	0.71	3.10	0.52	1.97
128	hexacosane	$C_{26}H_{54}$	2600	0.03	0.12	0.03	0.11
129	unidentified m/z ? [M+], 82 (100)	–	2636	0.10	0.44	–	–
130	unidentified m/z ? [M+], 81 (100)	–	2648	0.40	1.77	0.32	1.23
131	heptacosane	$C_{27}H_{56}$	2700	0.20	0.87	0.28	1.08
132	***[(3R,5R,8R,9S,10S,13R,14S,17R)-17-[(E,2R,5S)-5,6-dimethylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl] acetate***	$C_{30}H_{50}O_2$	***2724***	***1.63***	***7.12***	***1.72***	***6.56***
133	unidentified m/z ? [M+], 121 (100)	–	2766	0.02	0.09	–	–
134	octacosane	$C_{28}H_{58}$	2800	0.01	0.03	–	–
135	(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol; [stigmasterol]	$C_{29}H_{48}O$	2816	0.01	0.04	–	–
136	(6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene; [squalene]	$C_{30}H_{50}$	2829	0.01	0.05	0.03	0.11
137	unidentified m/z ? [M+], 82 (100)	–	2839	0.02	0.07	–	–
138	nonacosane	$C_{29}H_{60}$	2900	0.03	0.12	0.06	0.21
TOTAL				**100.00**	**437.03**	**100.00**	**381.03**
Total number of compounds/common compounds				**135/106**		**109/106**	
Total major compounds/common major compounds				**19/14**		**18/14**	
Share of major compounds, %				**86.20**		**82.63**	
Share of common major compounds, %				**73.39**		**77.91**	
Major compounds, µg/g of dry plant weight					**320.73**		**296.85**

Note: for some compounds, trivial or most commonly used names (depositor-supplied synonyms) are given in square brackets; Major compounds are highlighted in bold italics, the proportion of which, at least in one sample, exceeded 1%; «–» – the component is missing.

18), accounting for 86.2 and 82.63% of total VOCs content (Table **2**) respectively. The number of common major components was 14. There were no major compounds in the Lake Kuznechnoye sample, such as 9-methylandrostan-3-ol, ethyl octadeca-9,12-dienoate, and 7a,17a-dimethyl-5b-androstane-3a,17b-diol (two of which are ecdysteroids), which were detected in the Lake Suuri sample.

At the same time, some of the compounds that can be classified as major compounds in the metabolome of pondweed from the eutrophic lake Kuznechnoye were not such compounds in the LMWM composition of pondweed from the mesotrophic Lake Suuri, despite their presence: myristic acid, nonadec-1-ene, isophytol, and pentacosane.

Among the VOCs of the floating-leaf pondweed, compounds belonging to esters, alcohols, and diverse functional groups predominated (Table **3**). These groups also included 3 substances with the highest concentrations in the composition of the essential oil of the studied samples of *P. natans* from Lake Suuri and Lake Kuznechnoye: manool (alcohol, 16.12% and 19.61%, respectively), methyl athecate (ether, 11.21% and 10.03%, respectively), and (17-acetyl-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl) acetate (diverse functional groups, 10.19% and 8.73%, respectively). The content (%) of such groups as aldehydes, hydrocarbons, carboxylic acids, and ketones in the LMWM of floating-leaf pondweed from a eutrophic lake was approximately two times greater than in pondweed from a mesotrophic lake (Table **3**).

The assessment of the similarity of the component composition of *P. natans* essential oil samples as a whole revealed a high degree of similarity of LMWMs between the two analyzed habitats (Table **4**). When only the existence or absence of a component was considered (similarity by Jaccard and Sőrensen-Czekanowski indices), it was discovered that when all

Table 3: Comparative Content (% in Relation to whole Essential Oil) and Concentration (C, µg/g of Dry Plant Weight) of the Main Groups of Compounds in the LMWM of *P. natans* from Investigated Lakes

Compound Groups	Lake Suuri		Lake Kuznechnoe	
	%	C	%	C
Aromatic Hydrocarbons	0.06	0.26	0.10	0.37
Alcohols	**27.54**	**120.36**	**32.24**	**122.85**
Aldehydes	2.87	12.53	4.74	18.05
Hydrocarbons	2.02	8.85	5.18	19.74
Carboxylic acids	4.96	21.69	9.81	37.38
Esters	**33.92**	**148.23**	**22.92**	**87.33**
Unidentified	1.65	7.22	1.71	6.53
Ketones	1.35	5.92	3.48	13.28
Diverse functional groups	**25.60**	**111.89**	**19.76**	**75.29**
Nitrogen-containing	0.003	0.01	0.00	0.00
Chlorine-containing	0.02	0.09	0.06	0.22
TOTAL	100.00	437.03	100.00	381.03

Note: Values in bold type correspond to compounds that make up the first three places in the composition of the essential oil.

VOCs were considered, better similarity rates were found than when only major components were used in the analysis. The Morishita index produced the highest similarity scores when all VOCs concentrations in the LMWM composition were considered, only major components and groups of chemicals (Table **4**).

Table 4: The Similarity of the LMWM of *P. natans* from Investigated Lakes According to the Jaccard Similarity Indices (J), Sőrensen–Czekanowski Similarity Indices (Ks) and Morisita-Horn Index (Cmh)

Similarity Calculated from:	J	Ks	Cmh
all compounds	0.77	0.87	0.93
major compounds	0.61	0.76	0.92
groups of compounds	–	–	0.95

Note: «–» – not calculated.

DISCUSSION

P. natans had 138 VOCs discovered. In other studied species of the genus Potamogeton, this number may be higher, as, for example, in *P. perfoliatus* from the River Volga Delta (164 VOCs) [18], approximately the same (*P. perfoliatus* from the Svir Bay of Lake Ladoga, 134 VOCs) [17], or lower, as in sago pondweed (*P. pectinatus*) from Lake Ladoga (112 VOCs) and from lakes in Astrakhan Region, Russia (80-94 VOCs) [19].

It should be noted that the level of synthesis of VOCs in *P. natans* in lakes Suuri and Kuznechnoye is significantly higher (437.03 and 381.03 µg/g dry plant weight, respectively) than, for example, in *P. perfoliatus* from the lower zone of River Volga's delta and Svirskaja Bay of Lake Ladoga (150.7 µg/g dry plant weight and 42.5 µg/g dry plant weight, respectively), as well as in *P. pectinatus* (87.77-203.14 µg/g dry plant weight) from various biotopes in Lake Ladoga and lakes of Astrakhan Region, Russia [17-19]. It should be emphasized that all of these species were studied during the same stage of development (fructification).

Another distinguishing feature of *P. natans* LMWM is the predominance of a group of alcohols in its composition, which was the most abundant in plants in Lake Kuznechnoye and the second in terms of content in Lake Suuri (Table **3**). While in other pondweed species, fatty acids are frequently the majority group [18, 19].

It should be noted that LMWM of *P. natans* contains high concentrations of such a component as manool. Manool is a naturally occurring, commercially valuable labdane diterpene that has been used to synthesize a number of natural products [36, 37].

Manool and many of the products made from it have a variety of biological properties, including antibacterial, larvicidal, antifungal, antioxidant, anti-inflammatory, antitumor, cytotoxic, and hepatoprotective activity [36-44].

According to the information obtained regarding the high degree of similarity between the LMWM of *P. natans* in two quite different types of lakes (Table **4**), the LMWM in floating-leaf pondweed appears to have been sufficiently conservative. So, for example, a total of 198 VOCs were discovered over two years of research in rigid hornwort (*C. demersum*) in the Volga-Akhtuba floodplain lake with a variable trophic state, while only slightly more than 60% of the compounds were common for two years of research [21]. Evaluation by the similarity coefficients of Jaccard (0.46) and Sőrensen-Czekanowski (0.63) showed a low similarity of the samples [21], given that, according to general ideas, a similarity of more than 80% is considered high and less than 50% is considered low [45]. These similarity indices were substantially higher in the *P. natans* that we investigated, even in diverse water bodies (Table **4**).

The potential for using *P. natans* as an ecological indicator arises from the likelihood that its LMWM is more conservative than that of other pondweed species. In the case of a substantial change in its LMWM, one may apparently speak of some disturbing factor having a significant impact on the aquatic ecosystem.

Previously, it was demonstrated that fatty acids (including those discovered in *P. natans*) are pronounced allelochemicals with inhibitory properties, particularly against cyanobacteria [46-48]. The concentration and content of fatty acids in the floating-leaf pondweed from the eutrophic Lake Kuznechnoye were much higher (nearly two times) than in plants from the mesotrophic Lake Suuri (Table **3**). This could imply that pondweed actively produced fatty acids in reaction to the presence of cyanobacteria in order to suppress them.

The example of *P. natans*, which grew in the form of a small clump of several plants under severe competitive pressure from other macrophytes in the floodplain Lake Obvalovannoe in the Astrakhan region and under favorable conditions (large dense association) of Lake Suuri (Karelian Isthmus) (Figure **2A**), well confirmed the active change in the level of synthesis of certain VOCs by plants [8]. As a defense mechanism, the pondweed in the first case synthesized allomon - geranyl linolool in large quantities. Manool was produced in large amount in a prosperous population in Lake Suuri, although the quantity of geranyl linolool in the VOCs complex was exceedingly low. *P. natans* was not found in the second year of the Obvalovanny Lake survey, indicating that it was still entirely forced out by other plants. [8].

The importance of ecological and geographical factors in the content of some VOCs in LMWM aquatic plants was demonstrated by the example of ecdysteroids [49]. The strong similarity of the composition of *P. natans* LMWM from two closely spaced lakes suggests that geographically determined factors may be largely responsible for the formation of LMWM in aquatic plants even in lakes of different trophic types with vastly different living conditions.

According to research [50], macrophytes like *P. natans* , *N. lutea*, *Nymphaea alba* L., *Myriophyllum spicatum* L., and *Persicaria amphibia* (L.) Delarbre are among the most active producers of allelochemical fatty acids. As a result, they can have a significant allelopathic effect on cyanobacteria and phytoplankton as a whole. In these plants, the percentage of fatty acids in the volatile organic compounds can exceed 60-70 %.

Plants produce phytoecdysteroids as secondary metabolites to protect themselves from phytophagous insects and other pathogens [51-53].

Pondweed from the mesotrophic lake Suuri contained 11 ecdysteroids with a total concentration of 81.17 μg/g of dry plant weight (18.6% of the total content of VOCs), whereas *P. natans* from eutrophic Lake Kuznechnoye contained only 5 compounds with a total concentration of 54.55 μg/g of dry plant weight (14.32% of the total content of VOCs). Ecdysteroids found in Potamogeton plants have previously been shown to be protective against Crustacea and non-adapted phytophagous insects [49, 54]. High quantities of these chemicals in *P. natans* may imply that this plant is well-defended against herbivorous consumers. Moreover, apparently, the level of pondweed protection is higher in a mesotrophic lake compared to a eutrophic one. The appearance of plants (Figure **2 B**, **D**) also supports this.

Methyl jasmonate can control the amount of ecdysteroid production [55]. In our study, plants from Lake Kuznechnoye had greater concentrations of methyl jasmonate in pondweed leaves (Table **2**). However, as mentioned above, greater concentrations of ecdysteroids were found in pondweed from Lake Suuri. Due to some metabolic disorders, the more depressed overall state of plants in the eutrophic lake with a very high concentration of cyanobacteria may have prevented achieving a greater level of ecdysteroids.

CONCLUSIONS

As a result of the research, the qualitative and quantitative component composition of VOCs of the essential oil of the floating-leaf pondweed (*Potamogeton natans* L., Potamogetonaceae family) growing in various lakes of the Karelian Isthmus (North-West of the Russian Federation) in the fruiting stage was revealed in detail for the first time. The LMWM of *P. natans* contained 138 components, 128 of which were identified.

VOCs belonging to esters, alcohols, and various functional groups dominated the LMWM of floating leaf pondweed from mesotrophic and eutrophic lakes. A significant similarity was found between the component composition of the LMWM of floating leaf pondweed from mesotrophic and eutrophic lakes. Many of the substances found in the LMWM of *P. natans* can be attributed to biologically active compounds. This opens up prospects for the use of this plant for various economic applications as a valuable natural raw material.

Due to the characteristic of the floating leaf pondweed's substantial resistance of its LMWM to the factor of the trophic status of the lakes, it is feasible to use it as an ecological indicator of significant disruptions in aquatic environments.

Thus, as a result of the specific component composition of its LMWM, *P. natans* can serve as a natural renewable resource for obtaining valuable natural forms of VOCs (particularly manool and ecdysteroids) of plant origin for a variety of applications in pharmacology, medicine, cosmetology, the food industry, and so on.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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