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LIPID COMPOSITION OF *Potamogeton pectinatus* AS A FUNCTION OF WATER CONTAMINATION

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The lipid composition of Potamogeton pectinatus *L. collected in different parts of the Sok River in the Lower Volga basin was studied. Differences were found in the quantitative lipid composition of the plant as a function of habitat. Plants inhabiting contaminated waters typically had a lower content of total lipids and glycolipids with an increased content of neutral lipids in addition to a lower content of polyunsaturated acids.*

Keywords: submerged plants, lipids, water quality assessment.

The pondweed *Potamogeton pectinatus* L. (Potamogetonaceae, Potamogetonales, Liliopsida) is used to assess the biochemical situation of phytohydrocoenoses of fluvial rivers of the Lower Volga basin [1, 2]. The studied species is classified ecologically as an aquatic submerged plant [3].

Specimens were collected during flowering in the upper and middle parts of the Sok River.

The ecological situation of the river parts was assessed using the man-made impact [4], the water contamination index (WCI) [5], criteria for assessing the ecological situation [6], and the ecological situation index (ESI) of the aquifer according to hydrochemical indicators [7].

The Sok River in the upper reach is slightly affected by anthropogenic factors. This enables this part of the river to be considered as a standard and the biochemical characteristics of the plants, as background indicators characteristic of clean rivers. The waters are contaminated in the middle reach.

The biochemical situation of the plants was assessed from the content of total lipids, the fatty-acid composition, and the ratio of individual lipid groups within each class. Plants for analyses were collected from several places of a single habitat according to previous recommendations [8]. Lipids were extracted and analyzed using methodical instructions [9].

The content of total lipids (TL) was 9.7–7.5 mg/g crude plant mass. The level of TL was greatest for plants from the clean part in the upper reach of the Sok River.

The TL were represented by three classes: membrane glycolipids (GL) and phospholipids (PL) and reserve neutral lipids (NL). The TL were dominated by GL (39.7–42.5%) with PL also present (28.2–29.6%). The NL content was from 27.8 to 32.1% depending on the habitat (Table 1).

Lipids from plants collected in clean habitats had the greatest content of GL and a reduced level of NL. The amount of PL was the same for the plants and did not depend on the habitat.

The analysis of the relative content of individual GL in *P. pectinatus* cells, the main chloroplast lipids, showed that monogalactosyldiacylglycerols (MGDG) dominated in all plants. They made up 51.0-52.6% of the total GL. The content of digalactosyldiacylglycerols (DGDG) was 34.2–35.8%. The content of sulfolipids (SQDG) was the least at 13.2%. The fraction of MGDG in the GL decreased slightly under contaminated conditions because of the increased amount of DGDG.

Greater than 80% of the PL were so-called principal PL such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI). Table 1 shows that the fraction of PC was 38.2–40.1% of the total PL followed by PE (21.6–22.5%), PG (18.6–19.6%), PI (7.2–8.9%), PA (6.8–10.3%), and diphosphatidylglycerol (DPG) (2.4–3.8%). The variability in the basic membrane structural units such as the principal PL showed that the PL composition changed insignificantly depending on the habitat. The minor constituents such as PA and DPG typically varied most.

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TABLE 1. Lipid Composition of *P. pectinatus* as a Function of Habitat

Lipids	Upper reach of Sok River		Middle reach of Sok River	
	mg/g crude wt.	% of total	mg/g crude wt.	% of total
TL	9.7 ± 0.5	100	7.5 ± 0.6	100
GL	4.1 ± 0.2	42.5	3.0 ± 0.1	39.7
PL	2.9 ± 0.1	29.6	2.0 ± 0.2	28.2
NL	2.7 ± 0.2	27.9	2.5 ± 0.0	32.1
		Glycolipids		
MGDG	2.2 ± 0.4	52.6	6.4 ± 0.2	51.0
DGDG	1.4 ± 0.2	34.2	4.5 ± 0.0	35.8
SQDG	0.5 ± 0.0	13.2	1.6 ± 0.2	13.2
		Phospholipids		
PC	1.1 ± 0.1	38.2	0.8 ± 0.1	40.1
PE	0.6 ± 0.1	21.6	0.5 ± 0.2	22.5
PG	0.5 ± 0.2	18.6	0.4 ± 0.3	19.6
PI	0.3 ± 0.1	8.9	0.1 ± 0.4	7.2
PA	0.3 ± 0.0	10.3	0.1 ± 0.1	6.8
DPG	0.1 ± 0.3	2.4	0.1 ± 0.0	3.8
		Neutral lipids		
HC	0.3 ± 0.0	9.5	0.3 ± 0.2	11.0
SE	0.1 ± 0.2	5.7	0.2 ± 0.1	6.0
Wa	0.3 ± 0.2	11.6	0.2 ± 0.1	7.9
TAG	0.5 ± 0.4	17.5	0.6 ± 0.3	24.8
FFA	0.4 ± 0.4	13.9	0.4 ± 0.1	15.1
Al	0.3 ± 0.4	12.3	0.2 ± 0.0	9.7
FS	0.5 ± 0.2	19.3	0.4 ± 0.2	16.2
DAG	0.3 ± 0.1	10.2	0.2 ± 0.0	9.3

Wa, waxes; DAG, diacylglycerols; DGDG, digalactosyldiacylglycerols; DPG, diphosphatidylglycerols; GL, glycolipids; MGDG, monogalactosyldiacylglycerols; NL, neutral lipids; FFA, free fatty acids; Al, alcohols; FS, free sterols; SQDG, sulfoquinovosyldiacylglycerols; TAG, triacylglycerols; PG, phosphatidylglycerols; PI, phosphatidylinositols; PA, phosphatidic acids; PL, phospholipids; PC, phosphatidylcholines; PE, phosphatidylethanolamines; HC, hydrocarbons; SE, sterol esters.

The NL composition, in contrast with that of membrane lipids, turned out to be more variable. This was to a large extent due to such constituents as triacylglycerols (TAG) and waxes (Wa). The accumulation of TAG, one of the main sources of reserve energy, is a known reaction of plants to unfavorable conditions [10, 11]. This effect is due to increased hydrolysis of polar lipids and subsequent incorporation of the liberated acyl groups into TAG synthesis.

The functional features of lipids and the condition of membranes are known to be determined largely by their fatty-acid (FA) composition. The amount of unsaturated acids has an important influence on the membrane permeability and the activity of many membrane-bound enzymes [12]. According to GC analysis, more than 95% of the acids in the studied plants had a chain length from 16 to 18 C atoms. Diene and triene acids were the main unsaturated lipids in *P. pectinatus*. It is thought that the high degree of unsaturation in the FA is responsible for the ability of the plants to adapt to unfavorable environmental factors, e.g., low temperatures, because of the less dense packing of the molecules into the membrane bilayer.

Qualitative differences in the FA compositions of the *P. pectinatus* specimens were not observed. The quantitative compositions of plants from the middle reach of the Sok River showed a lower content of linolenic acid $C_{18:3}$. This indicated that the cell membranes of plants inhabiting this part were more saturated.

The FA composition (% of total acids) of *P. pectinatus* as a function of habitat is given below:

Fatty acid	*Upper reach, Sok R.*	*Middle reach, Sok R.*	*Fatty acid*	*Upper reach, Sok R.*	*Middle reach, Sok R.*
16:0	13.7	15.5	18:2	9.7	8.1
16:1	0.5	5.3	18:3	69.1	60.4
18:0	1.5	1.4	20:1	0.3	0.2
18:1	2.0	3.0	Others	2.6	6.1.

In general, the qualitative lipid composition of *P. pectinatus* inhabiting the Sok River was the same as that for aquatic plant life of a corresponding system. A characteristic feature of the lipid composition in cells of this plant species, one of the most important classes of biomolecules, was the domination of TL by GL. The principal GL with respect to quantity was MGDG. The contents of lipids making up most of the PL decreased in the following order: PC > PE > PG > PI. The NL were represented by eight constituents. Of these, more than half were di- and triacyl-substituted glycerin derivatives (TAG and DAG). The FA composition included (over 70%) unsaturated FA, mainly of the triene series.

In addition, the content of TL in plants inhabiting contaminated waters was 25% less than in plants from clean waters. The GL content in plant cells from contaminated areas was less than in plants from clean parts with an increased NL content. Differences were also seen in the quantities of FA. Thus, lipids of plant cells from contaminated habitats consistently had a lower content of polyunsaturated acids.

EXPERIMENTAL

Lipids were extracted from native tissues by $CHCl_3$:MeOH (1:1, v/v) [13] and were identified using specific reagents for separate functional groups [9]. PL were separated by two-dimensional TLC on glass plates (6 × 6 cm) with a fixed layer of silica gel using the following solvent systems: $CHCl_3$:MeOH:C_6H_6:NH_4OH (130:60:20:12; first direction); $CHCl_3$:MeOH:C_6H_6:Me_2CO:CH_3COOH (140:60:20:10:8, second direction) [14]. PL were detected by spraying with H_2SO_4 solution (10%) in MeOH with subsequent heating at 180°C for 15 min. The amount of PL was determined from the content of inorganic phosphorus [15].

GL were separated by TLC on plates (10 × 10 cm) with a fixed layer of silica gel using Me_2CO:C_6H_6:H_2O (91:30:8). GL were detected by spraying the plates with $12MoO_3 \times H_3PO_4$ (5%) in EtOH with subsequent heating at 120°C for 10 min. The amount of GL was determined using a Sorbfil densitometer (Russia). Calibration curves were constructed using standard MGDG (Larodan, Sweden) [14].

NL were separated by TLC on metal plates (10 × 10 cm) with a fixed layer of silica gel (Sorbpolimer, Russia) using MeC_6H_5:C_6H_{14}:HCO_2H (70:30:0.5) and C_6H_{14}:Et_2O:HCO_2H (60:40:1). The amount of NL was determined using a Sorbfil densitometer (Russia) using tripalmitate (Sigma) as a standard to construct calibration curves.

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