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LIPIDS FROM MACROPHYTES OF THE MIDDLE VOLGA

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Abstract—Lipid compositions from 14 macrophytic species found in the Middle Volga region belonging to different taxonomic groups but making up certain ecological groups based on the similarity of their environments, were studied. The contents of total lipids, neutral lipids, glyco- and phospholipids were determined and their respective fatty acid compositions analysed. Percentages of individual phospholipids were estimated. Common features in the qualitative and quantitative lipid characteristics of the studied water plants were revealed.

INTRODUCTION

The majority of publications on the lipid compositions from water plants deal with marine algae [1–8]. Freshwater plants are much less studied [9–11], although their role in the life of water ecosystems is equally important. Water plants, including macrophytes, are universally recognized as important participants in the natural processes of water self-purification [12, 13].

The present paper describes the lipid compositions of some representatives of higher plants found in water pools of the Middle Volga region. The macrophytic species selected for the purposes of our investigation, considering their life-style but regardless of their systematic position, can be classified into five ecological groups, in-depth free-swimming hydrophytes (I), submerged root-developing hydrophytes (II), on-surface free-swimming hydrophytes (III), root-developing hydrophytes with on-surface hydrophytes (III), root-developing hydrophytes with on-surface floating leaves (IV) and low-growing water plants with aerial shoots (V). Obviously, the first four groups include the hydrophytes proper, while the last one represents helophytic plants. This classification is based on the ecobiomorphological classification for macrophytes from the Middle Volga water pools and streams suggested by Papchenkov [14, 15].

It is well-known that many plants, e.g. algae [16] and lichens [17] can change their lipid contents, as well as other lipid characteristics, for instance, their fatty acid composition, under the influence of the environmental conditions [18]. In view of this, we collected all our macrophytic samples during one season, during the period of the most active vegetation from lakes located within the same climatic zone, in order to ensure a valid comparison of lipid ranges from water plants belonging to different ecological groups.

RESULTS AND DISCUSSION

The results of lipid analyses are given in Tables 1–6. They demonstrate certain similarities between lipid characteristics, as well as some differences due to different environmental conditions.

Total lipids (TL) accumulated in the studied macrophytes amounted to 36–178 mg g^{-1} dry wt for helophytes (Table 1). A higher lipid content (88–178 mg g^{-1} dry wt) was noted for the in-depth free-swimming hydrophytes, *Ceratophyllum demersum*, *Lemna trisulca* and for the submerged root-developing hydrophytes, *Elodea canadensis*, *Limmotium spongia*, *Potamogeton perfoliatus* and *Myriophyllum verticillatum*. The root-developing hydrophytes with floating leaves represented in our study by two species, *Nuphar lutea* and *Polygonum amphibium*, had lipid contents (36–44 mg g^{-1} dry wt) closer to water-plants with aerial shoots, *Alisma plantago-aquatica* and *Sagittaria sagittifolia*. Separation of lipids by column chromatography demonstrated the domination, of glycolipids (GL) (Table 1). The latter were found to vary from 40.0 to 53.7% in hydrophytes and from 40.4 to 43.8% in helophytes.

Generally, neutral lipid (NL) contents in the examined macrophytes varied from 24.7 to 40.9%. However, it should be noted that content of NL found in hydrophytes was somewhat lower (24.7–35.9%) compared to helophytes (31.6–40.9%). Phospholipids (PL) in hydrophytes and helophytes varied from 19.2 to 30.8% and from 18.7% to 27.9%, respectively.

Within each ecological group made up of several species, the range of variation for each lipid class was not large. This can be illustrated by the submerged root-developing group which was represented by the greatest number of plant species; these had 24.9–29.2% NL, 396–53.7% GL and 19.2–31.2% PL.

Table 1. Lipids from some freshwater macrophytes*

Ecological group	Species	TL	NL	GL	PL
I	*Ceratophyllum demersum* L.	115	35.9	38.2	25.9
	Lemna triscula L.	92	31.4	42.4	26.3
II	*Elodea canadensis* Michx.	88	24.7	44.5	30.8
	Limnombium spongia Bosch.	93	29.2	39.6	31.2
	Potamogeton perfoliatus L.	100	26.6	53.7	19.7
	Myriophyllum verticillatum L.	178	26.9	48.4	24.7
III	*Lemna minor* L.	88	29.3	30.7	40.0
IV	*Nuphar lutea* L. Smith	44	28.4	41.9	29.7
	Polygonum amphibium L.	36	33.7	41.7	24.6
V	*Alisma plantago-aquatica* L.	78	40.9	40.0	19.1
	Butomus umbellatus L.	66	36.0	44.9	19.1
	Sagittaria sagittifolia L.	123	37.5	43.8	18.7

*Average results based on two to three independent analyses.
TL, Total lipids, mg g^{-1} dry wt.
NL, Neutral lipids; GL, glycolipids; PL phospholipids.

Table 2. Phospholipids from some freshwater macrophytes*†

Species	PG	DPG	PE	PC	PI	PS	PA‡
Ceratophyllum demersum	13.4	—	19.1	36.0	9.5	4.9	17.1
Lemna trisulca	16.1	9.1	20.2	35.5	7.8	3.5	7.8
Elodea canadensis	11.3	—	18.8	47.6	11.4	8.4	2.5
Limnobium spongia	12.1	3.4	25.0	37.5	5.6	2.8	13.6
Potoamogeton perfoliatus	24.9	—	11.0	41.4	9.9	4.1	8.7
Myriophyllum verticillatum	15.7	—	16.7	38.0	18.8	4.5	6.3
Lemna minor	10.2	8.2	24.1	41.0	10.8	3.1	2.6
Nuphar lutea	12.2	7.1	20.6	37.6	12.6	3.7	6.3
Polygonum amphibium	13.0	—	15.9	46.9	12.2	1.3	10.7
Alisma plantago-aquatica	16.4	—	13.2	55.8	9.7	1.7	2.8
Butomus umbelatus	10.4	—	25.5	53.3	5.6	1.4	3.7
Sagittaria sagitifolia	21.9	—	10.9	42.7	7.1	5.3	12.1

PG, Phosphatidylglycerol; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PA, phosphatidic acid.
*Average results based on three parallel analyses.
†Values are given in % of total phosphorus.
‡High PA values may be due to partial lipid degradation occurring during transportation of samples and their processing before analysis.

Analyses of individual phospholipids revealed the presence of the following components: phosphatidylglycerol (PG), disphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylchloine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA) (Table 2). Quantitatively, the main phospholipid was PC ranging from 35.5 to 55.8%. We found no relationship between the level of PC and the character of an environment for the examined ecological groups of macrophytes, although, as a general rule, the level of PC in hydrophytes was slightly lower (35.5–47.6%) than in helophytes (42.7–55.8%). The second most abundant pholipids were PE (11.0–28.2%) and PG (11.3–24.9%). PI was present in all the examined species in the range 4.2–18.8%. PS in amounts from 1.3 to 8.4% was also found in most of the studied macrophytes.

Fatty acid (FA) compositions from the studied macrophytes, both for total lipid and individual classes, are presented in Tables 3–6. The composition and distribution of FAs should be noted for certain common features between the macrophytes despite their ecological classification. Thus among the general FA from all the studied species (Tables 3 and 4), the presence of palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and polyunsaturated fatty acids (18:3) is noted

Water plants developed from terrestrial plants through adaptation. As in higher terrestrial plants, freshwater macrophytes possess 16:0 which dominates their saturated fatty acids [5]. Acids with 20 or more carbon atoms were either not found or detected in small amounts in our experiments (except *L. trisulca* and *S. sagittifolia*), contrary to marine plants which are known to have such

Table 3. Fatty acids from total lipids of some freshwater macrophytes*

Species	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Ceratophyllum demersum	1.7	tr	36.7	8.1	1.7	3.5	23.5	24.4	—
Lemna trisulca	2.0	2.2	22.0	12.4	0.6	7.4	24.4	28.5	0.6
Elodea canadensis	1.5	3.7	24.2	3.9	3.7	8.5	18.8	34.8	0.6
Limnombium spongia	2.0	—	25.8	5.2	4.0	3.6	16.1	42.8	tr
Potamogeton perfoliatus	3.0	tr	63.8	6.3	2.8	9.7	2.3	11.8	tr
Myriophyllum verticillatum	3.5	2.7	25.6	21.2	1.2	4.7	17.4	23.6	—
Lemna minor	0.6	—	12.7	6.0	tr	2.4	28.7	49.0	0.6
Nuphar lutea	2.3	—	45.5	—	2.7	3.5	39.9	5.6	—
Polygonum amphibium	—	tr	32.4	0.6	tr	5.7	17.7	43.5	—
Alisma plantago-acquatica	tr	—	54.5	1.8	2.3	0.7	5.0	35.4	—
Butomus umbellatus	1.8	2.5	21.6	2.8	5.7	2.9	8.4	53.9	tr
Sagittaria sagittifolia	0.5	1.1	42.8	2.6	4.6	3.2	18.8	26.4	—

Average results based on three parallel analyses.
tr = Trace amounts, or less than 0.5%
— Not detected.
*Values given in % of total FA.

Table 4. Fatty acids from neutral lipids of some freshwater macrophytes*

Species	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Ceratophyllum demersum	3.0	0.9	29.4	12.6	2.5	7.3	16.6	27.5	tr
Lemna trisulca	5.1	1.5	24.3	15.5	4.7	29.3	13.4	6.2	—
Elodea canadensis	3.7	tr	41.0	12.1	1.6	7.1	15.1	18.8	—
Limnombium spongia	2.8	tr	52.3	8.5	4.6	18.6	6.6	6.2	—
Potamogeton perfoliatus	3.4	2.7	44.9	6.5	8.0	12.7	15.0	6.3	0.5
Myriophyllum verticillatum	4.2	—	43.0	9.2	3.2	6.9	20.4	13.1	—
Lemna minor	4.5	tr	38.2	16.2	5.6	7.6	8.2	19.6	—
Nuphar lutea	2.9	2.1	32.2	3.1	2.1	3.3	46.1	8.2	—
Polygonum amphibium	6.5	10.3	45.6	3.3	3.4	11.0	13.7	6.0	—
Alisma plantago-acquatica	2.8	2.5	63.6	2.8	4.9	9.9	8.5	4.9	tr
Butomus umbellatus	5.9	1.5	37.9	0.9	8.4	9.2	7.6	27.4	0.6
Sagittaria sagittifolia	2.3	0.9	45.2	3.4	7.4	12.7	16.3	10.8	0.9

*Footnotes as in Table 3.

Table 5. Fatty acids from glycolipids of some freshwater macrophytes*

Species	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Ceratophyllum demersum	—	—	5.7	tr	—	tr	4.2	89.9	—
Lemna trisulca	0.6	—	15.5	3.2	0.8	0.9	10.8	66.8	0.9
Elodea canadensis	tr	tr	6.2	1.4	—	—	8.4	84.0	tr
Limnombium spongia	tr	—	5.6	tr	—	0.9	1.8	91.4	—
Potamogeton perfoliatus	tr	0.9	12.2	1.3	0.8	1.2	12.2	70.3	0.7
Myriophyllum verticillatum	tr	—	12.6	2.4	tr	0.6	9.5	74.7	—
Lemna minor	—	—	5.3	0.6	0.9	0.8	3.4	88.9	—
Nuphar lutea	tr	—	11.9	tr	0.5	0.7	17.9	68.9	—
Polygonum amphibium	tr	0.6	11.4	tr	0.7	1.7	4.8	80.3	—
Alisma plantago-aquatica	tr	tr	12.1	1.1	0.7	0.9	12.4	72.3	tr
Butomus umbellatus	tr	tr	3.2	tr	0.7	0.6	1.0	93.6	—
Sagittaria sagittifolia	0.6	0.5	19.6	1.5	1.9	1.2	16.6	57.6	—

*Footnotes as in Table 3.

Table 6. Fatty acids from phospholipids of some freshwater macrophytes*

Species	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3
Ceratophyllum demersum	—	—	51.1	3.5	tr	0.7	40.9	3.6
Lemna trisulca	0.5	tr	46.5	5.6	4.3	4.0	28.4	10.1
Elodea canadensis	tr	tr	25.9	4.8	2.0	3.7	25.3	38.0
Limnombium spongia	—	—	55.8	0.9	2.1	0.6	10.3	30.3
Potamogeton perfoliatus	0.5	0.6	38.6	9.0	1.3	4.9	32.3	13.6
Myriophyllum verticillatum	—	—	35.5	tr	tr	0.5	58.4	5.1
Lemna minor	—	—	65.1	1.8	tr	—	10.7	22.4
Nuphar lutea	tr	—	59.0	tr	0.5	0.9	37.0	1.9
Polygonum amphibium	tr	0.6	31.8	5.2	1.4	13.6	30.3	16.7
Alisma plantago-acquatica	tr	—	38.9	10.3	2.8	—	18.5	29.3
Butomus umbellatus	tr	tr	28.3	tr	3.8	2.3	10.0	54.6
Sagittaria sagittifolia	—	—	57.1	8.7	—	—	18.3	16.0

*Footnotes as in Table 3.

acids at levels as high as 61.2% in certain species [8]. The relationship between saturated and unsaturated acids within the general lipids in the species studied did not correlate with their ecomorphological classification.

Comparison between the FAs from the neutral fraction (Table 4) and those from the total one showed that the range of acids remained the same for all the studied species. The ratio between the main acids was also preserved.

The acid distribution among glycolipids was different. The amounts of 16:0, 16:1 and 18:1 were decreased, while there was an increase in those of 18:3. Polyunsaturated acids (18:3) predominated in nearly all of the macrophyte species from different ecological groups (57.6–93.6%).

A characteristic of the FA distribution in phospholipids was an almost complete absence of 14:0, 15:0 and 20:0. The obtained data testify to the fact that despite the ecological and morphological differences between the examined water plants, only some of their lipid characteristics showed variation. The transition from hydrophytes to helophytes is characterized by a decrease in phospholipids among the major lipid classes, accompanied by an increase in PC. No significant differences in composition or distribution of fatty acids between the water plants proper, on the one hand, and the water plants with aerial shoots, on the other, were revealed.

EXPERIMENTAL

Samples of macrophytes were collected from freshwater lakes in off-town locations in the vicinity of Togliatti, during the beginning of July 1992. Plants were washed free from silt particles, shredded and fixed in $CHCl_3$–MeOH (1:1). Extraction of lipids was done after a 2 min homogenization in $CHCl_3$–MeOH (1:2) according to ref. [19]. Combined extracts were washed in 0.9% KCl. Total lipids were sepd by CC on silica gel L 100/250 [19]. Isolated lipid frs were analysed by TLC using authentic samples. Phospholipids were identified and quantified according to the method described in ref. [20]. Fatty acids were transformed into Me esters and analysed by FID-GC using a 3 m-long glass column filled with 10% PEGA on chromaton N-AW-HMDS, operated at 198°.

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