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Iwona KOSTRZEWSKA-SZLAKOWSKA

Centre for Ecological Research, Polish Academy of Sciences
Dziekanów Leśny, 05-092 Łomianki, Poland, e-mail: iwona.ks@wp.pl

SURFACE MICROLAYER IN LAKES OF DIFFERENT TROPHIC STATUS: DISSOLVED ORGANIC MATTER AND MICROBIAL COMMUNITY

ABSTRACT: The aim of this paper was to recognize the abundance and frequency of occurrence of neustonic organisms (i.e. bacteria and algae) and accumulation of organic matter in the surface microlayer of three lakes of various trophic status. Water samples of surface microlayer (0.5–0.6 mm) were taken (with Larsson plate) as well as from respective epilimnion layer (0.5 m deep). The samples were collected from shallow, humic (*Sphagnum* bog) lake (L. Flosek), shallow, eutrophic (L. Zelwążek) and deeper, mesotrophic lake (L. Kuc) in the period May–October during several years. The ratio of the organisms' density in the surface microlayer to that in deeper (0.5 m) layer was considered as the enrichment factor (Ef). Heterotrophic bacteria accumulation in the surface microlayer was more frequent in the humic lake (75% of samples), than in meso- and eutrophic lakes (64%). Mean Ef values for bacteria ranged from 1.3 to 1.4. Frequent, but not strong accumulation of dissolved organic matter measured as the absorbance A_{254} was noted in the surface microlayer. Dissolved organic carbon (DOC) measured in automatic analyzer showed much stronger accumulation in microlayer, particularly in humic lake. Concentration of chlorophyll *a* in the surface microlayer was found as the most fluctuating and the highest mean Ef value was found in the mesotrophic lake (Ef = 6.3). An attempt was undertaken to explain these differences between the lakes in terms of variable ratio between allochthonous and autochthonous

production in humic, mesotrophic and eutrophic lakes.

KEY WORDS: surface microlayer, dissolved organic matter, microbial community, enrichment factor

1. INTRODUCTION

The surface microlayer of water bodies possesses physical and chemical properties unique and different in comparison to the rest of the water column. It accumulates the various materials from the atmosphere and the bulk water. Buoyancy, electrostatic attraction, physical and chemical adsorption, and surface tension are interacting processes capable holding various material in this layer and contributing to considerable heterogeneity in its composition (Södergren 1987). The surface water undergoes greater fluctuations in temperature, wind, water currents, wave action, and intense solar radiation than deeper water. These specific conditions of life favor the development of some organisms and limit the distribution of others, and create an assemblage of organisms called neuston. The neuston organisms are the representatives of most major divisions of the bacteria, archaea and eukarya domains,

which either live, reproduce, or feed on the surface microlayer. Also larval or juvenile stages of many species of fish occur there (Hardy 1982). The accumulation of bacterioneuston has been reported in many sources; it is a typical phenomenon in this layer (Hatcher and Parker 1974, Norkrans 1980, Hardy 1982, Maki and Remsen 1989, Münster *et al.* 1998, Donderski *et al.* 1999). The phytoneuston community differs significantly from the phytoplankton community. It displays a lower species diversity and greater degree of dominance, greater biomass and primary production (Hardy 1973) but on the other hand – lower photosynthetic activities (Albright 1980). The range of Ef values (mostly measured as chlorophyll *a* concentration) appears to be very wide indicating the accumulation as well as the depletion of algae in this layer (Saijo *et al.* 1974, Carlson 1982, Danos *et al.* 1983, Estep and Remsen 1985, Södergren 1987, Münster *et al.* 1998, Kostrzewska-Szlakowska 2000).

This study was aimed to recognize in the surface microlayer the extent and frequency of accumulation of some neustonic components (bacteria, chlorophyll *a*) and organic matter (measured as DOC and absorbance A_{254}) in lakes of various trophic status.

Up-to-date studies allow to expect that: 1) like in case of nutrients (Hillbricht-Ilkowska and Kostrzewska-Szlakowska 2004) also the organic matter and bacterial density accumulation in the surface microlayer should be commonly observed; 2) this accumulation level should be different in humic, mesotrophic and eutrophic lakes due to the origin and chemical structure of the organic matter and due to different ratio of autochthonous to allochthonous organic matter supply; 3) organic matter accumulation in the surface microlayer should be especially intensive in humic lake due to higher supply of specific allochthonous matter in which refractory fraction dominates; 4) accumulation of bacteria should be also high and related to the trophic state of lakes; 5) concentration of chlorophyll *a* in the surface microlayer might be strongly variable due to extreme conditions in this layer.

2. STUDY SITES AND METHODS

Three lakes of different trophic status are selected for this study in the Masurian Lakeland (north-eastern Poland). Lake Flosek is a humic, shallow (mean depth 3.0 m), small (4.0 ha), mid-forest (pine-spruce) lake with sediments of the “dy” type. Western part of the lake adjoins the *Sphagnum* raised bog with *Ledum palustris* and *Droesera rotundifolia*. The lake water is low-productive with $TP \leq 50 \mu\text{g l}^{-1}$, chlorophyll $\leq 5 \mu\text{g l}^{-1}$ and a high water transparency in summer of 3–4 m. The colour of water is equal to $30 \mu\text{g Pt l}^{-1}$, which is equivalent to $<10 \text{ mg l}^{-1}$ DOC (Hillbricht-Ilkowska *et al.* 1998). Lake Zelwążek is a eutrophic, small (11.5 ha), shallow (mean depth 3.7 m), through-flow lake in the river system (Jorka river). Littoral zone occupies almost half of the lake surface. Its direct catchment has agricultural character. During summer periods in years 1996–1998 maximal concentration of TP in epilimnion was $84.0 \mu\text{g l}^{-1}$, and that of TN – 2.5 mg l^{-1} . The mean chlorophyll *a* concentration was $10 \mu\text{g l}^{-1}$, but the higher values of $40\text{--}50 \mu\text{g l}^{-1}$ were also recorded. Deeper layer of hypolimnion water is deoxygenated during summer (Hillbricht-Ilkowska 2002). Mesotrophic, mid-field Lake Kuc is the largest (98.8 ha) and deepest (mean depth 8.0 m). It has extensive submerged macrophyte zone dominated by charophytes (*Chara* sp.) and *Myriophyllum* sp. The mean summer concentration of TP in epilimnion was about $36 \mu\text{g l}^{-1}$ and that of chlorophyll *a* – $5.2 \mu\text{g l}^{-1}$ (Kufel 2001). For more details see in Hillbricht-Ilkowska and Kostrzewska-Szlakowska (2004).

Water samples from the surface microlayer (SM) were taken using the Larsson's plate (Larsson *et al.* 1974), 15×15 cm perforated plate made of hydrophobic Teflon. Samples of epilimnetic water were collected from the depth of 0.5 m (subsurface water – SSW) with a 1 litre Patalas type sampler (Hillbricht-Ilkowska and Kostrzewska-Szlakowska 2004). In each lake samples were collected in five repetitions on three sites. Water samples (from the surface microlayer – SM and the subsurface water – SSW) were collected monthly, from April till October. Samples from lake Flosek were taken in the years 1992–1993, from lake

Zelwążek – in 1995–1996 and from lake Kuc in 1997. In 1998, on three sampling occasions (May, August, October) samples were taken from all three lakes (for chemical analyses and bacterial abundance). In the laboratory, water volume was measured and the thickness of the surface microlayer was calculated. The thickness of sampled microlayer was 0.5–0.6 mm in all lakes.

All samples were analysed for following elements: 1/ chlorophyll *a* content was measured with spectrophotometer (Shimadzu model UV 160 A) following the procedures of Riemann (1980). Samples were filtered on glass fibre filters (Whatman GF/C, 2.5 cm). Until analyses they were kept refrigerated. The filters were sucked dry and extracted in 6 ml of 96% methanol during 24 h in darkness. The absorbance of the extracts were measured at 665 and 750 nm, before and after treating with 1N HCl. The Lorenz equation was used to calculate concentration in $\mu\text{g l}^{-1}$; 2/ water samples for bacterial analysis were treated following method of Porter and Feig (1980). The samples were kept in plastic tubes preserved with 37% formaldehyde (2% final concentration) and refrigerated until analysis. Subsamples (1 or 2 ml) were diluted with 10 ml of distilled water and incubated for 5 min. with 1 ml of DAPI (final concn. 10 μM ; 4'-diamidino-2-phenylindole). After that the samples were drawn through the black polycarbonate filters (0.2 μm , 25 mm, Poretics). The filters were placed on a microscope slide with a drop of nonfluorescing immersion oil (Cargille type A) and covered with cover slip. The NIKON Optiphot 2 epifluorescence microscope (1 000 \times magnification) was used. Up to 1000 cells were counted for each sample and calculated for 1 ml of the water. 3/ concentration of dissolved organic carbon (DOC; mg l^{-1}) was measured with Automatic Organic Carbon Analyzer (Shimadzu TOC-5000) in samples filtered through Whatman GF/C filters and acidified with 2N HCl to pH 2. Concentration of dissolved organic matter was measured spectrophotometrically in filtered (GF/C) water as the absorbance at 254 nm (Polish Standard Methods PN-84, C-04572).

The enrichment factor E_f ($= X_{SM}/X_{SSW}$) was calculated to compare concentration of a substance in the SM with concentration

of a substance in the SSW samples at a depth of c. 0.5 m (Hunter 1997). Mean enrichment factors for each pair of independent data were also analysed.

Statistical processing was made with the STATISTICA software. To compare SM and SSW data the T-test for independent variables was used. The enrichment factors of chlorophyll *a* in three lakes were compared with the Tukey test for different number of data (n ; ANOVA). All statistical analyses were made at $\alpha < 0.05$.

3. RESULTS

In the humic and eutrophic lakes chlorophyll *a* concentrations ($\mu\text{g l}^{-1}$) in the surface microlayer and subsurface water were distributed almost evenly along the diagonal line (Fig. 1). It means, that the chlorophyll *a* content in SM and SSW samples were similar. But in the mesotrophic lake strong accumulation of chlorophyll *a* in the surface microlayer occurred (Fig. 1). Here also E_f values higher than 1.0 prevailed (63% of samples; Table 1). In the humic and eutrophic lakes the concentrations of chlorophyll *a* in both layers were more or less equal (Fig. 1). Similar trend was observed when mean values of chlorophyll *a* concentration in both layers calculated for the whole data set are considered (Table 1). Mean chlorophyll *a* concentrations were higher (c. 20 $\mu\text{g l}^{-1}$) in both layers of eutrophic and in the surface microlayer of mesotrophic lake than in both layers in the humic and subsurface water of the mesotrophic lake (c. 5 $\mu\text{g l}^{-1}$) (Fig. 1, Table 1). Statistical analysis (t-test for independent samples) confirmed the significance of differences between concentrations of chlorophyll *a* in SM *versus* SSW only in the mesotrophic lake ($P < 0.05$; Table 1) where the mean E_f values for the whole data set was 6.3. In other lakes, E_f values were only slightly over 1 (Table 1). Statistical analysis confirmed also the significance of differences between accumulation rate of chlorophyll *a* in the surface microlayer of mesotrophic lake to these of the humic and eutrophic lakes. These data show, that the highest accumulation of chlorophyll *a* in the surface microlayer occurred only in the mesotrophic lake. In other lakes, the concentrations were similar in both layers.

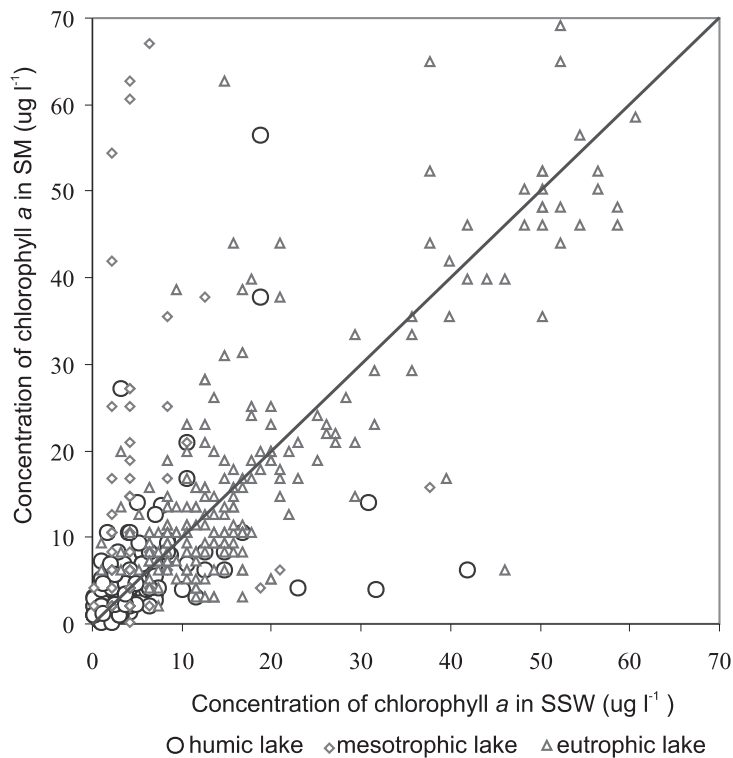


Fig. 1. Concentration of chlorophyll *a* ($\mu\text{g l}^{-1}$) in surface microlayer (SM) and subsurface water (SSW) in lakes under study.

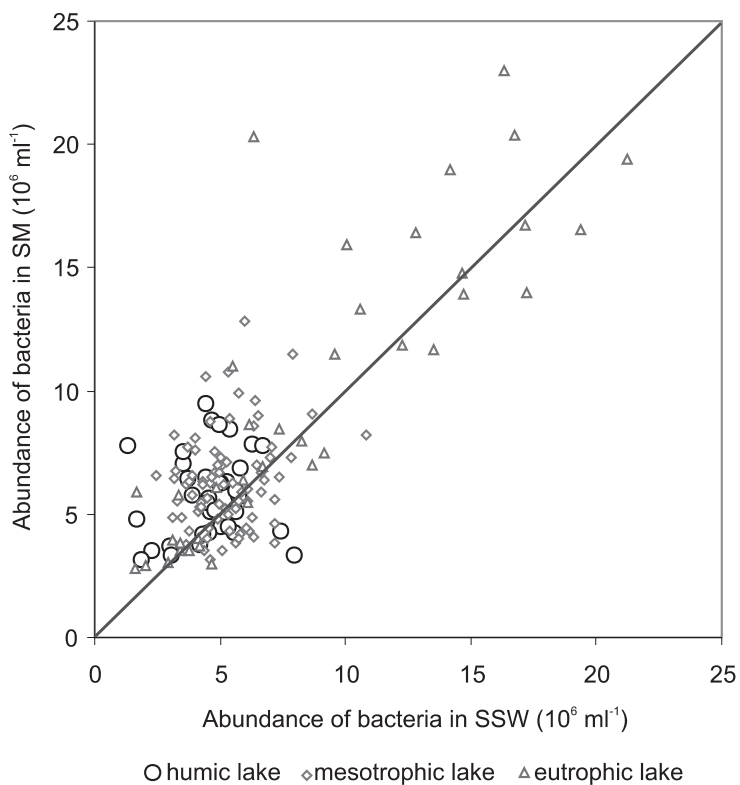


Fig. 2. Abundance of bacteria ($10^6 \text{ cells ml}^{-1}$) in surface microlayer (SM) and subsurface water (SSW) in lakes under study.

A weak tendency to bacteria accumulation was observed in the surface microlayer (Fig. 2). However, higher abundance of bacteria in the SM than in the SSW was observed at least in more than 64% of samples in all studied lakes (Table 2). The highest mean value of Ef was found in the humic lake and accumulation occurred there the most frequently. In other lakes, mean Ef values were slightly lower. The mean abundance of bacteria in SM and SSW of the humic and mesotrophic lakes were generally low ($4.6\text{--}6.7 \times 10^6$ cell ml⁻¹), while in the eutrophic one it was twice as much (Table 2). Although Ef values were not high (1.3–1.4), the statistical analysis (t-test) confirmed the significant differences in the accumulation of bacteria in the

surface microlayer in the mesotrophic and humic lakes. In the eutrophic lake the difference between the layers were insignificant (Table 2).

Two estimation methods of content of organic matter in lakes water gave different view. Absorbance data (A_{254}) in both layers of examined lakes were similar, so enrichment factors were slightly over 1 (Table 3). DOC concentration values in the SM were highly variable but generally higher than in SSW and mean enrichment factors of DOC in SM ranged from 7.7 to 26.6 (Table 3).

No correlation was found between the abundance of bacteria and concentration of organic matter (A_{254}) or dissolved organic carbon (DOC).

Table 1. Average values of concentration of chlorophyll *a* ($\mu\text{g l}^{-1}$) in the surface microlayer (SM) and subsurface water (SSW) in lakes under study; *n* – number of data; Ef – average values of enrichment factor in SM water and % of the samples, when the values for SM are greater than for SSW. In brackets – standard deviations (SD).

	Lake		
	humic	mesotrophic	eutrophic
<i>n</i>	128	76	207
SM	5.6 (± 6.0)	21.0 (± 35.0)	22.1 (± 23.2)
SSW	5.8 (± 6.6)	5.0 (± 5.0)	20.1 (± 14.9)
<i>P</i>	ns	***	ns
Ef (SD)	1.4 (± 2.2)	6.3 (± 12.3)	1.2 (± 1.0)
Ef > 1 (%)	44	63	42

ns – non significant

*** $P < 0.001$

Table 2. Average values of abundance of bacteria (10^6 cells ml⁻¹) for *n* data set in the surface microlayer (SM) and subsurface water (SSW); standard deviations (SD) in brackets. Ef – average values of enrichment factors and percent (%) of the samples when abundances in SM were greater than in SSW (Ef > 1).

	Lake		
	humic	mesotrophic	eutrophic
<i>n</i>	36	87	42
SM	5.7 (± 1.4)	6.7 (± 3.7)	10.6 (± 7.3)
SSW	4.6 (± 1.1)	5.3 (± 1.4)	8.8 (± 5.5)
<i>P</i>	**	**	ns
Ef (SD)	1.44 (± 0.9)	1.33 (± 0.8)	1.29 (± 0.6)
Ef > 1 (%)	75	64	64

ns – non significant

** $P < 0.01$

Table 3. Average values (for n samples) of enrichment factors: A_{254} – organic matter measured as the absorbance (at 254 nm) and DOC – dissolved organic carbon (measured in Automatic Analyzer) in lakes under study (data from May, August and October 1998; in brackets – standard deviation).

Lake	A_{254}	n	DOC	n
humic	1.24 (\pm 0.2)	34	26.60 (\pm 23.5)	8
mesotrophic	1.15 (\pm 0.1)	17	7.71 (\pm 9.1)	5
eutrophic	1.29 (\pm 0.2)	24	14.88 (\pm 14.8)	9

4. DISCUSSION

Enrichment factor values in the surface microlayer for chlorophyll a found in publications are quite variable. In lakes of similar (to the present study) trophic state (meso- and eutrophic), Saijo *et al.* (1974), found much lower accumulation of chlorophyll a in SM. On the other hand, Danos *et al.* (1983) estimated the value of chlorophyll accumulation of SM in experimental ponds equal to 6.6; it is comparable to the average value found in our mesotrophic lake. Estep and Remsen (1985) worked on the same ponds, found out lower enrichments factors – around 1.5. Münster *et al.* (1998) studied accumulation of chlorophyll a at the air-water interface in small, humic lakes used two sampling techniques; enrichment factor values ranged from 1.7 to 80.0. The differences in values between the samples were mostly related to the depth of the sampled layer and different properties of the sampling surface (glass-teflon). Higher enrichment factors of chlorophyll were noted for the SM of lower thickness collected by a rotating teflon – coated drum attached to especially constructed catamaran. The thicker SM sample could have the surface water diluted with subsurface water that has lower concentration of materials. Södergren (1987) in eutrophic and humic lakes found E_f values for chlorophyll ranged from 2.3 to 4.5. Danos *et al.* (1983) found out in small, experimental ponds chlorophyll accumulation in 66 and 42% of the samples. Estep and Remsen (1985) data from SM in ponds have shown that SM was enriched with chlorophyll a in only 40% of samples. Accumulation of chlorophyll a

in SM was obtained in half of the samples (Table 1).

Results presented in this paper and data from Japan lakes (Saijo *et al.* 1974) seem to indicate that the highest enrichment factors of accumulation of chlorophyll a in SM took place in mesotrophic lake. The variation of values of accumulation of chlorophyll a in SM found in literature references could be caused by several factors. Some of microlayer algae populations are adapted to intense solar radiation by reducing photosynthetic activities per biomass unit of the phytoneuston (Albright 1980). Results of Hardy and Apts (1984) from their studies on the sea-surface microlayer, indicated non-consistent enrichment of chlorophyll a while Carlson (1982) found that SM was consistently depleted of algae. Hardy and Apts (1984) found, that concentration of total active chlorophylls (chlorophylls $a+b+c$) and phaeopigments in surface microlayer was higher than in the bulk water. Their results suggest that photoinhibition, higher metal levels in the microlayer, or another form of stress probably leads to release of significant quantities of labeled extracellular products. In this study, values of enrichment factors of chlorophyll a displayed very variable pattern, resembling approximately to variability found by other researchers for lakes of high trophic state.

The results of this study show, that enrichment of bacteria in SM was not high (up to 1.4; Table 2). According to Hatcher and Parker (1974) concentration of bacteria in the surface microlayer samples from farm ponds was enriched by 2.6. Danos *et al.* (1983) obtained similar to my results on experimental ponds (1.6). Accumulation

of bacteria in SM in humic lakes displayed higher values of enrichment factor – up to 2.8 (Münster *et al.* 1998). Higher (up to 3.6) accumulation of bacteria in SM in eutrophic lake was also stated by Donderski *et al.* (1999). Frequency of accumulation of bacteria in the SM found in this study fell within the range found by other authors, from 75% in the humic lake to 64% in the rest of lakes (Table 2). The recalculated data of Danos *et al.* (1983), revealed that the accumulation of bacteria in experimental ponds occurred in 86% of the samples. One of the reason for bacteria accumulation in the surface microlayer could be the higher concentration of organic compounds originating there from both, air (dry and wet deposition) and bulk water (on gaseous bubbles and due to the Langmuir circulation; Falkowska 1996).

Mean enrichment value of dissolved organic matter measured with absorbance (A_{254}) amounted to 1.3, while dissolved organic carbon (measured in analyzer) ranged from 7.7 to 26.6 (Table 3). Similar results A_{254} – 1.8, DOC – 15.0 were obtained by Knulst *et al.* (1997) in small, mesohumic lake. Ef values for DOC found by Saijo *et al.* (1974) in lakes were lower: 2.0 in mesotrophic lake and 2.5 in eutrophic one. Concentration of dissolved organic matter in Scandinavian lakes (Södergren 1987), spectrophotometrically measured at 280 nm, was higher in the SM than in the subsurface water, and respective enrichment factor was up to 2.0. Münster *et al.* (1998) measured organic matter concentration at 254 nm, obtained slight accumulation in the surface layer of humic lakes (Ef 1.05–1.15). Knulst *et al.* (1997) stated, that the high organic content of SM will trap lipophilic (e.g. hydrophobic organic pollutants, sterols, glycerides) or ionizable compounds (e.g. trace metals) from the atmosphere or from the bulk water. Generally, the DOC measurements indicate the greater accumulation of organic matter in surface microlayer than the measurements of absorbance of organic matter at A_{254} .

The surface microlayer of humic lake seems to be dominated by high molecular weight organic compounds of allochthonous origin, blown by wind or delivered

in the runoff from the catchment basin, which could be trapped by surface tension and deposited there. But due to its chemical character and weight, difficult to decomposed by bacteria (Södergren 1987). It is why the Ef values for DOC and bacteria are the highest for the humic lake. In mesotrophic lake due to general low rate of autochthonous production – the accumulation of producers (measured with chlorophyll *a*) in surface microlayer could be more conspicuous. In eutrophic lake the higher rate of autochthonous production of low molecular weight compounds, does not allow to distinguish the surface microlayer in terms of chlorophyll, DOC and bacteria concentrations.

No significant correlation has been found between abundances of bacteria and concentration of dissolved organic matter in the surface microlayers of the studied lakes. Also Maki and Remsen (1989) did not discover a significant correlation between the abundance of bacteria and concentration of dissolved nutrients. Similarly, Tranvik (1990) found out no significant correlation between abundance of bacteria and dissolved organic carbon concentration in lakes. Hardy (1982) and Södergren (1984) suggested, that correlation could be stated between particulate organic carbon and abundances of bacteria, because the majority of neustonic bacteria are attached to seston organic particles.

Basing on the literature and my data, it could be stated, that surface microlayer of humic and mesotrophic lakes accumulate more nutrients, organic matter and bacteria in comparison with eutrophic lakes. The high molecular weight organic matter of allochthonous origin dominating in this layer being difficult to degrade by bacteria can stabilize the surface layer from the bulk water. This pattern could be easily seen in humic lakes and quite clear in mesotrophic one. The accumulations of examined elements are a common feature in these lakes. In eutrophic lakes intensive autochthonous production takes place. Nutrients and organic matter are equally concentrated both in the surface microlayer and the subsurface water, so enrichment factors are low. Also high and frequent accumulation

values of examined elements are the lowest in comparison to lakes of lower trophic state.

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