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EXPERIMENTAL ARTICLES

Microbial Decomposition of Organic Matter in the Bottom Sediments of Small Lakes of the Urban Landscape (Lithuania)

A. Krevs[a, 1] **and A. Kucinskiene**[a, b]

[a] *Nature Research Center, Vilnius, Lithuania*

[b] *Lithuanian University of Educational Sciences, Vilnius, Lithuania*

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Abstract—Bacterial abundance and the rates of sulfate reduction (SR) and total organic matter decomposition (D_{total}) were studied in the bottom sediments of nine lakes in the vicinity of Vilnius (Lithuania) during the ice-free seasons of 2006–2009. During the spring mixing of the water, aerobic processes of organic matter decomposition prevailed in the bottom sediments of most lakes, while anaerobic processes predominated (up to 80–90% D_{total}) in summer and early autumn. SR rates in the bottom sediments made up 0.16–2.6 and 0.09–2.0 mg S^{2-}/(dm^3 day) for the medium-depth and shallow lakes, respectively. The highest numbers of sulfate-reducing bacteria (up to 10^6 cells/cm^3) and SR rates were observed in summer. SR rate in medium-depth lakes increased with development of anaerobic conditions at the bottom and elevated sulfate concentrations (up to 96.0 mg/dm^3). In shallow lakes, where O_2 concentration at the bottom was at least 6.7 mg/L, SR rates increased with temperature and inflow of fresh organic matter, especially during cyanobacterial blooms. The average SR rates in the bottom sediments of the lakes of urbanized areas were 4 times higher than in the shallow lakes of protected areas. Accumulation of organic matter and its intensive decomposition during summer may enhance the processes of secondary eutrophication of these small and shallow lakes.

Keywords: small lakes, benthic bacteria, organic matter decomposition, sulfate reduction

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Increasing urbanization and recreational load over the last decade resulted in eutrophication of lakes close to the centers of population (especially of the small lakes with limited self-purification potential), causing an urgent environmental issue. Accumulation of organic matter (OM), its increasing sedimentation, and silt deposition in shallow lakes are among the negative effects of eutrophication. OM of the bottom sediments is degraded by both anaerobic and aerobic microorganisms; their activity depends on environmental conditions [1, 2]. Eutrophication of shallow lakes results in anaerobic processes, including sulfate reduction, playing the dominant role in OM mineralization. Release of environmentally toxic hydrogen sulfide is increasing [3].

In Lithuania, research in microbial ecology was previously carried out in large water bodies with significant anthropogenic load and in small lakes not influenced by intense anthropogenic impact and located in state-protected areas [4, 5].

The goal of the present work was to investigate the features of the bacterial processes of sulfate reduction and total (aerobic and anaerobic) decomposition of organic matter in the bottom sediments of small lakes of the urbanized area in relation to certain environmental conditions.

MATERIALS AND METHODS

The investigations were carried out during the ice-free periods of 2006–2009 (May, July, and September) in nine lakes of the (river) Neris (Viliya) basin located in the suburbs of Vilnius (Fig. 1). These lakes are of glacial origin, with the area of 3.5 to 88 ha, average depth from 1.5 to 9 m, and maximal depth from 3 to 17 m. During intensive agricultural activity of 1950s–1980s, allochthonous biogenic and organic compounds inflowed into the lakes from cultivated fields. Presently, decreased agricultural areas and increased individual construction activity in the basin resulted in higher anthropogenic load from the urbanized territories, which occupied 9 to 40% of the lake area, according to 2003 data [6]. Two of the lakes, Paezeris and Mazasis Gulbinas (M. Gulbinas) are most remote from the urbanized territories. The coastal zones of Lake Paezeris and Lake M. Gulbinas are surrounded by uncultivated fields and a forest belt, respectively, which prevent the inflow of eutrophying substances and polluting compounds to a significant degree.

[1] Corresponding author; e-mail: alinakrevs@gmail.com

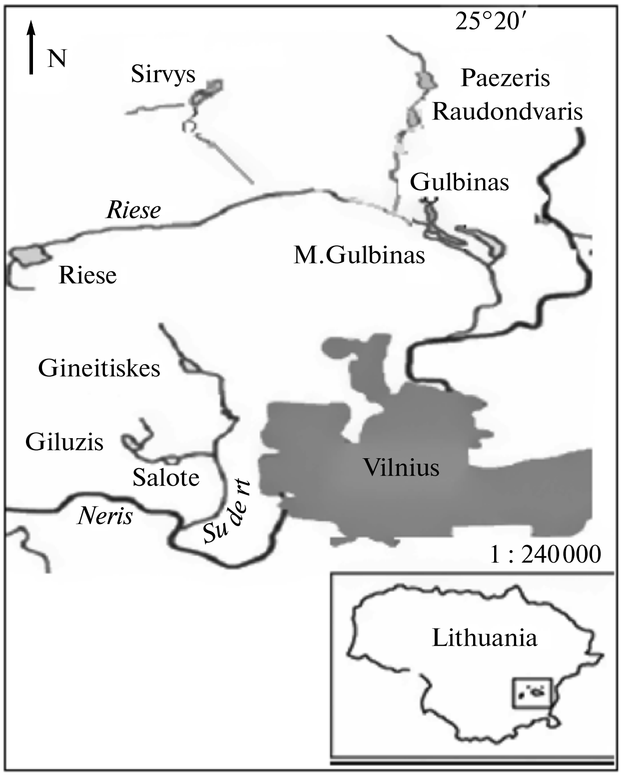

Fig. 1. Schematic location of the lakes.

Some lakes (Salote, Giluzis, and Gulbinas) are used for recreational purposes.

The water and bottom sediments were sampled in the deepest parts of the lakes. Water was collected with a Ruttner sampler. Bottom sediments were collected with a box grab, from which samples of the upper 5-cm layer for chemical and microbiological analyses were taken with sterile glass tubes.

Temperature and pH were determined in situ using a WTW MultiLine F/Set3 portable meter. Water transparency was determined with a Secchi disk. Oxygen concentrations were determined by the Winkler method. Organic carbon in the sediments was measured by the dichromate oxidation method [7], sulfates, by the colorimetric method, and hydrogen sulfide and acid-soluble sulfides, by the Volkov and Zhabina method [8].

Total bacterial numbers were determined on black polycarbonate membrane filters with 0.2 μm pore diameter (Millipore) by the fluorescence method with DAPI as the stain [9]. Bacterial cells were counted in 20–40 fields of view under an Olympus IX70 microscope (1000×) Sulfate-reducing bacteria (SRB) were grown in the Postgate medium with lactate [10].

Primary production (PP) was determined by the radiocarbon method [9]. Water samples (100 mL) were supplemented with 0.5 mL $NaH^{14}CO_3$ (2×10^5 Bq/mL). Experimental samples with the label (3 transparent and 3 darkened vials) were incubated in situ for 4 h. For total PP, radioactivity of the precipitate on 1.2-μm filters and of the filtrate was determined.

Total (aerobic and anaerobic) OM decomposition in the upper sediment layers was determined from the amounts of consumed O_2 and produced CO_2 in hermetically sealed stratometric tubes with a silt core and near-bottom water [11].

The rate of sulfate reduction (SR) was determined by the radioisotope method with $Na_2{}^{35}SO_4$ [11, 12]. Bottom sediment samples in glass tubes sealed hermetically with rubber stoppers and water samples in darkened 60-mL glass vials were supplemented with 0.1 mL of the $Na_2{}^{35}SO_4$ solution ($2–3 \times 10^6$ cpm). Experimental samples with the label were incubated in situ for 24 h and then fixed with 2 mL of 20 mM $Na_2MoO_4 \cdot 2H_2O$. The subsequent procedures were carried out in the laboratory. After oxidation, sulfide was distilled into alkaline 0.05 N $KMnO_4$. Labeled sulfides were converted to sulfates, and their radioactivity (as $BaSO_4$ precipitates) was determined on a Beckman Instruments scintillation counter using the Opti Phase HiSafe 3 scintillation cocktail (Wallac). Statistical treatment of the results was carried out using the SPSS 12 software package.

RESULTS

Physicochemical characterization of water and bottom sediments. According to complex parameters, trophic state of the lakes differed (Table 1). Shallow, low-running Lakes Gineitiskes and Sirvys, where cyanobacterial blooms occurred in summer, were hypereutrophic. The deepest Lake Giluzis and Lakes Paezeris and M. Gulbinas, which were most remote from the urbanized territories, had the lowest productivity, according to transparency and primary production (PP). In shallow lakes, the temperatures of the surface and near-bottom water were similar and varied from 9.6°C in May to 21°C in July (Table 2). In medium-deep lakes (9–17 m), thermal stratification was observed throughout the sampling period, with the temperature of near-bottom water not exceeding 5–13.6°C. The pH values of the water varied from 7.4 to 8.9 in the surface layer and from 7.2 to 8.5 near the bottom. In the stratified Lakes Giluzis and M. Gulbinas, microaerobic conditions near the bottom were observed throughout the sampling period. Anaerobic conditions near the bottom also developed in summer in the medium-depth Lakes Raudondvaris, Paezeris, and Gulbinas, where the depth of the sampling stations did not exceed 4.5 m. In shallow lakes, oxygen concentration at the bottom, while lower than at the surface, still was at least 6.7 mg/L.

The bottom sediments of the lakes were silts with high water content (83–94%). C_{org} content varied from 10.5 to 37.4% of dry sediments sample (Table 2).

Table 1. General characteristics of investigated lakes in summer 2006–2009

Lake	Area, ha	Max. depth, m	Trophic indices of the water				
			Transparency, m	P_{total}, mg/L	N_{total}, mg/L	DOC, mg/L	PP, mg C/(m^3 h)
Giluzis	22.5	17.3	3.0	0.124	0.761	11.7	49.2
Raudondvaris	3.5	9.0	1.4	0.022	0.612	13.3	52.3
Paezeris	5.3	9.0	2.5	0.022	0.858	8.2	43.7
Gulbinas	36.7	11.8	0.5	0.027	1.30	7.1	57.2
M. Gulbinas	10.1	16.5	3.0	0.023	1.26	5.9	39.6
Salote	9.8	4.4	1.8	0.061	0.761	16.7	82.5
Gineitiskes	13.2	3.0	0.5	0.26	0.896	37.0	465.2
Sirvys	85.1	4.0	0.6	0.113	2.44	22.0	329.4
Riese	88.1	5.0	0.6	0.055	2.14	12.3	213.8

Table 2. Physicochemical parameters of the near-bottom water layer and bottom sediments of investigated lakes in 2006–2009

Lake	Sampling time: May/July/September				
	Near-bottom water		Bottom sediments		
	T, °C	O_2, mg/L	C_{org}, %	S–SO_4^{2-}, mg/dm^3	$H_2S + HS^-$, mg/dm^3
Medium-depth lakes					
Giluzis	6.2/ 5.0/ 8.7	2.7/ 1.1/ 1.3	14.2/ 13.7/ 15.6	7.0/ 96.0/ 7.0	96/ 184/ 48
Raudondvaris	7.2/ 9.0/ 9.9	10.9/ 0.0/ 0.0	18.7/ 23.0/ 22.6	50.6/ 59.0/ 50.0	192/ 240/ 352
Paezeris	8.3/ 10.3/ 13.1	10.9/ 1.4/ 4.6	16.0/ 22.3/ 19.5	42.0/ 52.3/ 63.3	144/ 400/ 192
Gulbinas	11.9/ 13.6/ 11.9	8.0/ 0.0/ 7.2	10.5/ 12.0/ 11.8	30.6/ 38.7/ 18.5	104/ 272/ 80
M. Gulbinas	7.1/ 7.5/ 5.2	6.0/ 0.0/ 1.9	19.4/ 23.8/ 22.0	16.6/ 20.7/ 14.0	64/ 80/ 80
Shallow lakes					
Salote	13.1/ 20.9/ 16.4	8.0/ 6.9/ 10.2	33.0/ 35.8/ 37.4	47.0/ 54.3/ 23.6	48/ 48/ 16
Gineitiskes	9.6/ 20.6/ 15.4	11.0/ 6.7/ 8.8	19.8/ 23.2/ 30.8	17.3/ 24.0/ 7.3	64/ 80/ 48
Sirvys	17.4/ 21.1/ 15.8	8.0/ 7.7/ 7.0	17.8/ 17.3/ 18.0	27.0/ 30.0/ 30.0	112/ 192/ 160
Riese	10.8/ 18.4/ 13.4	9.9/ 9.6/ 9.6	13.6/ 17.0/ 15.7	27.0/ 52.0/ 46.6	144/ 144/ 32

The highest C_{org} content was found in peaty silts of Lake Salote, covered with underwater vegetation. After cyanobacterial blooms, C_{org} content in Lake Gineitiskes also increased to 30.8%. Sulfate concentrations in lake sediments varied from 7 to 96 mg/dm^3. The highest C_{org} concentrations were observed in summer and autumn, while the highest sulfate levels occurred in summer.

Abundance of benthic bacteria. The numbers of benthic bacteria and its seasonal distribution were nonuniform. The lowest average bacterial number for the observation period (1.7×10^9 cells/cm^3) was found in the sediments of the least productive lakes (Giluzis and Paezeris), while the highest value was detected in the hypereutrophic Lake Gineitiskes (3.5×10^9 cells/cm^3) (Table 3). SRB number varied from 10^2 to 10^6 cells/cm^3 in medium-depth lakes and from 0 to 10^6 cells/cm^3 in shallow lakes. In all lakes, SRB numbers were higher in summer and autumn.

Total decomposition of organic matter. According to the rates of CO_2 emission from silt cores into the water, the benthic microflora was most active in the summer: the rates of OM decomposition varied from 785 to 3864 mg C/(m^2 day) (Table 4). The parallel measurements of the oxygen uptake rate showed that aerobic OM decomposition predominated in May in the sediments of most shallow lakes. In summer and early autumn, in all lakes, independent on their depth, conditions were favorable for the benthic anaerobic microorganisms. During this period, anaerobic processes were responsible for an average of 80% D_{total} in shallow lakes and for 90% in medium-depth ones. The highest rates of destruction processes, 2970–3864 mg C/(m^2 day), were observed in Lakes Gineitiskes and Sirvys during the water bloom. The rates of CO_2 emis-

Table 3. Bacterial numbers in the bottom sediments of investigated lakes

Lake	TBN, 10^9 cells/cm^3	SRB, CFU/cm^3	
		May	July–September
Medium-depth lakes			
Giluzis	1.722 ± 0.317	10^3	10^4–10^3
Raudondvaris	2.304 ± 0.550	10^2	10^5–10^6
Paezeris	1.673 ± 0.392	10^3	10^5–10^6
Gulbinas	2.491 ± 0.083	10^4	10^5–10^5
M. Gulbinas	3.350 ± 0.544	10^4	10^5–10^5
Shallow lakes			
Salote	1.988 ± 0.448	10^3	10^4–10^4
Gineitiskes	3.512 ± 0.487	10^3	10^4–10^4
Sirvys	2.051 ± 0.333	10^5	10^6–10^6
Riese	2.632 ± 0.611	0	10^3–10^3

Note: TBN stands for the total bacterial number (averaged for the period of investigation), SRB stands for sulfate-reducing bacteria, and CFU stands for colony-forming units.

Table 4. Rates of microbial processes in the bottom sediments of investigated lakes during the ice-free period of 2006–2009 (D_a, D_{total}, and SR stand for aerobic decomposition, total decomposition, and sulfate reduction, respectively)

Lake	Sampling time: May/July/September		
	D_a, mg O_2/(m^2 day)	D_{total}, mg C/(m^2 day)	SR, mg S^{2-}/(dm^3 day)
Medium-depth lakes			
Giluzis	557/ 109/ 130	768/ 1632/ 1062	0.21/ 2.60/ 0.16
Raudondvaris	576/ 0/ 0	680/ 1603/ 2046	0.78/ 1.38/ 1.12
Paezeris	819/ 96/ 541	576/ 785/ 812	0.94/ 1.25/ 1.20
Gulbinas	780/ 288/ 789	780/ 2340/ 2436 (990*)	1.70/ 2.15/ 0.50 (1.22*)
M. Gulbinas	283/ 0/ 0	554/ 1402/ 1872	0.21/ 0.39/ 0.18
Shallow lakes			
Salote	198/ 256/ 339	1488/ 1536/ 954	1.28/ 1.58/ 0.21
Gineitiskes	51/ 317/ 614	648/ 2970/ 708	0.30/ 2.00/ 0.15
Sirvys	1731/ 2330/ 1237	762/ 3864/ 2498	0.70/ 0.91/ 0.82
Riese	1488/ 357/ 224	744/ 1339/ 1680	0.09/ 0.88/ 0.77

* July 2002.

sion by the bottom sediments of these lakes were on average 2.5 times higher than in other lakes.

Sulfate reduction. In different periods of observation, the rates of sulfate reduction in the bottom sediments were 0.16–2.6 mg S^{2-}/(dm^3 day) in medium-depth lakes and 0.09–2.0 mg S^{2-}/(dm^3 day) in shallow lakes. The highest SR values were observed in summer, especially in the stratified Lake Giluzis, where microaerobic conditions developed at the bottom and sulfate concentration increased up to 96 mg/dm^3. In medium-depth lakes, apart from the bottom sediments, sulfate reduction at the rates of 0.001–0.08 mg S^{2-}/(dm^3 day) was observed in the near-bottom water layers. In Lake Gulbinas, an increase of the sulfide zone and SR rates in the water column occurred parallel with increasing eutrophication (Fig. 2). In 2009, SR rate in the bottom sediments of this lake was 1.8 times higher than the values reported in 2002 (Table 4). Among the shallow lakes, a relatively high rate of the process, 1.58–2.0 mg S^{2-}/(dm^3 day) was revealed in Lakes Salote and Gineitises, where the temperatures at the bottom were high (21°C) and C_{org} concentrations higher than in other lakes of this group (23–37%).

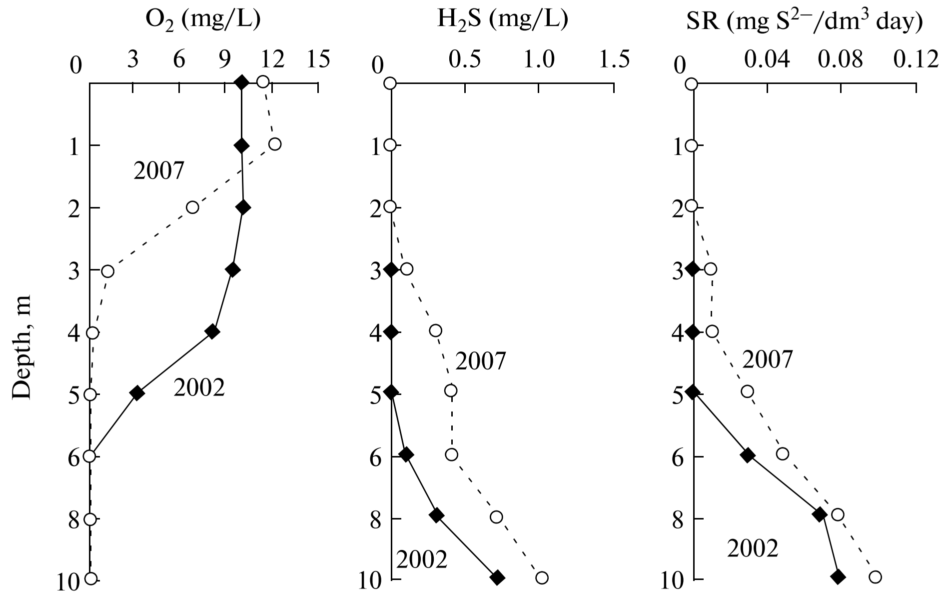

Fig. 2. Concentrations of oxygen, sulfide, and the rates of sulfate reduction in the water column of Lake Gulbinas in August 2002 and 2007.

The amounts of the terminal products of sulfate reduction (hydrogen sulfide and acid-soluble sulfides) varied from 16 to 400 mg/dm^3 (Table 2). The highest values (up to 400 mg/dm^3) were found in medium-depth lakes during the summer stratification. Oxygen deficiency at the bottom prevented the oxidation of H_2S, which accumulated in the bottom sediments as sulfides. In the bottom sediments of the shallow lakes, in spite of the relatively intense sulfate reduction in summer, significant oxygen levels near the bottom provide for H_2S oxidation. This is probably the reason why the concentrations of soluble sulfides in the sediments of these lakes were lower (up to 192 mg/dm^3).

Statistical analysis. In the lakes under investigation, a direct correlation was found between D_{total} rate and primary production in summer. This correlation was more pronounced in shallow lakes (Table 5). At the background of the general high OM levels in the bottom sediments, no reliable dependence of D_{total} and SR on the total C_{org} content was found. In shallow lakes, direct correlation was observed between decomposition processes and temperature and a negative correlation between these processes and oxygen concentration in the near-bottom water.

DISCUSSION

Investigations carried out in nine lakes in the vicinity of Vilnius, in the zone of intense anthropogenic activity, revealed that C_{org} content in the sediments of most lakes, especially in the shallow, highly trophic ones, increased by autumn. This tendency was especially pronounced in the hypereutrophic Lake Gineitiskes, where C_{org} content in autumn was 1.6 times higher than in spring. In summer, massive bloom of cyanobacteria *Microcystis* and *Anabaena* occurred

Table 5. Pearson correlation coefficient between the rates of decomposition processes in lake bottom sediments and environmental conditions (D_{total}, SR, and PP stand for total decomposition, sulfate reduction, and primary production, respectively)

Parameters	T, °C	O_2, mg/L	PP, mg C/(m^3 h)	C_{org}, %	$S{-}SO_4^{2-}$, mg/dm^3	H_2S, mg/dm^3
Medium-depth lakes						
D_{total}, mg C/(m^2 day)	NR	NR	0.62*	NR		
SR, mg S^{2-}/(dm^3 day)	NR	NR	NR	NR	0.76**	0.51
Shallow lakes						
D_{total}, mg C/(m^2 day)	0.67*	–0.63*	0.72*	NR		
SR, mg S^{2-}/(dm^3 day)	0.72*	–0.77**	NR	NR	0.47	NR

Note: NR stands for not reliable. * and **—indicate the confidence intervals of $P < 0.05$ and $P < 0.01$, respectively.

(chlorophyll *a* up to 162 μg/L). After the death and sedimentation of cyanobacteria, the bottom sediments of this shallow lake (3 m) were significantly enriched with labile OM. In this lake, as well as in Lake Sirvys, where mass development of *Planktothrix agardhii* occurred from August to September, the highest levels of the overall activity of benthic communities were also found, from 2970 to 3864 mg C/(m^2 day). The values of CO_2 emission from the bottom sediments of these lakes were comparable to those for other shallow eutrophic and hypereutrophic lakes [13]. Aerobic processes prevailed in the profundal of shallow lakes in spring. In summer and early autumn, anaerobic processes of OM decomposition predominated in all lakes, independent on their depth. Microaerobic and anaerobic conditions near the bottom, together with high OM content, resulted in activity of anaerobic benthic communities in medium-depth lakes. The oxidative conditions in the profundal of shallow lakes (O_2 > 6 mg/L) favored the development of aerobic microorganisms. However, in summer and early autumn aerobic microorganisms were active probably only in the upper, aerated surface layer, so that in the sediments of these lakes, similar to the stratified medium-depth lakes, anaerobic mineralization prevailed, which resulted in release of 1182–2500 mg C/(m^2 day).

The highest SRB numbers in lake sediments (summer–autumn) were from 10^3 to 10^6 cells/cm^3. These values were of the same order of magnitude as those for the bottom sediments of the Lithuanian karst lakes of the sulfate type and higher than in the lakes located in protected areas, where SRB number in summer varied from 10 to 10^3 cells/cm^3 [5, 14]. Sulfates and available OM are known to be the major factors determining the SRB activity, while temperature is only partially responsible for the seasonal SRB activity in lakes [15, 16]. Other works, however, state direct dependence between temperature and SR [17]. Environmental conditions affecting the SRB activity in the lakes under study varied. In medium-depth lakes, the rate of sulfate reduction increased with formation of anaerobic conditions near the bottom and increased sulfate content. The temperature in the near-bottom layer of these stratified lakes in May–September varied insignificantly and had little influence on the activity of SRB. In shallow lakes SR rates increased with the temperature of near-bottom water and probably depended on the qualitative composition of the products of fermentation of the detritus (especially during and after cyanobacterial blooms), rather than on the total OM content. Elevated SR rates during phytoplankton blooms were reported for the bottom sediments of some other shallow lakes [18]. In the shallow lakes studied in the present work, in spite of the oxidative conditions in the near-bottom water, the abundance and activity of SRB were similar to those in the stratified lakes with anaerobic hypolimnion. Occurrences of SRB in oxidative environments, as well as the possible oxidation of organic substrates by SRB with oxygen as an electron acceptor were discussed in other works [19]. The highest SR rates in the lakes under study reached 2–2.6 mg S^{2-}/(dm^3 day) and were within the range determined for the bottom sediments of the profundal of some brackish meromictic lakes and karst lakes [14, 20–22], although they were lower than the values known for shallow saline lakes with high levels of available organic matter [18]. Sulfate reduction in the bottom sediments of these lakes was, on average, four times more active than in the bottom sediments of shallow lakes unaffected by anthropogenic impact [5]. In the case of Lake Gulbinas (11 m), it may be seen that activation of the SRB activity and increase of the anaerobic sulfide water layer (from 3.5 m on) were among the negative effects of anthropogenic eutrophication.

Thus, our results showed that mineralization of a significant amount of OM accumulated at the bottom of the lakes under study was relatively intense. The highest rates of sulfate reduction were mostly associated with the seasonal influx of easily available substrates into the silts, with elevated sulfate content in some cases. OM accumulation in the bottom sediments and its active decomposition in summer, including sulfate reduction, may facilitate secondary eutrophication and promote an increase of the sulfide water layer in these small and shallow lakes. The changed forms of land use in the basin require further investigation of the functioning of microbial communities in the lakes of the urbanized environment.

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