

Microbial mineralization of organic matter in bottom sediments of small anthropogenised lakes

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The peculiarities of total (aerobic and anaerobic) organic matter (OM) mineralization and microbiological sulphate reduction intensity were studied in profundal bottom sediments of anthropogenised lakes situated in surroundings of Vilnius (Salotė, Gilužis and Gineitiškės) in May, July and September 2006. The research showed that organic carbon content varied from 13.7 to 37.4% of dry weight and the highest was estimated in Salotė (up to 37.4%) and Gineitiškės (30.8%). The intensity of OM mineralization varied from 648 mg to 2830.6 mg C/m² d⁻¹. Anaerobic decomposition prevailed in the total mineralization of OM and was most intensive in summer in Lake Gineitiškės. The process of terminal anaerobic OM mineralization – sulphate reduction was most intensive in Lake Gilužis (2.6 mg S²⁻/dm³ d⁻¹) and correlated with sulphate concentration. The accumulation of high amounts of OM in bottom sediments and its intensive mineralization may stimulate the processes of secondary eutrophication and a lower the recreation value, especially in such shallow lakes as Salotė and Gineitiškės.

Key words: organic matter, aerobic and anaerobic mineralization, sulphate reduction, bottom sediments, lakes

INTRODUCTION

Intensive human activity induces quantitative changes in limnetic systems of lake areas: it intensifies the overgrowth of aquatic macrophytes, broadens the littoral swamp area, decreases the biodiversity, causes water blooming (Correl, 1997; Haycock et al., 1996; Margaritora et al., 2005). These processes are especially dangerous in small lakes with a limited capacity of self-cleaning. To prognosticate ecological changes in such lakes, it is necessary to know their ecological state through a detailed investigation of the structure of biota and changes in its functioning. The increasing anthropogenisation highly influences the state of lakes situated in the Vilnius district. Eutrophication is one of the most negative results of the economic activities that cause accumulation of organic matter in bottom sediments and increase silting processes in shallow lakes. Aerobic and anaerobic microorganisms play the crucial role in the mineralization of organic matter in bottom sediments of water basins. Their activity highly depends on the surrounding conditions: in eutrophicated lakes, there prevails anaerobic and terminal anaerobic (sulphate reduction) organic matter (OM) mineralization resulting in an increased

release of hydrogen sulphate, a compound highly toxic for biota (Heyer, Kalf, 1998; Jonsson, 2001; Cook, 1992).

The aim of the study was to evaluate total (aerobic and anaerobic) and terminal anaerobic (sulphate reduction) organic matter mineralization processes in profundal bottom sediments of three anthropogenised lakes situated in the Vilnius district.

MATERIALS AND METHODS

Study area. The research was carried out in lakes Salotė, Gilužis and Gineitiškės situated in the surroundings of Vilnius and depending to the Sudervė river basin (Fig. 1).

The lakes, due to the expansion of Vilnius, at present lie in its territory and undergo an active anthropogenic impact. The largest and the deepest one is Gilužis: its area is 22.5 ha, length 830 m and width 260 m, the maximum depth reaching 16 m. The depth of the sampling site was 10–12 m. The banks of the lake are mostly steep. An intensive construction of new buildings takes place there. The area of Lake Salotė reaches 13 ha. It is a shallow lake; the depth of the sampling site was 2–3 m. A public beach and new houses are situated near this lake. The banks are low, and some of them are surrounded by swampy areas. Through an unnamed streamlet it

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Fig. 1. Location map of Salotė, Gilužis and Gineitiškės lakes

is connected with Lake Gilužis. These two lakes are situated in the Pilaitė microdistrict. Lake Gineitiškės is situated in the Zujūnai microdistrict. It is also shallow – the depth of the sampling site was approximately 3 m. An intensive construction of new buildings takes place in the district. This lake undergoes the highest impact of anthropogenisation.

Sampling and analysis. Water and bottom sediment samples were taken three times in each lake during different active vegetation periods (May, July and September) of 2006. Ruttner's sampler was used to collect water samples. Bottom sediment samples (0–5 cm) were taken with an Ekman grab. Temperature, pH, conductivity were measured *in situ* with a portable universal Multi Line F / Set-3 meter (WTW). Water transparency was measured with a Secchi disk. Dissolved oxygen concentration was estimated by Winkler's method (Merkienė, Čeponytė, 1994). Sulphate concentrations in water and in bottom sediments were determined by the turbid metric method (Merkienė, Čeponytė, 1994). This method is based on the formation of BaSO_4 crystals in a suspension and the subsequent measurement of its optical density. Measurements were carried out with a spectrophotometer at a wavelength of 400 nm. The method has a detection limit of about 2 mg/l. The total amount of organic matter in bottom sediments was measured by the dichromate oxidation method (Потапова, 1985). Hydrogen sulfide and acid-soluble sulfides (mg/dm^3 natural sediments) were determined by the method of Volkov and Zhabina (Волков, Жабина, 1980). Bottom sediment samples were taken to 100 ml flasks and fixed with aquatic $\text{ZnSO}_4 + \text{Na}_2\text{CO}_3$ solution. After treatment with gaseous nitrogen, sulfides were collected into alkaline CdSO_4 solution. The total content of hydrogen sulfides and acid-soluble sulfides was calculated after titration with sodium tiosulphate. The intensity of aerobic and total (aerobic + anaerobic) organic matter mineralization was de-

termined by the method of isolated columns (Кузнецов, Дубинина, 1989). Undistracted bottom sediment samples (5 cm) were taken to glass columns. Then the columns with samples and control (column without bottom sediments) were carefully filled with bottom water and exposed *in situ* for a day. Oxygen consumption ($\text{mg O}_2/\text{m}^2 \text{ d}$) and inorganic carbon release intensity ($\text{mg C}/\text{m}^2 \text{ d}$) were estimated after incubation as content differences between sample and control. Sulfate reduction intensity ($\text{mg S}^{2-}/\text{dm}^3 \text{ d}$) was ascertained using the $\text{Na}_2^{35}\text{SO}_4$ tracer technique (Кузнецов, Дубинина, 1989; Sorokin, 1999). 0.1 ml $\text{Na}_2^{35}\text{SO}_4$ solution (Amersham Pharmacia Biotech) of at least $2\text{--}3 \times 10^6$ imp/min radioactivity was added to duplicate or triplicate 20 ml glass tubes, which were pushed 0–5 cm deep into the sediment so that the sediment layer filled the tube beneath the plunger completely. The tubes were exposed *in situ* for a day. After the chemical treatment of samples, the filters were placed in vials containing 5 ml of Opti Phase Hi Safe 3 scintillation cocktail (Wallac Scintillation Products). Radioactivity was determined with a liquid scintillation counter (Beckman Instruments Inc).

The total number of bacteria in bottom sediments (cells / cm^3) was counted under the epifluorescence microscope after DAPI staining on black Millipore filters (0.2 μm) (Porter, Feig, 1980; Sherr, 1993). The abundance of heterotrophic bacteria (CFU/cm^3) was calculated from their colonies grown on $10\times$ diluted agar nutrient medium. The most probable number technique (MPN) was used to enumerate SRB ($10\times \text{CFU}/\text{cm}^3$) in bottom sediments. Postgate's "C" medium with lactate as the electron donor was used (Postgate, 1984). Six dilutions of bottom sediments were made in liquid media. Then 1 ml aliquots of each dilution were inoculated into the agar medium. After 3–4 weeks, black tubes were counted according to the MPN procedure.

The abundance of bacteria and the results of microbiological processes are given using the average data of three replications.

RESULTS AND DISCUSSION

The development of microorganisms in water basins is closely related to environmental conditions. The main abiotic indices formatting the development of microorganisms are water transparency, temperature, pH, gas regime (dissolved oxygen, hydrogen sulfide), organic and mineral substances (Кузнецов и др., 1985). The main physical-chemical indices evaluated in the lakes during different vegetation periods are presented in Table 1.

Water transparency depends mainly on the intensity of solar radiation, the content of particular and dissolved organic and mineral substances and the amount of plankton organisms. It shows the degree of water basin's eutrophication (Wetzel, 1983). The highest water transparency during the study period was determined in Lake Gilužis (2.7–3.7 m) and the lowest in Lake Gineitiškės (0.6–1.0 m).

Table 1. Physical-chemical indices in water of the Sudervė river basin lakes in 2006

Lake	Depth, m	Secchi, m	T, °C	pH	Conductivity, µS/cm	O ₂ , mg/l
May 2–4						
Gilužis	10.5	2.5	*11.1 / 6.2	8.46 / 7.53	425 / 482	9.92 / 2.72
Salotė	2.0	2.0	13.2 / 13.1	8.23 / 8.18	352 / 352	7.68 / 8.0
Gineitiškės	2.5	1.0	9.6 / 9.6	7.76 / 7.79	173 / 174	10.56 / 11.04
July 18–20						
Gilužis	12.5	3.0	21.8 / 5.0	7.99 /	373	7.52 / 1.1
Salotė	2.5	1.8	21.5 / 20.9	7.83 /	367 / 369	7.36 / 6.88
Gineitiškės	2.5	0.8	20.6 / 20.6	7.62 /	–	7.04 / 6.72
September 19–21						
Gilužis	11.0	3.7	16.3 / 8.7	8.34 / 8.54	386 / 482	9.76 / 1.28
Salotė	2.5	2.0	16.7 / 16.4	8.33 / 8.38	338 / 339	10.4 / 10.24
Gineitiškės	2.7	0.6	15.4 / 15.4	7.94 / 8.03	184 / 179	8.8 / 8.8

* In denominator – in the upper water layer, in numerator – in the bottom water layer.

The surface water temperature in the lakes varied from 9.6 (in May) to 21.8 °C (in June). In the deepest lake Gilužis, thermal stratification was characteristic during all study period. In this lake, water temperature near the bottom varied from 8.7 to 5.0 °C. The water pH in the lakes was slightly alkaline – optimal for the development of most microorganisms.

Electric conductivity varied greatly (from 174 to 482 µS/cm), the lowest index of this parameter being determined in Lake Gineitiškės. No distinct differences between surface and bottom water were determined.

A high concentration of oxygen (7.68–10.56 mg/l) was characteristic of surface water in all the lakes. Insignificant differences between oxygen concentrations in surface and bottom water layers were evaluated in lakes Salotė and Gineitiškės. Therefore, the microaerobic conditions were being formatted during all the periods of investigation near the bottom of the stratified Lake Gilužis.

The content of organic matter (C_{org.}) in bottom sediments of the lakes varied from 13.7% (Gilužis) to 37.4% (Salotė) of dry weight. Peat sediments and outflow from the banks mainly influenced the high content of organic matter in the bottom of Lake Salotė. Seasonal investigations revealed the highest content of organic matter in autumn, especially in

Lake Gineitiškės in which the content of C_{org.} in autumn was 1.6 times higher than in spring (Table 2). According to previous hydrochemical-biological studies (Paviršinio..., 1995; Kavaliauskienė, 1996), Lake Gineitiškės was assigned to hypereutrophic ones with low self-purification facilities. The biomass of algae might amount to 40 mg/l and chlorophyll *a* concentration to 161.6 µg/l in the cyanobacteria blooming period (Kasperovičienė et al., 2005). In autumn, after an intensive development of algae, due to sedimentation of detritus, the content of C_{org.} in bottom sediments of this shallow lake increased.

The concentration of sulphate ions in bottom sediments of the lakes varied from 7.0 to 96.0 mg/dm³. The highest concentration of this compound was determined in July and varied from 24.0 (Gineitiškės) to 96.0 mg/dm³ (Gilužis). This phenomenon could occur due to the intensive rain in July and surface outflow from the banks of the lakes. The development of sulphur oxidizing bacteria might influence the increase of sulphate concentration as well (Горленко и др., 1977).

The total amount of bacteria varied from 1.247 to 4.242 × 10⁹/cm³. The highest level, also of aerobic heterotrophic bacteria, was determined in spring in Lake Gineitiškės where aerobic microorganisms dominated in the

Table 2. Physical-chemical indices and the amount of benthic microorganisms in bottom sediments of the Sudervė river basin lakes in 2006 (TA – total amount; HB – heterotrophic bacteria; SRB – sulphate reducing bacteria; CFU – colony formatting units)

Lake	Humidity %	Corg. %	H ₂ S + HS ⁻ , mg/dm ³	S/SO ₄ , mg/dm ³	TA, 10 ⁹ /cm ³	HB, CFU/cm ³	SRB, CFU/cm ³
May 2–4							
Gilužis	83	14.2	96.0	7.0	1.247	2550	10 ³
Salotė	91	33.0	48.0	47.0	1.445	1625	10 ³
Gineitiškės	88	19.8	64.0	17.3	4.242	2900	10 ⁴
July 18–20							
Gilužis	89	13.7	184.0	96.0	1.738	2835	10 ⁴
Salotė	90	35.8	48.0	54.3	2.660	2275	10 ⁴
Gineitiškės	91	23.2	80.0	24.0	3.000	2050	10 ⁴
September 19–21							
Gilužis	93	15.6	48.0	7.0	2.182	2150	10 ³
Salotė	94	37.4	16.0	23.6	1.860	1700	10 ⁴
Gineitiškės	94	30.8	48.0	7.3	3.294	2400	10 ³

mineralization of organic matter: aerobic decomposition comprised 70% of its total mineralization (Table 2; Fig. 2A). The intensive aeration of bottom water determined the activity of these bacteria. The research showed a positive correlation between oxygen concentration and the intensity of aerobic decomposition of OM, thus indicating the activity of

bottom bacteria and a high amount of easily oxidizing substrate (Fig. 3A).

Environmental conditions for the activity of anaerobic bacteria were most favourable in July in bottom sediments of all the lakes studied (Fig. 2A). In summer, the anaerobic mineralization of OM comprised more than 90% of its total min-

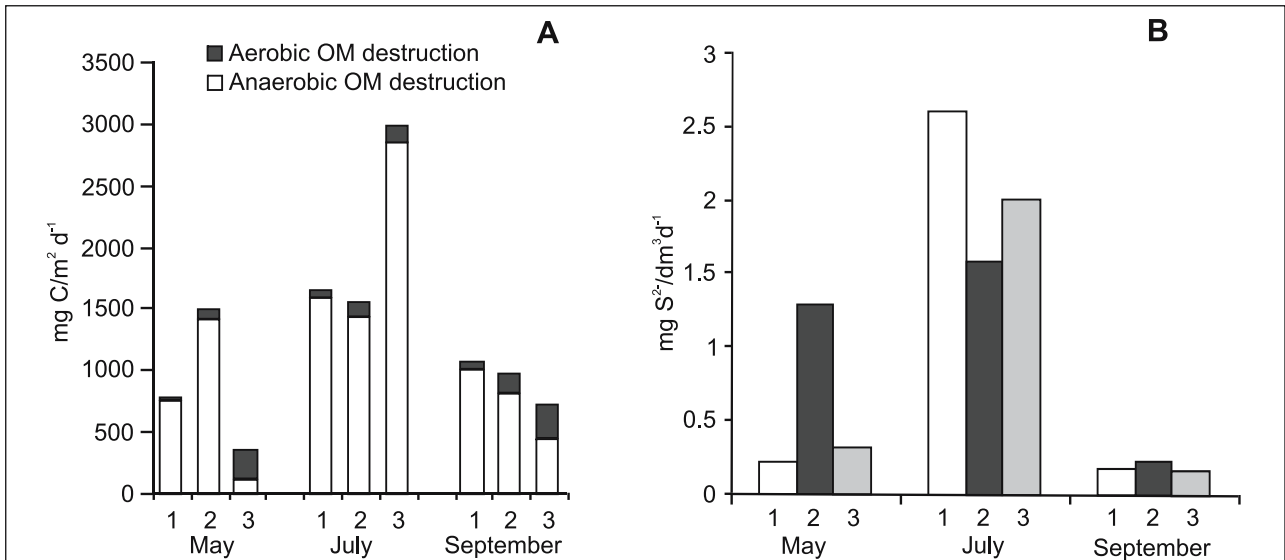


Fig. 2. Intensity of total (aerobic and anaerobic) mineralization of organic matter (A) and sulphate reduction (B) in bottom sediments during different periods of vegetation in 2006 (1 – Gilužis, 2 – Salotė, 3 – Gineitiškės lakes)

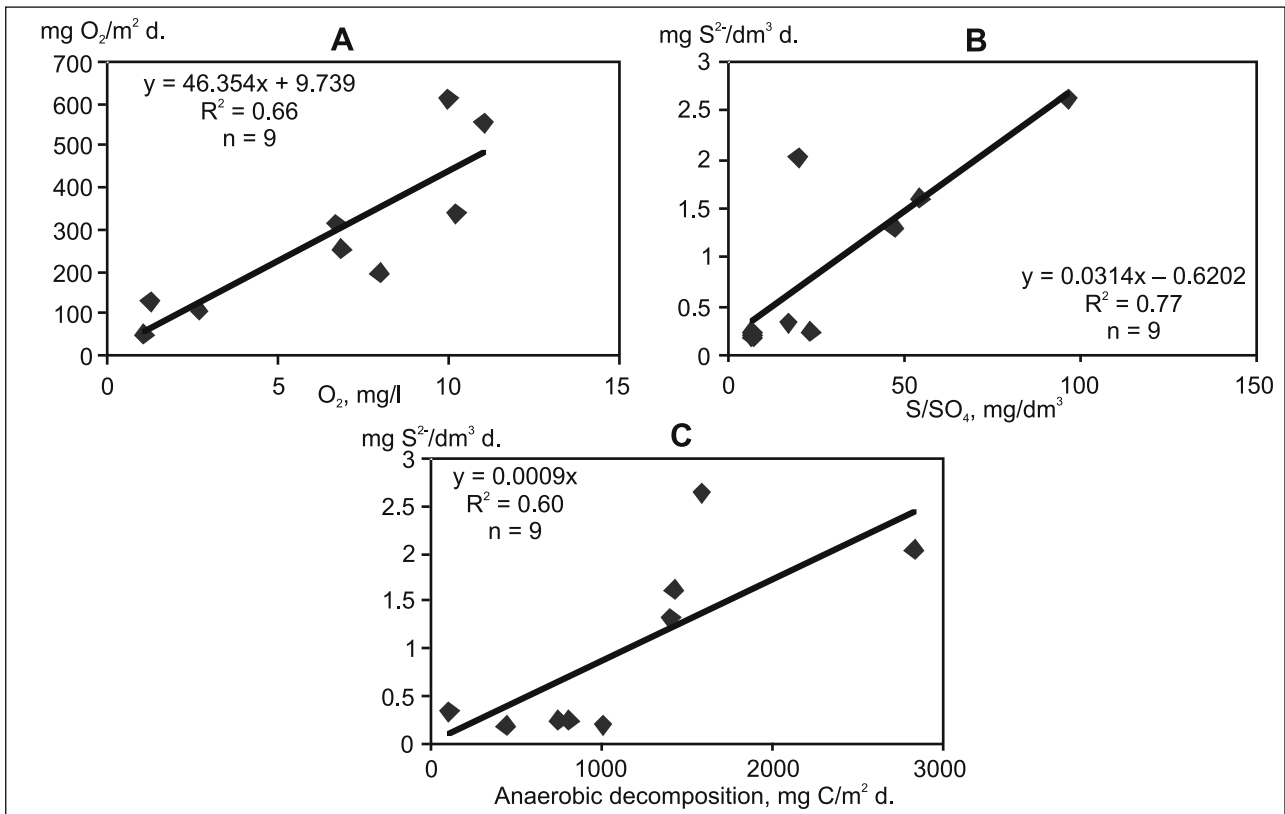


Fig. 3. Dependence between oxygen concentration and aerobic mineralization of OM (A), the amount of S / SO₄ and intensity of SRP (B), intensity of SRP and anaerobic mineralization of OM (C) in bottom sediments of the lakes in 2006

eralization. During this period, the most intensive process was determined in bottom sediments of Lake Gineitiškės (to 2830.6 mg C/m²d⁻¹) and was about twice as intensive as in the other two lakes. In shallow Gineitiškės and Salotė lakes, the development of aerobic benthic microorganisms was active in profundal parts (O₂ was about 7.0 mg/l), while in the presence of a high content of organic matter, their functioning took place probably only in a very thin upper layer of bottom sediments. In deeper, anaerobic sediments, the anaerobic organic matter mineralization processes prevailed. Similar results were also obtained in some other shallow eutrophic lakes (Дзюбан, 2007). Therefore, the most pronounced anaerobic OM decomposition was determined in Lake Gilužis (up to 97% of total mineralization) in which microaerobic conditions near the bottom had been forming during all periods of investigation. The lack of oxygen determined the development of anaerobic bacteria which played a crucial role in the decomposition of OM in Lake Gilužis.

Despite anaerobic conditions, the concentration of sulphates and OM are the other two main factors influencing the activity of sulphate-reducing bacteria (SRB) which play a crucial role in the terminal anaerobic decomposition of OM (Postgate, 1984). Oxygen of sulphates is used for breathing by these bacteria while water-soluble OM serves as a source of electron donor. The amount of SRB in bottom sediments was not high and varied from 10³ to 10⁴ CFU/dm³ (Table 2). The highest amount of SRB was registered in July (10⁴ CFU/dm³) in all the lakes. The physiological activity of SRB is pronounced in the sulphate-reducing process (SRP). Our investigations showed a positive correlation between the anaerobic decomposition of OM and the activity of SRB – the sulphate-reducing process (Fig. 3C). The most intensive SRP was determined in July in bottom sediments of all three lakes, especially in the stratified Lake Gilužis (2.6 mg S²⁻/dm³ d⁻¹) (Fig. 2B). This might occur due to some physical-chemical conditions near the bottom: a higher water temperature (Salotė and Gineitiškės), micro-aerobic conditions and sulphate concentration (Gilužis) as well as the flow of planktonic detritus to bottom sediments of the lakes. Changes in the structural composition of organic matter might have a stronger influence on the succession of SRB physiological groups and their activity than on their amount (Кузнецов и др., 1985; Карначук и др., 2006).

In spring and autumn, the decrease of sulphates as well as oxygen saturation near the bottom due to water mixing (lakes Salotė and Gineitiškės) lowered the intensity of SRP on the average four times compared with the summer period. Investigations had shown the strong positive correlation between concentration of sulphates and the intensity of sulphate reducing process (Fig. 3B).

The end product of SRP is hydrogen sulphide – a substance highly toxic for lake benthic fauna. The highest concentration of this compound was registered during summer stratification in bottom sediments of all the lakes studied (48–184 mg/dm³) when the most intensive SRP took place

(Fig. 2). The highest concentration of this compound was determined in the deepest lake Gilužis – up to 184 mg/l (Table 2).

During accumulation and intensive mineralization of OM (aerobic and anaerobic), the additional biogenic substances separate from sediments and get into bottom waters. This may stimulate the secondary eutrophication. The reassimilation of phosphorus occurs during anaerobic mineralization of OM, thus enriching bottom sediments with this compound (Мартынова, 1984; Holmer, Storkholm, 2001). In summer, in lakes of the river Sudervė basin, the SRP was about four times more intensive as compared with lakes situated in protected areas with a low anthropogenic impact (Krevš et al., 2006; Kučinskienė, Paškauskas, 2003). During an intensive SRP, the release of hydrogen sulphide, toxic for most hydrobionts, occurs as well and may have a negative impact on the quantitative and qualitative structure of benthic organisms. Such processes are very dangerous, especially to small lakes such as Gineitiškės and Salotė. Thus, for the preservation of their recreation value, the protection means are necessary for these lakes at the present and in the future as well.

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ORGANINIŲ MEDŽIAGŲ MINERALIZACIJA MAŽŲ ANTROPOGENIZUOTŲ EŽERŲ DUGNO NUOSĖDOSE

Santrauka

2006 m. gegužę, liepą ir rugsėjį mažų antropogenizuotų ežerų (Salotė, Gilužis ir Gineitiškės), išsidėsčiusių Vilniaus apylinkėse, profundalės dugno nuosėdose buvo tirtas bendras (aerobinės ir anaerobinės) organinių medžiagų (OM) mineralizacijos bei sulfatų redukcijos intensyvumas. Tyrimai parodė, kad organinės anglies kiekis ežerų dugno nuosėdose kito nuo 13,7 iki 37,4 % orasausio svorio. Didžiausias jos kiekis buvo nustatytas Salotės (iki 37,4 %) ir Gineitiškių ežeruose (iki 30,8 %). Organinių medžiagų mineralizacijos intensyvumas tirtųjų ežerų dugno nuosėdose kito nuo 648,0 iki 2830,6 mg C/m² per parą. Suminėje organinių medžiagų mineralizacijoje visų tirtų ežerų dugno nuosėdose vyravo anaerobiniai procesai, kurie intensyviausiai vyko vasarą, ypač Gineitiškių ežere. Sulfatų redukcijos intensyvumo tyrimai parodė, jog šis anaerobinis terminalinis organinių medžiagų skaidymo procesas intensyviausias buvo Gilužio ežere (iki 2,6 mg S⁻²/dm³ per parą) ir tiesiogiai priklausė nuo sulfatų koncentracijos. Didelio organinių medžiagų kiekio kaupimasis dugno nuosėdose bei intensyvi OM mineralizacija gali skatinti šių ežerų, ypač negilių Salotės ir Gineitiškių, antrinės eutrofikacijos procesus ir mažinti jų rekreacinę vertę.

Raktažodžiai: organinė medžiaga, aerobinė ir anaerobinė mineralizacija, sulfatų redukcija, dugno nuosėdos, ežerai