Diurnal variations of trace metals and heterotrophic bacterioplankton concentration in a small boreal lake of the White Sea basin

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Abstract – This work represents a concerted effort aimed at understanding the microbiological and chemical evolution of a small boreal lake during the diurnal cycle of photosynthesis. We studied diurnal variation of ~ 40 dissolved macro- and trace elements, organic carbon and bacterial population dynamics in the surface and bottom water layer of the shallow Vilno Lake in the White Sea basin. Four-days continuous measurements with 6 h sampling steps both at the surface (0.5 m) and on the bottom (4.0 m depth) during no-bloom periods revealed constant concentrations (within ± 20–30%) of all major elements (Na, Mg, Si, K, Ca), organic and inorganic carbon and most trace elements (B, V, Cr, Fe, Cu, Ga, As, Rb, Y, Zr, Sb, Cs, Ba, all REEs, Hf, Pb, Th, U). At the same time, the concentration of some biologically important trace metals (Mo, Mn, Co, Cd) was subjected to variations partially reflecting the change of bacterioplankton concentration. This work enables two types of element behavior to be distinguished during photosynthesis in the water column – constant concentration and sinusoidal variations – depending on their speciation in solution and their affinity to aquatic microorganisms.

Key words: Boreal lake / trace metals / speciation / heterotrophic bacteria / diurnal cycle

Introduction

Establishing functional relationships between the biological activity of microorganisms and fluxes and speciation of trace metals is among the major objectives of the environmental geochemistry. Quantitative characterization of these relationships in water systems requires detailed analysis of microbial population, phytoplankton, dissolved organic carbon and major and trace elements. Boreal lakes represent an important and highly attractive subject of research in view of various environmental issues raised in the subarctic region, including, for example, pristine concentrations of metals and trace elements that are most likely to be affected by the human impact (Roederer, 1991; de Caritat et al., 1996). Another important environmental issue is the fate of organic carbon in lakes that is likely to significantly control the CO2 balance in high latitudes (Smith et al., 2004; Shirokova et al., 2009). High concentrations of dissolved organic matter (DOM) and thus, the colloidal status of most trace elements (TE) is the most important characteristic feature of TE geochemistry in European Russian Arctic zone (Pokrovsky and Schott, 2002; Pokrovsky et al., 2005). This change in metal speciation (i.e., the proportion of organo-mineral colloids and humic/fulvic complexes of trace metals, size distribution in colloidal fraction) between different water reservoirs or in the water column is expected to be controlled by: (1) biological processes of primary production/mineralisation and the release of organic exudates, both on diurnal (photosynthesis) and monthly (summer bloom, spring and autumn overturn) scales occurring in the water column; and (2) hydrological conditions of the watershed that controls the delivery of allochtonous organic matter from the soil reservoirs via riverine inflow, which in turn, depends on intra-soil processes, watershed lithology, and, if present, anthropogenic and atmospheric input.

Diurnal variation of major hydrochemical and biological parameters in the lake waters have been studied since the beginning of the last century (i.e., Rose et al., 1925; Rose et al., 1925;
Hutchinson, 1957; Eiler et al., 2006). Less in known about the fate of trace metals, except those involved in redox cycles linked to photoreduction that has been extensively investigated (McMahon, 1969; Emmenegger et al., 2001). There is a large body of literature on trace element geochemistry in temperate lakes (e.g., Hamilton-Taylor and Willis, 1990; Noel et al., 1990; Balistreri, 1992; Vioillier et al., 1995, 1997; Hamilton-Taylor et al., 1996, 2005; Nriagu et al., 1996; Falkner et al., 1997; Albéric et al., 2000), including detailed seasonal observations. However the diurnal patterns of trace metal concentration remain poorly investigated. In contrast, diurnal variations of trace elements were extensively studied on marine and coastal systems (Luengen et al., 2007) and in rivers (Brick and Moore, 1996; Jones et al., 2004; Gammons et al., 2005a, 2005b). In the latter, these variations are known to be induced by change in environmental conditions such as temperature and biological activity and are strongly controlled by adsorption processes (e.g., Nimick et al., 2005; Parker et al., 2007). However, relationships of chemical and biological parameters kinetics with temperature and photochemical regime of redox sensitive elements are virtually unknown for the lakes of high latitudes especially those that do not bear any anthropogenic pressure. At the same time, compared to rivers, small lakes represent a far better object for testing the diurnal variations of chemical and biological parameters due to the stable functional processes of primary production–bacterial mineralization. In lakes, such links are minimally influenced by short-term variations of external factors such as hydrology, human activity, allochthonous soil and groundwater inputs. In boreal waters, most trace elements and oligo-metals are associated with organo-mineral colloids of allochthonous origin such as soil humic and fulvic acids. The bioavailability of these colloids for autotrophic phytoplankton is rather low (Xue and Sigg, 1993). In contrast, heterotrophic bacterioplankton is known to mineralize dissolved organic matter of both allochthonous and autochthonous origin (Tranvik, 1988, 1989, 1994; Kritzberg et al., 2004) and thus can potentially liberate or consume associated trace metals.

In the present work, we monitored the trace element and bacterioplankton concentration in the water column of a small lake Vilno of the White Sea basin. We characterized colloidal speciation and analyzed concentrations of ~40 major and trace elements and two groups of heterotrophic bacteria in the surface and bottom layers over four days of continuous observation in order to address two following question: (1) Are there diurnal variations of trace element concentration at different depths, and (2) Is there a link of these variations with major hydrochemical parameters and the heterotrophic bacterio-plankton concentration in the water column? An additional motivation for this work was to test whether a single sampling of the small lake surface may serve as representative of trace elements concentration for a longer period of observation. It is anticipated that addressing these objectives will improve our knowledge of factors controlling the biogeochemical cycles of trace elements and biologically important heavy metals migration in lake systems of high latitudes.

**Materials and methods**

We studied a small non-stratified Lake Vilno located in the Arkhangelsk region (Russia) within the state protected territory of Kenozersky National Park (61°31’N; 38°07’E, Fig. 1). The studied area can be considered as pristine environment with natural concentrations of elements because of the absence of any industrial or agricultural activity and the limited accessibility. The watershed lithology is represented by glacial moraine (products of granite-gneisses erosion) and carbonate rocks. The lake has overall surface area of 2.57 km² and an average depth of 3 m. There is no inlet in this lake and only one small outlet. Sampling was performed on 17–22 July 2007 at essentially anticyclone conditions with in-situ measurements of pH, temperature, redox potential and dissolved oxygen. Water samples were collected from the surface (0.5 m depth) and from the bottom (4.0 m depth) each 6 h and immediately filtered through acetate cellulose membrane (0.22 μm) filters.

The pH was measured using a combined Schott-Geräte electrode calibrated against NIST buffer solutions (pH = 4.00 and 6.86 at 25 °C), with an accuracy of ±0.02 pH units. Clean trace-metal technique was employed throughout samples and collecting, filtration and analysis. Samples were collected on the monitoring station from a PVC boat having no metal parts submerged in the water using a polycarbonate water sampler for ultraclean water collection (Aquatic Research®). Plastic gloves were always used during handling of the samples. The water samples were immediately filtered through sterile, Minisart® filter units (Sartorius, acetate cellulose filter) with pore sizes of 0.22 μm. The first 20 mL of the filtrate was systematically
discarded. The biological availability of trace metal is known to be fully controlled by their speciation in aqueous solution. For example, permeation of free ions through the biological membrane is determined by ion transport channels having approx. 600 Da molecular size. Conventional filtration through a 0.22 μm membrane cannot, therefore, account for the bioavailability of metals, and thus fine size separation procedure via ultrafiltration/dialysis is necessary. In order to better characterize trace element speciation in studied lake, we performed in-situ dialysis at the surface (0.5 m depth). Dialysis experiments were performed using 20–50 mL precleaned dialysis bags placed directly in the lake water. Similar technique has been used to account for metal speciation both in the water column and in the pore waters (Carignan et al., 1985; Alfaro-De la Torre et al., 2000; Gimpel et al., 2003). The duration of this dialysis procedure was between 24 and 48 h. Pokrovsky et al. (2005) showed in laboratory experiments that, for both 1 and 6–8 kDa membranes, an equilibrium distribution is attained within 6–12 h, in agreement with the manufacturer’s specifications. At the same time, Carignan et al. (1985) argued that much longer equilibration time is necessary to achieve equilibrium dialysis distribution in porewaters. However, we would like to stress that diffusion control on intra-membrane transport in extremely stagnant pore waters is much slower than that in well-agitated, shallow water column of the lake. For dialysis experiments, EDTA-cleaned trace-metal pure SpectraPor 7™ dialysis membranes made of regenerated cellulose and having pore sizes of 1 kDa (approximately 1 nm) were thoroughly washed in 0.1 M double-distilled HNO₃, ultrapure water, filled with ultrapure MQ deionized water and then placed into natural water. The efficiency of the dialysis procedure was evaluated by comparing major anion concentrations (e.g., Cl⁻ and SO₄²⁻) or neutral species (H₂SiO₄) not associated with colloids between the dialysis bag and the external solution. These concentrations were always identical to within ±10%, suggesting an equilibrium distribution of dissolved components.

Filtered or dialysed solutions for cations and trace element analyses were acidified (pH = 2) with ultrapure double-distilled HNO₃ and stored in HDPE bottles previously washed with ultrapure 0.1 M HCl and rinsed with MilliQ deionized water. The preparation of sampling bottles was performed in a clean bench room class A 10000. Filtered samples for DOC analyses were collected in a pyrolysed sterile Pyrex glass tube. For all major and most trace elements except Zn, concentrations in blanks were below detection limits. Zn concentration in blanks was sometimes 2–3 times higher than the detection limits; however, it was still a factor of 5 to 10 lower than the sample concentration. Aqueous silica concentrations were determined colorimetrically (molybdate blue method) using a Technicon autoanalyzer II, with an uncertainty of 2%. Alkalinity was measured by potentiometric titration with HCl by automated titrator (Metrohm 716 DMS Titrino) using a Gran method with a detection limit of 10⁻⁵ M and an uncertainty of 2%. DOC was analysed using a Carbon Total Analyzer (Shimadzu TOC 6000) with an uncertainty better than 3%. Major and trace anion concentrations (Cl⁻, SO₄²⁻, F⁻, NO₃⁻ and PO₄³⁻) were measured by ion chromatography (HPLC, Dionex ICS 2000) with an uncertainty of 2%. Calcium, magnesium, sodium, and potassium concentrations were determined with an uncertainty of 1–2% using a Perkin-Elmer 5100PC atomic absorption spectrometer. Trace elements (TE) were determined without preconcentration by ICP-MS Agilent 8000. Indium and rhenium were used as external standards. The international geostandard SLRS-4 (Riverine Water Reference Material for Trace Metals certified by the National Research Council of Canada) was used to check the accuracy and reproducibility of each analysis (Yeghicheyan et al., 2001). We obtained a good agreement between replicated measurements of SLRS-4 and the certified values (relative difference <10% SD on repeated measurements), except for B and P (30%).

Chlorophyll a was measured by spectrophotometry at six different wavelengths after extraction from 0.45 μm filter using 90% acetone. For this, from 1 to 1.5 L of the lake water was processed. Typical analytical uncertainty of Chl a determination is ±10% in the concentration range 0.1 to 10 μg.L⁻¹. Samples for microbiological analyses were collected in sterile 250-mL flacons and stored less than 2 h in a portable cooler at 5 °C. Active bacteria number count (colony forming units, CFU·mL⁻¹) was performed using Petri dishes inoculation (0.1 to 1.0 mL of lake water in three replicates) performed in specially organized microbiological field laboratory. For this, internal space of metal-free camping tent was maximally cleaned and sterilized by UV irradiation. All manipulations were performed in the vicinity of open flame on thoroughly alcohol- and detergent-treated microbiological table. Samples were inoculated on Nutrient Agar (5 g.L⁻¹ beef extract, 5 g.L⁻¹ gelatine peptone, 15 g.L⁻¹ bacteriological agar, pH = 6.8 ± 0.2 at 25 °C) to determine the total number of heterotrophic bacteria. Difco® agar (granulated powder, Lot No 6290083) inoculation was used to assess the number of oligotrophic bacteria. Inoculation of blanks was routinely performed to assure the absence of contamination from external environments.

Results and discussion

Element speciation in colloids

In-situ dialysis procedure revealed that >50% of trivalent and tetravalent elements are in colloidal form (1 kDa–0.22 μm fraction), whereas alkaline and alkaline-earth metals, neutral species (Si, Sb, As) and anions (Mo, V) are essentially present in their inorganic (ionic) forms. Specifically, according to their association with these colloids, two groups of elements can be distinguished: (i) species that are weakly affected by size separation procedure and present in the form of true dissolved inorganic species (max 20–30% of colloidal fraction) such as Ca, Mg, Li, Na, K, Cs, Si, Ba, Sr, Rb, As, Sb,
Mo and Mn; and (ii) elements significantly affected by dialysis procedure with ≈ 40–50% being concentrated in colloids (1 kDa – 0.22 μm): Al, Ti, Ni, Co, Y, REE, Pb, Zr, Hf, Th, U). A stack diagram of element proportion in colloidal form is illustrated in Figure 2A. The affinity of chemical elements towards colloidal fraction is fairly well documented in many boreal rivers and streams (e.g., Ingri et al., 2000; Pokrovsky and Schott, 2002; Pokrovsky et al., 2006; Dahlqvist et al., 2007; Vasyukova et al., 2010), and is most likely linked to their presence in the form of Fe-rich, organic-matter stabilized aggregates of large (10 kDa – 0.22 μm) molecular weight. This is further illustrated by ultrafiltration pattern of TE partitioning in colloids assessed from the experiments performed at the wetland surface water adjacent to the lake Vilno (Fig. 2B). There is a strong decrease of insoluble trace element concentration with decrease of Fe concentration in colloids that occurs between 10 kDa and 0.22 μm. Further decrease also occurs upon filtration through 1 kDa membrane.

**Element concentration variation during the diurnal photosynthesis cycle**

Water temperature variations both at 0.5 and 4 m depth were rather minor (±1–1.5°) although followed some diurnal cycle (Fig. 3A). It is well known that pH is the most sensitive parameter of the primary production–mineralization processes. Indeed, this parameter demonstrates clear diurnal periodicity, both at the bottom and on the surface where it changes from 6.7 to 7.2 (Fig. 3B). Note that the phytoplankton bloom in lakes of studied region usually occurs in August and thus the present study conducted in July is not addressing the biogeochemistry of the lake water column during the bloom period. Dissolved oxygen concentration demonstrated non-systematic variations in the course of observation and no clear maximum or minimum was detected neither during the afternoon period or night measurements except a global minimum at the beginning of third day of observation (Fig. 3C). The DOC remained constant with non-systematic (±30%) fluctuation from the average value of 8.9 ± 0.3 mg.L⁻¹ (Fig. 3D). Carbonate alkalinity was subjected to minimal variations remaining constant within 435 ± 5 μmol C.L⁻¹ (30 measurements at 0.5 and 4.0 m depth, not shown). Chlorophyll a (5 measurements at 0.5 m depth) varied from 3.0 to 4.1 μg.L⁻¹ with a minimum value observed in the morning of the third day of observation. These measurements are compatible with the absence of bloom and relatively weak primary production during the observation period. Apparently, the pool of allochtonous dissolved organic carbon was very weakly affected...
by autochtonous production of phytoplankton exometabolites which could feed the microbial loop of the system.

During four days of continuous observations on both depths, we have not detected significant variation \( (i.e., \geq 30\%) \) of most major and trace elements \((B, Na, Mg, Si, K, Ca, V, Cr, Co, Fe, Cu, Ga, As, Rb, Sr, Y, Zr, Sb, Cs, Ba, all REEs, Hf, Pb, Th, U)\) concentrations as illustrated for some of them in Figure 4. At the depth of 4 m, we observed some non-systematic variations of Al, Ti, Mo and Pb concentrations that never exceeded 30–50\% deviation from the average value. Overall, we observed a quite high stability of chemical composition of the lake water, which signifies that the role of external fluctuations, internal lake processes, anthropogenic or atmospheric contaminations were negligible during full period of observation. This result is important from methodological point of view as it suggests that for most major and trace elements dissolved in the water column, a single one-time sampling from the surface at any period of the day is sufficiently representative for a larger temporal and spatial range of observation.

Three typical oligoelements-heavy metals \((Fe, Zn, Cu)\) demonstrate highly contrasting behavior in the course of observation. While Cu and Fe concentrations remain rather stable \((\pm 20–30\% \text{ deviation from average})\) over a full period of sampling, Zn concentration is subjected to significant variations achieving one order of magnitude \( (Fig. \, 5)\). These variations are not compatible with those of pH as it can be seen from the comparison of diurnal pH variations demonstrating at least three day-time maxiums over the 4-days observation period \( (Fig. \, 3B)\) and variations of Zn concentration demonstrating only one day-time maximum. The variation of Zn concentration in the surface water of lake Vilno is almost an order of magnitude higher than that of the Chl a. The independence of Zn on pH and other macroscopic parameters of solution variations is at first glance surprising because Zn uptake by phytoplankton is strongly pH-dependent \((Gélabert \, et \, al., \, 2006; \, Tripathi \, et \, al., \, 2009)\) and Zn concentration during the bloom often follows that of the Chl a \((Luengen \, et \, al., \, 2007)\). Moreover, the pH is known to be the governing factor of metal adsorption on the surface of photosynthetic microorganisms such as diatoms or cyanobacteria \((Gélabert \, et \, al., \, 2007; \, Pokrovsky \, et \, al., \, 2008a, \, 2008b)\). However, we would like to underline that relatively small pH variations \((from \, 6.7 \, to \, 7.2)\) may not be sufficient to control the adsorption and uptake process from the bulk of lake water; rather, these processes are dominated by local pH gradients in the pristine layer around the photosynthesizing cells.

It is possible that the absence of bloom during studied period does not allow testing these dependences for selected boreal lake. For copper, even during the bloom, this element behaves independently on biological and hydrochemical parameters \((Luengen \, et \, al., \, 2007)\). Independence of Cu concentration \((unlike \, Zn)\) from that of chlorophyll and biogenic components has been also reported for England lakes during the diatom bloom period \((Reynolds \, and \, Hamilton-Taylor, \, 1992)\). To our knowledge, such information on boreal lakes rich on dissolved organic matter is lacking. Our dialysis results demonstrate that almost 90\% of Fe is in colloidal form. One can suggest that the strong complexation of Fe and Cu with colloidal organic matter of essentially allochtonous origin
from the adjacent swamps precludes their prompt response to the variation of biological activity in the diurnal cycle. Zn is known to be less complexed with organic colloids and thus it is more labile during short-term variations of the environmental parameters in the water column. Unfortunately, due to contamination during dialysis procedure, we were not able to measure the proportion of colloidal fraction of Cu and Zn in studied lake. However, from the knowledge of these metals speciation in adjacent boreal zones, they are most likely to be present in small-size organic (fulvic) complexes and simple ions, respectively (Vasyukova et al., 2010). The trace elements are likely to be involved in two major processes controlling their concentration in the lake water: uptake by growing phytoplankton and release during degradation of allochthonous and autochthonous organic matter by heterotrophic bacterioplankton. Our previous results support the possibility of utilization by heterotrophic bacteria of colloidal allochthonous organic matter in boreal surface waters of Arkhangelsk region and Northern Siberia (Shirokova et al., 2008, 2009). By consuming allochthonous dissolved organics, heterotrophic bacteria are also capable of uptaking oligo-elements (limiting nutrients) or releasing biologically-indifferent trace elements. However, even though colloidal-bound trace elements such as trivalent and tetravalent metals are potentially bioavailable for aquatic biota via heterotrophic bacteria pathway, the constant pattern of their concentration with time over period of observation signify the negligible role of intra-lake biological processes (i.e., occurring within the water column) in geochemistry of these elements.

Several trace elements clearly demonstrate diurnal variations in concentration during the observation period (Figs. 6A–6D). These are the biologically important elements such as Mn, Co and Mo, and also Cd. The latter is known to substitute Zn in the carbonic anhydrase of diatoms in case of Zn limitation conditions (Lane et al., 2005) and both are significantly affected by the trophic transfer along the foodwebs (Twiss et al., 1996). Peak concentrations of these elements occurred during the second night of monitoring. This coincided with a decrease in concentrations of both eutrophic and oligotrophic bacterioplankton (Fig. 7), and, consequently, may imply a direct link between these elements and heterotrophic bacteria activity. For example, phytoplankton cells and their large-size exometabolites mineralization by heterotrophic bacteria, or the degradation of bacterial biomass may release oligoelements in soluble (<0.22 μm) form.
in the water column as it is known for Zn and Cd from laboratory experiments on degradation and elemental release rates from phytoplankton debris (Lee and Fisher, 1992; Wang and Guo, 2001). However, our data do not allow quantitatively resolve the correlation coefficient between these parameters due to limited number of measurements. The four considered elements are quite contrasting in terms of their colloidal speciation in solution. While Co and Cd are 80–90% complexed with colloids, Mn and Mo are linked to colloidal fraction by not more than 20–30% of their total concentration (Fig. 2A). Therefore, for biologically-important elements, the affinity to plankton biomass is apparently more important for diurnal cycle variation pattern than the speciation in the lake water. It is important to note that both surface and deep lake waters depicted the diurnal variation of these biologically important oligo-elements and bacterioplankton concentration. This strongly implies that the vertical distribution of all hydrochemical and biological parameters is quite homogeneous and not subjected to summer stratification. This is consistent with the low depth of this lake having high surface area and the absence of bloom event during the sampling period. Another important factor known to control trace element variations along the depth profile in lakes is redox process of Fe and Mn oxides dissolution and precipitation (i.e., Davison, 1993). The Mn, Mo, Co and Cd concentration increase after the second night of observation (Fig. 6) is concomitant with dissolved oxygen decrease (Fig. 3C) and may be caused by partial reductive dissolution of Mn oxides formed in the water column or may be linked to the diffusion flux of metals from the sediments as it is observed in neighboring seasonally-stratified lakes (Pokrovsky et al., 2009). In this regard, Mn, Mo and Co peaks could reflect a benthic response to bacterial biomass accumulation at the sediment interface (e.g., Taillefert et al., 2002).

Overall, results of this work enable two types of element behavior during photosynthesis in the water column to be distinguished: those exhibiting constant concentration (all major elements, anions and neutral molecules, trivalent and tetravalent trace elements tightly linked to organic or organo-mineral colloids) and those demonstrating sinusoidal variations (biologically important heavy metals, oligoelements). Two factors that control to which group the chemical element belongs are: element speciation in solution controlled by the presence of colloids and the element affinity to aquatic microorganisms.

Fig. 6. Diurnal variations of Mn (A), Mo (B), Cd (C) and Co (D) concentration at 0.5 and 4.0 m depth of Lake Vilno.

Fig. 7. Diurnal variations of eutrophic (A) and oligotrophic (B) bacterioplankton concentration (colony forming units per mL) at 0.5 and 4.0 m depth of Lake Vilno.
Conclusions

We observed a highly constant concentration of many trace and major elements at the depth of 0.5 and 4.0 m of non-stratified lake during bloom-free period of continuous 4-days observation. Among ~ 40 studied major and trace elements, only Zn, Mo, Mn, Co and Cd may be linked to some variations of bacterioplankton concentrations, whereas most of trivalent and tetravalent elements, Fe and Cu are strongly complexed with organo-mineral colloids, behave independently of biological and hydrochemical parameters of the water column. It is anticipated that single, one-time sampling from the lake surface (0.5 m) can be sufficiently representative for a large number of dissolved components. This approach should be extended to verify the functional relationships between geochemical (concentration and speciation of trace element) and microbiological (bacterioplankton and phytoplankton concentration, primary productivity and respiration) parameters during winter conditions and bloom periods in the boreal lake.

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