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Spatio-temporal distribution of organic and inorganic pollutants from Lake Geneva (Switzerland) reveals strong interacting effects of sewage treatment plant and eutrophication on microbial abundance

Florian Thevenon, Neil D. Graham, Aline Herbez, Walter Wildi, John Poté *

University of Geneva, Institute F.A. Forel, 10 route de Suisse, CP 416, 1290 Versoix, Switzerland

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Variation with depth and time of organic matter (carbon, nitrogen, phosphorus), inorganic pollutant (mercury), as well as bacterial abundance and activity, were investigated for the first time in sediment profiles of different parts of Lake Geneva (Switzerland) over the last decades. The highest organic contents (about 32%), mercury concentration (27 mg kg^{-1}), bacterial abundance (in order of 9×10^9 cell g^{-1} dry sediment), and bacterial activity (1299 Relative Light Units (RLU)) were found in the highly polluted sediments contaminated by the waste water treatment plant (WWTP) discharge, which deposited during the period of cultural eutrophication. Such data, which contrast with the other sampled sites from deeper and more remote parts of the lake, prove that the organic matter and nutrients released from the municipal WWTP have considerable effects on bacterial abundance and activities in freshwater sediments. In fact, the relatively unpolluted deepwater sites and the coastal polluted site show large synchronous increases in bacterial densities linked to the anoxic conditions in the 1970s (lake eutrophication caused by external nutrient input) that subsequently increased the nutrient loading fluxes. These results show that the microbial activities response to natural or human-induced changing limnological conditions (e.g., nutrient supply, oxygen availability, redox conditions) constitutes a threat to the security of water resources, which in turn poses concerns for the world's freshwater resources in the context of global warming and the degradation of water quality (oxygen depletion in the bottom water due to reduced deep waters mixing). Moreover, the accumulation of inorganic pollutants such as high mercury (methyl-mercury) concentration may represent a significant source of toxicity for sediment dwelling organisms.

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1. Introduction

Sediment constitutes an important compartment of aquatic ecosystems where anthropogenic (e.g., chemicals, nutrients) and natural (e.g., living organisms, plant or animal debris and mineral material) products accumulate with time. As a result, sediments are a heterogeneous compartmentalised system that can be described in physical, chemical or biological terms. More precisely, sediments contain a great variety of microorganisms endowed with biological and physico-chemical reactivities, and represent a complex habitat of microorganisms (e.g., bacteria, cyanobacteria, viruses, fungi, algae, and protozoans), which play a major role in aquatic food webs as well as nutrient cycling and decomposition of organic matter.

Anthropogenic activities and, in particular urban effluent water from waste water treatment plants (WWTP) constitute the main sources of aquatic environment contamination by inorganic and organic matters which include organic components, heavy metals, and hydrophobic organic compounds. Moreover, the increasing contamination of sediments is a big concern in numerous aquatic ecosystems (Förstner and Wittmann, 1979; Pardos et al., 2004; Schwarzenbach et al., 2006; Vörösmarty et al., 2010), especially for freshwater lakes that have undergone oxygen depletion in their bottom water during warm periods (that reduced the vertical mixing and the oxygenation of deep waters) and/or lake reservoirs affected by anthropogenic changes in their watershed (e.g. construction of hydroelectric dams that may reduce the turbidity currents and therefore oxygen input to the deep lake basin) that indirectly increase organic matter concentration and nutrient flux from the bottom sediments.

It is puzzling that although the diversity and activity of microbial communities may be sensitive indicators of pollution in aquatic ecosystems, some studies observed either a reduction or an increase in abundance along with sediment contamination (Sandaa et al., 1999; Sorci et al., 1999), or even no significant variation

* Corresponding author. Tel.: +41 22 379 03 21; fax: +41 22 379 03 29.
E-mail address: john.pote@unige.ch (J. Poté).

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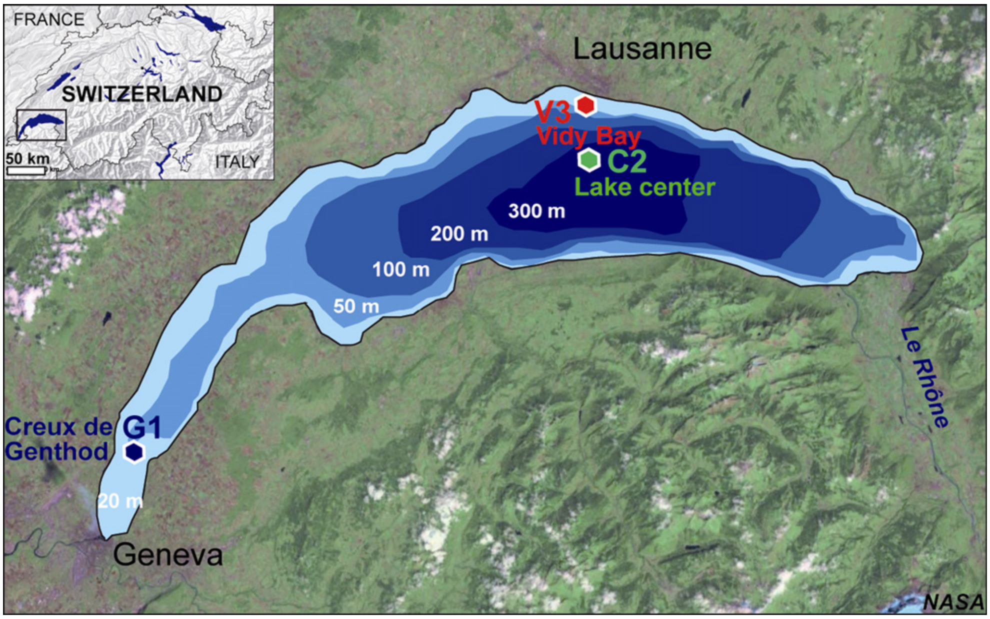

Fig. 1. Map of Switzerland (upper left) with the bathymetric map of Lake Geneva showing the three sample sites: The bay of Vidy (V3, close to the outlet pipe of the wastewater treatment plant discharge), the Creux de Genthod region (G1), and the lake centre (LG C2).

(Gillan et al., 2005). In fact, sedimentary OM content constitutes a major determinant of heterotrophic bacterial activities in sediments, and can be one of the most important limiting factor for bacterial and benthic microorganism abundances (e.g. Goedkoop et al., 1997; Fisher et al., 2002; Polymenakou et al., 2007).

Lake Geneva is the largest freshwater reservoir in Western Europe, with a volume of 89 km^3 and a maximum depth of 309 m. The lake was considered eutrophic in the 1970s and has become mesotrophic in the 1980s after a drastic reduction of phosphorus inputs (Dorioz et al., 1998). Approximately 700 000 people are supplied with water from Lake Geneva. The city of Lausanne, located on the northern shore, discharges the largest volume of treated wastewater into the nearby Bay of Vidy. The WWTP was built in 1964 for 220 000 eq.-inhabitants. The WWTP effluent is released into the Bay of Vidy at 700 m from the shore and 40 m depth. As a consequence, Vidy Bay is the most contaminated area of Lake Geneva. Previous data document the accumulation of contaminants in sediments close to a recreational area on the bay's shoreline, as well as significant related ecological impacts and health risks (Loizeau et al., 2004; Pardos et al., 2004; Wildi et al., 2004). Several studies have already focused on the physico-chemical characteristics of surface sediments of Vidy Bay, and on the spatial distribution of OM, faecal indicator bacteria, heavy metals, and hydrophobic organic compounds (e.g. Poté et al., 2008; Haller et al., 2009). However, there is still a lack of crucial information on bacterial abundance, diversity, and functional characterization in these sediments.

Untreated (or only partly treated) wastewaters including industrial and domestic effluents contain relatively high concentrations of OM, nutrients and other contaminants which may have adverse effects on aquatic living organisms e.g., on the sedimentary microbes. Sediment contamination by heavy metals and other micropollutants might cause potential adverse effects to ecosystems and also pose human health risks (e.g. Verweij et al., 2004; Wang et al., 2004). The main environmental risk is remobilization of the contaminants and their return to the hydrosphere either by sediment re-suspension or by infiltration into the groundwater (Eggleton and Thomas, 2004; Wildi et al., 2004). Therefore, the release of different contaminants from wastewater or their accumulation in sediments should be examined extensively. To the present day, polluted aquatic ecosystems have not been investigated in this sense, and only a few studies have been carried out on sedimentary records to assess the temporal distribution of inorganic pollutants such as mercury (Hg) and of both OM content and bacterial activities. The objective of this work is to assess the vertical distribution of Hg, OM and sedimentary microbial activities in contrasting polluted environments of Lake Geneva. Its aim is to test the influence of polluted sediments abnormally enriched in OM and heavy metals on the temporal-microorganism abundance and activities in the sediments. For this task, three sites in Lake Geneva were selected on the basis of their contrasting pollution levels and OM contents (Fig. 1): (i) site V3, nearby the WWTP outlet pipe discharge location into the Bay of Vidy, (ii) site G1 close to the Creux de Genthod in the Small Lake, and (iii) site C2 in the centre part of the Large Lake. Sediment cores were continuously analysed for total and active culturable extracted bacteria cells, and ATP for living microorganisms using a commercial ATP essay. For a better understanding of the obtained results, these analyses were complemented by a detailed sediment analysis of the organic fraction that includes the quantification of OM, organic carbon (Corg), total nitrogen (N), and total phosphorus (P). Total mercury (Hg) analysis was performed to reconstruct changes in anthropogenic inorganic inputs (heavy metal contamination).

2. Materials and methods

2.1. Study site description and sampling procedure

The boat "La Licorne" of the Institute F.-A. Forel was used for sediment coring (Fig. 1): (i) in the Bay of Vidy, near the outlet pipe of the WWTP of Lausanne, at 40 m water-depth (core V3: 100 cm-length), Swiss coordinates X: 534682, Y: 151538, distance to coast ~700 m. This site represents the most contaminated site of Lake Geneva in heavy metals, hydrophobic organic compounds and organic matter content, (ii) in the Creux de Genthod region at 50 m water-depth (core G1, 80 cm-length), Swiss coordinates X: 502613, Y: 122938, distance to coast ~1000 m, and (iii) at 304 m-water depth in the centre of the large lake (core C2: 130 cm-length), Swiss coordinates X: 534504, Y: 146178, distance to coast ~5500 m.

After their collection, the cores were brought to the Institute F.-A. Forel, opened, and sliced into 2 cm thick sections for microbiological analysis. The sediment samples were placed in sterile plastic containers (stored in an icebox) and treated in the laboratory within 24 h. The opened sediment cores were stored in a cold-room at 4 °C until their sampling for chemical analysis.

2.2. Organic matter (OM), total nitrogen (N) and total phosphorous (P) analyses

Sedimentary OM content was estimated from loss on ignition (LOI) at 550 °C for 30 min in a Salvis oven (AG Emmenbrücke, Luzern, Switzerland). Corg concentration was determined by titrimetry following acid oxidation: N content was determined by the Kjeldhal's method (APHA, 1985), and P content was measured with a spectrophotometer (Helios Gamma UV–Vis Thermo Electroporation, Thermo scientific, USA) at 850 nm. The sediment sample preparations for P analysis were performed as describe below: 50 mg of dry sediments were diluted in 5 mL HCl (1 N) and introduced in centrifuge tubes. The mixture was ultrasonicated (at ambient temperature) for 16 h and centrifuged (4000 rpm) for 20 min. The supernatant was mineralised for 45 min at 130 °C after addition of $K_2S_2O_8$ solution (5%). The P concentration was performed by measuring the absorbance of the blue complex obtained after reduction of molybdophosphoric acid according to the method described by Murphy and Riley (1962), Harwood et al. (1969) and Burrus et al. (1990).

2.3. Sediment chronology

The age-depth relationships of sediment cores have been estimated using artificial (cesium, ^{137}Cs) and natural (radiocarbon, ^{14}C) radionuclides. The presence of anthropogenic Cs (measured by gamma spectroscopy) in the record from the Vidy Bay (core V3), as well as the absence of the maximum radionuclide fall-out from atmospheric nuclear tests (1963/64) in this core, both indicate that sediments deposited after 1964; i.e., after the implementation of the municipal sewage treatment plant of the city of Lausanne. The Cs profile of the core from the Creux de Genthod (core G1) locates the maximum radionuclide fall-out from atmospheric nuclear tests (1963/64) about 18 cm core depth. For core C2 (lake centre), the age model is based on two linear interpolations between three tie points: the coring year (2010 at 0 cm core depth), the maximum Cs activity (1963/64 at 16 cm core depth) and one ^{14}C date (ETH-40161) performed on a leaf founded at 78 cm in core C2 (130 cm core long), yielding a radiocarbon age of 365 ± 30 years BP (before present = 1950). Calibration using OxCal v3.10 program (Bronk Ramsey, 2001) gives a calendar age of 1540 ± 53 AD.

2.4. Mercury (Hg) analysis

Before analysis, sediment samples were sieved at 63 μm mesh size and air-dried at ambient temperature. The total Hg analysis was carried out using atomic absorption spectrophotometry for mercury determination (Advanced Mercury Analyser; AMA 254, Altec s.r.l., Czech Rep.) following the procedure described by Hall and Pelchat (1997) and Ross-Barraclough et al. (2002). To summarize, this method is based on sample combustion, gold amalgamation and atomic absorption spectrometry.

2.5. Extraction and quantification of bacteria cells

The extraction of bacteria cells from sediments was performed using Nycondez density gradient centrifugation as previously described (Lindahl, 1996; Bertrand et al., 2005; Poté et al., 2010) but with minor modifications; briefly, 100 g (wet weight) of freshwater sediments and 500 mL of 0.2% sodium hexametaphosphate $(Na_2(PO_3)_6$, E. Lotti S.A, Switzerland) were dispersed in 1 L sterile plastic bottles and mixed for 1 h using the agitator rotary printing-press Watson-Marlow 601 controller (SKAN, Switzerland) at room temperature. The sample was then centrifuged at 750*g* for 15 min at 15 °C in a 3 K^{-1} (Sigma, centrifuge). The supernatant was then centrifuged at 7500*g* for 30 min at 10 °C. The microbial cell fraction-containing pellet was resuspended in sterile 0.8% sodium chloride solution by vortexing. Twenty-five milliliters of the homogenous solution was transferred to an ultracentrifuge tube containing 11 mL of Nycodenz solution (Axis-Shield, Oslo, Norway) at 1.3 g mL^{-1} density. Bacterial cells and sediment particles were separated by high-speed centrifugation (15 000*g* for 1 h at 10 °C) in centrikon T-1080 (Kontron, Instrument, ultracentrifuge). A white layer of bacterial cells was obtained at the interface between the Nycodenz-soil mix particles and the overlying aqueous layer. This white layer was carefully recovered, and mixed with an equal volume of sterile ultra pure water, then centrifuged at 7500*g* for 20 min at 10 °C. To remove traces of Nycodenz, pelleted bacteria were resuspended in 20 mL sterile ultra pure water and centrifuged for 20 min at 7500*g*.

Quantitative estimation of recovered bacterial cells were performed using DAPI (4′,6′-diamino-2-phenylindole hypochloride) staining couples with fluorescence microscopy (DDC: Dapi direct counts) and by counts on cultivation method (IBC: indirect bacteria cell counts). The results are expressed in number of cells (or colony-forming units for cultivation) g^{-1} dry sediment. All analyses were conducted in triplicate for each set of conditions. SigmaStat 3.0 software (Systat Software, Inc., USA) was used for the statistical analysis of the data. Spearman Rank Order Correlation has been conducted using SigmaPlot 11.0 (Systat Software, Inc., USA).

2.6. ATP microorganism assays

The test of microorganism activities in sediments was performed using a commercial ATP test Uni-lite®NG luminometer (Floralsolution, Nederland) according to the manufacture's instruction. The measurement of the Uni-lite NG Luminometer is based on the reaction between a chemical reagent present in the Clean-Trace measuring pens and adenosine tri-phosphate (ATP), which is present in all living cells (including bacterial and fungal cells). One milliliter of fresh sediment was homogenized with 1 mL of deionized water. The mixture was then centrifuged and the supernatant was brought into contact with a reagent combination luciferin/luciferase. The Uni-lite NG Luminometer measures the intensity of the emitted light and expresses it in Relative Light Units (RLU).

3. Results

3.1. Time-depth distribution of OM, N, and P

The LOI, Corg, N, and P contents for the three sites are reported in Table 1 and in Fig. 3 as a function of sediment depth. The highest concentrations of these organic parameters were measured in the sediments deposited close to the outlet pipe of the WWTP (core V3, Fig. 1) during the second part of the twentieth century. Here, OM content rose from about 15% before ca. 1970 (bellow 18 cm) to about 20–30% (0–18 cm) following the eutrophication period

Table 1
Distribution of organic matter (OM%), total organic carbon (Corg), total nitrogen (N) and total phosphorous (P) (concentration mg kg^{-1} dry sediments) in sediments of three sampled sites.

Depth (cm)	Bay of Vidy (LG V3)				Creux de Genthod (LG G1)				Lake centre (LG C2)			
	OM	Corg[a]	N[b]	P	OM	Corg[b]	N[c]	P	OM	Corg[b]	N[c]	P
0–3	30.12	1.44	1.39	4553	6.7	6.86	2.49	921.81	6.53	5.98	2.53	992.84
4–6	28.66	1.48	1.35	5617	7.21	6.79	2.36	790.07	6.44	5.85	2.54	748.48
7–9	22.74	1.44	1.33	23 075	7.3	6.39	2.38	777.03	6.19	5.65	2.52	696.13
10–12	22.95	1.47	1.28	19 667	5.98	6.32	2.56	740.67	5.44	5.65	2.34	859.42
13–15	32.14	1.42	1.27	24 783	6.09	6.45	2.28	764.25	5.82	5.32	2.43	989.40
16–18	20.23	0.89	0.45	27 220	6.31	6.17	2.25	715.61	5.72	5.37	2.41	699.42
19–21	15.62	0.78	0.43	15 982	5.84	6.29	3.45	617.75	5.72	5.40	2.50	696.09
22–24	18.98	0.75	0.35	23 279	4.17	6.45	2.36	553.18	5.46	5.45	2.35	669.46
25–27	16.57	0.68	0.32	25 295	3.63	6.12	2.17	504.49	5.38	5.34	2.24	738.80
28–30	15.06	0.67	0.34	20 864	3.46	6.06	2.09	529.29	4.82	4.84	2.10	690.01

[a] Concentration at mg kg^{-1} dry sediments $\times 10^5$.
[b] Concentration at mg kg^{-1} dry sediments $\times 10^4$.
[c] Concentration at mg kg^{-1} dry sediments $\times 10^3$.

(after ca. 1970; Fig. 2). These levels are much higher than values measured at deep lake sites, ranging from ca. 2% to 8%. By comparison, a relatively moderate increase in OM, Corg, N, and P contents was inferred from cores C2 and G1. For example, at the large lake centre (core C2), the LOI concentration varied from about 3% before 1850 to ca. 4–6% after the 1960s. According to these lower levels of pollution, a similar pattern of variation was observed for all organic and inorganic parameters at the deepest parts of the small and large lake (cores G1 and C2, respectively), thereby reflecting homogeneous sources, depositional processes, and sediment preservation in the deep water and coastal areas of Lake Geneva.

3.2. Bacterial abundance and distribution

With regard to the OM records, the highest bacteria cell counts were observed after ca. 1970 (0–18 cm) in the sediments deposited close to the outlet pipe of the WWTP (core V3, Table 2 and Fig. 2). The corresponding total number of bacteria (DDC) and heterotrophic bacteria (IBC) counts of bacteria cells abruptly increased after ca. 1970. The DDC counts significantly varied ($P < 0.05$) with time, ranging between 6.4–6.7 $\times 10^8$ cell g^{-1} dry weight before ca. 1970 (18–30 cm) to 8.7–9.7 $\times 10^9$ cell g^{-1} dry weight afterwards. Similarly, The heterotrophic bacteria quantified by IBC count varied between 1.5 $\times 10^6$ cfu g^{-1} dry weights (sediment older than ca. 1970) to 3.8 $\times 10^7$ (sediment younger than ca. 1970). In contrast, no significant variation for DDC counts was observed on the two sediment records from the central parts of the lake (Table 2). Nevertheless, IBC counts in these sites show an increase in bacteria with time, from in order of 10^6 to order of 10^7 cfu g^{-1} dry weight. In all cases, the IBC bacterial densities represent less than 3–5% of total DDC ($P < 0.05$), indicating that the bacterial densities enumerated by DDC count were higher than the number obtained by IBC plate counting.

3.3. Microorganism activity

The microorganism activities measured using ATP tests (RLU) generally increased exceedingly after ca. 1970 for all sampling sites

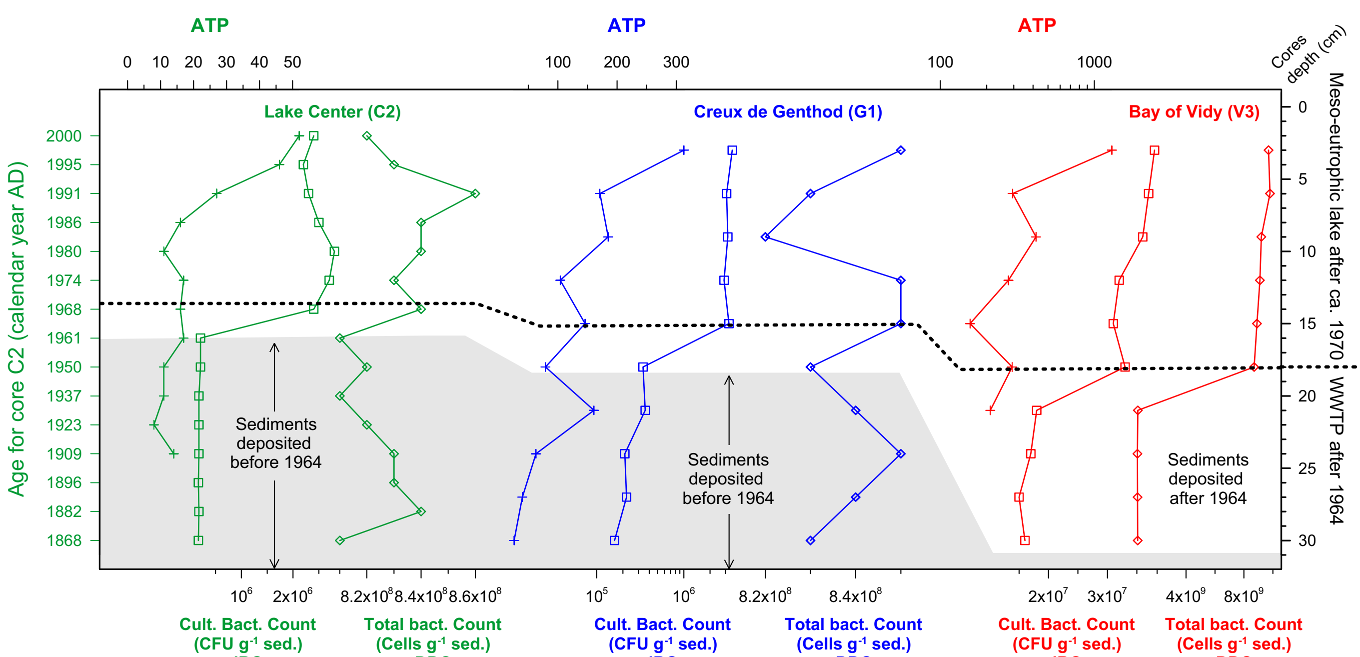

Fig. 2. Adenosine tri-phosphate (ATP), direct bacteria count (DDC; total bacteria (cells g^{-1} dry sediment)) and indirect bacteria count (IBC culturable bacteria count (colony-forming units cfu g^{-1} dry sediment)) for cores C2 (lake centre), G1 (Creux de Genthod), and V3 (Bay of Vidy), expressed as a function of depth in cm (right axis), with the corresponding age (calendar) of core C2 on the left axis. The shaded area highlights the sediment deposited before the WWTP implementation (in 1964).

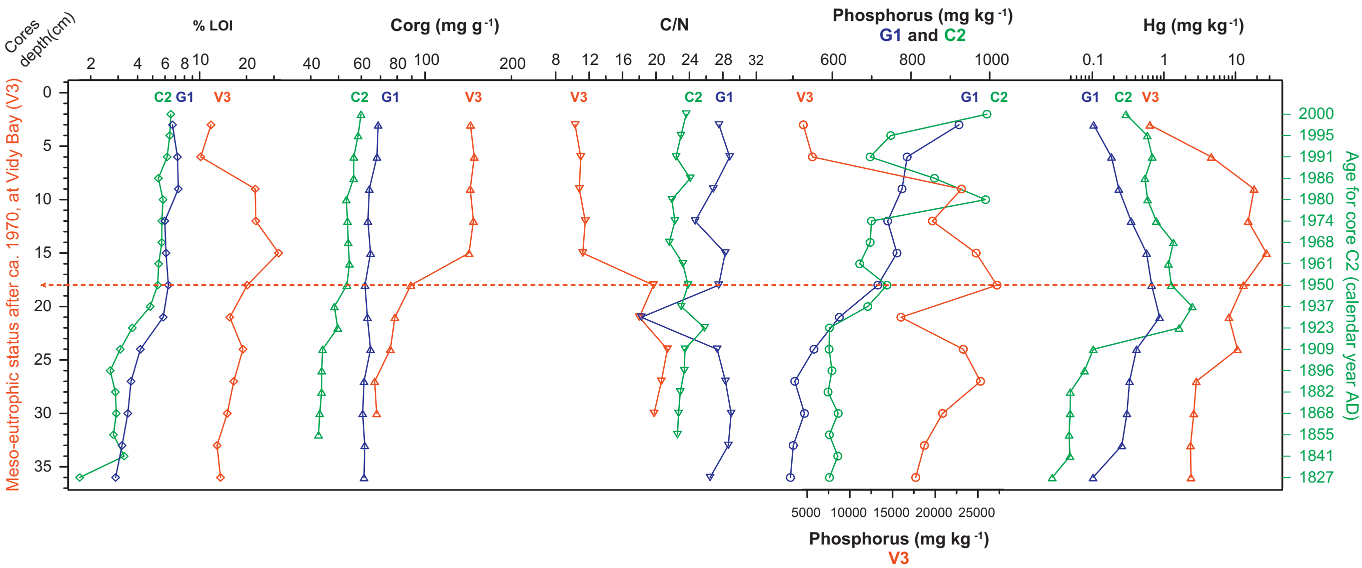

Fig. 3. Loss on ignition (LOI) and organic carbon content (Corg), C/N ratio, phosphorus, and mercury concentrations expressed as a function of depth in cm (left axis), with the corresponding age (calendar) of core C2 on the right axis.

($P < 0.05$). The recent sediment from the Bay of Vidy (core V3) and the Creux de Genthod region (core G1) presented much higher RLU values than those observed in the sediment samples from the lake centre (core C2). The highest values were observed in the upper sediments of core V3, with values increasing from 182 RLU (before ca. 1970) to 1299 (after ca. 1970). In core G1, the values increased from 26 to 313 RLU over the same time period. In core C2, the measured values were much lower but nonetheless increased from 11 to 52 RLU (Table 3 and Fig. 2).

3.4. Mercury (Hg) distribution

The maximum Hg contamination occurred during the second part of the twentieth century, with the highest concentrations of metals recorded between ca. 1850 and 1970 in the remote sites (cores C2 and G1), and between ca. 1970 and 1990 in the Vidy Bay (core V3) (Fig. 3). Afterwards, the anthropogenic Hg input continuously declined, pointing out the efficiency of environmental policies on industrial heavy metal emissions (e.g., implementation of sewage treatment plants). The agreement between the LOI and the Hg profiles on Fig. 3 demonstrated the dominant anthropogenic origin for the organic compounds and trace metals recently deposited into Lake Geneva (Fig. 3). Such a pattern was particularly evident at Vidy Bay (core V3), where the maximum values for all the analyzed parameters were observed after ca. 1970, pointing to the concomitant effect of the WWTP effluent water discharge into the lake during the period of eutrophication. We note that the Hg content in the sediment deposited into the Bay of Vidy during the second part of the twentieth century were about ten times higher than those deposited synchronously into the central parts of the lake (Fig. 3). These former levels were also ten times higher than the preindustrial ones inferred from cores C2 and G1, pointing out the very important Hg contamination of Lake Geneva during the late nineteenth century, due to increasing industrial (and domestic) untreated wastewater releases.

3.5. Spearman Rank Order Correlation between parameters

The results of the Spearman rank (no parametric) product moment correlation are found in Tables 4 and 5 for core G1 and V3, respectively. The parameters used were, organic matter (OM) content, DAPI (4′,6′-diamino-2-phenylindole hypochloride) direct count (DDC), indirect bacterial count (IBC), (RLU), and total mercury (Hg) concentration. Note that in the following description, strongly correlated is indicated by correlation coefficients > 0.8 (bolded values in Tables 4 and 5).

4. Discussion

4.1. Organic matter, mercury and bacteria distribution

In a former study based on the surface distribution of OM from 25 sites of Lake Geneva, Poté et al. (2008) showed that LOI in surface sediments varied from 4% to 8%, except nearby the WWTP

Table 2
Quantification (including standard deviation) of bacteria by DDC (DAPI (4′,6′-diamino-2-phenylindole hypochloride) direct count, cell g^{-1} dry sediments) and by IBC (indirect bacteria count: colony-forming units (cfu g^{-1} dry sediment). Standard deviations are indicated in parentheses.

Depth (cm)	Bay of Vidy (LG V3)		Creux de Genthod (LG G1)		Lake centre (LG C2)	
	DDC	IBC	DDC	IBC	DDC	IBC
0–3	9.7×10^9 (2.5×10^8)	3.8×10^8 (2.3×10^7)	8.5×10^8 (6.7×10^7)	3.6×10^6 (4.1×10^5)	8.2×10^8 (6.2×10^7)	2.4×10^6 (5.3×10^5)
4–6	9.8×10^9 (3.1×10^8)	3.7×10^8 (1.5×10^7)	8.3×10^8 (4.6×10^7)	3.1×10^6 (8.2×10^5)	8.3×10^8 (8.3×10^7)	2.2×10^6 (7.2×10^5)
7–9	9.2×10^9 (1.7×10^8)	3.6×10^7 (3.4×10^6)	8.2×10^8 (2.8×10^7)	3.2×10^6 (9.3×10^5)	8.6×10^8 (4.1×10^7)	2.3×10^6 (6.5×10^5)
10–12	9.1×10^9 (7.2×10^8)	3.2×10^7 (3.2×10^6)	8.5×10^8 (9.3×10^7)	2.9×10^6 (7.4×10^5)	8.4×10^8 (6.3×10^7)	2.5×10^6 (3.9×10^5)
13–15	8.9×10^9 (5.3×10^8)	3.1×10^7 (5.1×10^6)	8.5×10^8 (4.8×10^7)	3.3×10^6 (7.4×10^5)	8.4×10^8 (7.4×10^7)	2.8×10^6 (8.4×10^5)
16–18	8.7×10^8 (6.4×10^7)	3.2×10^7 (2.5×10^6)	8.3×10^8 (1.3×10^7)	3.4×10^5 (7.4×10^4)	8.3×10^8 (8.7×10^7)	2.7×10^5 (7.7×10^4)
19–21	6.7×10^8 (4.3×10^7)	1.8×10^7 (3.7×10^6)	8.4×10^8 (5.3×10^7)	3.6×10^5 (7.4×10^4)	8.4×10^8 (2.9×10^7)	2.4×10^5 (2.8×10^4)
22–24	6.4×10^8 (5.2×10^7)	1.7×10^7 (1.6×10^6)	8.5×10^8 (7.6×10^7)	2.1×10^5 (7.4×10^4)	8.1×10^8 (6.3×10^7)	2.1×10^5 (6.4×10^4)
25–27	6.5×10^8 (5.3×10^7)	1.5×10^7 (2.4×10^6)	8.4×10^8 (6.5×10^7)	2.2×10^5 (7.4×10^4)	8.2×10^8 (2.7×10^7)	2.1×10^5 (4.3×10^4)
28–30	6.6×10^8 (6.2×10^7)	1.5×10^7 (6.1×10^6)	8.3×10^8 (2.4×10^7)	1.6×10^5 (7.4×10^4)	8.1×10^8 (5.1×10^7)	1.8×10^5 (5.6×10^4)

Table 3
Distribution of microorganism activities using ATP tests (in Relative Light Units (RLU)) and mercury (mg kg^{-1} dry matter).

Depth (cm)	Bay of Vidy (LG V3)		Creux de Genthod (LG G1)		Lake centre (LG C2)	
	RLU	Hg	RLU	Hg	RLU	Hg
0–3	1299	1.64	313	0.11	52	0.29
4–6	295	7.60	171	0.19	46	0.58
7–9	418	27.18	185	0.23	27	0.67
10–12	277	15.06	104	0.35	16	0.54
13–15	156	7.02	146	0.57	11	0.59
16–18	293	12.88	79	0.67	17	0.78
19–21	211	8.10	161	0.87	16	1.35
22–24	209	10.65	63	0.41	17	1.16
25–27	176	2.82	40	0.33	11	1.27
28–30	182	2.62	26	0.30	11	2.50

Table 4
Spearman Rank Order Correlation matrix of selected parameters in sediment core G1, at Creux de Genthod.

	OM	DDC	IBC	RLU	Hg
Depth	−**0.903**	−0.0572	−**0.891**	−**0.903**	**0.903**
OM		−0.273	**0.794**	**0.855**	−**0.709**
DDC			0.191	0.0127	−0.356
IBC				**0.891**	−**0.806**
RLU					−**0.673**
Hg					

(n = 10, statistically significant coefficients ($p < 0.05$) are in bold).

Table 5
Spearman Rank Order Correlation matrix of selected parameters in sediment core V3, in the Bay of Vidy.

	OM	DDC	IBC	RLU	Hg
Depth	−**0.830**	−**0.939**	−**0.952**	−**0.770**	−0.115
OM		**0.745**	**0.721**	0.333	0.0303
DDC			**0.915**	**0.721**	0.0303
IBC				**0.867**	0.127
RLU					0.176
Hg					

(n = 10, statistically significant coefficients ($p < 0.05$) are in bold).

outlet discharge. Similar values were observed in the sediment samples from the remote site C2 (3.5–7.2%) and G1 (4.8–6.5%). The mean sedimentation rate over the last 50 years was twice higher in core V3 (~0.6 cm/year) as compared to core C2 (~0.3 cm/year). In fact, the sedimentation rate and the concentration of pollutants in sediments are inversely related to the distance from the shore and to the WWTP outlet pipe discharge location, respectively (V3 > C2 > G1).

The WWTP source for OM deposited in sediment was clearly evidenced at site V3, with organic proxies (LOI and Corg) subsequently increasing after ca. 1970. In particular, the OM varied exceedingly at different times ($P > 0.05$), with the minimum value of 15% observed in sediment deposited before ca. 1970. Accordingly, this value was much higher than those measured in surface sediments at the others sites, confirming (i) the presence of abnormally high levels of pollution during the middle of the twentieth century due do the excessive inputs of wastewater effluents into Vidy Bay, and (ii) that the central parts of the lake were relatively exempt from WWTP effluent water inputs that mostly accumulated in the Bay of Vidy (Wildi et al., 2004; Poté et al., 2008).

The WWTP source for OM deposited into the Bay of Vidy was also evidenced by the C/N ratio, which is a good indicator of organic carbon sources (Meyers, 1994). Before the 1970s, C/N ratios around 20–28 evidenced the predominance of terrestrial inputs for all sites. Indeed, vascular land-plants have C/N ratios of 20 and greater, while phytoplankton, the major source of autochthonous OM in lakes, produces ratios from 4 to 9. However, a major drop in C/N ratio occurred in V3 around 1970, concurrent with a significant rise in OM (LOI, Corg, and P contents on Fig. 3). Relatively similar C/N ratios (~11) have been reported elsewhere for sediments enriched by sewage-derived OM (Sweeney and Kaplan, 1980; Andrews et al., 1998; Ruiz-Fernandez et al., 2003). These results therefore demonstrate the anthropogenic origin of the OM deposited into the Bay of Vidy during the cultural eutrophication period of Lake Geneva, whereas the C/N ratios of remote sediments continued to attest to the predominance of terrestrial inputs, without significant effect of WWTP effluents.

Despite a higher variability, the large values of P measured at Vidy (20–30 times larger than at other sites) also evidenced the anthropogenic contribution to OM deposition. Increases in P contents were inferred after ca. 1925 in the deepest part of the lake (core C2), and especially from the 1970s (Fig. 3), when many freshwater Swiss lakes (e.g. Morat, Greifensee) became eutrophic due to excessive release of P-untreated wastewater and use of phosphate in detergents. In opposition to the P concentrations measured in the water column at the centre of Lake Geneva (CIPEL, 2005), P concentrations at C2 and G1 were seen to increase during the last decades, most probably as a consequence of internal P supplies and a warmer climate effect on lake's stratification.

Our previous study (Poté et al., 2008) showed that only Hg still presents a high concentration in the surface sediments of the Bay of Vidy. This is exemplified by the maximum measured value of 27.18 mg kg^{-1} surround WWTP outlet discharge. Compared to the values of 8.65 mg kg^{-1} in 2005 and 4.28 mg kg^{-1} in 1996, as explained by Poté et al. (2008), the concentration of Hg in the sediment of the Bay of Vidy are more than 20 times higher than the inactive values of the Canadian Sediment Quality Guidelines (CCME EPC-98E, 1999; Beelen, 2003). In comparison with the Mean Probable Effect Concentration Quotients value (MacDonald et al., 2000; Long et al., 2006), the concentration of Hg in the sediments of the Bay of Vidy may therefore represent a significant source of toxicity for sediment dwelling organisms.

The Nycondez gradient centrifugation can be a useful alternative procedure to extract and to quantify purified bacteria cells from complex matrices such as soils and freshwater sediments, especially for environmental matrices enriched in organic matter (McDaniel and Capone, 1985; Courtois et al., 2001; Maron et al., 2006; Poté et al., 2010). In this study, this method has been applied to extract and quantify bacterial cells since the sediments from our sampling sites presented significant changes in organic matter content and micropollutants abundance, which could have biased the quantification of bacteria exposed to different sediments.

In the present study, DDC and IBC counts were applied to assess the vertical depth distribution of bacteria in the sediment. The culturable bacterial densities (IBC) represented less than 5% of total DDC (total cell counts), but followed the same pattern of variation than total cell counts (Fig. 2). Vertical distribution of bacterial abundance in sediments could have varied with different states of the lake, in particular within the eutrophication period that affected Lake Geneva in the 1970s and early 1980s. Some former studies showed that the total bacterial abundance have increased within recent times (e.g. Fisher et al., 2002; Haglund et al., 2003), whereas Wobus et al. (2003) reported no significant influences of sediments depth within the uppermost 5 cm. Interestingly, the data obtained in this study confirmed these results (Table 2 and Fig. 2): The bacterial abundance using both DDC and IBC counts increased with time at site V3 together with a high sedimentary OM content attributed to sustained anthropogenic organic pollutant inputs. For sites G1 and C2, only bacterial abundance by IBC counts strikingly increased with sediment depth, whereas no significant variation was observed on the bacterial abundance using DDC counts over time. Such results therefore strengthened the hypothesis that the heterotrophic bacterial abundance in sediment is strongly correlated with the quality and quantity of OM available (Fisher et al., 2002; Polymenakou et al., 2007). The two case studies presented here, therefore demonstrated the strong interacting effects of sewage treatment plant and cultural (or climate-driven) eutrophication on microbial abundance.

Furthermore, the results of this study confirm previous studies showing that the area around the WWTP outlet pipe in Vidy Bay is heavily contaminated with various organic and inorganic pollutants (Hg). Such data, which contrast with the other sampled sites from deeper and more remote parts of the lake, prove that the organic matter and nutrients released from the municipal WWTP have considerable effects on bacterial abundance and activities in freshwater sediments.

4.2. Bacterial activities

ATP data increased with time for core V3, with the highest values (1299 RLU) recovered in the surface sediments. Although the ATP values increased with time for all the sampled sites, no correlation was found with OM content or with bacterial abundance (Tables 1–3, and Fig. 3). For example, the comparison of the upper sediment samples from cores C2 and V3 exhibited 6.7% and 6.5% of OM, ATP 313 and 52 RLU, and IBC count 8.5×10^8 and 8.2×10^8 cell g^{-1} dry sediments, respectively. As explained above, total bacterial count did not increased with time at these sites, but ATP nonetheless increased from 11 to 52 RLU in core C2.

It has been demonstrated that bacterial activities correlated positively with OM content, but negatively with the total number of bacteria in sediments (e.g. Wobus et al., 2003). It is also known that the nutrient and OM content in wastewater can stimulate the growth and activities of microorganisms in soils and can change the microbial communities and abundance in lake sediments. (Tam, 1998; Nam et al., 2008). The results of the present study concerning core V3 furthermore confirm this hypothesis for sediments exposed to the effects of WWTP effluent waters. In addition, many studies showed that bacterial activity and biomass are generally higher near the sediment surface and decrease with sediment depth (e.g. Haglund et al., 2003); this hypothesis is confirmed by this study in relatively non contaminated sediment deposits (cores C2 and G1).

In addition to the influence of the external source of OM on bacterial activity (WWTP at Vidy), this investigation pointed out the major influence of internal organic supplies related to the lake's eutrophication. In fact, the release of abundant external organic matter involved the deterioration of the water quality from Lake Geneva and anoxic conditions in the 1970s and early 1980s. Our study demonstrated that this change in trophic state had a major impact on polluted environments (core V3), but also on non-polluted environments such as the remote parts of the lake (cores C2 and G1, Fig. 2), which were more depleted in nutrients loading before the cultural eutrophication. Indeed, there was a great rise in IBC at that time in non-polluted environments that contrasted with ATP and DDC profiles. Such result indicated that the heterotrophic bacterial activity was greatly enhanced by the trophic state of the lake and the higher flux of labile OM to the sediments. Therefore, high increase in microbial activity in remote (and coastal) parts of the lake were certainly driven by the regional variation in organic compounds available (P, N, Corg) which have increased with the lake's eutrophication.

Based on the results of this study, we recommend further research focusing on microbial community composition and specific activities of microorganisms inhabiting similar environments. Such an urgent and challenging task will improve our understanding how organic and inorganic pollutions are impacting microbial communities and activities within lake sediments over time.

4.3. Spearman Rank Order Correlation between parameters

With respect to core G1 (Table 4) from Creux de Genthod, it can be seen that there is a strong, mutual and negative correlation between OM, IBC, and RLU with depth. In contrast, Hg has a strong mutual and positive correlation with depth. These findings indicate that with increased time (shallower depth), there is an increase in OM, IBC, and RLU, while there is a decrease in Hg content related to the recent treatment of industrial wastewaters. In addition, there is a strong positive correlation between RLU and OM, and RLU and IBC. These findings indicate that there is an increase in ATP levels as OM and the indirect bacterial count increase. A strong negative correlation was also found between the IBC and Hg, showing that as Hg levels increase, the indirect bacterial count decreases. In addition, there is a negative correlation between both RLU and OM with Hg, along with a positive correlation between IBC and OM. These findings indicate that, at this site (the deepest part of the Small Lake), higher concentrations of Hg are found in the samples with less organic matter and lower ATP levels and, that the indirect bacterial count increases with OM content. This is also substantiated with the correlation with depth where both Hg and OM showed strongly mutual, yet opposite correlations (positive and negative, respectively) with depth. This indicates that older sediment results in less OM and greater levels of Hg at this site. It should be noted that DDC did not correlate significantly with any measured factor at this site.

As for core V3 (Table 5), from the Bay of Vidy, it can be noted that there is a strong negative correlation between depth and the variables OM, DDC, and IBC and a negative correlation between RLU and depth. These findings indicate that with increased depth there is a decrease in OM, DDC, and IBC, with a likely decrease in RLU. There are also strong positive correlations between both DDC and RLU with IBC. These findings indicate that as the direct and indirect bacterial count increase, so does the amount of measureable ATP. These findings are in agreement with the correlations of these factors with depth. In addition, there are positive correlations between OM and DDC, OM and IBC, and RLU and DDC; hence, when an increase in OM, DDC, or IBC is found, there will be most likely, an increase in the other two related parameters (i.e. DDC and IBC with OM, OM and DDC with IBC, and IBC and OM with DDC). These results differ from those found in core G1 in that there are no strong correlations found between Hg and any other parameter and that there are correlations between the DDC and other measured parameters, attesting that the bacterial activity was not dependent of inorganic metal inputs but primarily affected by organic material releases and lake trophic state.

Multiple factor analysis (using Pearson correlation) revealed that the microbial abundance and the environmental variables were correlated in-time in polluted and non-polluted sites, which suggests that in addition to environmental parameters, organic pollution may be one of the factors affecting microbial community structure and activities.

5. Conclusion

This research demonstrates the effects of the WWTP effluent water and changes in lake trophic state on the time distribution of OM, bacterial abundance and activity in freshwater lake sediments of Lake Geneva. The sediments near the WWTP outlet presented very high OM concentrations after the sewage treatment plant implementation in 1964, because of the local release of abundant organic products. This local situation contrasts with the general trend observed the central parts of the lake, showing increasing organic (LOI) and inorganic (Hg) pollutant inputs after the Industrial Revolution of 1850, and decreasing heavy metal accumulation after the improvement of restoration measures such as the implementation of sewage treatment in the second part of the 20th century. Our findings indicate a much higher bacterial abundance and activities in the sediments exposed to WWTP inputs than in the sediments from the remote deep lake environments. Therefore, one can conclude that the external organic matter inputs considerably influence bacterial abundance and activity in sediments. One important finding of this study is that microbial abundance and activities do not necessarily decrease with sediment depth, since the quality and quantity of OM highly varied throughout the past, along external (e.g., weathering processes, anthropogenic inputs) or internal (e.g., primary productivity, redox conditions) factors that strongly influenced the presence and activities of microorganisms within freshwater sediment time-profile.

We present here the first study regarding the time distribution of sediment organic matter content, bacterial abundance and ATP assays in the contaminated and uncontaminated sediments of the Bay of Vidy, a part of Lake Geneva, Switzerland. The results of this study will serve as a reference point for future research in polluted aquatic areas affected by wastewater inputs and by warmer climate impact on oxygen depletion in the bottom waters. However, given the long history of pollution in the Bay of Vidy, more research on microbial community composition and specific microorganism activities should be performed, in order to improve the understanding of how organic pollution and eutrophication may affect microbial communities and activities. Such an approach will help to apprehend the problem of pollution in Vidy Bay and to guide future decisions on the improvement of the lake water quality.

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