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Accumulation of Clinically Relevant Antibiotic-Resistance Genes, Bacterial Load, and Metals in Freshwater Lake Sediments in Central Europe

Naresh Devarajan,[†] Amandine Laffite,[†] Neil D. Graham,[†] Maria Meijer,[†] Kandasamy Prabakar,[‡] Josué I. Mubedi,[§] Vicky Elongo,[∥] Pius T. Mpiana,[⊥] Bastiaan Willem Ibelings,[†] Walter Wildi,[†] and John Poté*[,†,§,⊥]

[†]University of Geneva, Faculty of Sciences, Earth and Environmental Sciences, Institute F. A. Forel and Institute of Environmental Sciences, Case Postale 416, 1290 Versoix, Switzerland

[‡]Postgraduate and Research Department of Zoology, Jamal Mohamed College, Tiruchirappalli, 620020 Tamil Nadu, India

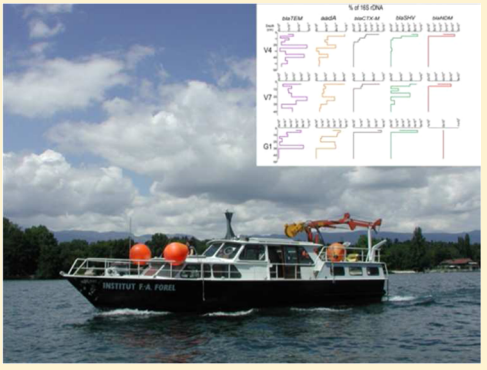
[§]Université Pédagogique Nationale, Croisement Route de Matadi et Avenue de la Libération, Quartier Binza/UPN, Boîte Postale 8815, Kinshasa, République Démocratique du Congo

[∥]Université de Kinshasa, Faculté des Lettres et Sciences Humaines, Département des Sciences de l'Information et de la Communication, Boîte Postale 243, Kinshasa XI, République Démocratique du Congo

[⊥]University of Kinshasa, Faculty of Science, Department of Chemistry, Boîte Postale 190, Kinshasa XI, Democratic Republic of the Congo

Supporting Information

ABSTRACT: Wastewater treatment plants (WWTP) receive the effluents from various sources (communities, industrial, and hospital effluents) and are recognized as reservoir for antibiotic-resistance genes (ARGs) that are associated with clinical pathogens. The aquatic environment is considered a hot-spot for horizontal gene transfer, and lake sediments offer the opportunity for reconstructing the pollution history and evaluating the impacts. In this context, variation with depth and time of the total bacterial load, the abundance of faecal indicator bacteria (FIB; *E. coli* and *Enterococcus* spp. (ENT)), *Pseudomonas* spp., and ARGs (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{NDM}, and *aadA*) were quantified in sediment profiles of different parts of Lake Geneva using quantitative PCR. The abundance of bacterial marker genes was identified in sediments contaminated by WWTP following eutrophication of the lake. Additionally, ARGs, including the extended-spectrum ß-lactam- and aminoglycoside-resistance genes, were identified in the surface sediments. The ARG and FIB abundance strongly correlated ($r \geq 0.403$, $p < 0.05$, $n = 34$) with organic matter and metal concentrations in the sediments, indicating a common and contemporary source of contamination. The contamination of sediments by untreated or partially treated effluent water can affect the quality of ecosystem. Therefore, the reduction of contaminants from the source is recommended for further improvement of water quality.



INTRODUCTION

The question of the environmental and human risks of an increasing release of bacteria carrying antibiotic-resistance genes (ARGs) into the natural environment has been a subject of intense scientific and political debate over the past decade. The high concentration of antibiotics (Abs) found in water, sediments, and soils can be the consequence of shifts in the original use of Abs in hospitals and farms for treating or protecting against bacterial infection.[1] The use of a wide variety of antimicrobials in human and veterinary medicine, including aquaculture, has led to the widespread emergence of antibiotic-resistant pathogens.[2] The waste water treatment plant (WWTP), which receives the mixture of effluent from hospitals, communities, and animal farming as well as industrial and agricultural runoff, is considered a major source of antibiotic-resistant bacteria (ARB) and ARGs for the aquatic environment and a hot-spot for horizontal gene transfer (HGT).[2−5] The pathogenic bacteria are able to acquire the resistant genes from the environment and environmental bacteria through gene-transfer mechanisms and could represent a potential threat to human and animal health.[6] ß-lactams are the most consumed Abs globally, and about 1000 ß-lactamase enzymes have been identified, conferring resistance to several ß-lactams. This

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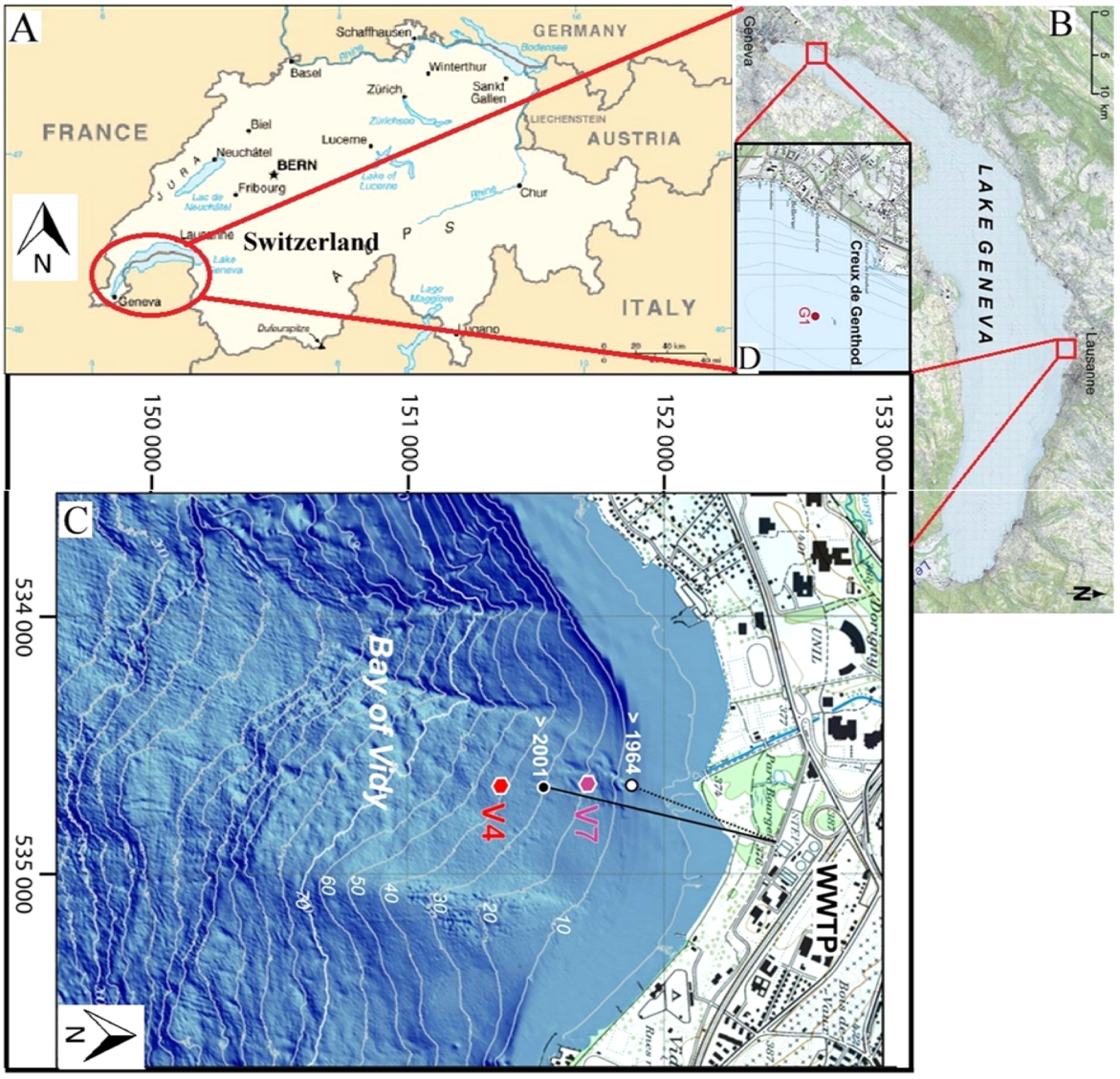

Figure 1. (A) Location of Lake Geneva at the border of France and Switzerland. (B) Location map of Lake Geneva with the cities of Lausanne and Geneva. (C) Vidy Bay and the position of cores V4 (red dot) and V7 (pink dot) and the 1964 (white circle) and 2001 (black circle) WWTP outlet pipe locations. (D) Creux de Genthod region (control site) with the location of core G1.

indicates the rapid evolution of bacterial resistance to ß-lactams in soil and aquatic environments.[3,7,8]

Freshwater sources (rivers and lakes) are a major public resource but also are typically the final destination of treated and untreated effluent water. WWTPs were not originally designed to have a specific impact on removal of resistant bacteria or antimicrobial residues, and little is known of the effects of WWTPs on microbial contaminants.[5,9]

Lake Geneva is a temperate lake with a surface area of 580.1 km^2, a volume of 89 km^3, and a maximum depth of 309.7 m. The lake provides drinking water but also receives the wastewater from urban developments. The lake was eutrophic during the 1970s, but after reduction of phosphorus inputs during the 1980s, the lake has become mesotrophic.[10] The largest WWTP is located in the city of Lausanne, and it releases its effluent into Vidy Bay. From 1964 to 2001, the WWTP effluents were released into the lake about 300 m from the shore, at a water depth of 15 m. In 2001, the WWTP outlet pipe was extended by the authorities to a distance of 700 m from the shore at a 35 m water depth (Figure 1). The reason for and the effect of the new and old outlet pipes are well-described in Poté et al.[11] The release of effluents from this WWTP has made Vidy Bay a heavily polluted site.[11] Long-term monitoring data are available on the composition and distribution of microbial and aquatic biota, including fecal indicator bacteria (FIB), as well as on the amount and distribution of organic matter, metals, micropollutants, and hydrophobic organic compounds in the lake.[11−16] In recent years, the surface sediments of the lake were studied for the presence of some ARGs (*sul1*, *sul2*, *tet*(*B*), *tet*(*M*), *tet*(*W*), and *qnrA*) and ARB.[5,9,17,18] However, there is a paucity of information on the spatiotemporal variation in the distribution of ARGs (ß-lactam- and aminoglycoside-resistance genes) in the lake sediment.

To our knowledge, very little data are available on the concentration and periodic accumulation of ß-lactam-resistance genes in the lake sediments. Yet, the assessment of ARGs and their persistence overtime are critical for devising and evaluating strategies to mitigate ARG propagation.[5,19] ARB and ARGs are ubiquitous in nature and can occur in high

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concentrations in industrial, community, clinical, and farming wastewaters that are being released into freshwater ecosystems.[5,9,20] Studying the sediments allows us to address the persistence of ARGs and the potential impact of the emergence of resistant bacterial strains from WWTP to freshwater sediment microbes. Thus, the sediments offer a unique opportunity for reconstructing the pollution history and evaluating the impacts using quantitative data.

This research has been performed in Lake Geneva and has the following purposes: (i) to evaluate the sediment quality through physicochemical parameters including total oganic matter (OM) content, particle grain-size distribution, and metal concentrations (Cr, Mn, Fe, Co, Ni, Cu, Zn, Ag, Cd, Pb, and Hg), and (ii) to quantify the FIB (*E. coli* and *Enterococcus* (ENT)), *Pseudomonas* spp. (P.spp), and ARGs (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{NDM}, and *aadA*) in sediment profiles. The data were statistically analyzed and correlated in order to determine whether the WWTP effluent waters can affect the distribution of ARGs, FIB, and toxic metals in sediments and to interpret the time origin of pollution as well as the potential impact on lake water quality.

MATERIALS AND METHODS

Study Site and Sampling Procedure. Vidy Bay is located at the northern shore of Lake Geneva at the city of Lausanne and accounts for the 0.3% of the total lake volume.[21] Approximately 1−3 $m^3\ s^{-1}$ of urban wastewater is treated by this WWTP in Lausanne from ca. 214 000 inhabitants, including wastewater from several healthcare centers. The Centre Hospitalier Universitaire Vaudois (CHUV) is one of the largest and the most important facilities in terms of capacity and Abs consumption.[17,18,22] The main building accounts for 71% of the CHUV sewage outputs. On an average day, ca. 4.7 × 10^{-3} $m^3\ s^{-1}$ of raw sewage is disposed from this building to the municipal sewer system of Lausanne.[22] The WWTP receives on average 107 734 $m^3\ day^{-1}$ of wastewater and discharges 86 631 $m^3\ day^{-1}$ of treated sewage (typically 1−3 $m^3\ s^{-1}$ and up to 5−6 $m^3\ s^{-1}$) directly into Vidy Bay.[23] The other sources of contaminants to Vidy Bay include the Chamberonne River from its natural drainage basin and inputs of some untreated wastewater from damaged urban collectors.[14,21] The other major input, the River Flon, also collects surface and wastewater from the western part of the city, which is usually treated at the WWTP but released into the lake at a 10 m depth during floods when the input to the WWTP exceeds 4−5 $m^3\ s^{-1}$. One of the drinking water supplies for the city of Lausanne is lake water, taken at St-Sulpice, a pumping station located just 3.8 km downstream from the WWTP outlet. It provides 58% of drinking water for the city of Lausanne at an average rate of 385 L s^{-1} and taken from a 45 m depth. This water pumping station meets the water needs of 127 000 inhabitants (approximately) of the city of Lausanne.[11,17,23]

The boat *La Licorne* of the Institute F. A. Forel was used for the collection of core sediments from Lake Geneva in May 2014 at the following locations: (i) in Vidy Bay near the present WWTP outlet-pipe discharge (core V4, 60 m water depth, 55 cm in length, Swiss coordinates X = 534 682, Y = 151 410), (ii) in between the two outlet pipes (core V7, 35 m water depth, 40 cm length, Swiss coordinates X = 534 426, Y = 151 512), and (iii) in the Creux de Genthod area at a 51 m water depth (core G1, 45 cm length, Swiss coordinates X = 502 565, Y = 123 544). The Creux-de-Genthod area of Lake Geneva is a coastal area, but on the basis of the study by Thevenon et al.,[5] this area could be considered as WWTP-pollution-free. After collection, the cores were opened and sliced into 3 cm thick sections for the first 15 cm and into 5 cm thick sections for the remainder of the core. For chemical analysis, the sediment samples were frozen, freeze-dried, and ground into a fine powder. For the DNA extraction, the samples were stored in sterile plastic cups at 4 °C until used.

Sediment Grain Size and Total Organic Matter and Water Content. The particle grain size was measured with a Laser Coulter LS-100 diffractometer (Beckman Coulter, USA) following the method described by Loizeau et al.[24] The sediment total OM content was estimated by the loss on ignition at 550 °C for 1 h in a Salvis oven (Salvis AG, Switzerland).

Metal Analysis. Sediment samples were lyophilized at −45 °C after homogenization and air-drying at ambient temperature. The metals, including Cr, Co, Ni, Cu, Zn, As, Cd, and Pb, were determined by quadripole-based inductively coupled plasma mass spectrometry (ICP-MS, model 7700 series, Agilent, USA) following the method described previously.[11,15] Total variation coefficients of triplicate sample measurements were smaller than 10%, and chemical blanks for the procedure were less than 2% of the sample signal. The metal concentrations of sediments were expressed in ppm (mg kg^{-1} dry weight sediment). The total Hg analysis was quantified with an atomic absorption spectrophotometer (AAS; AMA 254, Altec s.r.l., Czech Republic) following the method described by Ross-Barraclough et al.[25] The detection limit (3 SD blank) was 0.005 mg kg^{-1}, and the reproducibility was better than 95%.

Total DNA Extraction. Total DNA from sediment samples was extracted using Ultraclean Soil DNA Kit (Mo Bio Laboratories, USA) by following manufacturer's instructions. The isolated DNA was stored at −20 °C until used. DNA extraction was performed with two replicate samples (from the same section of the sediment core) to compensate for heterogeneity. The DNA samples were diluted to 10× and 50× to avoid inhibitors to the PCR reaction.

Positive Control Plasmid Construction. The positive control target genes (*E. coli*, *uidA* gene; ENT and P.spp., 16S rRNA) were amplified from *E. coli* ATCC25922, *E. faecalis* ATCC29212, and *P. aeruginosa* ATCC27853 and ARGs (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{NDM}, and *aadA*) from well-characterized laboratory strains available at Institute F. A. Forel, Universtiy of Geneva, using the primers described in Table 2s (Supporting Information). The PCR products were purified using Genelute Gel extraction kit (Sigma-Aldrich, USA), and cloned into the pGEM-T vector (Promega, USA) according to the manufacturer's instructions. Briefly, 4 μL of each ligated product was transformed into chemically competent *E. coli* DHα cells. Plasmids from selected transformants were purified by Sigma-Aldrich Genelute HP plasmid extraction kit (Sigma-Aldrich, USA). The inserted PCR products in each plasmid were sequenced (GenBank numbers: KP172294−KP172300) for confirmation, and the concentration of plasmids was measured using Qubit assay kit (Life Technologies, Switzerland) following the manufacturer's instructions and used as standards for subsequent quantitative PCR (qPCR) reactions.

qPCR Quantification of FIB and ARGs in Sediments. The abundance of FIB and ARGs in sediments samples collected from Lake Geneva was quantified with an Eco qPCR system (Illumina, Switzerland) using KAPA SYBR FAST qPCR Master Mix Universal Kit (KAPA Biosystems, USA). The

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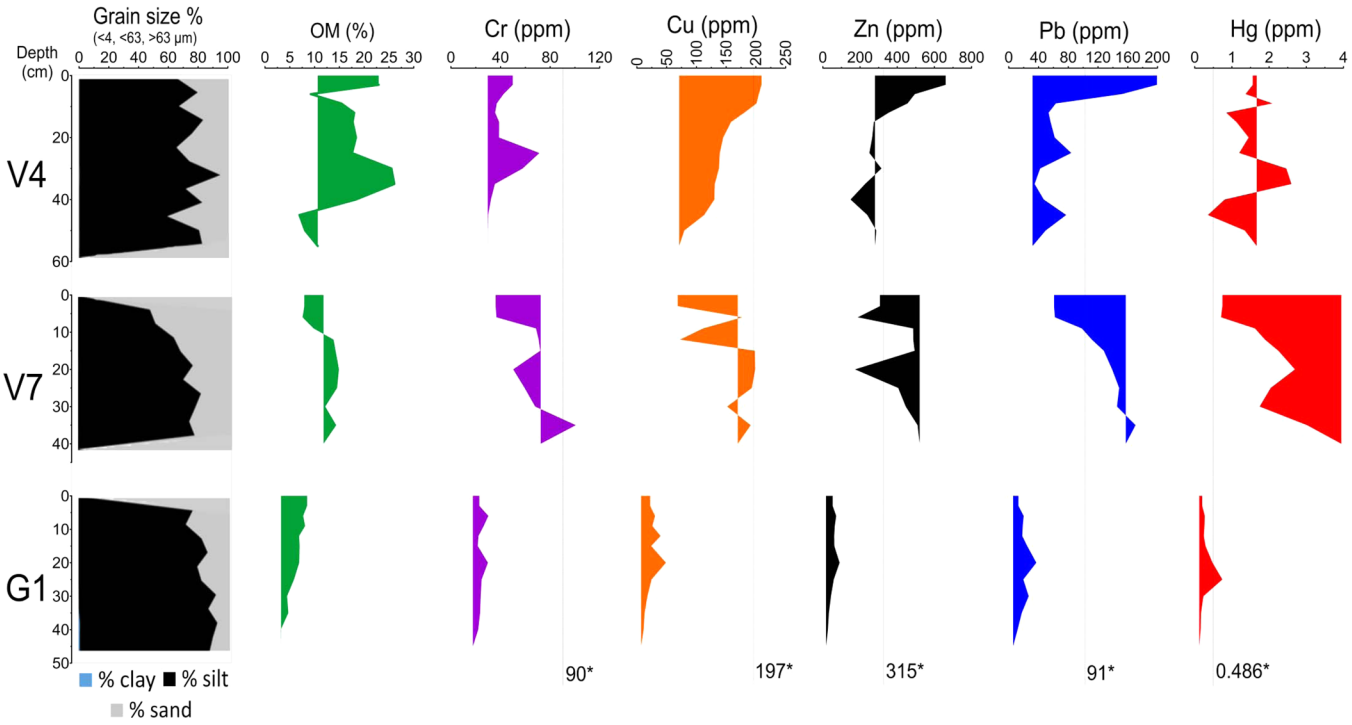

Figure 2. Graphical representation of physical chemical parameters of the sediment cores including grain size, total OM content, and Cr, Cu, Zn, Pb, and Hg concentrations. *, metal values above the recommended PEL (probable effect level) values as recommended by CSQG.

primer sequences and reaction conditions are provided in Table 2s (Supporting Information). The following cycling parameters were applied: 10 min at 95 °C for the polymerase activation; followed by 40 cycles of 95 °C for 30 s, optimal T_m (Table 2s, Supporting Information) for 30 s, and 72 °C for 30 s. The melting-temperature-curve profile was obtained using the following conditions: 95 °C for 30 s, optimal T_m (Table 2s, Supporting Information) for 30 s, followed by 95 °C for 30 s.

All the reactions included negative (with no template DNA) and positive controls (10-fold serial dilutions of pGEM-T plasmid with respective target gene insert). All negative controls resulted in either no amplification or a threshold cycle (C_t) higher than the most diluted standard (pGEM-T plasmid). A sample was considered to be below the limit of detection (LOD) or negative for a target gene if ≥2 out of 3 technical replicates were negative or if sample C_t values were ≥C_t of negative controls. Samples above LOD were considered to be below the limit of quantification when the standard deviation of C_t values of methodological triplicates was 40.5 and their C_t value was higher than the C_t of the most diluted standard whose standard deviation of C_t values was ≤0.5. For each reaction, the efficiency of the assay was calculated by using the measured slope of the standard curve ($E = 10^{(-1/\text{slope})} - 1$). The absolute copy number of each reaction was quantified by referring to the corresponding standard curve obtained by plotting the copy number of the constructed pGEM-T plasmid versus threshold cycles. The serial 10-fold dilutions of plasmid DNA containing the respective target gene copies were used for the standard curve. To emphasize the relative abundance of the resistance genes, the concentrations of the gene copy numbers were presented as percentage of the ratio = (copy number of a gene)/(copy number of 16S rDNA) for each sample.

Data Analysis. The target genes were normalized to sediment sample dry weight, and we refer this as "gene concentration" from here on. The gene copy numbers were normalized to 16S rDNA copy numbers and are reported as percentages as an indicator of relative abundance to bacterial/ARGs population. We refer to this as the "ARGs/bacterial 16S rDNA abundance" hence forth. For all measured parameters, triplicate measurements were performed on selected samples. The data was subjected to a Spearman Rank Correlation test to investigate possible relationships using SigmaStat 11.0 (Systat Software, Inc., USA). The data was subsequently subjected to a principle component analysis (PCA) using a correlation matrix performed with R Statistical Software, version 3.1.2 (The R core team) and the ade4 package.[26] The correlation matrix form of PCA was used because the measured parameters had different scales of measurement. The correlation-matrix-based PCA normalizes each measured parameter allowing for direct comparison. The average of the qPCR efficiencies calculated from the slopes of standard curves for each assay and the representation of derivative melt curve analysis are presented in Figure 7s (Supporting Information). Previous studies have demonstrated that the copy number of 16S rDNA per bacterial genome can vary among bacteria,[27] but in environmental samples, its quantification has previously been used to estimate the overall bacterial abundance and to normalize selected genes to the total bacterial population.[18,28]

RESULTS AND DISCUSSION

Grain Size, Organic Matter, and Water Content. The grain size distribution, total OM, and the water content are given in Table 1s (Supporting Information) and Figure 2. The sediments collected at all three sampling sites have a silty−sand composition with less than 1% of clay. The values of silt and sand ranged between 58.8−94.2/46.2−78.8/70.5−91.0 and 5.5−41.2/21.1−53.7/7.8−29.5 for the V4, V7, and G1 sites, respectively. Previous studies showed that total OM in the surrounding sediments varied between 4−8%, except for the sampling sites closer to the WWTP outlet.[11,16] Similar results

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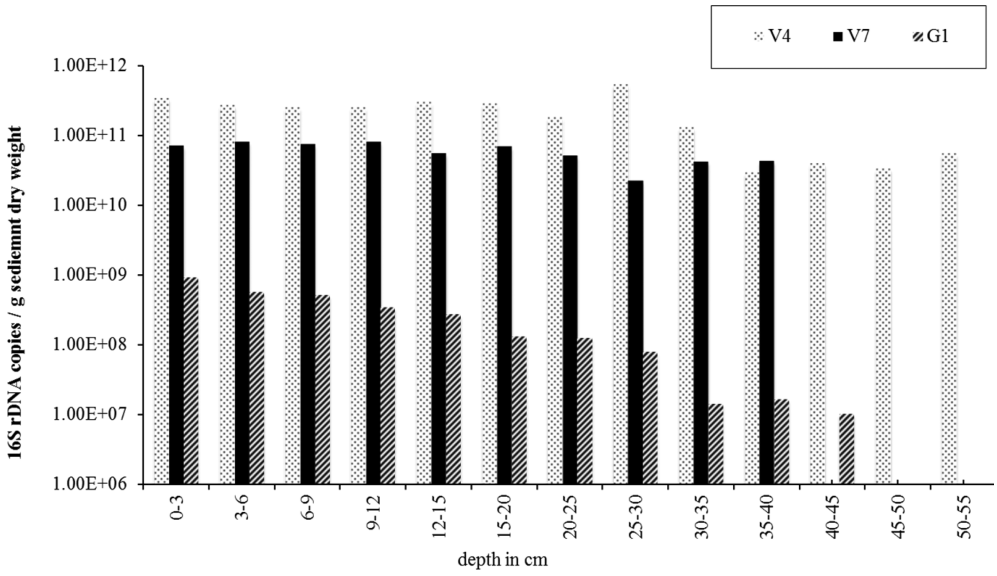

Figure 3. qPCR quantification of 16S rDNA in the sediment core samples at different depths. Values are expressed on a log scale for the copy number per gram of sediment dry weight normalized to the DNA extraction yield.

were also observed in this study with the organic matter values ranging between 3.1−8.5% at control site (G1) and 6.9−26.1% at WWTP outlet site (V4). At this site, the organic matter content showed a similar pattern of variation with the lowest concentrations for the bottom part of the sediment core (preindustrial period) increasing values after the beginning of the WWTP discharge (in 1964) and values that tripled at a 40−45 cm depth during the eutrophication period.[5] The mean grain size of sediment particles in the upper part of core V4 ranged from 17.7−46.7 μm, which presents a slight decrease to 25.2−46.7 μm down from 50 cm; this difference can be linked to the installation of the WWTP in 1964.[5,14] However, the increase in grain size from the bottom of the cores to the surface (from 26 to 71 μm and from 14.4 to 40.7 μm) and the increase in sand (from 21 to 53% and from 1.8 to 40.7%) in cores from V7 and G1, respectively, indicates the coarser sediments (high-energy environment caused by wave action) and the source contributions (the Chamberonne River delivers coarse sediments close to its mouth, V7 sample).[21] The water content along the core samples depended on the total OM content because we observed an increase in water content with an increase in organic matter along the core samples depth.

Metal Analysis. The values of metals are presented in Table 3s (Supporting Information) and Figure 2 (Cr, Cu, Zn, Pb, and Hg), ranked according to sampled core depth. The metal values (mg kg^{-1}) range between 17−100 (Cr), 155−319 (Mn), 7127−21130 (Fe), 3.2−6.2 (Co), 18.4−33.5 (Ni), 7−209.5 (Cu), 17−660.7 (Zn), 0.05−4.4 (Ag), 0.07−3.4 (Cd), 5.8−199.2 (Pb), and 0.12−3.9 (Hg). Deeper sediments were observed to have higher concentrations of metals at the V7 site compared to the surface of the core. The same is true for core V4, with the exception of its very surface layer having a greater concentration of metals. The concentrations of metals at the G1 site were compared to natural background values reported by Arbouille et al.[12] All the metals, except Hg, were comparable to natural background values, indicating that the accumulation of metals at the control site is a natural process. This observation indicates that the WWTP is a primary input source of metals to Vidy Bay. After the extension of the WWTP outlet pipe to the V4 site, the contaminants have started to accumulate more in the surface sediments of this area to concentrations that now exceed those at the bottom of the sediment core, corresponding to the time period before extension of the WWTP outlet. An evaluation of the potential deleterious effects of the metals toward benthic fauna, applied on consensus-based guidelines for sediment quality,[29] gives an estimate of the potential hazard these sediment may represent to the biota. The metal concentrations were primarily compared to the Canadian Sediment Quality Guidelines (CSQG) for the Protection of Aquatic Life,[30] and Cr, Cu, Zn, Pb, and Hg at V4 and V7 were always found to be above the CSQG guidelines. The values of the metals were one (Cr, Cu), two (Zn, Pb), and eight (Hg) times greater than the probable effect levels (PEL) recommended by the CSQG.[30] The pattern and amplitude of Hg accumulation has changed since the prolongation of the WWTP outlet pipe, and the surface area of strongly contaminated sediments has decreased from 1.3 to 0.8 km^2.[11] Previous studies have shown higher Hg concentrations in the sediments of Vidy Bay, with maximum values of 27.18 mg kg^{-1} surrounding the WWTP outlet pipe,[11,16] in comparison with historical Hg values measured in Vidy Bay, which range from 4.28 mg kg^{-1} in 1996 to [16] 8.64 mg kg^{-1} in 2005,[11] and 1.56 mg kg^{-1} (this study) in 2014. Hence, a reduction in Hg deposition with time is evident. However, the deeper, older sediments of Vidy Bay remain contaminated with high Hg concentrations (V7 at 40 cm = 3.9 mg kg^{-1}). The fine sediment deposits, with higher water content, form bars and embankments, which are unstable and subject to erosion via waves, currents, movement by gravity, and anthropogenic activities.[11,12] According to these parameters, it is evident that the sediments of Vidy Bay are heavily contaminated with metals and represent a significant potential threat to the ecosystem.

Quantification of Bacterial Population. The average gene copy numbers of the bacterial marker genes in the sediment samples are presented in Figure 8s (Supporting Information) for *E. coli*, ENT, and P. spp. The total bacterial load (on the basis of 16S rDNA gene copy numbers) in the sediment samples (Figure 3) were in the range of 1.01×10^7−5.5×10^{11} copy numbers per g^{-1} dry sediment. The bacterial density varied: 4.4×10^4−1.8×10^{10}, 7.6×10^3−1.1×10^{10}, and

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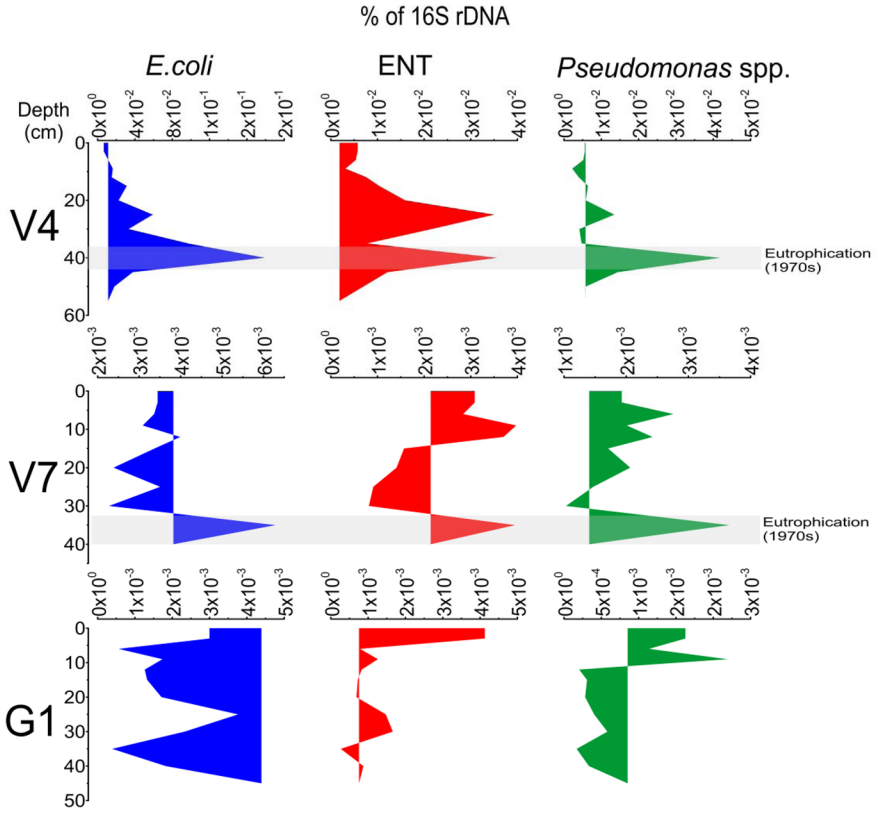

Figure 4. qPCR quantification of the bacterial population for selected groups (*E. coli*, ENT, and P.spp.) at different depths in the core sample. Values are expressed as the percentage of 16S rDNA bacterial population.

8.6×10^3–2.4×10^9 for *E. coli*, ENT, and P. spp., respectively. We repeat that in order to avoid inconsistencies among qPCR assays, including suboptimal efficiency, we use 16S-rDNA-normalized values (Figure 4). The highest abundance of bacterial populations was recorded in the core samples at the 40 and 35 cm depths of the V4 and V7 cores, respectively. This observation indicates that the bacterial load increased dramatically in the sediments during the 1970s in Vidy Bay, probably due to both eutrophication of the lake and the signature of the WWTP.[5] The vertical distribution of the *E. coli*, ENT, and P. spp. in the V4 and V7 cores shows the influence of the WWTP after 1964 and supports the impact of the eutrophication that influenced the increase the bacterial load in the sediments of Vidy Bay. *E. coli* and ENT are common indicators of pathogens in aquatic environments and can persist in the aquatic environment but preferably accumulate in the sediment rather than remaining in the watercolumn.[13,14] Also, bacteria can survive longer in sediments, which provide favorable conditions for growth and protection from sunlight inactivation and protozoan grazing.[31] Environments outside the gastrointestinal tract of warm-blooded animals (such as water, sediments, and soils) have previously been shown to represent a secondary habitat for FIB.[32] Therefore, it can be hypothesized that sediments can act as reservoirs of metabolically active FIB. According to the metal analysis and the organic matter content data, it is evident that the G1 sampling site reflects the natural background values for Lake Geneva. In a comparison between the control site and the WWTP influenced site (V4) at the surface layer, there is a 720-, 700-, and 1080-fold increase in the bacterial load for the studied *E. coli*, ENT, and P. spp. bacterial markers, respectively. Hence, it is evident that the contaminated sediments of Vidy Bay constitute a reserve of bacterial populations that persist in certain areas of the bay. Resuspension of these sediments could affect water quality and could pose a potential health risk to humans and aquatic life.

Quantification of Antibiotic-Resistance Genes. The ARGs conferring resistance to ß-lactam (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, and bla_{NDM}) and aminoglycoside (*aadA*) that were selected for the qPCR study were based on the following criteria: (i) clinically relevant genes (human risk); (ii) genes conferring resistance to frequently used Abs (penicillins and aminoglycosides) and newer extended-spectrum ß-lactams (carbapenems and cephalosporins); (iii) ARGs previously reported in mobile genetic elements; and (iv) ARGs not previously studied in Vidy Bay.[5,17,18]

The relative abundance of ARGs in the sediment core samples are presented in Figure 5. In the cores, bla_{TEM} and *aadA* genes were detected at all the sampling sites with relative abundances ranging from 2.6×10^{-5} to 2.8×10^{-3} and from 1.5×10^{-5} to 1.1×10^{-3}, respectively. These genes have previously been found to be abundant in sewage and effluent-receiving systems.[5] This enriched pattern of *aadA* and bla_{TEM} genes may be related to the long-term clinical usage of penicillin and aminoglycosides. The high abundance of bla_{TEM} genes at both the control and contaminated sites could be explained by their ubiquitous presence as housekeeping genes, which has previously been shown to occur frequently among soil bacteria.[8] The relative abundance of $bla_{CTX\text{-}M}$ and bla_{SHV} genes ranged from 3.2×10^{-6} to 1.7×10^{-3} and from 1.4×10^{-6} to 8.9×10^{-4}, respectively, and these genes were detected primarily in the top layers of the core samples (15–20 cm depth). The relative abundance of the bla_{NDM} gene ranged between 6.07×10^{-6} and 1.2×10^{-6} copy numbers and was

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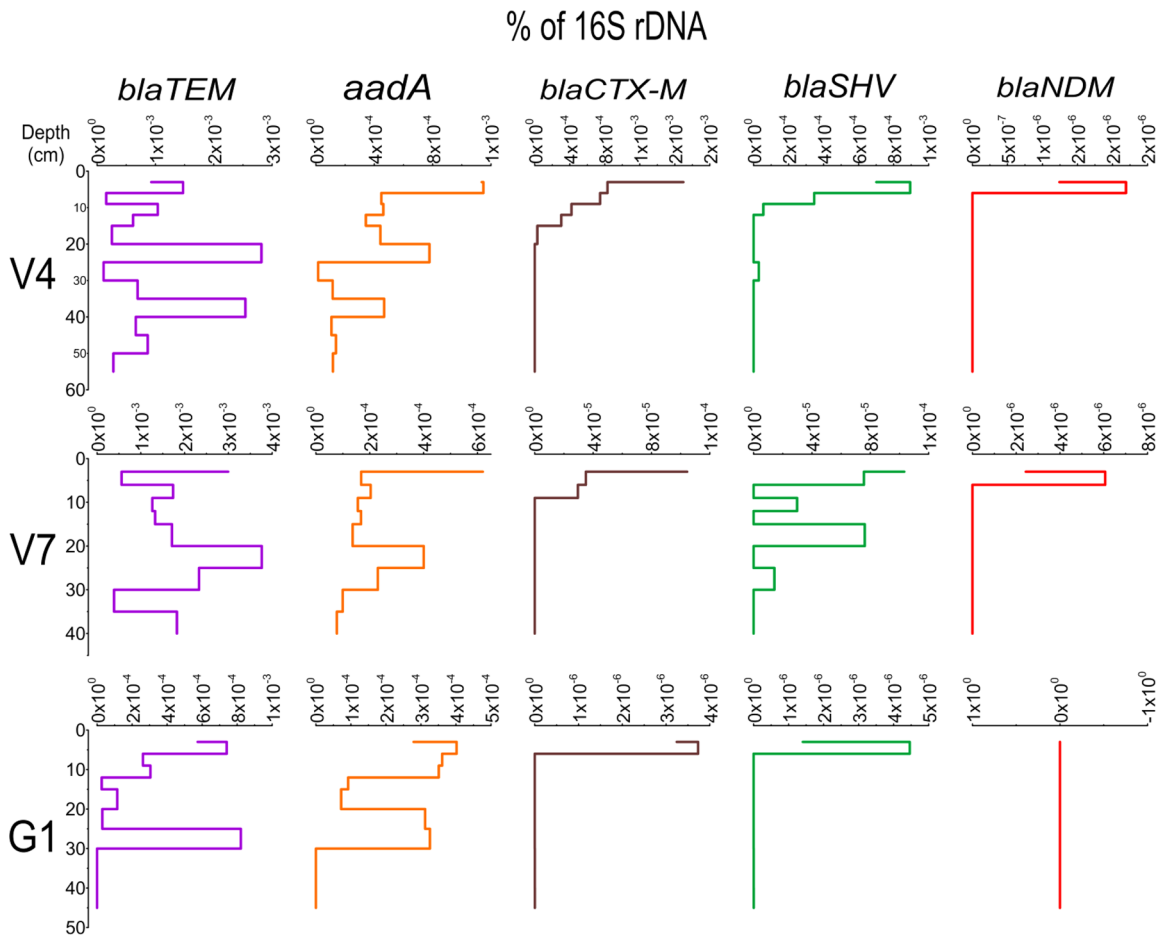

Figure 5. qPCR quantification of the selected antibiotic resistance genes (bla_{TEM}, *aadA*, bla_{CTX-M}, bla_{SHV}, and bla_{NDM}) along the length of the sediment core, expressed as a percentage of 16S rDNA bacterial population.

identified only in the surface layer of the core samples (V4 and V7). The relative abundance of extended-spectrum ß-lactamases (ESBLs) was higher in sediment samples collected at the vicinity of WWTP-outlet-pipe discharge than at the control site. Likewise, elevated abundance of ARGs was observed in the surface layers of the V4 core, indicating the recent increase in ESBLs in Vidy Bay. The relative abundances of the studied ARGs provides a possibility to compare data between ARGs quantified in various other WWTPs.[18,28] According to Lartigue et al.,[33] the spread of CTX-M-type ESBL producers in the community of acquired *E. coli* infections in Switzerland, in a manner similar to that which is observed in other countries, highlights the difficulties in controlling these resistance genes and their carriers in the environment. The persistence of ESBLs in the sediments of freshwater systems, as found in our study, could pose a further potential threat to the humans and aquatic life.

Statistical Correlations. Total organic matter content, metal concentrations (with the exception of Mn, Co, and Ni), bacterial-indicator genetic markers, and ARGs were, for the most-part, significantly, mutually, and positively correlated (Table 4s, Supporting Information). Strong negative correlations were found among depth, grain size, total bacterial load, and the relative abundance of bacterial indicators and ARGs ($r \geq -0.1310$, $p < 0.05$, $n = 34$). Likewise, there were strong positive correlations among total OM, metal concentrations, and bacterial-load/bacterial-indicator genetic markers/ARGs ($r \geq 0.403$ were observed, $p < 0.05$, $n = 34$). This implies that contaminants likely originate from a common source and were transported and deposited by a common carrier to the receiving lake ecosystem. The relationship between the contaminant content and the grain size is not as strong as would be expected from an existing general transport-and-deposition model of hydrophobic pollutants.[34] This supports the notion that contaminants, attached to both large organic and small inorganic particles, behave in a similar manner with respect to their mobility and sedimentation. A recent study in Vidy Bay has demonstrated that organic and inorganic particles at the sediment−water interface of Vidy Bay appeared to be unstable and play a role in the transport of contaminants over long distances.[35]

Inspection of Figure 6 shows the contaminated sites (V4 and V7) to be oppositely and separately clustered to the control site on separate axes of the first-principle component, substantiating the high prevalence of metals, bacterial indicators, and ARGs at sites V4 and V7 as compared to the control site, G1. Figure 6A shows the relationship between the eigenvectors of each variable measured. Here, the segregation between metals is apparent because Co, Ni, and Mn are separated from the other metals analyzed. Also, grain size is noted to be segregated from both groups of metals and is directly opposed to the Co, Ni, and Mn eigenvectors along the second-principle component. This infers that as the grain size increases, the concentrations of Ni, Mn, and Co tend to decrease, whereas the other measured parameters (metals, bacterial marker genes, and ARGs) will increase with the total OM content at Vidy Bay.

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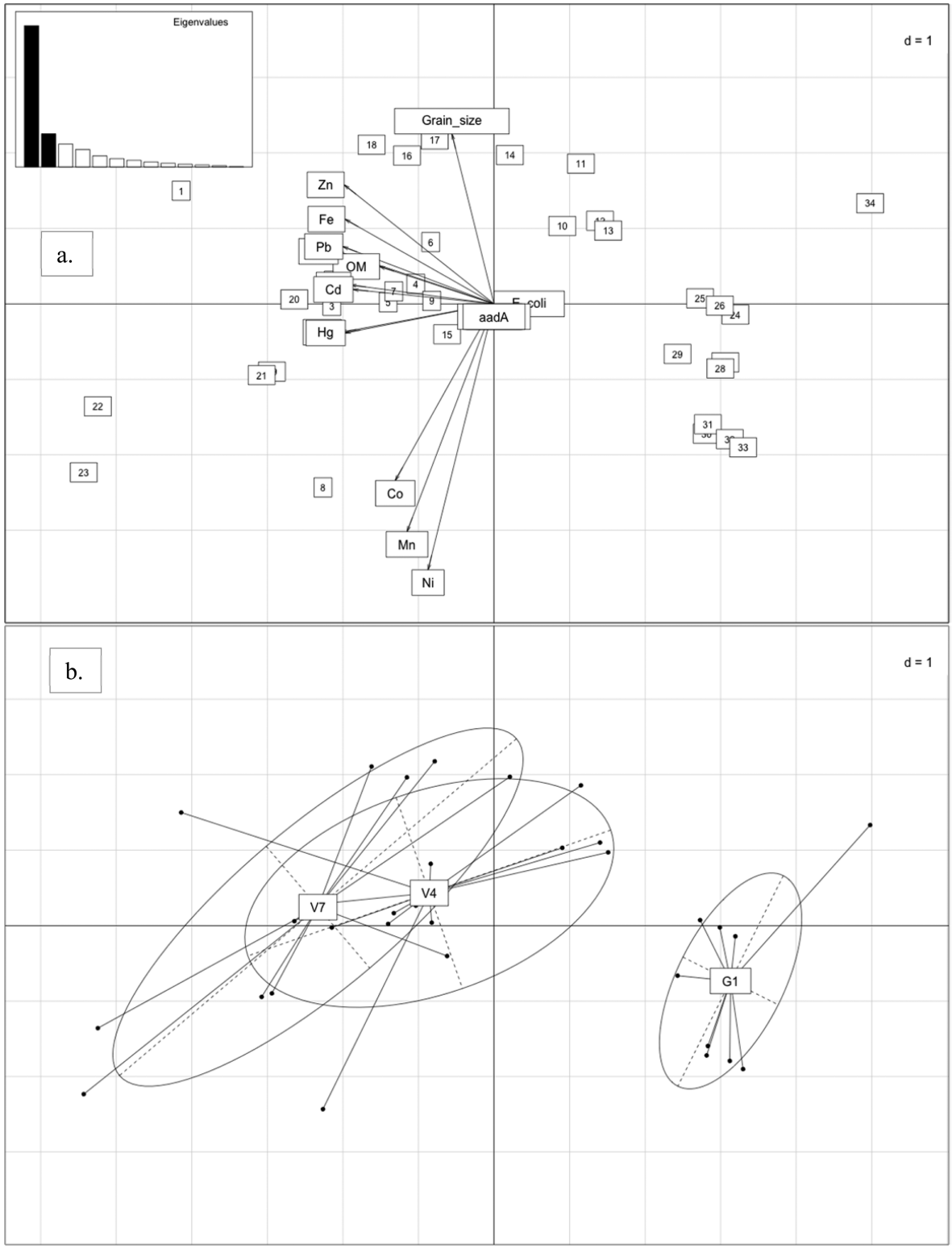

Figure 6. Scatter plot analysis on the eigenvectors/values for the studied parameters. Principle component is the *x* axis, with the second component being the *y* axis. Right (top) side is positive and the left (bottom) is negative. (a) Scatter of the PCA results. (b) Classification on the basis of the sampling site.

Factors of ARGs Contamination. In the natural ecosystem, the selective pressures to which bacteria are exposed may favor the emergence and spread of ARB. The discharge of Abs and ARB into the aquatic environment could favor the spread of ARB/ARGs to nonresistant bacterial communities through HGT.[36] A study by Zuccato et al.[37] reported an average of 352.5 g of Abs per 1000 inhabitants per year being discharged to the receiving lake by the WWTP of Lugano (located in Switzerland 300 km away from our study site). The study also averaged the penicillin discharge at 37 mg per 1000 inhabitants per day in the WWTP effluent. The total antibiotic consumption in Switzerland increased between 2004 and 2008,

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and Switzerland has an antibiotic usage close to the European mean.[38]

It is well-known that metals can coselect for ARGs because the mechanism often favors resistance to both and the genes are located on the same mobile elements.[39] The physiochemical results demonstrate a high contamination in the sediments of Vidy Bay with the metal concentrations being in excess of the natural background values. The correlation matrix (Table 4s, Supporting Information) shows the positive correlation values found among the bacterial indicators, ARGs, and the studied metals (expect Mn, Co, and Ni) with coefficient values ranging from 0.475 to 0.923. With positive correlation values and $p < 0.05$, one could argue that the bacterial load tends to increase with an increase in metal levels, and this coselection could be a relevant factor influencing the selection of ARGs in the sediment of Vidy Bay. However, the physicochemical sediment properties could also influence the accumulation and play an important role in the selection of ARGs. Sediments with a high content of fine particles (clay and silt) can bind DNA and protect it from degradation, aiding its transport to water-saturated soils and groundwater.[40] This is also suggested from the correlation between the total OM and ARGs with correlation coefficients ranging between 0.574 and 0.846. Sorbed to flocs, suspended solids, and/or activated sludge, Abs are partially removed from WWTPs. Using this sludge as fertilizers could favor the selection of antibiotic resistance with the release of antibiotic-laden sludges to the environment.[41]

In this study, we investigated the accumulation of total OM, metals, abundance of bacterial population, and ARGs in sediment profiles from two different parts of Lake Geneva. The elevated levels of metals in Vidy Bay indicate the negative effects of effluent releases to the lake that would pose a threat to the ecosystem. Our study also supports the hypothesis that the natural environment (i.e., sediments) can act as a reservoir for ARGs, and as such, changes to the ecosystem may support the emergence of unknown resistances in the bacterial community.[1,42] The identification of *aadA* and bla_{TEM} genes in the sediment layers deposited before the start of the twentieth century also supports the fact that ARGs have been an emerging contaminant in the aquatic ecosystem for more than a century.[5] It implies that these genes were carried by bacteria as structural integrated reservoir genes even before invention of ß lactams.[7,20] According to European directive 271/1991/EC,[43] Switzerland and other European countries monitor the treatment efficiency of WWTPs by implementing selected water quality measures. These parameters, however, are not designed to estimate threats associated with the spread of Abs/ARGs/ARB. In Switzerland, many of these WWTPs have been considered for, or are already undergoing, modernization. It is important to consider instituting advanced protocols for ARB/ARG removal from effluents when planning this modernization.[44] Given the level of complexity, the evaluation of ARB/ARG removal or selection requires more intensive case studies.[5,18] It has been demonstrated that the contamination of surface as well as deep sediments by untreated or partially treated effluent water can potentially affect the water quality.[21,35] Therefore, the reduction of contaminants released to the receiving water system is highly recommended for further improvement of the water quality.

■ ASSOCIATED CONTENT

Supporting Information

Physical sediment parameters, primers used in this study, metal concentration in sediment cores, Spearman Rank Order correlation matrix between parameters, qPCR efficiencies, and the total gene copy numbers. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b01031.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +41 22 379 03 21. Fax: + 41 22 379 03 29. E-mail: john.pote@unige.ch.

Notes

The authors declare no competing financial interest.

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