



# Comparative geochemical evaluation of toxic metals pollution and bacterial communities of industrial effluent tributary and a receiving estuary in Nigeria<sup>☆</sup>

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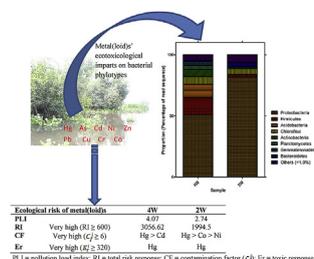
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## HIGHLIGHTS

- Two ecosystems in Lagos, Nigeria were polluted with Cd and Hg from human activities.
- Mercury posed very high ecological risks on the estuary (2W) and its tributary (4W).
- Proteobacteria, Firmicutes, and Acidobacteria predominated both ecosystems.
- 755 OTUs in tributary but absent in estuary shape metal (loid)s' bioremediation.
- 2938 OTUs missing in 4W but found in 2W indicated natural attenuation in 2W

## GRAPHICAL ABSTRACT



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## ABSTRACT

Toxic metals/metalloid contaminations of estuarine sediments due to compromised tributaries arouse significant interest in studying bacterial community that triggers natural attenuation processes. Geo-accumulation index ( $I_{geo}$ ), contamination factor (CF), pollution load index (PLI), and Hakanson potential ecological risk index (RI) as a sum of risk factors (Er) were used to quantify toxic metal/metalloid-pollution status of Lagos Lagoon (2W) and 'Iya-Alaro' tributary (4W) sediments in comparison with pristine 'Lekki Conservation Centre' sediment (L1-B). Bacteriology of the ecosystems was based on culture-independent analyses using pyrosequencing. 2W and 4W were extremely contaminated with mercury ( $I_{geo} > 7$ ), whereas, cadmium contamination was only observed in 4W. The two ecosystems were polluted with toxic metal based on PLI, where mercury (Er = 2900 and 1900 for 4W and 2W, respectively) posed very high ecological risks. Molecular fingerprinting revealed that Proteobacteria, Firmicutes, and Acidobacteria predominately contributed the 20 most abundant genera in the two ecosystems. The 240 and 310 species present in 2W and 4W, respectively, but absent in L1-B, thrive under the metal concentrations in the polluted hydrosphere. Whereas, the 58,000 species missing in 2W and 4W but

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found in L1-B would serve as indicators for systems impacted with metal eco-toxicity. Despite toxic metal pollution of the ecosystems understudied, bacterial communities play vital roles in self-recovery processes occurring in the hydrosphere.

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## 1. Introduction

The problem of environmental pollution is escalating everyday due to natural sources and anthropogenic activities emanating from increase in huge population, industrialization and urbanization (AMAP/UNEP, 2013; ATSDR, 2008). Amid notorious environmental stressors associated with wastewaters from industrial processes are metals and metalloids, which are released into estuarine environment unnoticed in low concentration but large quantities. Nevertheless, wastewaters from metalliferous processes do introduce high concentrations of metals and metalloids in moderate quantities into the environment. The threat of metal/metalloid pollution to public health and wildlife due to compromised estuarine/ocean health has led to an increased interest in developing systems that can remove or neutralize its toxic effects in estuaries (Valls and de Lorenzo, 2002). Some bacteria have evolved mechanisms enabling them to cope with high concentrations of toxic metals and metalloids in the ecosystem, where the toxicants serve as either electron donors or electron acceptors (Valls and de Lorenzo, 2002). Intrinsically, metal- and metalloid-resistant bacteria play an important role in controlling the speciation and bioavailability of metals and metalloids, participating in their cycling and ultimate applications in bioremediation strategies (Oyetibo et al., 2017a, 2017b; 2013).

In natural, unperturbed ecosystems, normal oscillations in diversity and abundance of microorganisms occur as a function of environmental vagaries (Pinto et al., 2015). Alterations of environmental conditions upon discharge of pollutants, mounts pressure on the ecosystem and thus select microorganisms with specific traits for adaptation to the polluted system. However, it is easy to imagine that the elevated concentrations of heavy metals (HMs) and metalloids in the environment could further alter the subsisting microbial processes (Hemme et al., 2010) through both their long-lasting presence and toxic potential (Desai et al., 2009; Patel et al., 2015; Turpeinen et al., 2004; Vishnivetskaya et al., 2011). The influence of the toxic metals and metalloids may or may not be additive, and it can decrease the genetic population of the microbiome (Desai et al., 2010; Jomini et al., 2015), altering the ecological balance (Desai et al., 2009). The interference of HMs and metalloids with microbiological processes lead to drastic changes to the abundance and diversity of microbial populations, and thus affect the health of estuarine systems, those of autochthonous organisms therein, and the public health in general (Pinto et al., 2015). However, functional or structural bacterial diversity are considered as efficient bio-indicators of estuarine quality (Desai et al., 2009; Jones et al., 2009) because they respond faster and are more sensitive to subtle environmental changes than higher organisms.

There have been many studies on microbial community of a number of polluted environments across the globe using culture-independent methods (Barbato et al., 2016; Desai et al., 2009; Margesin et al., 2011; Obi et al., 2016; Patel et al., 2015). Such studies have been without considering the direct impact of toxic metals and metalloids. There is a significant interest in investigating contaminating HMs and metalloids along with community of bacteria present in a HM/metalloid-polluted estuary and its main attendant tributary. A priori to ecotoxicological impact of HMs and

metalloids is emergence of tolerant bacterial communities and their possible roles via functional genes redundancy in the natural attenuation processes. A posteriori biocatalysis of such bacterial community driven attenuation processes would lead to eco-friendly bioremediation campaign of the polluted matrixes. This study, therefore, aims at assessing the diversity and abundance of bacterial community in the sediments of a major industrial wastewater tributary, and the estuary that receives the wastewater in correlation with the toxic metals and metalloids dynamics in the environments. We shall integrate geochemical and culture-independent (SSU rRNA gene analysis) bacteriology, to observe major differences in the bacterial community structure between the tributary and the estuarine sediments and postulate tolerant strains that may drive natural attenuation phenomena in the hydrosphere.

## 2. Materials and methods

### 2.1. Study sites description and sampling

Lagos in Nigeria, with its adjoining conurbation, is the largest city in Africa and one of the fastest growing in the world. It comprises four islands separated by creeks of estuaries that finally empty their contents into the Atlantic Ocean. Among the creeks is Lagos Lagoon (2W) whose major tributary is 'Iya-Alaro' (4W) that daily receives several thousand litres of industrial wastewaters. The pristine environment used in this study is 'Lekki Conservation Centre' (L1-B) of the Nigerian Conservation Foundation, which is dedicated to nature conservation in Nigeria. The coordinates of the sampling locations were N6° 27' 56.898" E3° 22' 55.801" (2W), N6° 34' 47.413" E3° 22' 23.815" (4W) and N6° 26' 13.982" E3° 32' 9.232" (L1-B). Water and sediment (0–5 cm depth) samples (triplicate sampling at each location) were collected, using 10 composite sampling technique. Sediment and filtered-water (using No. 1 Whatman filters) samples were immediately and aseptically stored in airtight sterile bags at –20 °C for subsequent analyses.

### 2.2. Geochemical and physical parameters analyses

Physico-geochemical parameters of the samples were determined by standard methods earlier described (AOAC, 1990; Eaton et al., 1995; Nelson and Sommers, 1982). Parameters determined *in situ* were pH and temperature, while *ex situ* assays include moisture content, texture, total organic carbon (TOC), total organic matter (TOM), nitrate, phosphate, sulphate, chlorides, total solids, total dissolved solids (TDS), cation-exchange capacity (CEC), acidity, alkalinity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) as applicable. Nine HMs including Pb, Cu, Zn, Cd, Co, Ni, and Cr, and a metalloid (As) were quantified via inductively coupled plasma mass spectrometry (ICP-MS) (ELAN 9000, PerkinElmer SCIEX, Boston, MA, USA) after dry sample (0.1 g) digestion with HNO<sub>3</sub>/HCl (4:1, v/v) in a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria). Multiple standard solutions prepared in 3% nitric acid (Manufactured under UL ISO 9001 Quality system XSTC-331, SPEX CertiPrep; Metuchen, NJ, USA) were used for the calibration and an internal standard of indium, <sup>115</sup>In, was used at a

final concentration ( $10 \mu\text{g kg}^{-1}$  in 3% nitric acid solution) for the quantification. Total Hg was determined directly without any pre-treatment using a fully automated thermal vaporization mercury analysis system, Mercury/MA-3000 (Nippon Instrument Corp., Osaka, Japan). The atomic absorbance of the atomized Hg was measured at a wavelength of 253.7 nm. The instrument was calibrated with standard Hg solution (BDH, Leicestershire, England) at concentrations ranging  $0.1\text{--}100 \text{ mg L}^{-1}$ .

### 2.3. Community DNA isolation and purification

Total community genomic DNA (cgDNA) was extracted from the sediment (0.3 g dry weight) using FastDNA<sup>®</sup> Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer instructions. To remove interference of humic substances in the sediment DNA with polymerase chain reactions (PCR), 40 mg of skim milk per gram of sediment sample was added to a lysing matrix (Takada and Matsumoto, 2005). A FastPrep<sup>®</sup> Cell Disruptor FP120 (Qbiogene, Heidelberg, Germany) was operated at 6.5 speed for 30 s to achieve a harsh cell wall disruption of the sediments. Successful extraction of cgDNA was checked with 0.8% (w/v) agarose gel electrophoresis and visualized in UV light upon staining with ethidium bromide.

### 2.4. Pyrosequencing of bacterial 16S rRNA genes

The amplification of the V1 to V3 regions of the prokaryotic 16S rRNA gene from cgDNA using the barcoded primers 9F and 541R as described elsewhere (Suh et al., 2015), library preparations, and pyrosequencing by using a 454 GS FLX Titanium Sequencing System (Roche, Branford, CT, USA) according to the manufacturer's instructions were conducted at ChunLab Inc., Seoul National University, Seoul, Korea. Processing of pyrosequencing data, and detection of chimeric sequences were as earlier reported (Suh et al., 2015). Bioinformatics analyses of pyrosequencing data were performed using CLcommunity<sup>™</sup> software package (ChunLab Inc., Seoul, Korea) following manufacturer's instructions. Taxonomic assignment was carried out by comparing the sequence reads against the EzTaxon-e database (<http://eztaxon-e.ezbiocloud.net/>), using a combination of the initial BLAST-based searches and additional pairwise similarity comparisons according to Nguyen et al. (2016). The 454 pyrosequencing reads obtained in this study have been deposited in the DDBJ's short read archive database under accession number **PRJDB4999**.

### 2.5. Statistical analyses

Means and standard errors of the means (SEM) of triplicate experimental datasets were performed using the Prism 5 software program (GraphPad Software, San Diego, CA, USA). The estimated coverage of the constructed 16S rRNA gene libraries was calculated as  $C = \left(1 - \frac{n}{N}\right) \times 100$  according to Kemp and Aller (2004), where  $n$  is the number of Singletons after assembly, and  $N$  is the total number of sequences in the initial dataset. Richness and diversity statistics of the bacterial community including abundance-based coverage estimator ( $S_{ACE}$ ), the bias-corrected Chao1 ( $S_{Chao1}$ ) and the Shannon-Weaver diversity index were estimated using pre-calculated program of CLcommunity<sup>™</sup> software package. All statistical tests were considered significant at  $p < 0.05$ .

## 3. Results

### 3.1. Geochemistry and pollution status of the environments

Virtually all the geochemical parameters determined for 2W and

4W samples exceeded the limit recommended by Federal Ministry of Environment, Nigeria (Table 1), unlike those of L1-B sample that were all below the recommended limit (Table S1) and thus used as geochemical background ( $B_n$ ) values of HMs and metalloid. Notably, 4W contained fewer suspended solids than the 2W, but astronomically higher COD ( $550 \text{ mg L}^{-1}$ ) and BOD ( $360 \text{ mg L}^{-1}$ ) were obtained in the waters of 4W as against  $65 \text{ mg L}^{-1}$  and  $32 \text{ mg L}^{-1}$ , respectively, recorded for the 2W. The pollution indicators for toxic metals and metalloid in the two environments are shown in Table 1. The indices include geo-accumulation index ( $I_{geo}$ ), contamination factor (CF), pollution load index (PLI), and Hakanson potential ecological risk index (RI) (Hakanson, 1980).

The  $I_{geo}$  is defined as:  $I_{geo} = \log_2 \left( \frac{C_n}{K \times B_n} \right)$ , where  $C_n$  is the concentration of metal  $n$  and  $B_n$  is as indicated above. The factor  $K$  is the background matrix correction factor due to lithospheric effects, which is usually defined as 1.5 according to Muller (1969). It was observed that the 2W and 4W were extremely contaminated with Hg ( $I_{geo} > 7$ ) as presented in Table 1 (see Table S2 for  $I_{geo}$  rankings), but there was no contamination with As, Cu, Pb, and Cr. However, moderate to heavy contamination with Cd, and moderate contaminations with Zn, Ni, and Co were observed in the sediment of 4W. On the contrary, 2W sediment was practically uncontaminated with Cd, but moderate to heavy contamination of Ni and Co were observed. The PLI is defined as the  $n$ th root of the multiplications of the  $CF_{metals}$ :  $PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_n)^{\frac{1}{n}}$ , where  $CF = \frac{C_n}{B_n}$ ,  $C_n$  and  $B_n$  are as earlier described,  $n$  represents number of HMs and metalloid, and  $CF$  is the contamination factor. Although, 4W ( $PLI = 4.1$ ) was more polluted than 2W ( $PLI = 2.7$ ), 2W and 4W were both polluted with metals.

Hakanson potential ecological risk index (RI) is defined as:  $RI = \sum_m E_r^i$ , where  $E_r^i = T_r^i \cdot CF$ ;  $CF$  is as earlier explained for each HM/metalloid, while  $T_r^i$  denotes toxic-response factor for a given substance (see Table S2). Summation of all risk factors for HMs and metalloid in sediments is RI. The relationships between evaluation indices, the degree of pollution and potential ecological risk are shown in Table 1. The contamination index expressed as  $CF$  ( $C_f^i$ ) for 4W ranged from 0.74 to 580, while it was 0.59–380 in 2W sediment. Thus, five HMs were found contaminating 4W sediment in the order  $\text{Hg} > \text{Cd} > \text{Ni} > \text{Zn} > \text{Co}$ , unlike in the estuarine (2W) sediment that had only three HMs contaminating the ecosystem in the order of  $\text{Hg} > \text{Co} > \text{Ni}$ . Mercury posed the highest risk to the two polluted ecosystems, where an average ecological risk of 580 was obtained in 4W sediment. Consequently, the potential ecological risks of the contaminating toxic metals revealed that Hg ( $E_r = 2900$  and 1900 for 4W and 2W, respectively) pose very high risk in both ecosystems, while considerable Cd ( $E_r = 110$ ) toxicity was only observed in 4W sediment, unlike low potential ecological toxicities of other metals observed in the two ecosystems. Overall, the two ecosystems are under very high ecological risk due to HMs and metalloid contamination since  $RI > 600$  (Table 1).

### 3.2. Bacterial taxonomy

After quality filtering, trimming, and removal of all chimeric reads of pyrosequencing, a total of 87,000, 5100 and 3400 reads were obtained from L1-B, 2W and 4W, respectively, and their respective taxonomic assignments created by the EzTaxon-e database are presented in Table S3. The composition and relative abundance of bacteria among the samples clearly varied at phylum level as depicted in Fig. 1. Twelve identified phyla composed those taxa that have  $\geq 1\%$  sequence reads in L1-B, unlike in 2W where Acidobacteria, Actinobacteria, and Gemmatimonadetes were apparently included in the reads whose relative abundance were less than 1%. Moreover, Verrucomicrobia, Nitrospirae, Spirochaetes and Chlorobi had relative abundance less than 1% of the taxonomic

**Table 1**

Geochemistry of environmental samples and Degree of sediment contamination with heavy metals and metalloid in industrial wastewater tributary (4W) and the receiving estuarine environment (2W) based on pollution indices and potential ecological risk factors.

Parameter	4W				2W			
	Water		Sediment		Water		Sediment	
Texture	ND		Sandy clayey		ND		Silty clayey	
Moisture content (%)	ND		4.1		ND		7.1	
pH	7.0		6.6		5.1		6.5	
Total organic carbon (TOC) (%)	ND		4.6		ND		6.2	
NO <sub>3</sub> <sup>-</sup> -N	2.3 <sup>a</sup>		44 <sup>b</sup>		1.9 <sup>a</sup>		86 <sup>b</sup>	
PO <sub>4</sub> <sup>3-</sup> -P	0.1 <sup>a</sup>		45 <sup>b</sup>		0.0		52 <sup>b</sup>	
Total solids (mg L <sup>-1</sup> )	760		ND		12,000		ND	
Total dissolved solids (mg L <sup>-1</sup> )	200		ND		6100		ND	
Acidity (mg L <sup>-1</sup> )	24		ND		14		ND	
Alkalinity (mg L <sup>-1</sup> )	3.1		ND		BDL		ND	
Chemical oxygen demand (COD; mg L <sup>-1</sup> )	550		ND		65		ND	
Biochemical oxygen demand (BOD; mg L <sup>-1</sup> )	360		ND		32		ND	
Metal (loid)s	4W				2W			
Pollution index	MC	I <sub>geo</sub>	PI	Er	MC	I <sub>geo</sub>	PI	Er
Hg	140	8.6	580	2900	95	8.0	380	1900
Cd	8.6	2.5	8.6	110	0.59	-1.4	0.59	7.2
As	11	-1.0	0.74	7.5	16	-0.48	1.1	11
Cu	69	-0.12	1.4	6.9	41	-0.86	0.83	4.1
Pb	89	-0.23	1.3	6.4	44	-1.3	0.62	3.1
Cr	77	-0.82	0.85	2.7	94	-0.52	1.1	3.3
Zn	680	1.4	3.9	8.6	250	-0.05	1.5	3.3
Ni	200	1.4	3.9	20	330	2.1	6.3	32
Co	7.5	1.3	3.8	19	15	2.3	7.4	37
Ecological risk								
PLI		4.1			2.7			
RI		3100			2000			
CF	Very high (RI ≥ 600)	Hg > Cd			Hg > Co > Ni			
	Considerable (3 ≤ C <sub>f</sub> <sup>i</sup> < 6)	Ni > Zn > Co			–			
	Moderate (1 ≤ C <sub>f</sub> <sup>i</sup> < 3)	Cu > Pb			Zn > Cr > As			
	Low (C <sub>f</sub> <sup>i</sup> < 1)	Cr > As			Cu > Pb > Cd			
Er	Very high (E <sub>f</sub> <sup>i</sup> ≥ 320)	Hg			Hg			
	High (320 > E <sub>f</sub> <sup>i</sup> ≥ 160)	–			–			
	Considerable (160 > E <sub>f</sub> <sup>i</sup> ≥ 80)	Cd			–			
	Moderate (80 > E <sub>f</sub> <sup>i</sup> ≥ 40)	–			–			
	Low (E <sub>f</sub> <sup>i</sup> < 40)	Ni > Co > Zn > As > Cu > Pb > Cr			Co > Ni > As > Cd > Cu > Cr > Pb > Zn			

All values represent mean of triplicate analyses.

4W = 'Iya-Alaro' Creek (tributary of industrial wastewater); 2W = Lagos Lagoon, an estuary where contents of 4W empties; I<sub>geo</sub> = geo-accumulation; CF = contamination factor (C<sub>f</sub><sup>i</sup>); PI = contamination index; Er = toxic response; PLI = pollution load index; RI = total risk response; ND = not determined; BDL = below detection level; MC = measured concentrations of metals in sediments (mg kgdw<sup>-1</sup>).

<sup>a</sup> Unit of NO<sub>3</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P in water sample is mg L<sup>-1</sup>.

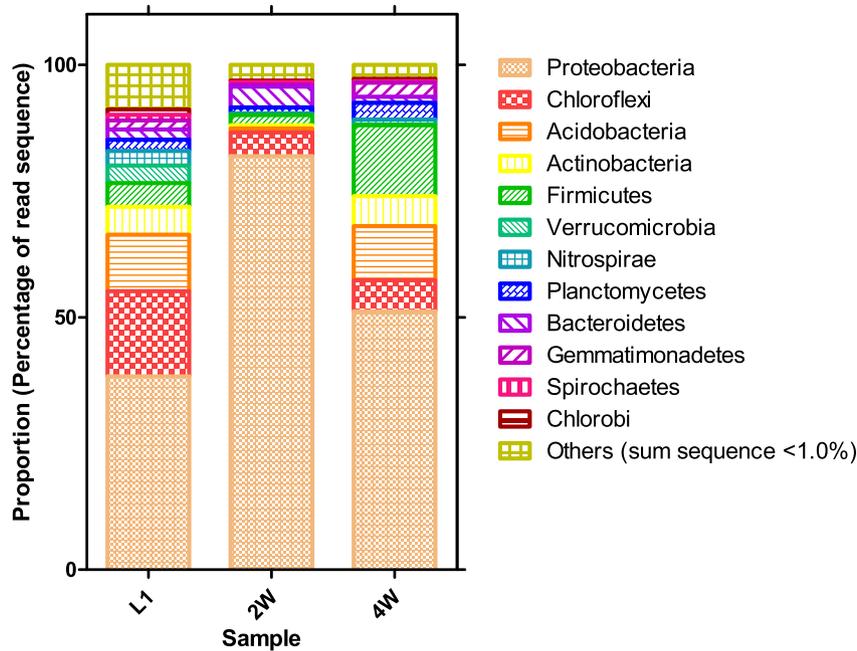
<sup>b</sup> Unit of NO<sub>3</sub><sup>-</sup>-N and PO<sub>4</sub><sup>3-</sup>-P in sediment sample is mg kg<sup>-1</sup>.

composition of 2W and 4W. Interestingly, a number of novel lineages (yet to be identified phyla) were among the group of reads tagged 'Others' (see Table S4). However, the average taxonomic composition of the ecosystems based on their phyla and species are represented with double-pie chart (Fig. S1).

### 3.3. Bacterial community compositions and comparison

Heat map analysis of the pyrosequencing data at the phyla level revealed bacterial composition and abundance in the sediments of L1-B, 2W and 4W, where the most abundant phyla include Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria and Firmicutes (Fig. 2). Proteobacteria dominated other phyla in all the environments, connoting 38%, 82% and 51% of the total valid sequences in L1-B, 2W and 4W, respectively. The sediment of 4W contained 14% unlike 2.0% in 2W, and 4.6% in L1-B sequence reads

that were identified as Firmicutes. Whilst, 6.1% (4W) and 4.7% (2W) of the respective total valid sequence reads were Chloroflexi as against 17% of sequence reads in L1-B. The composition of Acidobacteria was 11%, 0.79%, and 11% of valid reads in 4W, 2W and L1-B, respectively. Other notable phyla whose relative abundance is higher than 0.2% from each environment include Bacteroidetes, Actinobacteria, Planctomycetes, Gemmatimonadetes, and Nitrospirae (Table S4). While 44 phyla present in the pristine L1-B sediment were found missing in the sediments of both 2W and 4W, the phyla found in 4W but absent in 2W include Bacteria\_uc, GQ246394\_p, WS5, OP9, Deinococcus-Thermus, NKB19, and Elusimicrobia. On the contrary, more phyla were found in 2W that were absent in 4W. The phyla and their abundance (in parenthesis) include UT06 (7), WM88 (1), Lentisphaerae (2), Synergistete (4), Caldithrix\_p (17), JN537925\_p (1), SAR406 (2), Chlorophyta (1), Cloacamonas\_p (6), WS1 (2), OP8 (23) and Tenericutes (5). Table 2



**Fig. 1.** Phylum-level comparison of bacterial taxonomic composition of 'Iya-Alaro' Creek (4W) and Lagos Lagoon (2W) sediments. Phyla that are less than 1% of the total sequence reads were treated as 'Others'.

shows the 20 most abundant genera (only classified, with sum relative abundance  $\geq 1.8\%$ ) from all the sediment categories. Interestingly, no genus of Actinobacteria (despite been among the five dominating phyla) was among the 20 most abundant genera that predominantly spanned the four classes of Proteobacteria. However, a yet to be identified genus, *GU127739\_g* (Verrucomicrobia) was among the dominant genera that was present in L1-B but absent in the two polluted ecosystems, whereas *HQ190481\_g* (Acidobacteria) was among dominating genera that was only present in the 4W but absent in both L1-B and 2W. Moreover, *Magnetovibrio*, *FN820314\_g*, and *Sedimenticola* were exclusively found in 2W sediment. Using Taxon XOR (Taxon Exclusive Or) Analysis, 58,000 species were present in L1-B but absent in the polluted ecosystems. Whereas, 240 and 310 species were present in 2W and 4W, respectively, but absent in the pristine sediment (L1-B). Considering the two polluted ecosystems, 4200 species were found in the sediment of 2W, but missing in 4W, while 2500 species were present in 4W but absent in 2W.

### 3.4. Bacterial community diversity

Operational taxonomy units (OTUs) was depicted with rarefaction curves, based on Taxonomy-dependent clustering (TDC) followed by a taxonomy-based clustering (TBC) (see Fig. S2), allow comparison of OTU richness in the two polluted ecosystems. The curves did not reach an asymptote, showing that 4W reached higher number of OTUs per sequence reads than the curve of 2W. The 95% confidence intervals of the rarefaction curves of the samples from the two polluted environments did not overlap. The richness and diversity estimations presented in Table 3 revealed >70% of the sequences in the ecosystems represented the bacteria present in the sediments based on Goods libraries coverage estimator. Although there were marginal differences in the richness and diversity of bacterial species in the two polluted aquatic systems, 2W was still observed to contain richer (based on Ace, Jack-Knife and Chao1 estimators) but less diverse (through NPSannon and Shannon indices) bacterial species than 4W. The pristine L1-B

expectedly contained richer and more diverse bacterial species than the polluted ecosystems. Furthermore, 4W was observed to be more complex than 2W based on Simpson's diversity index, while L1-B contained the most complex bacterial community.

### 3.5. Relationships among bacterial communities

Comparing the bacterial communities of the polluted ecosystems with that of pristine, using Fast UniFrac metric, the OrthoANI values and unweighted pair group method and arithmetic mean (UPGMA) dendrogram revealed that L1-B community is distantly related to the polluted sediments (Fig. 3a). Further, evolutionary relatedness of the taxa in the ecosystems, upon definition of OTUs by CD-HIT and the radius of the sample determined by Shannon, is depicted by PCoA coordinates showing that L1-B was ordinated farther away from 2W to 4W, while 2W and 4W are somehow dissimilar since they did not ordinate alike (Fig. 3b).

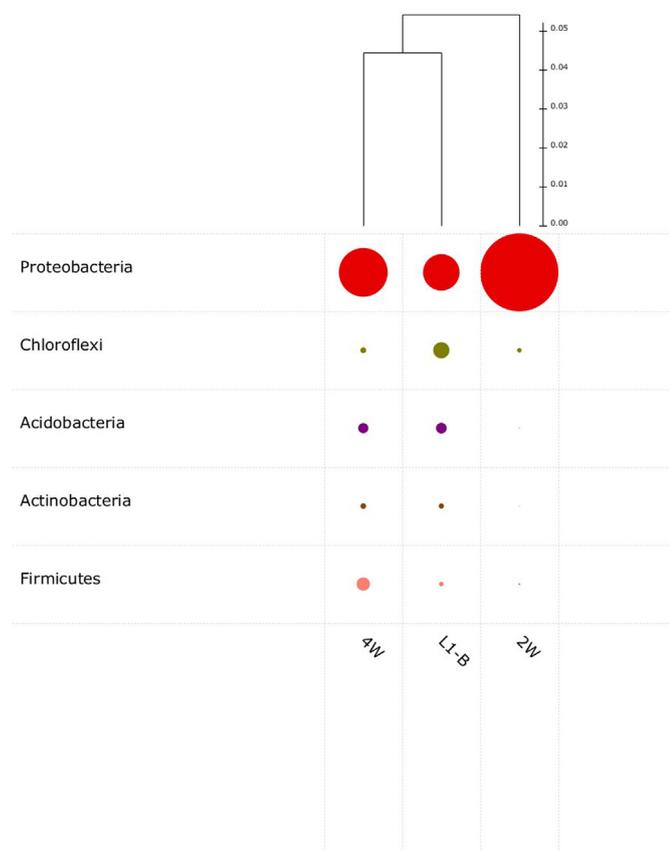
## 4. Discussion

A significant environmental concern is the manner and nature of wastewater discharges without adherence to environmental protection legislatures. Contamination of estuarine environments is a consequence of the integrity of water channels that empty into it. This is the case with 4W that serve as the main industrial tributary that feed estuarine 2W. It is evident, from the higher geochemical values (above recommended limit), that the sites (2W and 4W) are exposed to heavy anthropogenic activities. Recent reports stipulated that hydrosphere is influenced by prevailing physico-chemical characteristics (Fortunato et al., 2013; Wemheuer et al., 2014; Zhang et al., 2016). The abiotic factors revealed that 4W contained higher concentrations of pollution indicators, like COD, than 2W as evidence of exposure to heavier loads of chemical compounds. Pollution status of 4W due to industrial wastewater discharge has been reported (Oyetibo et al., 2010).

Disparity in the physical and chemical contents between 4W and 2W may be connected to the volume of water and closeness to

Heat map(Circle)

Full size of circle = 81.87%



**Fig. 2.** Heat map (circle) representing abundance of four bacterial families that is above the cut off. Each taxon and its proportion are represented by a circle (area). The full size of circle equals 81.87%.

**Table 2**

Taxonomic composition of 20 most abundant genera from the sediments.

Taxonomic rank				Relative abundance of 16S rDNA sequence reads (% of total reads)			
Genus	Order	Class	Phylum	L1-B	2W	4W	SUM
<i>Sulfurimonas</i>	Campylobacteriales	Epsilonproteobacteria	Proteobacteria	0.003	30	0.0	30
<i>Magnetovibrio</i>	Rhodospirillales	Alphaproteobacteria	Proteobacteria	0.0	9.0	0.0	9.0
<i>Clostridium</i>	Clostridiales	Clostridia	Firmicutes	1.1	0.9	6.8	8.8
<i>Dyella</i>	Xanthomonadales	Gammaproteobacteria	Proteobacteria	0.07	0.3	8.3	8.6
<i>FN820314_g</i>	FN820314_o	Gammaproteobacteria	Proteobacteria	0.0	7.8	0.0	7.8
<i>Rhodospirillaceae_uc</i>	Rhodospirillales	Alphaproteobacteria	Proteobacteria	0.22	4.8	0.058	5.0
<i>Pseudomonas</i>	Pseudomonadales	Gammaproteobacteria	Proteobacteria	0.087	0.53	4.2	4.8
<i>Rhodanobacter</i>	Xanthomonadales	Gammaproteobacteria	Proteobacteria	0.006	3.7	0.67	4.4
<i>EU786132_g</i>	EU786132_o	Betaproteobacteria	Proteobacteria	1.5	0.02	2.6	4.1
<i>Sedimenticola</i>	Chromatiales	Gammaproteobacteria	Proteobacteria	0.0	3.3	0.0	3.3
<i>Sideroxydans</i>	Gallionellales	Betaproteobacteria	Proteobacteria	2.9	0.0	0.087	3.0
<i>Pseudolabrys</i>	Rhizobiales	Alphaproteobacteria	Proteobacteria	1.8	0.12	0.82	2.8
<i>DQ123621_g</i>	Rhizobiales	Alphaproteobacteria	Proteobacteria	0.0	0.02	2.6	2.6
<i>Koribacter</i>	Acidobacteriales	Acidobacteria_c	Acidobacteria	0.35	0.0	2.1	2.5
<i>HM243779_g</i>	HM243779_c	HM243779_o	Acidobacteria	2.4	0.079	0.0	2.4
<i>GQ500701_g</i>	Anaerolineales	Anaerolineae	Chloroflexi	1.2	0.16	0.99	2.3
<i>HQ190481_g</i>	HQ645210_c	HQ645210_o	Acidobacteria	0.0	0.0	2.2	2.2
<i>Bacillus</i>	Bacillales	Bacilli	Firmicutes	1.2	0.02	0.96	2.2
<i>Romboutsia</i>	Clostridiales	Clostridia	Firmicutes	0.18	0.46	1.4	2.0
<i>GU127739_g</i>	Pedospaera_o	Verrucomicrobiae	Verrucomicrobia	1.8	0.0	0.0	1.8

**Table 3**

Alpha diversity of bacterial richness and diversity in the sediments.

Index	L1-B	2W	4W
Valid reads	83,000	5100	3400
Actual OTUs	8700	1300	1300
Estimated			
Ace	8900 ± 18	6200 ± 260	5400 ± 210
Chao1	8700 ± 7.0	3700 ± 270	3100 ± 190
JackKnife	9100	5700 ± 520	4000.0 ± 250
NPS Shannon	8.0	5.7	6.6
Shannon	7.8 ± 0.009	5.3 ± 0.04	6.3 ± 0.04
Simpson	0.002	0.029 ± 0.001	0.008 ± 0.001
Goods Lib. Coverage	100%	82%	74%

OTUs = Operational taxonomic units, determined at 97%.

L1-B = Pristine Lekki Conservation sediment.

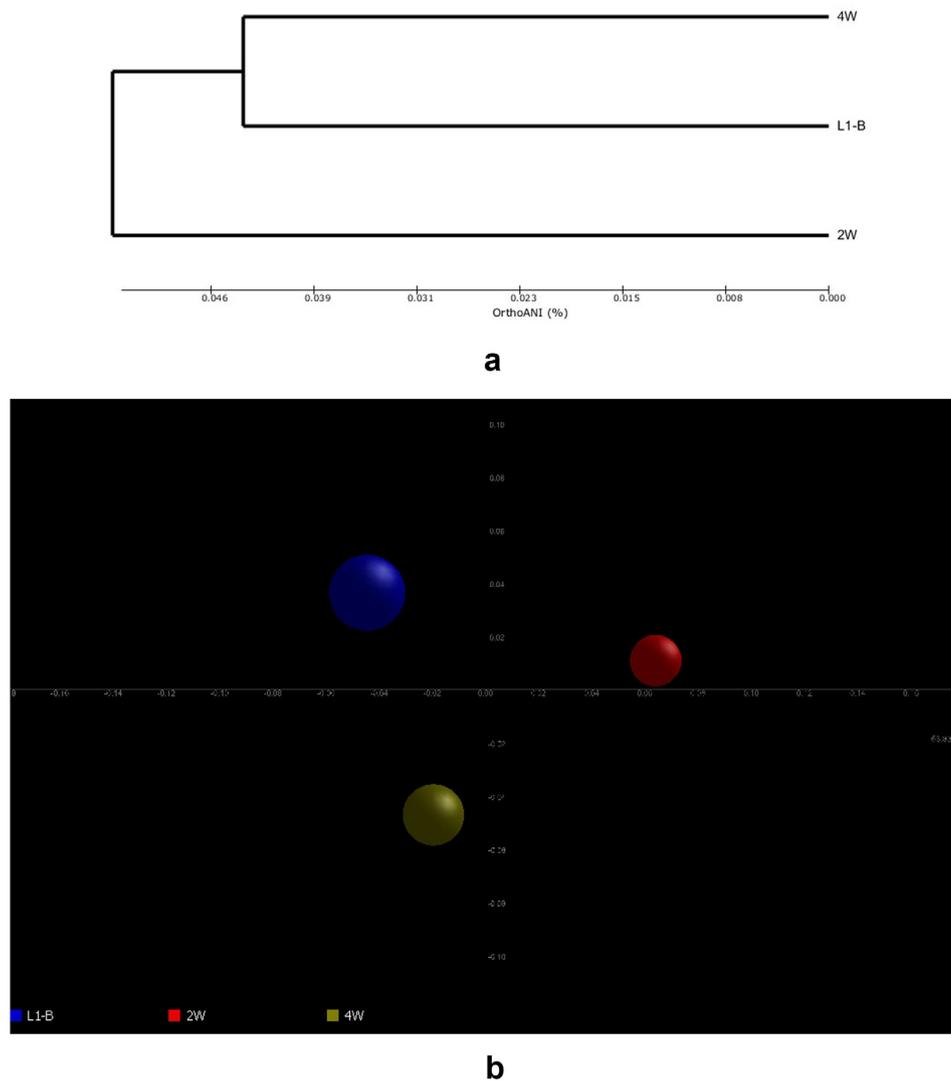
2W = Lagos lagoon (an estuary where contents of 4W empties).

4W = 'Iya-Alaro' Creek (tributary of industrial wastewaters).

Richness and diversity were determined with CD-HIT clustering method. Values represent mean (± standard error mean) of replicate analyses.

source point of chemical contaminant. 4W contained smaller volume of water, and it is closer to point of contamination, diluting its content until it finally empties into 2W that provides larger surface area for dilution of 4W contents. HM- and metalloids-pollution indicators revealed that 4W was more laden with toxic concentrations of HMs and metalloids than the receiving estuarine environment (2W). Based on the Canadian Sediment Quality Guidelines (Environment Canada, 1999), the two environments were polluted with toxic metals, except Cd concentration in 2W that was below the threshold. Also, Fowler (1990) adjudged estuarine sediment to be contaminated when Hg concentration is above  $5 \mu\text{g g}^{-1}$ . However, ecological risk indexes established that 4W was more polluted than 2W, implicating Hg as the major eco-toxicant in both polluted ecosystems that drives the functionality of bacterial community in the surface sediments in corroboration with the submission of Nethaji et al. (2016).

Toxic concentrations of HMs and metalloid oscillate the diversity and number of microorganisms that play pivotal role in biogeochemical cycling of elements, organic matter demineralization and biochemical degradation. The number of sequence reads in



**Fig. 3.** The relatedness of bacterial communities between the polluted sediments (2W and 4W) and the pristine sediment (L1-B), using Fast UniFrac analysis and revealed by a) dendrogram showing inter-community relatedness calculated with OrthoANI and clustered with UPGMA; b) principal coordinate analysis (PCoA) of L1-B, 2W and 4W based on weighted UniFrac parameters that are normalised and treated unclassified OTUs as different. OTUs are defined by CD-HIT and radius is Shannon.

the two environments indicates the active activities of bacteria existing in the sediments since the amount of rRNA in an ecosystem roughly correlates with the bacterial abundance and activity. Higher number of reads in the sediment of 2W than sediment of 4W depicts higher bacterial population is available for self-recovery processes that occur in the system. Taxonomic composition of bacterial community in the two polluted ecosystems revealed that 4W contains higher genus diversity than 2W with respect to higher number of genera designated as 'Others'. In this study, we observed high ecophysiological indices in the two polluted sites, which reflected the richness and diversity estimators that are in tandem with the rarefaction curves of OTUs in the ecosystems. This equally indicates high bacterial diversity due to adaptation of the bacterial community to the prevailing conditions at 4W and 2W (Jurburg and Salles, 2015).

Dominance of Proteobacteria in the present study agrees with reports of other locations of Lagos estuarine bodies (Obi et al., 2016), extreme environments (Mondav et al., 2017) and metal-contaminated sediments across the globe (Hemme et al., 2010; Patel et al., 2015; Rossa et al., 2014; Zhang et al., 2016). Low abundance of Proteobacteria (51% of total reads) along with higher

abundance of Firmicutes (14%) and Acidobacteria (11%) in 4W sediment against 82%, 2.0%, and 0.79%, respectively, for sediment of 2W indicated metal pollution-induced shift in bacterial communities with respect to corresponding 38%, 4.6% and 11% in the pristine L1-B sediment as earlier reported (Hemme et al., 2010). Apparent absence of Epsilonproteobacteria in 4W (but constituted dominant class in 2W) connotes the probable sensitivity of Epsilonproteobacteria to ecotoxicology of metals, particularly Hg, causing their extinction in contrary to the dominance of this class in 2W where total risk response of metal was less (RI: 4W = 3100; 2W = 2000). However, class Betaproteobacteria (signifying 9.4% and 0.55% of total sequence reads of 4W and 2W sediments, respectively) and class Clostridia (4W, 11%; 2W, 1.7%) are assumed to comprise HMs-resistant strains that are expectedly playing significant role in biogeochemical transformation of the contaminating toxic metals (Hg, for emphasis) in 4W ecosystem.

Furthermore, higher abundance of Firmicutes in 4W (480 sequence reads) than 2W (100 sequence reads) may be due to higher toxic response ( $E_r^1$ ) of Hg in 4W (2900) than 2W (1900) that induces reduction of  $Hg^{2+}$  to  $Hg^0$  during self-recovery processes in the polluted ecosystems. High population of Firmicutes observed in

this study corroborates the reports of Desai et al. (2009), indicating that long-term Hg-induced perturbation results in community shifts towards Firmicutes dominance. Many genera of Firmicutes, strains of *Bacillus* for example, have reportedly utilised *mer* operon transcripts and other physiological traits to detoxify Hg<sup>2+</sup> ions and organo-mercury in contaminated hydrosphere, lowering the mercury concentration in the environmental media (Narita et al., 2004). Moreover, strains of *Bacillus* previously isolated from 4W were reported to have degraded petroleum hydrocarbons in matrixes containing toxic concentrations of metals unhindered (Oyetibo et al., 2017b, 2013). Other notable phyla, including Bacteroidetes, Actinobacteria, Planctomycetes, Gemmatimonadetes, and Nitrospirae, with higher relative abundances than 0.2% in the present study, have variously been found dominating extreme environments (Jiang et al., 2006; Nguyen et al., 2016).

Microbial diversity and composition have been reportedly affected by anthropogenic pollution (Pinto et al., 2015), hydrological regime fluctuation and allochthonous nutrients input (Sakami et al., 2016). The ratio between Proteobacteria and Acidobacteria may indicate the nutrient status in a particular ecosystem as discussed previously (Nguyen et al., 2016). In this study, the ratio between Proteobacteria and Acidobacteria in 4W sediment (4.8) was marginally lower than that of 2W (100), indicating that oligotrophic conditions are more prevalent in 2W than 4W. This also is an indication that 2W is more nutritive than 4W, which is usually determined by increased acidity due to high metal contamination in 4W than 2W. A shift in microbial community of environmental matrixes due to acidification emanating from anthropogenic pollution has been reported (Quadros et al., 2016). A higher population of Acidobacteria in 4W than 2W can be explained based on their acidic tolerance or preference since 4W consistently undergo acidification due to regular discharge of metal-laden effluents from industrial process. The more acidic an environment therefore is, the more abundance the Acidobacteria that would possibly exist. The Acidobacterial groups can tolerate and utilise a broad range of substrates and are reportedly found to prefer copiotrophic conditions, while some others are known to thrive well in oligotrophic niches (Mannisto et al., 2013; Naether et al., 2012; Ward et al., 2009). The abundance of the phylum Chloroflexi in the polluted ecosystems (more population in 2W than 4W) has been reportedly dominating in other estuarine system in Lagos (Obi et al., 2016). The major classes of *Caldlineae* (4W, 0.49%; 2W, 1.1%) and *Anaerolineae* (4W, 3.2%; 2W, 2.6%) within the phylum Chloroflexi in this study are known to mediate anaerobic oxidation of ammonium during biogeochemical cycling of nitrogen in the ecosystems.

In Table 2, where 20 most abundant genera in the two polluted matrixes were presented along with the pristine ecosystem, it is noteworthy that majority of the genera belong to four classes of Proteobacteria (Epsilonproteobacteria, Alphaproteobacteria, Gammaproteobacteria, and Betaproteobacteria). Conspicuously absent in 4W sediment were *Sulfurimonas*, *Magnetovibro*, *Sedimenticola*, and yet to be identified *FN820314\_g* and *HM243779*, which were predominantly found in 2W community. It may be concluded that these genera are intolerant to Hg toxicity and thus play little or no role in Hg biotransformation. Their dominance in 2W sediment may be due to natural attenuation already taking place in 2W, lowering the metal pollution, unlike in 4W where the total risk index of HMs and metalloids is extremely high (RI: 4W, 3100; 2W, 2000), causing the disappearance of the genera. However, the higher predominance of *Dyella* (4W, 8.3% of total reads; 2W, 0.3% of total reads), *Clostridium* (4W, 6.8% reads; 2W, 0.91%), *Pseudomonas* (4.2% for 4W; 0.53% for 2W) and unidentified *DQ123621\_g* and *EU786132\_g* (4W, 2.6%; 2W, 0.02% of total reads for each genus) in 4W sediment than sediment of 2W signifies higher tolerance of

many species in these genera to metals toxicity and definitely suspected to be involved in biogeochemical cycling and biodegradation of Hg in the ecosystem.

As a whole, the influence of toxic metals, as suggested, can lead to a decrease in the genetic population of bacteria domain when the metals occur at high concentrations. This often leads to evolution of multi-resistant strains whose functionalities are not affected by metal toxicities (Oyetibo et al., 2017b, 2013). The presence and activity of autochthonous bacteria in such polluted systems give insight into bacterial populations for effective stimulations that are required for bioremediation treatments. Therefore, the 760 OTUs present in 4W but absent in 2W, as revealed by Taxon XOR analysis through CD-HIT function, indicates the OTUs must be metallophilic bacteria that exhibit high resistance to HMs and contribute immensely to biogeochemical detoxification of HMs. These 760 OTUs may be the targets for biostimulation during decommissioning processes of the polluted environments. On contrary, the whopping 2900 OTUs found in the sediment of 2W, but missing in the sediment of 4W may have been due to ecotoxicological consequences of discharged HM- and metalloid-rich industrial effluents, causing extinction of the OTUs in 4W ecosystem. These 2900 OTUs may serve as bacterial indicator for the sediment of hydrosphere that is highly polluted with HMs (precisely Hg). Previous studies have found that microbial diversity decreased immediately after exposure to Hg and that adaptation to Hg stress may result in a recovery of diversity upon filling up of the empty niches with metal-resistant species that maintain ecological stability (Desai et al., 2010, 2009). Similarity calculations indicated that the change in microbial diversity was not due to a reversion towards the pre-exposure community but mainly due to the appearance of new dominating species (Turpeinen et al., 2004).

## 5. Conclusion

Our data suggest that the bacterial communities in the studied polluted ecosystems respond to long-term HM pollution with dominance of toxic metal-resistant phylotypes and extinction of sensitive strains. The toxic metals, mercury in particular, in the polluted environments drive the niche structure through changes in bacterial community structure and selection for resistant phylotypes that thrive under the metal concentrations reported, which may shape the bioremediation of the polluted hydrosphere. Thus, natural attenuation of the toxic metals and metalloids are possible in the hydrosphere through the activities of the dominant bacteria due to functional superfluity in the HM- and metalloid-polluted ecosystems.

## Conflicts of interest

Each of the Authors has no competing moral or financial interest in relation to the work described.

## CRediT authorship contribution statement

**Ganiyu O. Oyetibo:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. **Keisuke Miyachi:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing. **Yi Huang:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Wakako Ikeda-Ohtsubo:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Mei-Fang Chien:** Data curation, Methodology, Validation,

Visualization. **Matthew O. Ilori:** Conceptualization, Data curation, Methodology, Project administration, Supervision, Validation, Writing - review & editing. **Olukayode O. Amund:** Conceptualization, Project administration, Supervision, Validation, Writing - review & editing. **Ginro Endo:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing - review & editing.

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## Appendix A. Supplementary data

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