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Taxonomic profiling of bacteria and fungi in freshwater sewer receiving hospital wastewater

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ABSTRACT

Consistent discharges of hospital wastewaters (HWWs) pose ecological risk to the biome of the receiving environment with cumulative effect on its healthiness. Understanding the taxonomic profile of microorganisms in the impacted systems is required to establish taxa that are bio-indicators of toxicants, and provide possible taxa for mitigating ecotoxicity of the HWWs. Geochemistry, pollution status and ecotoxicity of heavy metals (HMs) in HWW-impacted sewer (LU) were assessed. The microbiome profiling was based on 16S rDNA and ITS of 18S rDNA metagenomes. The degree of HMs contamination exceeded 50 and HMs pollution load index of LU was severe (1,084), which consequently exerted severe risk (1,411,575 toxic response factors) with very high toxic responses of Co, Cu, Pb, and Cd. Eco-toxicological impact of the HMs on LU skewed microbiome towards Proteobacteria (43%), Actinobacteria (18%), and about 5% apiece for Chloroflexi, Acidobacteria, Plantomycetes, and Bacteroidetes. Likewise, the relative abundance of in LU inclined towards Ascomycota (59%), Basidiomycota (17%) and unclassified Eukarya_uc_p (16%). Exclusively found in LU sediments were 44,862 bacterial species and 42,881 fungi taxa, while 72,877 and 53,971 species of bacteria and fungi, respectively, were found missing. Extinction and emergence of bacteria and fungi taxa in LU were in response to HMs ecotoxicity and the need for natural attenuation processes respectively. The profiled taxa in LU may be plausible in bioremediation strategies of the impacted system, and in designing knowledge-based bioreactor system for the treatment of HWWs before discharge into the environment.

1. Introduction

Wastewaters from hospitals like domestic wastewaters are effluents of large volume of water from different units and services of hospitals. The quantity and characteristics of the hospital wastewaters (HWWs) is proportional to the type of units and services in tandem with the economy and size of the facilities. For example, 400–1200 L wastewaters per bed were reportedly generated every day in developed countries, but far lesser volume of wastewaters was associated with developing countries (Kumari et al., 2020). HWWs generally contain biological agents (Smiech et al., 2020), biologically active organic micro-pollutants (Castillo Meza et al., 2020), drug metabolites (Ngigi et al., 2020), chemical disinfectants, X-ray contrast agents, halogenated solvents (Castillo Meza et al., 2020), and metals (Alam et al., 2020) that are of great importance to the receiving environments. The components of HWWs do originate from substances used for medical, laboratory and research purposes along with excreta from patients in terms of drug metabolites. While the organic pollutants in HWWs have chemotherapeutic origins, the heavy metals (HMs) components are sourced from dental amalgam and medical devices residues causing rise in the HMs levels in the HWWs, which have recently drawn global attentions (Zhang et al., 2020). The HMs as toxicants in HWWs could therefore be categorized along with bioactive organic micro-pollutants earlier reported by Castillo Meza et al., 2020 as contaminants of emerging concern from health facilities that impact the receiving environments.

Treatment of HWWs before discharge into the environment in most developing countries is generally unpopular (Adeolu and Adewoye, 2019). However, treatment of HWWs, where applicable across the globe, targeted the degradation and removal of the organic compounds (Kumari et al., 2020) and infectious pathogens (Smiech et al., 2020). Some examples of worldwide designs for HWWs treatments include membrane bioreactors, advanced oxidation process based on UV/H_2O_2/O_2 (Mejia-Morales et al., 2020), thermal disinfection (Smiech

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List of abbreviations:

UV	ultra violet
UV–Vis	ultra violet visible
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
ITS	internal transcribed spacer
dsDNA	double stranded deoxyribonucleic acid
HMs	heavy metals
HWWs	hospital wastewaters
OTUs	operational taxonomic units
BOD	biochemical oxygen demand
COD	chemical oxygen demand
NCBI	national center for biotechnology information
UPGMA	unweighted pair group method with arithmetic mean
Taxon XOR	Taxon Exclusive Or Analysis

et al., 2020), biosorption using nano-materials (Pham et al., 2020), activated sludge, nanofiltration, and reverse osmosis (Kumari et al., 2020). In these treatment designs, the organic components of HWWs get degraded to form sludge while the HMs components remained intact or partially bound to organic matter in high concentrations. Such non-treatment or incomplete eradication of eco-toxicants in the HWWs makes HWWs emerging point sources for HMs pollution in the environments where the wastewaters are discharged. Moreover, the use of HMs and their oxides as chemical agents in advanced oxidation processes during degradation of HWWs' organic components (Mejia-Morales et al., 2020) causes increase in HMs concentrations in the sludge (Zhang et al., 2020). Notable metals found in appreciable quantities in every discharge of HWWs include Hg, Cd, Zn, Pb, Cr, Fe, and Cu (Emmanuel et al., 2005; Zhang et al., 2020).

Upon discharge of HWWs, the HMs contained thereof contaminate receiving environments and become hazardous to the ecosystems (Bastami et al., 2012; Salas et al., 2017). The concentrations of HMs in such receiving ecosystems continuously increase as more HWWs are discharged since HMs are non-degradable, thereby biomagnifies along the trophic level (Liu et al., 2019). The toxic metals do reportedly alter the biodiversity of the receiving ecosystem due to direct eco-toxicological impact on the biome with consequent extinction of susceptible taxa and dominance of tolerant strains (Oyetibo et al., 2017). However, selected microbial taxa adapt to the HMs-enriched ecosystems with evolution and horizontal transfers of HMs-resistance genes, which are known to exist together with antibiotic-resistant genes into a single integron in microbiomes (Castillo Meza et al., 2020; Paulus et al., 2019). Discharge of HWWs in contrast to domestic wastewater, triggers proliferation and spread of resistant pathogens leading to complex cross-selection patterns that constitute challenges to public health (Paulus et al., 2019). It has been found that bacterial integrons often combine antibiotic resistance genes and genes conferring HMs sequestrations in environmental matrixes, leading to complex co-selection dynamics between the two groups of genes (Oyetibo et al., 2010). Multiple-resistant hospital strains of enterobacteria, for example, have been observed to carry other resistance genes compared to those coding for resistance to streptomycin, chloramphenicol or spectinomycin in their integrons even when the antibiotics were not used in the hospital settings for decades (Leverstein-van et al., 2002).

Intrinsically, there is dearth of information about toxic metal pollution status and microbiome of freshwater that daily receive load of HWWs with ultimate goal of ameliorating the HMs ecotoxicity. Efforts have beleaguered impact of antibiotics and pharmaceutical molecules on microbiota of environments receiving HWWs with respect to resistome, without reverence to the dynamics of HMs eco-toxicity (Buelow et al., 2020). Understanding the correlation of HMs dynamics in terms of their eco-toxicological consequences on the biome, via microbial community structure, is cogent to designing eco-friendly microbial-based decommissioning strategies that would completely ameliorate HWWs-impacted environment. The study area has consistently been exposed to HWWs, with no known pre-disposal waste treatment protocol, for more than 50 years. Consequently, it is postulated that the toxicants in the HWWs would have affected the microbiota as an indication of ecotoxicological impact of the HWWs on the receiving freshwater environment. Profile of microbial assemblage in the HWWs-impacted milieu that could describe the impact of HWWs toxicants and those taxa that could drive eco-friendly amelioration of attendant stressors is scarce. Therefore, the present study is sought to fill the gap with the following specific objectives: 1) to determine the HM pollution status of freshwater that consistently receives HWWs; 2) to profile the bacterial and fungal taxa in the freshwater; and 3) to integrate the geochemistry and microbiome with goal of adopting strains that would serve as biotechnological tool for designing efficient integrated HWWs treatment system.

2. Materials and method

2.1. Study area and sampling

A freshwater sewer (LU) at Idi-Araba, Surulere, Lagos, Nigeria (N6° 30′ 55″ E3° 21′ 17″) receives loads (>100,000 L) of wastewater from a tertiary healthcare facility every day. The hospital is among top ten largest reference health facility in Nigeria and attends to more than 2000 outpatient cases and 120 new emergencies on a daily average even as 50 new patients are admitted every day to more than 600 beds available. The only treatment plant available to the facility had gone moribund for decades, whereby the generated HWWs are discharged into LU from various channels without any known form of treatment. However, the pristine freshwater (L1) is at Nigerian Conservation Foundation (NCF) with coordinates N6° 26′ 14″ E3° 32′ 9″, and has no history of pollution. Ten composite points at each location were sampled for surface sediments using Ven Veen Grab from each of the sites into clean containers and was transported in ice chest (approx. 0 °C) to the laboratory for analyses, or otherwise stored at −40 °C until further analysis.

2.2. Physico-chemistry, heavy metal assay, and pollution index analyses

The methods earlier reported by Oyetibo and colleagues (2010, 2019) were used to determine texture, pH, conductivity, and other physico-chemical parameters of the sediments. Parameter determined *in situ* was pH by using pH meter, while *ex situ* assays include moisture content, texture, total organic carbon (TOC), total organic matter (TOM), nitrate, phosphate, sulphate, chlorides, and cation-exchange capacity (CEC). For HMs analyses, sediment (1 g) was digested in ratio 3:1 aqua regia (HCl, HNO_3), allowed to stay overnight and subjected to 150 °C until there was disappearance of brown fumes. Along with the digestion of the sediment samples, 0.5 g each of SO-2 (reference material of Canada Center for Mineral and Energy Technology) was digested with 10 ml concentrated HNO_3 at 30 (±2) °C on a water bath for 2 h. The reference digests were filtered into a 10 ml volumetric flask and made up to the mark, such that the SO-2 digests form soluble nitrate of the metals in solution. The digests were resuspended with 5 ml $HClO_4$ (70–72%) and was then assayed for Cd, Co, Cu, Ni, Pb, and Zn using an AAnalyst 200 flame atomic absorption spectrophotometer (PerkinElmer, Canada) equipped with deuterium lamp background correction and an air-acetylene burner. The wavelengths used for cadmium, lead, cobalt, copper, zinc and nickel were 228.8, 283.3, 240.7, 324.8, 213.9 and 231.1 nm analytical lines, respectively. The operational conditions of the equipment at appropriate lamps currents were: 1.0 nm spectral bandwidth, acetylene flow rate at 1400 ml min^{-1}, and nebulizer flow rate was 5 ml min^{-1}. Sample flow-rate of 7 ml min^{-1} and background corrected atomic absorption were also adjusted. At least one blank

solution was run for each sample in order to evaluate the metal contamination by the reagents used. Prior to measurements of the sample digests, percentage recovery as calculated using standard methods was not less than 88% for cobalt and up to 94% for copper. The detection limits of the instrument during measurements at 95% confidence interval were (per liter) 0.002 mg, 0.010 mg, 0.020 mg, 0.050 mg, and 0.020 mg for Cd, Cu, Ni, Pb, and Zn, respectively. All the chemicals used including the metals standards for instrument calibration before measurement were of analytical grades purchased from Sigma-Aldrich (Germany). Measurements of samples and blank solution were in three replicates.

The geochemical indexes of the measured HMs were determined to evaluate the degrees of contamination, accumulation, pollution and ecotoxicity of the HMs as previously described (Oyetibo et al., 2019). The indexes include:

$$\text{Contamination factor (CF)}, \mathrm{CF} = \frac{Cn}{Bn} \quad (1)$$

where C_n is the concentration of the metal n and B_n is the natural local background concentration of metal n. The B_n for each heavy metal was the mean of triplicate measured concentrations of freshwater sediments from "Nigerian Conservation Foundation (NCF)" where there is no history of anthropogenic heavy metal pollution.

$$\text{Geo} - \text{accumulation index}\left(\mathrm{I_{geo}}\right): I_{geo} = log_2\left(\frac{C_n}{K \times B_n}\right) \quad (2)$$

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 as defined by Muller in 1969 (Oyetibo et al., 2019) was introduced to minimise the effect of possible variations in the B_n (background values).

$$\text{Pollution load index (PLI)}: \; PLI = (CF_1 \times CF_2 \times CF_3 \times \ldots \times CF_n)^{\frac{1}{n}}, \quad (3)$$

where CF is the contamination factor as described before.

$$\text{Degree of contamination}(\mathrm{C_d}), \; C_d \sum_{i=1}^{n} CF \quad (4)$$

$$\text{Potential ecological risk factor (Er)}, \; E_r^i = T_r^i.CF \quad (5)$$

where T_r is the toxic response factor for a given substance (see table A1)

Table 1
Physicochemical status of the impacted freshwater sewer receiving hospital wastewater and a pristine environment.

Parameter	LU	L1	NESREA	UNEP
Soil Texture	Sandy loam	Silt	NA	NA
pH	6.30	5.75	6.5–8.5	6.6–8.5
Conductivity (μS/cm)	320	260	240	380
Nitrate (mg kg^{-1})	19.19	17.3	45	0.16
Phosphate (mg kg^{-1})	21.85	85.9	0.1	4.5
Chloride (mg kg^{-1})	744	–	250	20
Sulphate (mg kg^{-1})	15.82	9.43	100	500
Ammonia (mg kg^{-1})	7.06	17.6	0.50	0.21
Moisture Content (%)	4.22	14.7	–	–
Total Organic Carbon (%)	1.28	1.25	–	–
Total Organic Matter (%)	2.21	2.16	–	–
Oil and grease (mg kg^{-1})	0	BDL	10	–
Total Petroleum Hydrocarbon TPH (gravimetric) (mg kg^{-1})	0.105	BDL	–	–
Cation Exchange Capacity, CEC (meq 100^{-1})	2.78	BDL	–	–

LU = Lagos University Teaching Hospital, L1 = Pristine environment (Nigeria Conservation Foundation), NESREA = National Environmental Standards and Regulations Enforcement Agency, Nigeria, UNEP = United Nations Environment Programme, BDL = Below detectable limit.

$$\text{Potential ecological risk index}\,(RI), \; RI = \sum_{i}^{m} E_r^i, \quad (6)$$

2.3. Community DNA isolation, purification and quantification

Genomic DNA extraction from 0.5 g (approx.) of sediment sample from each location was achieved with Fast DNA® Spin Kit for Soil (MP Biomedicals) using FastPrep® Cell Distruptor FP120 (Qbiogene, Heidelberg, Germany) at 6.5 speed for 30 s following manufacturer's instruction. Possible interference of humic substances in the DNA was removed by addition of skim milk (20 mg per 500 of sample) to the sample in lysis matrix based on recommendations of Takada and Matsumoto (2005). DNA was purified and visualized in an ethidium bromide stained 1% (w/v) agarose gel using UV trans-illumination, while quantification was via UV–Vis photo-spectrometry using Epoch™ Spectrometer system (BioTek, Winooski, VT, USA).

2.4. PCR amplification, library preparation and pyrosequencing

The genomic DNA was amplified at the V3–V4 of the 16S rRNA using the primers 341F and 805R for bacteria with some archaea; and ITS2 region using primer set ITS3-Mi (forward) and ITS4-Mi (reverse) for eukarya (ChunLab Inc., Seoul, South Korea). The first and second PCR recipe and conditions were according to existing protocol. The purified amplicons of 1st PCR were tagged with Illumina indices and adapters from a Nextera® XT Index Kit (Illumina, San Diego, CA, USA). Libraries were constructed at ChunLab Inc. using the Illumina MiSeq platform, where quality of the constructed libraries were checked with Agilent 2100 Bioanalyzer System (Agilent Technologies, Palo Alto, CA, USA) using a DNA 7500 chip at ChunLab Inc. (Seoul, South Korea) and thereafter quantified using Quanti-iT™ PicoGreen™ dsDNA Assay kit (Invitrogen) according to the manufacturer's instructions. Short DNA fragment was removed using CleanPCR™ (CleanNA, Netherlands), and sequencing was performed using Illumina, MiSeq Reagent Kit v2 (500-cycles) of Illumina MiSeq platform at ChunLab Inc., Seoul National University, Seoul, Korea.

Metagenome raw reads were processed beginning from quality check and filtering of low quality (<Q25) reads using Trimmomatic 0.32 software (Bolger et al., 2014). The pair-end sequence of the same strand of PCR amplicon were merged based on overlapping sequence information using PANDAseq software (Masella et al., 2012). ChunLab's pipeline in-house algorithms were used to remove 16S rRNA PCR primer sequences, and UNITE (https://unite.ut.ee) was used to analyse ITS2 gene. Non-specific amplicons were identified and removed using the HMMER program based search to exclude Singleton sequences (Eddy, 2011), while sequences denoising were performed with DUDE-Seq software (Lee et al., 2017), sequences were de-replicated and non-redundant reads were extracted via UCLUST-clustering (Edgar, 2010). UCHIME (Edgar et al., 2011) was used for detection and removal of chimera, while the remaining non-chimeric sequences were clustered into operational taxonomic units (OTUs) using UCLUST (Edgar, 2010). Query sequences that were matched with the reference sequences in EzBioCloud database (https://www.ezbiocloud.net/) by ≥ 97% similarity were considered to be at the species level while <97% similarity cut-offs were used for genus or higher taxonomic levels. The sequencing metadata obtained and used in this study have been deposited in the NCBI's sequence read archive (SRA) database under BioProject and SRA accession number **PRJNA604115**.

2.5. Statistical analyses

Statistical analyses used, unless otherwise stated, were performed using the prism 5 software program (GraphPad Software, San Diego, CA, USA). The estimated coverage of the constructed gene libraries were calculated as $C = 1 - (\frac{n}{N}) \times 100$ (Kemp and Aller, 2004), where n is the

number of Singletons after assembly, and *N* is the total number sequences in the initial dataset. Rarefaction curves were obtained by plotting the number of observed OTUs versus the number of sequences along with calculations of Good's coverage coefficient in order to determine the level of sequencing depth. Richness and diversity statistics of the bacterial community including abundance-based coverage estimator (S_{ACE}), the bias-correlated Chao1 (Scha o1), and the Shannon-Weaver diversity index were estimated using pre-calculated program of CLcommunity™ software package (ChunLab Inc.) in order to assay taxonomic diversity. Phylogenetic structure diversity was calculated by summing the shortest distance between the nodes of the system diagram in order to quantify the differences among species. Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree was created using Mega Software (Mega 6).

3. Results

3.1. Physico-chemistry and heavy metal pollution of sewer sediments

The physico-chemical properties of the sewer sediment that receive loads of HWWs in comparison with that of pristine freshwater were as presented in Table 1. The sediment appeared not rich in nutrients but high conductivity as against the recommended limits. Geochemical analysis revealed appreciable presence of Cd, Co, Cu, Pb, and Zn in the sediment of sewer receiving HWWs as shown in Table 2. The HMs pollution status of the sediments receiving the HWWs (Table 2) and ecological risk factors of the HMs in the sewer (LU) to autochthonous life (Table 3) were as determined via calculated indexes. Attempt to assess HM pollution and ecotoxicity informed simultaneous use of several indexes based on concentration of metals in unpolluted natural local background (L1) as presented in Tables 2 and 3 Anthropogenic addition of HMs (determined as CF) to the sewer sediments indicated that Co, Cu, Pb, Cd, Ni were very high, where Co (with $C_f^i = 200{,}010$) remains the highest, and Zn moderately contaminated ($1 \leq C_f^i < 3$) the sediment at 2.04 factor. Generally, degree at which the sediment of sewer receiving HWW was contaminated with HMs was extreme at 54 ($16 \leq C_d < 32$). The level of HM pollution in the sewer sediment as resolved via I_{geo} revealed varied degree of pollution, where Zn pollution was moderate, Ni pollution was high, and pollution with Cd, Pb, Co and Cu were severe. As such, HMs pollution load index of LU sediment was extreme at 1084 ($1 < PLI \leq 10$). The ecological risk assessment of the HMs in the LU sediments as defined by RI was extreme at 1,411,575 (very high: $RI \geq 600$) taking into account the toxic response (Er) of the sediments to HMs (Table 3). Toxic response of the sediment ecosystem to Co, Cu, Pb and Cd was very high ($E_r^i \geq 320$), while toxic responses to Ni and Zn were moderate ($160 > E_r^i \geq 80$) and low ($E_r^i < 40$), respectively (Table 3).

3.2. Taxonomic profile of bacteria and fungi

The total valid sequence reads for LU and L1 were 56,101 and 86,967, respectively, after quality filtering, trimming, and removing all

Table 3
Potential ecological risks of heavy metals in freshwater sewer that receives hospital wastewaters.

	Determinants	Interpretation
Contamination factors		
C_d	**($16 \leq C_d < 32$)**	**Very high ($C_d \geq 32$):** 54
PLI	**$1 < PLI \leq 10$**	1084
CF	Very high ($Cfi \geq 6$)	Co > Cu > Pb > Cd > Ni
	Considerable ($3 \leq Cfi < 6$)	
	Moderate ($1 \leq Cfi < 3$)	Zn
	Low ($Cfi < 1$)	
Ecological risk factors		
RI	Very high ($RI \geq 600$)	1,411,575
Er	Very high ($Eri \geq 320$)	Co > Cu > Pb > Cd
	High ($320 > Eri \geq 160$)	
	Considerable ($160 > Eri \geq 80$)	Ni
	Moderate ($80 > Eri \geq 40$)	
	Low ($Eri < 40$)	Zn

CF = contamination factor (Cfi); Er = toxic response; C_d = degree of contamination; PLI = pollution load index; RI = total risk response.

Table 4
Alpha diversity of microbiome evenness, richness and varieties of species in the sediments.

	Bacteria		Eukarya (Fungi)	
	L1	LU	L1	LU
Actual				
Valid reads	83,019	55,793	67,121	51,481
OTUs	8693	6390	1446	1113
Estimated richness				
ACE	8850.7	6538.3	1460.3	1130.5
HCI	8881.5	6568.8	1469.4	1140.9
LCI	8820.6	6508.6	1451.4	1120.4
Chao1	8723.4	6423.7	1448.3	1116.3
HCI	8738.3	6439.8	1454.5	1123.4
LCI	8713.4	6412.8	1446.7	1114.1
JackKnife	9126	6775	1485	1157
HCI	9126	6775	1485	1157
LCI	9126	6775	1485	1157
Estimated diversity				
NPShannon	7.96	7.83	4.52	4.82
Shannon	7.777	7.641	4.499	4.788
HCI	7.790	7.655	4.519	4.805
LCI	7.765	7.626	4.479	4.772
Simpson	0.0016	0.0016	0.0855	0.0259
HCI	0.0017	0.0017	0.0873	0.0264
LCI	0.0016	0.0016	0.0836	0.0254
Good's Lib. Coverage (%)	99.5	99.2	99.9	99.9

Clustering of OTUs found was achieved with CD-HIT and the open reference method as all taxa were selected for analysis; HCI = High Confidence Interval (95%); LCI = Low Confidence Interval (95%); OTUs = Operational taxonomic units determined at 97%.

Table 2
Geochemistry, and heavy metal pollution indexes of heavy metals in freshwater sewer that receives hospital wastewaters.

Metal	Geochemistry (mg $kgdw^{-1}$)			Pollution indexes		
Concentration	LU	L1	NESREA limit	I_{geo}	PI	Er
Cd	0.315	0.0013	0.003	7.3	242	2965
Co	2.001	0.0001	0.0001	13.7	200,010	1,000,050
Cu	7.072	0.0001	0.025	15.5	70,620	353,100
Ni	0.0021	0.0001	0.02	3.81	21	105
Pb	1.107	0.0001	0.04	12.85	11,070	55,350
Zn	25.155	12.312	0.0123	0.49	2.04	4.57

All values represent mean of triplicate analyses.
LU = Freshwater sewer that consistently receive hospital wastewater; L1 = Pristine freshwater where there is no known history of anthropogenic activities; NESREA = National Environmental Standards and Regulations Enforcement Agency, Nigeria; I_{geo} = geo-accumulation; PI = contamination index; Er = toxic response.

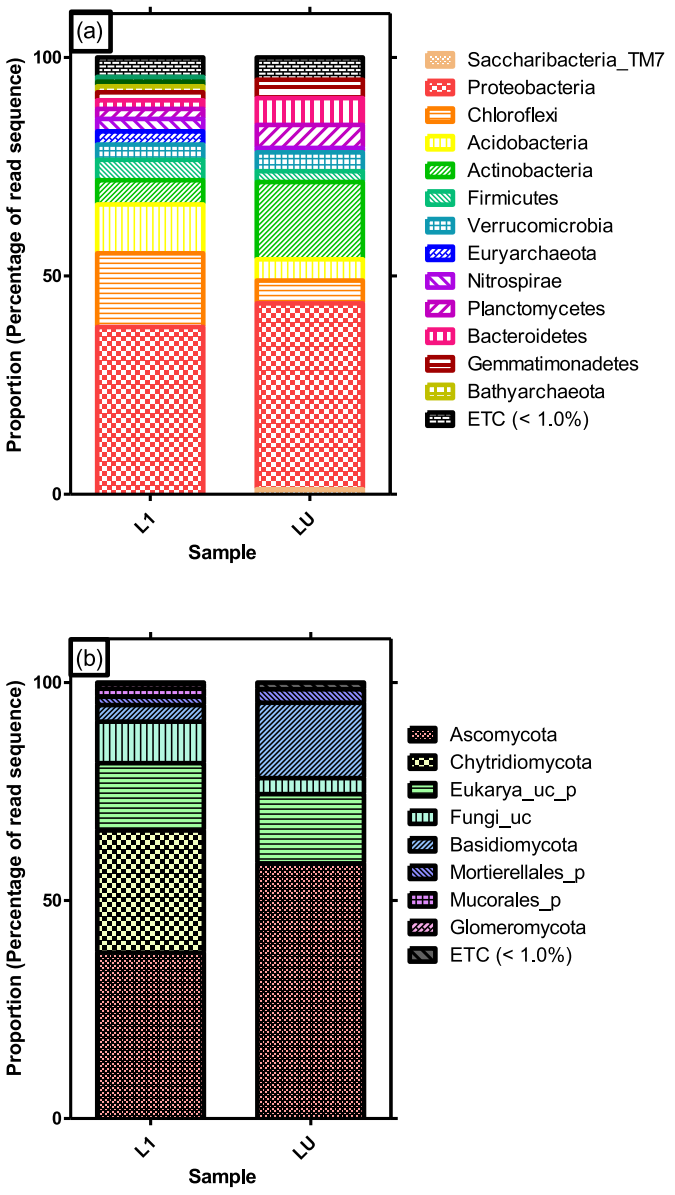


Fig. 1. Taxonomic composition of bacteria (a) and fungi (b) phyla present in the sediments of pristine environment (L1) and hospital wastewater impacted freshwater (LU). ETC represents aggregation of phyla whose sum sequences are less than 1%.

chimeric sequences (Table 4). Based on taxonomic groupings depicted in the supplementary material (Table A2) were displayed in Fig. 1 as taxonomic composition and relative abundance of bacteria (Fig. 1a) and fungi (Fig. 1b) phyla. Proteobacteria apparently dominate the impacted LU (43%) and the pristine L1 (38%). The relative abundance of Chloroflexi in the HWW impacted LU (5%) appeared to be lesser than that of L1, as similar observation was vivid with Acidobacteria (LU: 5%; L1: 11%). In the contrary, notable higher abundance of Actinobacteria (LU: 18%; L1: 6%), Plantomycetes (LU: 5%; L1: 2%), and Bacteroidetes (LU: 6%; L1: 2%) in relation to the total valid sequence reads were observed in the HWW impacted sediments in comparison with the pristine environment. Also observed among the sequence reads, were some phyla of Archaea in the pristine L1 but apparently not found in the impacted LU sediments. Fungi composition revealed that relative abundance of fungal phyla was skewed in LU where Ascomycota (59%), Basidiomycota (17%) and unclassified Eukarya_uc_p (16%) dominated the total valid sequence reads. The dominant fungal sequence reads of the pristine L1 were not only Ascomycota (38%), and Eukarya_uc_p (15%); but also Chytridiomycota (28%) that was completely absent in the impacted LU, as well as unclassified Fungi_uc that constituted 10% of the fungal sequence reads in pristine L1 but 4% in the HWW impacted LU. Other fungal phyla with relative abundance greater than 1% found in the pristine L1 but missing in the sediments of LU include Mucorales_p and Glomeromycota. Nevertheless, circular Heat map delineation of bacterial and fungal taxa and their phyla proportion in the impacted LU at cut off above 5% confirmed abundance of Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria among the bacteria taxon, while Ascomycota, Eukarya_uc_p, Basidiomycota, and Fungi_uc dominated the fungi kingdom (see Fig. A1).

3.3. Diversity and phylogeny of bacteria and fungi

A higher number of OTUs per sequence reads were consistently observed in the pristine L1 than the impacted LU as depicted in the asymptotic rarefaction curves (Fig. 2). UCLUST clustering described the detected OTUs in the rarefaction curves, such that 95% confidence intervals between the curves did not allow the bacterial and fungal OTUs in LU and L1 to overlap. Founded on UCLUST algorithm, richness and

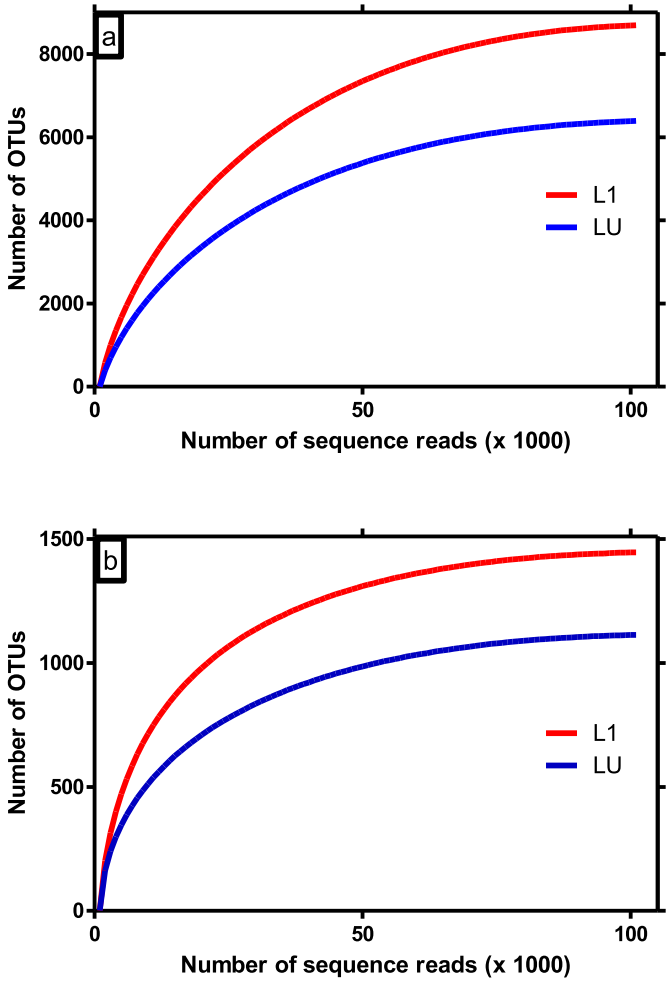


Fig. 2. Rarefaction curve of the operational taxonomy units (OTUs) of the bacterial (a), and fungal (b) sequence reads associated with the sediments. Clustering of OTUs in sediments was based on taxonomy-dependent clustering and taxonomy-based clustering (TDC-TBC), which first identified sequences at species level using a similarity-based identification method that hits against the EzBioCloud database (https://www.ezbiocloud.net/), and the sequences that cannot be identified (sequences with <97% similarity) were then subjected to a TBC to be assigned OTUs. Confidence intervals of the curves were 95%.

diversity of the bacteria and fungi were estimated as presented in Table 4. Not less than 99% of the sequences in sediments represented bacteria and fungi present in the sediments of both impacted LU and pristine L1 as portrayed by Goods libraries coverage estimator. The estimated richness via ACE, Chao1 and JackKnife described the impacted LU to contain less rich OTUs of bacteria and fungi than the pristine L1. Interestingly, fungal OTUs in the impacted LU appeared to be more diverse than those in the sediment of pristine L1 as estimated with NPShannon, Shannon and Simpson determinants. However, diversity of bacterial OTUs was observed to be higher in L1 than LU by using same diversity estimators.

Evolutionary relationships of the most abundant taxa as OTUs of bacteria and fungi in the impacted LU were delineated as contigs and calculated using UPGMA to infer dendogram (Fig. 3). Prominent among the bacterial contigs were methylotrophs, *Rhodospirillaceae, Laceyella sacchari, Solibacterales, Gemmatimonadales, Chloroflexi, Actinomyces, Asanoa hainanensis, Actinoplanes, Cytophagaceae* and *Chitiniphagaceaeis*. Whilst most of the dominant fungal contigs were not yet classified, those identified include contigs named as *Aspergillus fumigatus, Penicillium oxalicum*, coprophilous fungi, *Talaromyces pinophilus, Arthrographis kalrae, Cochliobolus australiensis, Mortierella* and *Trichosporon*. Based on Taxon XOR (Taxon Exclusive Or) Analysis by UCLUST function, bacteria and fungi taxa (20 most abundant species) that were exclusively found in pristine L1 and HWW-impacted LU sediments were presented in Table 5. It was revealed that 72,877 and 44,862 bacterial species were exclusively found in the sediments of L1 and LU, respectively. Moreover, 53,971 fungal species found in L1 sediment were found missing in HWW impacted sediment, while 42,881 fungi taxa existing in HWW impacted sediment were not found in the sediment of pristine L1. Notwithstanding, the bacteria taxa exclusively found in both sediment remained unclassified unlike the fungi taxa.

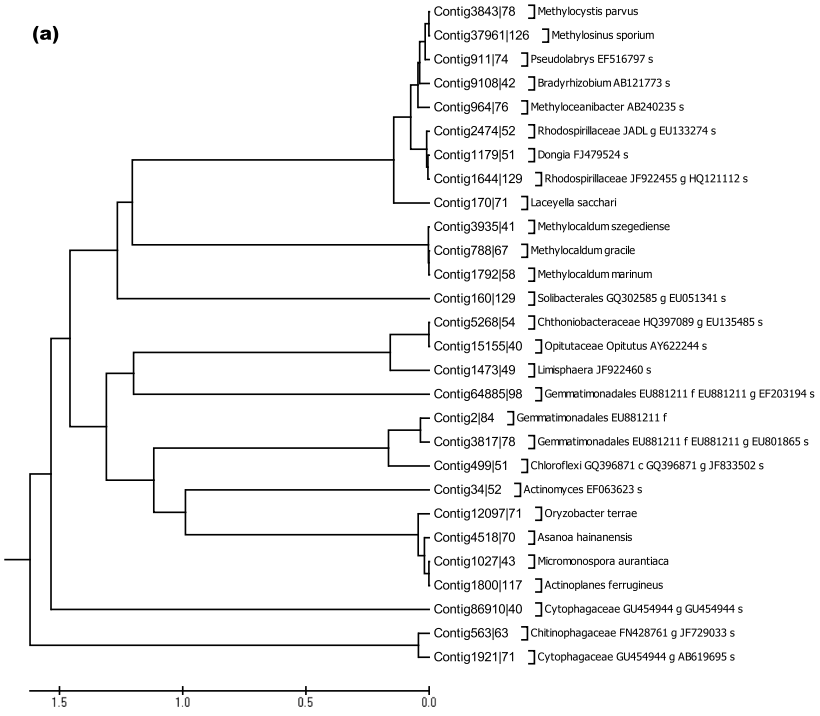
4. Discussion

The concept of assessing HWW hazards involves characterization of geochemistry and microbiome of the environments that receives the wastewater. Of paramount concern to freshwater bodies is the indiscriminate discharge of hospital wastewater without any treatment leading to release of toxic pollutant such as metals into freshwater bodies. The unregulated discharge HWW into environment is apparently the situation in LU as evident with the HM concentrations found in the sediments that is above recommended limits. HMs is collectively considered as one of the most important factors capable of influencing the aquatic environment (Rameshkumar et al., 2019). The high metal concentrations obtained are due to inability to treat effluents from hospital before being discharged into the environment. The high concentration of Pb and Cu reported in the present study agreed with previous studies (Adekoya et al., 2006; Ndimele et al., 2011). The importance of pH to biochemical functions and bioactivities in hydrosphere cannot be overemphasized, as optimum pH level of 7.0–8.5 was recommended (Rameshkumar et al., 2019). Acidic pH level of the impacted LU may be connected to the nature of the HWW, triggering the fluxes of acidophilic and acid-tolerant organisms in LU. In a related study, Bala and Mukherjee (2010) observed a pH range of 5.34–8.67, and concluded that it was due to metal pollution. Anions produced by dissociation of carboxyl and hydroxyl groups in total organic matter do result in competitive adsorption with phosphate on the surface of the sediments and thus explains the low nutrients recorded in LU.

Attempts to illuminate the pollution status and ecological risks of the HMs contained in the impacted LU via risk analysis and characterization established extreme pollution with Co, Cu, Pb and Cd exerting extremely high toxicities to the ecosystem. The HM pollution status as determined by I_{geo} and PI, cum severe degree of HM contamination that by far exceeded 32 indicated the HWW-impacted LU is severely polluted with all the HMs determined (except Zn and Ni). A very high contamination with Cd observed in the HWW-impacted LU is similar to earlier observation in sewerage impacted with industrial wastewaters (Oyetibo et al., 2019). The additional high contamination indexes of Co, Cu, Pb and Ni is an indication that HMs may be more associated with HWWs than many industry-based wastewaters. The pollution indexes of Pb and Cd were worrisome since they have no metabolic relevance to biome than exerting ecotoxicity to autochthonous organisms in the sediment of the impacted LU. The astronomically high total risk response (1,411, 575) of the impacted LU to the HMs is unprecedented and would definitely have adverse effect on autochthonous organisms in the hydrosphere. High levels of Pb and Cd in sediments have previously been reported to impact residing organisms (Laffite et al., 2016). As such, the toxic responses of Pb and Cd exceeding the very high threshold are suggested to definitely alter microbiome taxonomic profile in the HWW-impacted LU. This was depicted with a lesser total valid sequence reads and actual OTUs obtained in the HWW-impacted LU than those of the pristine L1 sediment as evidence of microbial abundances and activities.

Dominance of Proteobacteria in the two ecosystems is not unusual, but its higher relative abundance in LU than L1 may be connected to the oscillatory effect of the toxic HMs in the sediments of HWW-impacted LU. The expected diverse bacterial phyla in pristine L1 unlike skewed phyla present in the impacted LU must be due to lack of anthropogenic activities. Thus, HWW is suggested to have impaired taxonomic composition of the bacterial community in the LU ecosystem. Similarly, fungal taxonomic composition was also tilted in the impacted LU as against the pristine L1 where many fungal phyla indicated bereft ecotoxicological fluxes (Bai et al., 2019). Decrease in diversities of bacteria and fungi in the HWW-impacted LU due to toxic doses of HMs corroborates with previous reports (Oyetibo et al., 2019). A similar decrease in composition of Chloroflexi and Acidobacteria along with the abundance of unclassified Fungi_uc in LU is presumed to be as a result of high contamination of HMs that stemmed biodiversity of bacteria and fungi. The relative richness of Chloroflexi and Acidobacteria in LU was clearly reduced, while Actinobacteria increased in LU even in presence of metal pollutant. In addition, Chloroflexi is important in transforming chemical and biological contaminants present in sediments. Members of Ascomycota, Chytridiomycota etc have been found previously in freshwater sediments (Lin et al., 2019; Vargas-Gastélum et al., 2019). Ascomycota is commonly reported as the most abundant phylum in sediments (Zhang et al., 2016; Barone et al., 2018) and this agrees with the results obtained in the present study.

Phylogenies of most abundant sequences as contigs of bacteria and fungi revealed evolutionary relatedness of the taxonomic profile. Among the bacterial contigs were species that utilize methane including *Methylocystis* and *Methylosinus* genera that are pivotal to global methane cycle and biotechnological production of value-added bioproducts via methane utilization (Nguyen et al., 2018). Dominance of the methanotrophs and other anaerobes including *Rhodospirillaceae* that are active in biogeochemical cycling of nitrogen and sulfur; *Solibacterales* that reduce nitrates; and anoxygenic phototrophs, *Chloroflexi* that use halogenated organics as electron acceptors are evident that anaerobiosis prevails in the HWW-impacted LU. The anaerobiosis conditions must be due to high BOD/COD of HWWs, which must have deprived the impacted LU ecosystem of available oxygen and thus extinct autochthonous aerobic organisms. Similar phenomenon may have been responsible for abundance of contigs named as *Aspergillus fumigatus, Penicillium oxalicum*, coprophilous fungi, *Talaromyces pinophilus, Arthrographis kalrae, Cochliobolus australiensis, Mortierella* and *Trichosporon.* These fungal species are variously of ecological importance in HWW-impacted LU. For example, contigs represented by *Aspergillus fumigatus* is pathogenic (Deshmukh et al., 2020), implying the impacted LU may be reservoir for microbial pathogens; *Penicillium oxalicum* was reportedly associated with chromium bioremediation (Luo et al., 2020) and consequently may be involved in HMs sequestration in the impacted LU; and *Trichosporon* that is useful in degradation of lignocellulose (Yu et al., 2019) as evidence of coexistence of complex organic compounds with HMs in the



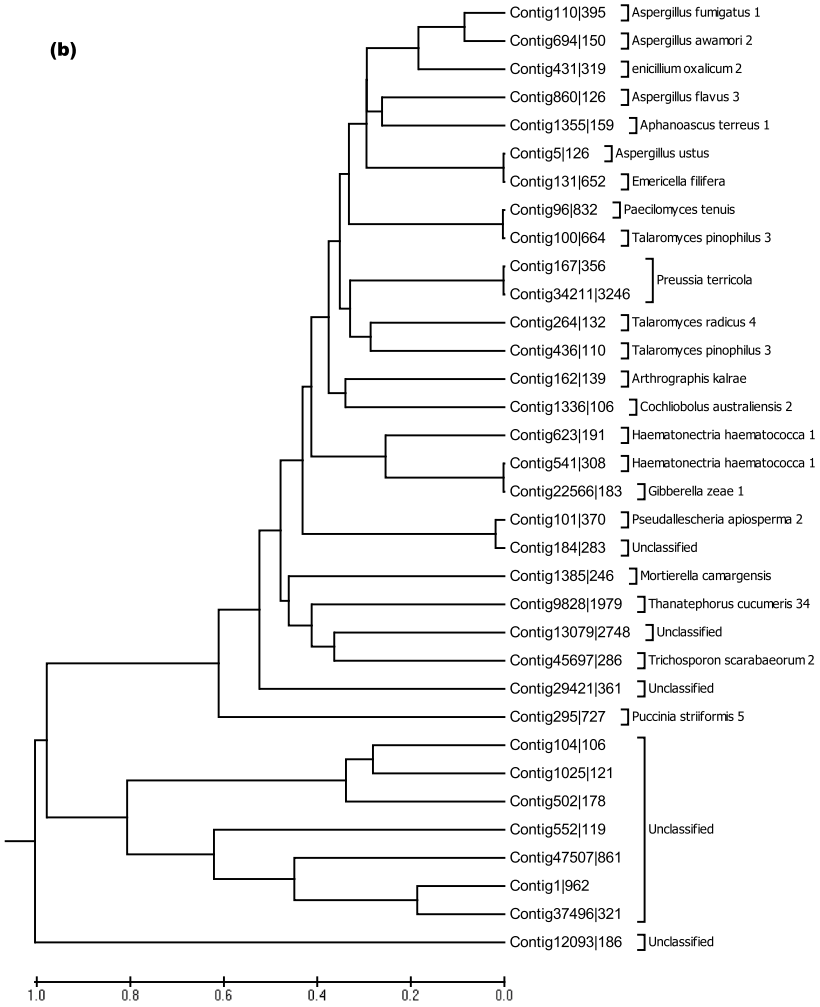


Fig. 3. Evolutionary relationships of most abundant bacteria (a) and fungi (b) taxa based on operational taxonomic units (OTUs) delineated by the OrthoAni scale as dendogram. The evolutionary history was inferred using the UPGMA. The optimal tree with the sum of branch length = 14.12054688 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 28 nucleotide sequences. Codon positions included were 1st+2nd+3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 402 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Table 5
Twenty (20) most abundant species of bacteria and fungi exclusively found in sediments of the pristine L1 and hospital wastewater (HWW) impacted LU.

Fungi				Bacteria			
Pristine L1		HWW impacted LU		Pristine L1		HWW impacted LU	
Taxa	No. of species	Taxa	No. of species	Taxa	No. of species	Taxa	No. of species
Boothiomyces macroporosum	18,007	*Preussia terricola*	4421	*AB186832_s*	847	*FJ479524_s*	353
Viridispora diparietispora	3214	*Coprinopsis cordispora_2*	3522	*FJ705115_s*	699	*HQ190573_s*	303
Rhizophagus irregularis_21	1712	*Thanatephorus cucumeris_34*	2868	*Limnobacter litoralis*	544	*EF203194_s*	256
Penicillium corylophilum	1642	*Thermomyces lanuginosus_4*	2285	*EF632757_s*	413	*Nocardioides aquiterrae*	211
Preussia terricola	976	*Haematonectria haematococca_1*	1768	*DQ386858_s*	408	*Methylocaldum szegediense*	203
Roussoella hysterioides_1	848	*Cryphonectria parasitica_2*	1196	*FJ538119_s*	406	*GQ500796_s*	194
Bipolaris micropus	618	*Sporendonema casei*	1155	*AB240284_s*	392	*JF265748_s*	176
Phialemonium dimorphosporum_4	613	*Aspergillus puniceus*	1142	*AY913276_s*	349	*EF018629_s*	169
Kazachstania telluris_1	569	*Arthrographis kalrae*	1091	*DQ125834_s*	296	*HQ910286_s*	166
Lasiodiplodia pseudotheobromae_2	486	*Cortinarius junghuhnii_1*	1054	*AB254784_s*	286	*EU335275_s*	159
Saccharomyces cerevisiae_1	415	*Verrucaria macrostoma*	504	*GQ402557_s*	264	*JF833502_s*	140
Rhizophagus irregularis	379	*Mortierella verticillata_2*	471	*AB240304_s*	255	*EF648040_s*	134
Penicillium coffeae	379	*Paraphaeosphaeria pilleata*	463	*AY163571_s*	252	*Streptomyces nanshensis*	130
Resinicium mutabile	361	*Trichosporon dulcitum*	449	*GU208301_s*	247	*AY622244_s*	129
Umbelopsis isabellina_4	282	*Pseudallescheria boydii_1*	398	*AY328730_s*	210	*FM207908_s*	129
Mucor laxorrhizus	274	*Paraconiothyrium brasiliense_3*	391	*FN391848_s*	200	*DQ413095_s*	126
Mortierella kuhlmanii	261	*Candida floris*	371	*EU735703_s*	193	*JF703442_s*	126
Umbelopsis versiformis	255	*Phomopsis phaseoli_2*	343	*AB240284_s*	191	*Plantactinospora mayteni*	124
Sporothrix stylites_1	248	*Conoplea fusca*	338	*EU155996_s*	183	*AB630582_s*	121
Hyphomucor assamensis_2	231	*Scolecobasidium terreum*	305	*GU208301_s*	182	*Jahnella thaxteri*	115

impacted LU. Large number of bacterial (72,877) and fungal (53,971) species found in L1 but missing in the impacted LU depicted susceptible species that were extinct due to HMs ecotoxicity. However, those species of bacteria (44,862) and fungi (42,881) that were exclusively found in the impacted LU may be invading strains from the HWW and possibly other sources that have adapted to the prevailing conditions and would drive natural attenuation of the impacted LU.

5. Conclusion

The findings in the present study established that discharge of HWW into sewer of freshwater impacted the environment with toxic loads of HMs that exerts ecotoxicity to the microbiome. Pollution indexes revealed the HWW-impacted sediment was severely contaminated with Co, Cu, Pb and Ni, while ecological risk assessment established that extreme ecotoxicity was exerted by Co, Cu, Pb and Cd. The impact of the toxic doses of the HMs on microbiome indicated drift in the taxonomic profile of the autochthonous bacteria and fungi with dire losses of microbial richness and diversity. Fewer phyla were observed in the HWW impacted sediment and the taxa were predominantly species that tolerate anaerobic conditions. Extinction and emergence of bacteria and fungi taxa in the impacted sediment were in response to HMs ecotoxicity and need for natural attenuation processes, respectively, which can be explored further for the bioremediation strategies of the HWW impacted sewer. The profiled taxa in the impacted sediment may be applicable in designing knowledge-based bioreactor system for the treatment of HWWs before discharge into the environment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110319.

Authors' contribution

VHO participated in sample collection, experimentation, data collation and manuscript preparation; GOO participated in experimental design, supervising experimentation, interpretation of data, and manuscript preparation; OOA participated in experimental design, and supervising experimentation.

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