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SEASONAL SURVEY OF BACTERIAL SPECIES AND THEIR BIOREMEDIATION POTENTIALS IN LEACHATES FROM A NIGERIAN MUNICIPAL SOLID WASTE DUMPSITE

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ABSTRACT: *Myriads of microorganisms present in dumpsite leachates produce varieties of extracellular enzymes that can degrade toxic compounds to innocuous products. The present study involved a seasonal survey of bacterial species and their bioremediation potentials in leachate from the Ikueniro open solid waste dumpsite situated in Uhunmwode Local Government Area, Edo State, Nigeria. Bacterial isolation and enumeration of the leachate samples was carried out using pour plate technique. Phenotypic techniques and 16S rRNA gene analysis identified the bacterial isolates. The bacterial mastermix for bioremediation of the leachate consisted of a consortium of bacterial strains isolated from leachate discharging from the Ikhueniro dumpsite. The bioremediation efficiency of the leachate was deduced by measurement of heavy metals and other chemical analytes using standard methods prescribed by the American Public Health Association. The bacterial species isolated from Ikueniro dumpsite such as Bacillus licheniformis strain EGBON & OKORIE 103 and Klebsiella aerogenes strain EGBON & OKORIE 108 were deposited in the United States NCBI GenBank. A consortium of these bacterial strains served as inocula for the bioremediation setup. A huge removal of ammonia from the leachate was observed during the 28-day bioremediation experiment, amounting to a mean bioremediation efficiency of 80.65%. The removal of the heavy metals from the raw leachate by the bacterial mastermix in increasing order was as follows: nickel<copper<zinc<lead<iron<cadmium<arsenic<mercury. Bioremediation of the leachate using indigenous bacteria promises to be an effective tool for management of toxicants from leachates of open solid waste dumpsites.*

KEYWORDS: Leachate, Bioremediation, Heavy metals, Bacterial species, Toxicants.

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INTRODUCTION

Leachate could be defined as any contaminated liquid that is generated as a result of water percolating through a solid waste dumpsite that contains soluble, suspended or miscible materials that areremoved from the waste [1]. Many factors influence the characteristics of dumpsite leachate [2]. The factors include precipitation, age, weather variation, waste types and compositions. The constituents of dissolved organic pollutants in the leachates could include acids, aldehydes and alcohols; while those of the inorganic pollutants could include phosphorous, sulphate, ammonium and heavy metals [3, 4]. Most frequent heavy metal constituents of leachates include iron, lead, nickel, cadmium, arsenic, chromium, copper and mercury; while non-metals include phenols, polyethene, pesticides, plasticizers, halogenated organic compounds such as polychlorinated biphenyls and dioxins, as well as aromatic hydrocarbons such as toluene, ethylbenzene, benzene and xylene. All these could constitute serious health hazards if present in excess of the permitted values. Genotoxic compounds are heavy metals which include zinc, copper, mecury, asenic, lead, nikel and cadmium. Exposure to these genotoxic compounds could cause genomic instabilities and/or epigenetic alterations which can result in several diseases including cancer [5]. This epigenetic disruption has been reported from studies on leachates from Ajakanga dumpsite, Nigeria [6, 7].

The leachates discharged from the dumpsites often emit smelly odours carrying volatile health risk pollutants to humans residing close to the dumpsite [8]. Gases emitted could include carbon dioxide (CO_2), methane (CH_4), nitrogen dioxide (NO_2) and sulphur dioxide (SO_2) [9].

The remediation of dumpsite leachates using microorganisms is a cost-effective approach when compared to the conventional physicochemical treatment processes [10]. Myriads of microorganisms present in the leachates produce a variety of extracellular enzymes that can degrade toxic compounds to innocuous products. Studies have found that certain bacteria, fungi and actinomycetes belonging to the phyla *Firmicutes, Actinobacteria*and *Proteobacteria* possess efficient degrading potential [11]. These leachate-degrading microorganisms are also known to be tolerant of heavy metals. Leachates from young dumpsites (≤ 10 years old) are more effectively treated with biological treatments [12], while leachates from intermediate or mature dumpsites (≥ 10 years of age) are best treated with physiochemical methods [13, 14, 15].Low cost and simplicity in operation make bioremediation the method of choice in the treatment of leachates.

The biological treatment techniques that are used to remove pollutants such as heavy metals and organic constituents in leachates include sequence batch reactors, trickling filter, rotating biological contactor, upflow anaerobic sludge blanket, aerated lagoon, activated sludge and sequencing batch reactor [9, 16, 17, 18, 19]. The present study used the trickling filter technique for bioremediation of raw leachate from the Ikhuniro dumpsite.Matthews *et al.*[20] used passive aeration trickling filters to treat landfill leachates from Wales, England, and were able to measure the effects of leachate characteristios and temperatue on the rates and process dynamics of trickling filters. There results revealed a significant reduction of ammonium-nitrogen in the bioremediated leachate when compatred to the raw leachate. Aluko and Sridhar [21] also carried a study to evaluate the trickling filter biological treatment

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of raw leachate from a municipal solid waste dumpsite in Ibadan which continuously discharged into Omi stream and its tributaries in Ibadan, southwest Nigeria. The study reported that leachate treatment using the trickling filter system produced effluents with significant reductions in suspended solids (SS) (73.17%), turbidity (71.96%), biochemical oxygen demand (76.69%) and ammonia (59.50%). Aluko and Sridhar [21] confirmed that the effluents produced by this biological treatment trickling filter technique were better in quality though the mean residual concentrations for colour, suspended solids and dissolved solids; though $BOD_5$and iron were above the national regulatory standards for discharge into surface water bodies. Ther findings of Aluko and Sridhar [21] were in accordance with the study of Mondal and Warith [22] who worked on treatment of landfill leachate in Ontario, Canada using the trickling filter technique.The trickling filter is an oxygenated, aerobic treatment method and easy to construct with simple operating method. It is majorly used for the treatment of domestic wastes.This simple trickling filter process involves the passage of the leachates over the surface of a fixed bed, consisting of the media in which biological films grow; thus helping to stabilize the organic matter in the leachates[20, 23].

The dumpsite investigated in the present study is the Ikhueniro open solid waste dumpsite located in Benin City, Edo State, Nigeria. The Edo State Waste Management Board (EDSWMB) is saddled with the operation of this dumpsite. The dumpsite occupies a land area of approximately 2 hectares. The wastes are haphazardly deposited in this dumpsite without segregation. However, some private individuals do sometimes visit the dumpsite to sort metal and plastic wastes for recycling. The Ikhueniro dumpsite is generally characterized by spontaneous fires largely as a result of spontaneous combustion that is very rampant during the dry season. There are no reports on studies in Ikhueniro open solid waste dumpsite in respects of physicochemical parameters as well as bacterial and fungal composition in the leachates and surrounding biotic and abiotic components of the environment. Also bioremediation studies of the leachate are lacking. This study hopes to develop a cost-effective bioremediation protocol that can remove organic and inorganic pollutants from the leachates for probable integration into the Ikhueniro dumpsite.

MATERIALS AND METHODS

Description of study area (Ikhueniro dumpsite)

The Ikhueniro open solid waste dumpsite occupies an area of approximately 20000 square meters (200 m × 100 m) situated in Uhunmwode Local Government Area OF Edo State. It lies within longitude of 6.6342^{o}N and latitude of 5.9304^{o}E, and wastes are dumped all year round. Pictorial presentations describing some sections of the Ikhueniro dumpsite is shown in Figure 1.The percentage composition of these wastes were estimated at 70%, 5%, 10%, 4%, 3%, 4% and 4% for the food wastes materials, metals, papers, textiles, plastics, glass and other miscellaneous wastes, respectively.The communities surrounding the Ikhueniro dumpsite include Aduhenhen, Irighon, Iguomon, Okeze, Iguevbiahiamwan, Irhue, Igueuwanghe, Ugiemwan, Igieduwa, Oghehe and Ikhuen. The distance of these neighboring communities to Ikhueniro dumpsite ranged from 20km to 150km.The Ikhueniro dumpsite has a tropical climate, with an annual temperature that ranged from 23.78^{o}C to 29.98^{o}C, and an annual precipitation that ranged from 22.44 mm to 332.95 mm. There is a well-defined raining season which occurs from April to October and the dry season that occurs from

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November to March. The soil type around the Ikhueniro dumpsite is generally regarded as sandy clay loam soil. Conventional fishing (Catfish) activities and crops farming by the local inhabitants are undertaking at the vicinity of the Ikhueniro dumpsite. Most of the vegetation planted by farmers around the vicinity of the Ikhueniro dumpsite includes Cassava (*Manihot esculenta*) Okro (*Abelmoschus esculentus*) Pepper (*Capsicum annuum*) Tomatoes (*Solanuum lycopersicum*) and Garden egg (*Solanuum malegenum*) plantations. The wild vegetations seen growing at the Ikhueniro dumpsite were *Capsicum annuum*, *Solanuum. lycopersicum*, Water melon (*Citrullus lanatus*), Melon (*Cucumis melo*), garden egg (*Solanuum malegenum)* and Pawpaw (*Carica papaya*) plants, particularly during the rainy season.

Figure1: Pictorial presentations describing some sections of the Ikhueniro dumpsite

Sampling periods

The seasonal periods of field sample collections were July to October 2019 for rainy season; and November 2019 to February 2020 for dry season. Samples were collected two different times each month, making a total of 8 rainy season samples and eight dry season samples. The sites of field samples collection from the Ikhueniro dumpsite, as well as surrounding communities and the adjoining Okhuahe River are shown on the map in Figure 2. Triplicate samples of raw leachate were randomly scooped from the dumpsite to a depth of 15 cm on each visit to the Ikhueniro dumpsite. All the samples collected from the Ikhueniro dumpsite were stored in ice-pack and transported to the laboratory for bacteriological analysis.

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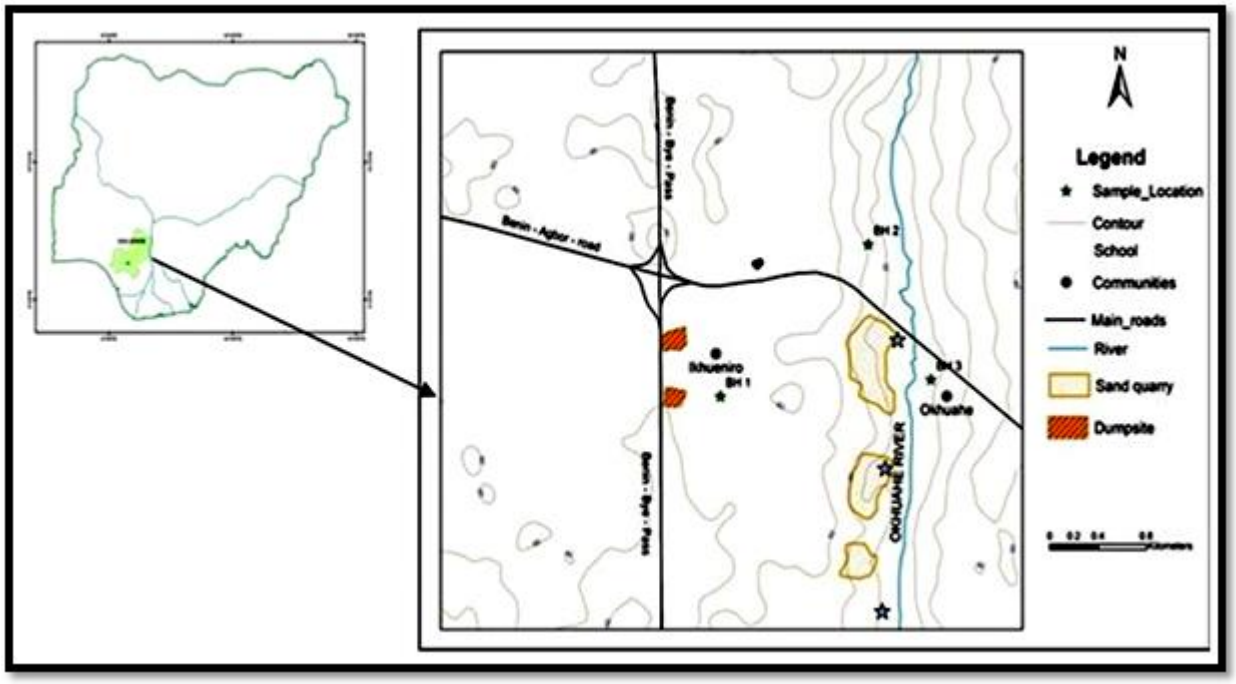

Figure 2: Map showing sites of field sample collection, neigbouring communities and the adjoining Okhuahe River

Isolation of leachate-degrading bacteria

Bacterial isolation and enumeration of the samples was carried out using the pour plate technique [24]. Serial dilution of samples was made up to 10^{-10}, with the first dilution of the leachate samples made by mixing 25 ml of leachate with 225 ml of sterile 1.5% peptone water in a sterile tube. One millilitre of each the serially-diluted samples was separately poured into duplicate sterile Petri dishes and then respectively mixed with 15ml of nutrient agar (NA) and MacConkey agar (MA) media. The NA and MA Petri dishes were subsequently incubated at room temperature for 72 hours. After incubation, bacterial colonies were counted with the colony counter and counts on NA and MA Petri plates were respectively reported as total heterotrophic plate count (HPC) and total coliform count (TCC). The counts were expressed as colony-forming units per milliliter (CFU/ml) of the leachate sample.

Genus-level identification of bacterial colonies

The phenotypic techniques employed for the genus-level identification of bacterial isolates obtained from all sampling sites were done with standard methods [25]. The phenotypic tests included the Gram-staining, coagulase, catalase, oxidase, citrate, methyl red, Voges-Proskauer and haemolysis tests.

Species-level identification with molecular methods

Species-level identification employed a technique which involved partial 16S rRNA gene analysis that was performed with polymerase chain reaction (PCR) and sequencing methods [26]. Zymo-Spin column was used to extract the DNA templates as prescribed by the manufacturer (Zymo Research Corporation, USA). The ultra-pure DNA template used to

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perform the polymerase chain reaction (PCR).Universal 16S rRNA bacterial primers [27F-AGAGTTTGATCMTGGCTCAG; 1492R- GGTTACCTTGTTACGACTT] [27]often employed for bacterial taxonomy was used to determine the presence of 16S rRNA gene. The PCR protocol was carried out with a 50 µl reaction mixture containing 2 µL of template DNA, 10mM Tris-HCL (pH 8.3), 50mM KCl, 2 mM $MgCl_2$, 200 mM of each of the deoxynucleoside triphosphates (dNTPs) (Fermentas Inc., USA), 2 U of GoTaq Hot Start Polymerase (Promega, USA) and 0.5 µM of each primer. Amplification was done on a GeneAmp PCR system 9700 (Applied Biosystems) with the following cycling conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles, with each cycle consisting of denaturation at 94°C for 45 seconds; annealing at 55ºC for 60 seconds; extension at 72ºC for 120 seconds; and a final extension at 72°C for 300 seconds. Ten microliters of the amplified product was analyzed by gel electrophoresis on a 2% agarose prepared in Tris-Borate-EDTA buffer containing 0.5 µg/ml of ethidium bromide at 100 V for 1 hour. The DNA band in the gel was subsequently visualized and documented on the gel documentation system (Applied Biosystems). A molecular marker (100 base pair ladder) was run concurrently. DNA sequencing of the amplicon was performed with the dideoxy-chain termination method [28]. The amplicon was cleaned up with ExoSAP-IT (ThermoFisher Scientific). The purified amplicon was then subjected to cycle sequencing with the Big Dye Terminator version 3.1 (Applied Biosystems) using standard cycling conditions. The purified cycle sequencing product was separated by capillary electrophoresis on an ABI 3730×I DNA analyzer. The sequence was then quality checked and proofread with Sequencher version 4.10.1 (Gene Codes Corporation, USA).

The taxonomic classification of isolates was confirmed by a comparison of the experimentally determined nucleotide sequence against the sequence database (rRNA_typestrains/prokaryotic_16S_ribosomal_RNA). The sequence comparison was performed with the BLASTN 2.8.0 + program [National Center for Biotechnology Information (NCBI)]. The identity of a bacterial species was confirmed if the query 16S rRNA sequence of the bacterial isolate matched with the reference (subject) 16S rRNA sequence of a bacterial species in the GenBank in terms of sequence similarity and sequence identity.

Phylogenetic analysis

16S rRNA gene sequences of representative bacterial strains collected from Ikhueniro dumpsite were compared with reference strains from other environmental sources to determine the extent of the evolutionary relationship between these strains with a view to inferring their ancestral lineages. Alignment of the sequences was performed with MUSCLE bioinformatics tool [29] *via* MEGA version 6 software [30]. The phylogenetic tree was then generated using the Neighbor-Joining algorithm in MEGA- version 6 software. The robustness of the groupings in Neigbor-Joining analysis was assessed with 10,000 bootstrap iterations.

Preparation of the bacterial consortium (bacterial mastermix)

The bacterial mastermix consisted of a consortium of bacterial strains isolated from leachates discharging from the Ikhueniro dumpsite. To prepare the consortium, isolated bacterial strains were separately inoculated into nutrient broth medium and incubated at a temperature of 35 ºC for 18 hours in a shaker incubator set at 150 revolutions per minute (rpm). After

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incubation, the bacterial cells were then harvested by centrifuging the cultured broth at 5000 rpm for 15 minutes to pellet out the bacterial cells. The pelleted bacterial cells were further re-suspended in 0.9% sodium chloride solution (normal saline). The turbidity of each of the bacterial suspensions was adjusted to an optical density of 0.1 (OD_{600nm} = 0.1), with equivalent concentration in the range of 10^9 cells per milliliter of suspension. Equal ratios of the suspensions from each of the bacterial strains were mixed to constitute the mastermix used as inoculum for the bioremediation protocols [31].

Bioremediation setup

The bioremediation protocols were as described [32, 33]. The columns in five separating funnels were packed with 400 g sterile soil (soil subjected to dry heat at 170^{o}C for 1 hour) to a height of 20 cm. The columns in three of the separating funnels were inoculated with a 250ml microbial mastermix (OD_{600nm} = 0.1), consisting of a consortium of bacterial strains isolated from leachates collected from the Ikhueniro dumpsite. The other two columns were not inoculated with the bacterial mastermix, and served as control. The inoculated soils in the three columns were incubated for 48 hours at room temperature after which the liquid were allowed to drain off. Raw leachate (200 ml) collected from the Ikhuniro dumpsite was separately mixed with the cultured soils in the three test columns, as well as with the uncultured soils in the two control columns. The bioremediation setups were held at room temperature with intermittent agitation for 28 days, after which leachate effluent samples were collected from the inoculated and uninoculated columns for analysis. Leachate samples were analyzed before and after all bioremediation treatments for biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia, phosphate, nitrate, copper, iron, zinc, nickel, lead, cadmium, arsenic and mercury.

Bioremediation efficiency (BE)

BE was deduced based on the percentage removal of heavy metals and other chemical constituents from raw leachate collected from the Ikhueniro dumpsite using the following formula:

$$\text{BE (\%)} = \frac{(\text{Co}-\text{Ce})-(\text{Co}-\text{Ci})}{\text{Co}} \times 100$$

Co is the initial concentrations of analytes in the raw leachate collected from the Ikhueniro dumpsite. *Ce* is the residual concentrations of analytes in the columns inoculated with the bacterial mastermix. *Ci* is the residual concentrations of analytes in the uninoculated columns.

Measurements of the analytes

The measurements of heavy metals and other chemical analytes were carried out according to the standard methods prescribed by the American Public Health Association (APHA) [34]. All the samples were acidified using nitric acid for reducing the pH to less than 2 to prevent the precipitation of metals. Flame atomic absorption spectrometer (FAAS) supplied by Perkin Elmer was used for the analysis of the heavy metal constituents in the leachate samples. Calibration standards were prepared from AAS grade reagent for all the heavy metals of interest.

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Statistical analysis

The NCSS ver. 12 data analysis software was used to carry out descriptive statistics of the datasets obtained from the study. Shapiro-Wilk normality test and Levene's test of homogeneity of variance, as well as the analysis of the variance (ANOVA) by the parametric Student's t-test and non-parametric Mann Whitney U (Wilcoxon rank-sum) test were also performed with NCSS ver. 12. The test of the hypothesis was considered statistically significant if the achieved level of significance (p) was less than 0.05.

RESULTS

Bacterial counts

The bacterial counts in leachates collected from the Ikhueniro dumpsite is presented in Table 1. The mean HPC of the raw leachate during the rainy and dry season were estimated at 7.41 ± 0.16 $\log_{10}$ CFU/ml and 5.52 ± 0.15 $\log_{10}$ CFU/ml, respectively. The HPC datasets of the raw leachate during the rainy and dry seasons were normally distributed ($p > 0.05$) with equal variance ($p > 0.05$). Student's t-test showed a significant difference ($p < 0.05$) between the mean HPC values of raw leachate during the rainy season when compared to those obtained during the dry season. The non-parametric Mann Whitney U (Wilcoxon rank-sum) test showed that there was a significant difference ($p < 0.05$; $p = 0.002$) between randomly. The mean TCC of the raw leachate during the rainy and dry season were reported as 5.80 ± 0.12 $\log_{10}$ CFU/ml and 3.59 ± 0.15 $\log_{10}$ CFU/ml, respectively. The TCC datasets of the raw leachate during the rainy and dry seasons were not normally distributed ($p < 0.05$) with equal variances ($p > 0.05$). The non-parametric Mann Whitney U (Wilcoxon rank-sum) test showed a significant difference ($p < 0.05$) between the randomly selected TCC values of the raw leachate during the rainy season and those obtained during the dry season.

Bacterial taxonomy

Tables 2 present the phenotypic and molecular characterizations of bacterial isolates obtained from the raw leachates. The bacterial isolates that were found in these leachates were mainly identified as *Micrococcus luteus*, *Staphylococcus saprophyticus*, *Bacillus licheniformis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Serratia marcescens*, *Enterobacter cloacae*, *Streptococcus pneumonia* and *Klebsiella aerogenes*. During the rainy and dry seasons, the most abundant bacterium in the raw leachate was *B. licheniformis* (22.92% and 52.08% during the rainy and dry seasons, respectively) and the least occurred bacterium was *S. marcescens* (2.08%) during the rainy season, while it was *E. cloacae* (2.08%) during the dry season. Representative bacterial strains isolated from the raw leachate were subsequently deposited in the United States National Center for Biotechnological Information (NCBI) GenBank database. Representative *B. licheniformis* strain EGBON 103, *E. coli* strain EGBON and OKORIE 102, *E. cloacae* strain EGBON and OKORIE 107, *K. aerogenes* strain EGBON and OKORIE 108, *M. luteus* strain EGBON and OKORIE 105, *P. vulgaris* strain EGBON and OKORIE 104, *S. aureus* strain EGBON and OKORIE 101, *S. marcescens* strain EGBON and OKORIE 106, *S. pneumoniae* strain EGBON and OKORIE 109 isolated from the leachate were deposited in the NCBI GenBank under accession numbers MZ768961, MZ768960, MZ768996, MZ769002, MZ768991, MZ768963, MZ768959, MZ768992, MZ769059.

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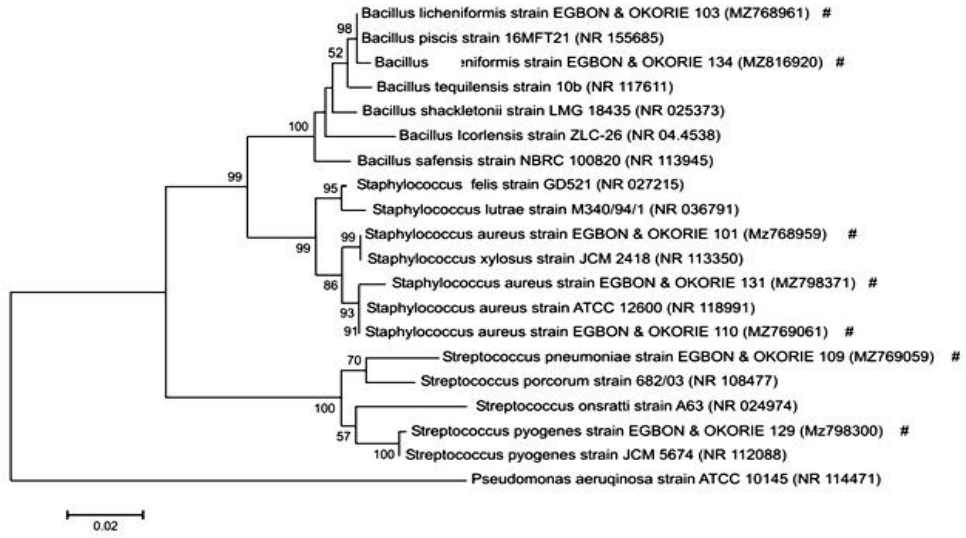
Phylogeny

Phylogenetic trees highlighting the evolutionary relatedness of bacterial strains, belonging to the phyla Firmicutes, Actinobacteria and Proteobacteria, that were found in the dumpsite/adjoining river and reference bacterial strains that were isolated from other environmental sources in the world are shown in Figures 1 to 3. As shown in Figure 1, *B. licheniformis* strain EGBON and OKORIE 103 (MZ768961) and *B. licheniformis* strain EGBON and OKORIE 134 (MZ816920) isolated in the present study were found to have shared a common ancestry with *B. piscis*strain 16MFT21 (NR 165685) isolated from the muscle of the Antarctic fish *Dissostichus mawsoni*, with a 98% likelihood. There was 99% likelihood that *S. aureus* strain EGBON 101 (MZ768959) and *S. xylosus* strain JCM 2418 (NR 113350) collected from Japan evolved from a common ancestor. A common ancestor was also inferred to be the origin of evolution for *S. pyogenes* strain EGBON and OKORIE 129 (MZ798300) and *S. pyogenes* strain JCM 5674 (NR _112088), a bacterium isolated from raw milk obtained from Japan, with a 100% likelihood. There was an 83% likelihood that *M. luteus* strain EGBON and OKORIE 111 (MZ769063) and a South Korean-derived *M. luteus* strain NCTC 2665 (ON222729) evolved from the same ancestry, with 83% likelihood (Figure 2). Figure 3 showed that *P. vulgaris* strain EGBON 104and OKORIE (MZ768963) and *P. vulgaris* strain ATCC 29905 (MN326681) from India shared a common ancestry, with a likelihood of 91%; while there was 82% likelihood that the Japanese-derived *S. marcescens* strain NBRC 102204 (NR 114043) and *S. marcescens* strain EGBON 106 and OKORIE (MZ768992) originated from a common ancestry. *E. cloacae* strain EGBON and OKORIE 107 (MZ768996) and *E. cloacae* strain DSM 30054 (NR 117679) collected from India were also found to share a common ancestry, with a 94% likelihood.

Figure 1: Phylogenetic tree highlighting some bacterial strains of the Phylum Firmicutes constructed with the neighbour-joining method



 # is used to indicate some novel bacterial strains of the Phylum Firmicutes isolated from the Ikhueniro dumpsite. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50% are displayed on branches.

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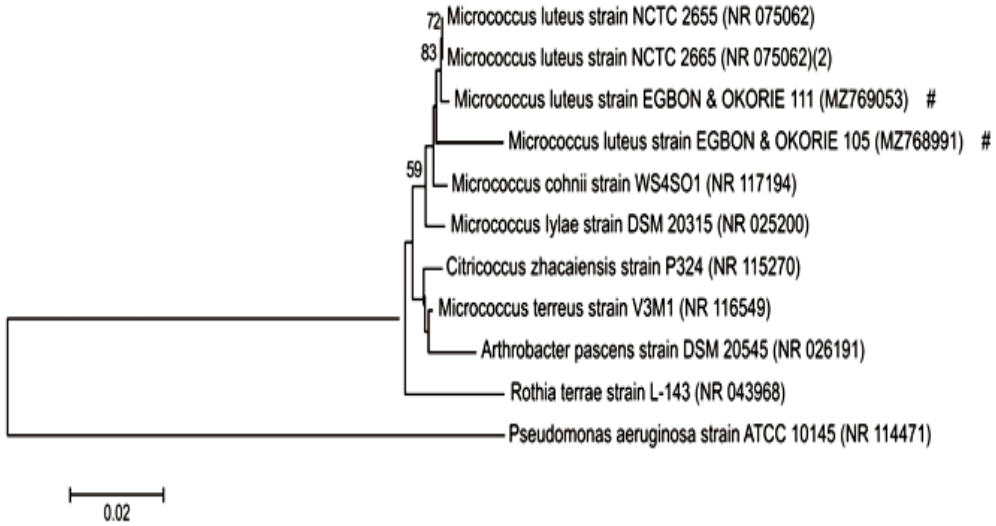
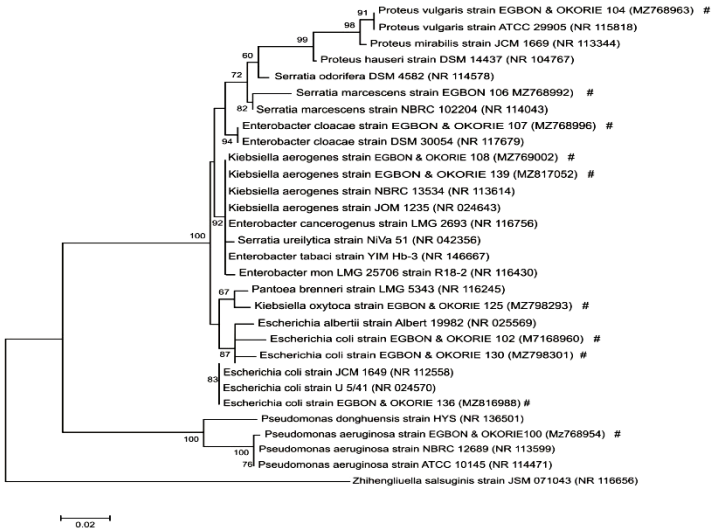
Figure 2: Phylogenetic tree highlighting some bacterial strains of the Phylum Actinobacteria constructed with the neighbour-joining method



 # is used to indicate some novel bacterial strains of the Phylum Actinobacteria isolated from the Ikhueniro dumpsite. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50% are displayed on branches.

Figure 3: Phylogenetic tree highlighting some bacterial strains of the Phylum Proteobacteria constructed with the neighbour-joining method



 # is used to indicate some novel bacterial strains of the Phylum Proteobacteria isolated from the Ikhueniro dumpsite. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50% are displayed on branches.

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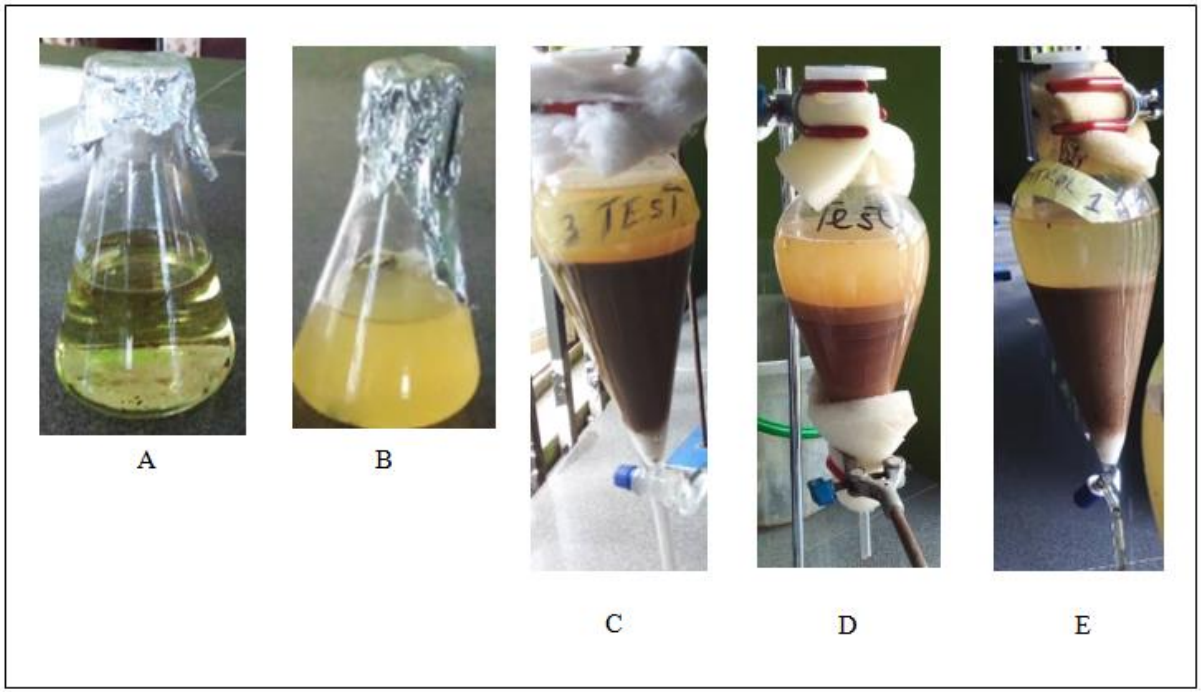
Bioremediation potential of the isolated indigenous bacterial strains

A pictorial presentation of the bioremediation setup is shown in Figure 4. The bioremediation efficiency of the bacterial consortium from Ikhueniro dumpsite on its raw leachates during the period of the bioremediation experiment is presented in Table 3. A huge removal of ammonia from the leachates was observed during 28-day bioremediation, amounting to a mean bioremediation efficiency of 80.65%. However, nitrate removal from the leachates appeared to be low, as shown by a mean bioremediation efficiency of 5.95% at day 28. Amongst the heavy metals present in the raw leachate, mercury was mostly removed from the leachate by the bacterial mastermix during the 28-day bioremediation, amounting to a bioremediation efficiency of 45.05%. Nickel was the heavy metal that was least removed by the bacterial mastermix during the 28-day bioremediation experiment, with the efficiency of bioremediation of nickel estimated at 14.71%. The removal of the heavy metals in the raw leachate by the bacterial mastermix in increasing order was as follows: nickel<copper<zinc<lead<iron<cadmium<arsenic<mercury.

Figure 4: The bioremediation setup



A: the sterile un-inoculated broth used for propagating the bacterial consortium. B: turbid broth after inoculation with the bacterial consortium. C and D: test columns containing the bacterial consortium. E: control column without the bacterial consortium.

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DISCUSSION

The dumpsite investigated in the present study is the Ikhueniro open solid waste dumpsite located in Benin City, Edo State, Nigeria. The Edo State Waste Management Board (EDSWMB) is saddled with the operation of this dumpsite. The mean HPC (Mean HPC = 7.41 ± 0.16 $\log_{10}$ CFU/ml and 5.52 ± 0.15 $\log_{10}$ CFU/ml during rainy and dry seasons, respectively) and TFC (Mean HPC = 5.47 ± 0.17 $\log_{10}$ CFU/ml and 4.29 ± 0.13 $\log_{10}$ CFU/ml during rainy and dry seasons, respectively) of the leachate reported in the present research agreed with those reported by Michaela *et al.* [35] who analyzed leachates from dumpsites in Onitsha, Nigeria. The mean TCC of the leachate recorded during the rainy and dry seasons (Mean TCC =5.80 ± 0.12 $\log_{10}$ CFU/ml and 3.59 ± 0.15 $\log_{10}$ CFU/ml during rainy and dry seasons, respectively) were at variance with the TCC of leachates from a dumpsite in Poland reported in the study of Szulc *et al.* [36]. Other authors have reported varying microbial load in leachates from different dumpsites. Azuna *et al.* [37] carried out a study on bacteriology of leachates from Government-approved dumpsites in Port Harcourt and Environs, Rivers State, Nigeria during the rainy and dry seasons. They reported that during the dry season, mean total coliform counts (TCC), total heterotrophic bacteria counts (THC) and total heterotrophic fungi counts (TFC) were 161±70.71 CFU/g, 173±19.80 CFU/g and 98.5±19.09 CFU/g, respectively. During the rainy season, mean TCC, THC, TFC were reported as 4290±1598 CFU/g, 390000±710000 CFU/g, 125000±105000 CFU/g.The seasonal variations show that the leachates during the rainy season had higher bacteria counts which may be due to increased washing of bacteria into the leachates, as well as the effects of anthropogenic activities. Microbial processes in dumpsites must be put in the context of changing societal practices [38]. Components such as hazardous wastes, plastics, garden waste, construction and demolition wastes, industrial wastes, recyclable materials and the presence of various contaminants of emerging concern have varied substantially through time, including both potential substrates for microbial metabolism as well as potential inhibitors of microbial growth [39]. The microbes that were isolated from the leachate were similar to those that were reported in the study of Szulc *et al.* [36] who investigated an illegal landfill in Central Poland, as well as those of Nasir *et al.* [40] who used 16S rRNA gene Sanger sequencing technique to identify bacterial species present in raw leachate from Gosa dumpsite, Abuja, Nigeria.The bacteria isolated from leachates obtained at the Ikhueniro dumpsite included *E. coli*,*P. aeruginosa*, *B. licheniformis*, *S. aureus*,*S. saprophyticus*, *S. pneumoniae*, *Micrococcus luteus*, *P. vulgaris*, *S. marcescens*, *K. aerogenes* and *E. cloacae*. These bacterial isolates are of public health significance. *S. aureus* produces myriads of enterotoxins [41]. *Micrococcus* species are ubiquitous in soil and water. *Enterobacter* species are dominant coliforms, though some non-fecal *Enterbacter* species inhabit vegetations and soil. The preferred carrier matrix for bacterial consortium used in the present research was soil. This was based on its low cost and extensive use in previous bioremediation studies of toxic compounds [42, 43]. The huge removal of ammonia observed at the end of 28-day bioremediation in the present research agreed with the removal efficiency of ammonia reported in the study of Morris *et al.* [11] who carried out bioremediation of leachates collected from Powerstown Landfill, Co Carlow, Ireland using isolated bacterial strains. Several studies have reported that ammonia is frequently present at elevated concentrations in both young and mature dumpsite leachates [44, 45, 46, 47]. As indicated by Bashir *et al.* [45], ammonia eradication is of prime concern since its level continues to surge as the dumpsite ages. Further studies such as the recirculation of leachates through column systems in association with the use of physicochemical treatment such as reverse osmosis or ammonia stripping are advocated to

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remove ammonia to the stringent levels that are stipulated by regulatory agencies. This advocacy is particularly important because discharged leachates during the methanogenic stage in dumpsites frequently do not effectively respond to biological treatment, hence the need to implement physicochemical treatment strategies. Ammonia is known to be very toxic to microorganisms, thus a potential inhibitor of the biological degradation process; which is why it is necessary to use a microbial consortium that can adapt to high levels of ammonia during bioremediation studies. The concentrations of zinc and cadmium in the raw leachates from the Ikhueniro dumpsite that was used for bioremediation study significantly agreed with the values reported by Fauziah *et al.* [48] who worked with dumpsite leachates from Malaysia. Values of cadmium and iron in the raw leachates significantly agreed with those (cadmium = 0.50 mg/l and iron = 10.22 mg/l) recorded in the study of Asibor *et al.* [49] who worked with leachates from a dumpsite in Delta State, Nigeria. However, values of mercury, arsenic and nickel in the raw leachates were at variance with values obtained from the Malaysian dumpsite [48]. Values of all the heavy metals in the raw leachate significantly exceeded the limits set by FEPA, the Nigerian regulatory agency. The nature of solid wastes in the dumpsites significantly contributed to the high concentration of metals recorded at the Ikhueniro dumpsite. Galvanized materials and other metal scraps seen at the Ikhueniro dumpsite might most likely have resulted in the high concentration of heavy metals in the leachates. A myriad of mechanisms such as the influx and efflux mechanisms, inclusive of formation of metal complexes by precipitation and adsorption are used by microbes to remove heavy metals from leachates [50]. Nickel was least removed from the leachate by the leachate-degrading bacterial mastermix during the 28-day bioremediation experiment. However, in the study of Fazzino *et al.* [51] who worked with leachates from Italy, nickel was relatively rapidly removed from the leachates with an efficiency of approximately 60% at the 30th day of the bioremediation operation. The significant difference in efficiencies of bioremediation in both the test and control columns in the present study further confirmed that microbes have the potentials to remediate heavy metals in leachates. The variations observed in heavy metals removal in the columns are most probably due to the specificity of microbes to specific heavy metals, as well as differential tolerances to heavy metals.

CONCLUSION

Microbes, heavy metal and other chemical contaminations are widespread in leachates due to poor waste management system at the Ikhueniro dumpsite. This contributes to high levels of heavy metals above the limits set by regulatory agencies. Certain microorganisms have the ability to survive and transform heavy metals to less toxic forms. From this study, it can be concluded that microbes have higher potentials for remediating heavy metals and other toxicants from dumpsite leachates, as observed in the significant removal of mercury, arsenic, cadmium and lead, as well as ammonia from the leachate at the Ikhueniro dumpsite.

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Table 1: Bacterial counts in the raw leachates

Season of sampling	Mean HPC (Log_{10} CFU/ml) N = 8	Mean TCC (Log_{10} CFU/ml) N = 8
Rainy (July – Oct 2019)	7.41 ± 0.16	5.80 ± 0.12
Dry (Nov 2019 – Feb 2020)	5.52 ± 0.15	3.59 ± 0.15

N: number of samples examined. HPC: Heterotrophic plate count. TCC: Total coliform count. Mean count represented as mean ± standard error of mean.

Table 2: Phenotypic and molecular characterizations of bacterial isolates obtained from the raw leachates

Season of sampling	Representative isolates	Morphology on Petri plates	Microscopy	Biochemical examinations									Molecular tests		Identified bacteria	Frequency of occurrence	
				Co	Ca	Ox	Ci	In	Mr	Vp	La	Ma	16S similarity	16S identity		F	P (%)
Rainy	1	Yellow colony on NA plate	Gram positive cocci	-	+	+	+	-	+	-	-	+	93 – 97%	92 – 94%	*Micrococcus luteus*	2/48	4.17
	2	Yellow colony on NA plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	-	+	98 - 100%	99 - 100%	*Staphylococcus saprophyticus*	10/48	20.83
	3	Dry colony on NA plate	Gram positive rods	-	+	-	-	-	-	+	-	+	94 - 99%	95 - 99%	*Bacillus licheniformis*	11/48	22.92
	4	Mucoid colony on NA plate	Gram negative rods	NP	+	-	-	+	+	-	+	-	98%	100%	*Escherichia coli*	4/48	8.34
	5	Green colony on NA plate	Gram negative rods	NP	+	+	+	-	-	-	-	-	100%	99%	*Pseudomonas aeruginosa*	3/48	6.25
	6	Swarming colony on NA plate	Gram negative rods	NP	+	-	+	+	+	-	-	-	100%	97%	*Proteus vulgaris*	2/48	4.17
	7	Red colony on NA plate	Gram negative rods	NP	+	-	+	-	-	-	-	-	100%	98%	*Serratia marcescens*	1/48	2.08
	8	Mucoid colony on NA plate	Gram negative rods	NP	+	-	+	-	-	+	-	+	95 - 99%	97 - 99%	*Enterobacter cloacae*	4/48	8.34
	9	Mucoid colony on NA plate	Gram positive cocci in chain	-	-	-	+	-	+	-	+	-	94 - 99%	92 - 99%	*Streptococcus pneumoniae*	7/48	14.58
	10	Mucoid colony on NA plate	Gram negative rods	NP	+	-	+	+	-	+	+	+	99 - 100%	99 - 100%	*Klebsiella aerogenes*	4/48	8.34
Dry	1	Dry colony on NA plate	Gram positive rods	-	+	-	-	-	-	+	-	+	95 - 100%	95 - 99%	*Bacillus licheniformis*	25/48	52.08
	2	Yellow colony on NA plate	Gram positive cocci in clusters	+	+	-	+	-	-	+	-	+	96 - 100%	98 - 99%	*Staphylococcus saprophyticus*	4/48	8.34
	3	Mucoid colony on NA plate	Gram negative rods	NP	+	-	+	-	-	+	-	+	99 - 100%	100%	*Enterobacter cloacae*	1/48	2.08
	4	Green colony on NA plate	Gram negative rods	NP	+	+	+	-	-	-	-	-	97 - 100%	99 - 100%	*Pseudomonas aeruginosa*	10/48	20.83
	5	Yellow colony on NA plate	Gram positive cocci	-	+	+	+	-	+	-	-	+	99%	100%	*Micrococcus luteus*	3/48	6.25
	6	Mucoid colony on NA plate	Gram negative rods	NP	+	-	-	+	+	-	+	-	98 - 100%	97 - 99%	*Escherichia coli*	2/48	4.17
	7	Mucoid colony on NA plate	Gram positive cocci in chain	-	-	-	+	-	+	-	+	-	99 - 100%	100%	*Streptococcus pneumoniae*	3/48	6.25

CO: Coagulase test. CA: Catalase test. OX: Oxidase test. CI: Citrate test. IN: Indole test. MR: Methyl red test. VP: Voges Proskauer test. LA: Lactose fermentation test. MA: Mannitol fermentation test. +: Positive results. -: Negative results. F: Fractional prevalence. P: Percentage prevalence. NP: Not performed

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Table 3: Bioremediation efficiency of the bacterial isolates

Analytes	Period of bioremediation				
	Day 0	Day 7	Day 14	Day 21	Day 28
	Mean bioremediation efficiency N = 3 %	Mean bioremediation efficiency N = 3 %	Mean bioremediation efficiency N = 3 %	Mean bioremediation efficiency N = 3 %	Mean bioremediation efficiency N = 3 %
Ammonia	0.00 ± 0.00	29.27 ± 0.55	44.95 ± 0.63	59.03 ± 1.15	80.65 ± 0.47
Nitrate	0.00 ± 0.00	0.79 ± 0.40	1.59 ± 0.40	1.98 ± 0.40	5.95 ± 0.69
Copper	0.00 ± 0.00	4.04 ± 1.51	6.62 ± 2.28	10.78 ± 1.58	16.77 ± 2.66
Iron	0.00 ± 0.00	4.49 ± 2.40	16.41 ± 1.59	28.20 ± 2.67	29.06 ± 2.60
Zinc	0.00 ± 0.00	4.09 ± 0.81	11.44 ± 0.55	15.81 ± 1.03	18.38 ± 1.78
Nickel	0.00 ± 0.00	7.84 ± 2.60	8.83 ± 2.94	12.75 ± 3.54	14.71 ± 1.70
Lead	0.00 ± 0.00	5.21 ± 1.04	15.21 ± 1.56	19.58 ± 0.83	28.13 ± 1.80
Cadmium	0.00 ± 0.00	3.00 ± 3.00	23.33 ± 3.33	25.33 ± 2.91	30.00 ± 1.16
Arsenic	0.00 ± 0.00	10.69 ± 1.67	27.67 ± 0.63	33.96 ± 2.18	38.37 ± 1.66
Mercury	0.00 ± 0.00	10.81 ± 1.56	31.53 ± 1.80	36.04 ± 0.90	45.05 ± 2.39

N is the total number of test/control columns. Mean bioremediation efficiency represented as mean ± standard error of mean.

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