App. Envi. Res. 39 (2): 29-40

Applied Environmental Research

Journal homepage : http://www.tci-thaijo.org/index.php/aer

Bioaccumulation of Heavy Metals using Selected Organisms Isolated from Electronic Waste Dumpsite of two South-Western States in Nigeria

A.K. Akintokun[1], O.O. Onatunde[1,*], O.B. Shittu[1], I.C. Okeyode[2], M.O. Taiwo[1]

[1] Department of Microbiology, Federal University of Agriculture, Abeokuta, Nigeria
[2] Department of Physics, Federal University of Agriculture, Abeokuta, Nigeria
[*] Corresponding author: Email: dejionatunde@yahoo.com; Phone: +2347037355973

Article History
Submitted: 3 February 2017/ Accepted: 19 April 2017/ Published online: 30 June 2017

Abstract

Heavy metals from electronic wastes can accumulate to alarming concentrations in soils, causing significant detrimental impacts on human life and the environment. Bioaccumulation of heavy metals by bacteria and fungi has been a major focus of most bioremediation studies owing to the excellent metal-binding properties. The current study was conducted to isolate the most promising Zn, Cu and Pb tolerant microorganisms from contaminated soils, and to assess their metal accumulating abilities. *Bacillus licheniformis, B. polymyxa, Pseudomonas aeruginosa, Micrococcus roseus, Aspergillus niger* and *A. flavus* were selected for the bioaccumulation study, based on their known tolerance to heavy metals. *Bacillus licheniformis* was most efficient in the removal of Cu (71.3%) and Pb (70.1%). Pb accumulation for *Aspergillus flavus* was 65.76%. Zn accumulation for *Pseudomonas aeruginosa* and *Aspergillus niger* were 74.1% and 78.3%, respectively. This study concluded that all these microorganisms have potential for bioremediating soil environments contaminated with heavy metals.

Keywords: Bioaccumulation; Electronic waste; Heavy metals

Introduction

Electronic waste (also referred to as wastes of electric-electronic equipment, or WEEE) is the fastest growing waste stream in municipal wastes, with an annual growth rate of about 3-5 %. [1]. These e-wastes are considered as high degree pollutants and are toxic to the environment [2]. Electrical and electronic equipment contain hazardous materials that are harmful to human health and the environment if not disposed of properly. Some elements, though harmless in their natural state, can present hazards when used in manufacture of electronic equipment (e.g. chromium becomes chromium IV) [1].

Several researchers have recorded higher concentrations of Cu, Zn and Pb in different

e-waste dumpsites which consist primarily of ferrous and non-ferrous metals, plastics, glass, wood and plywood, printed circuit boards (PCB), concrete and ceramics, rubber and other items. For example, several studies reported high concentration of these three heavy metals as among the most concentrated metals found in environmental samples from different locations [3-7]. However, resistance to toxic metals in bacteria probably reflects the degree of environmental contamination with these metals [8].

Heavy metal pollution is of great concern due to their bioaccumulation in microorganisms, then onward up the food chain to plants, animals and humans; they are responsible for a wide range of physiological disorders [9]. Before advancement in technology, chemical methods such as the use of tartaric acid were used to remove heavy metals and also physical extraction was employed; however, these methods are expensive and relatively ineffective. In recent years, scientists have turned to a new approach in remediation of heavy metals from the environment using microorganisms.

Microbes play a key role in transformation, decomposition and bioaccumulation of heavy metals, although in higher concentrations, heavy metal ions can react to form toxic compounds in cells [10]. Microorganisms can also convert toxic metals into insoluble substances that are easy to dispose of [11]. To survive under metal-stressed conditions, microorganisms have evolved several mechanisms to tolerate uptake of heavy metal ions [12]. These include the efflux of metal ions outside the cell, accumulation and complexation of the metals inside the cell, and reduction of the heavy metal into a less toxic state.

Biological methods can be more effective and cost-efficient than traditional remediation methods, and can result in a higher rate of removal of pollutants. The active mode of metal accumulation of living cells is in most cases referred to as bioaccumulation which relies on intrinsic biochemical and structural properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity. Bioaccumulation is defined as the uptake of toxicant by living cells and its transport into the cell. It is a growth-dependent process mediated only by living biomass [13]. The aim of this study was to isolate and characterize organisms from e-waste dump sites contaminated with heavy metals, and to study the heavy metals resistance pattern and the biodegradation potential of the organisms.

Materials and methods

1) Study area

Heavy metal contamination caused by electronic waste was evaluated at dumpsites in Lagos and Ogun State. Soil samples were collected from 3 major e-waste dumpsites. The first location was Alaba, located at 6º28’00”N 3º10’59”E., in the Ojo local government area, Lagos State. The popular Alaba international market- the largest electronics market in West Africa- is located here. The second site-Ikorodu- is located at 6º33’09”N 3º32’08”E- a heavily populated major city, also located in Lagos State. Ogij, the third site, is located at 6º40’59”N 3º30’40”E, an urban area in Ogun State, south-western Nigeria. The control soil sample was obtained from Ogba (6º34’08”N 3º19’59”E), a residential area free from e-waste dumpsites. Most of the wastes found at these 3 dumpsites originate from imported second-hand products, including electronic products such as communications and broadcasting equipment, computers, televisions, videos, home appliances, refrigerators, video games, generators, satellite receivers etc.

2) Collection of samples and preparation of metal stock solutions

Soil samples from Lagos State Environmental Protection Agency (LASEPA) e-waste dump site, Alaba International market waste dump and

e-waste dump site in Ikorodu were separately collected into polythene bags. The samples were taken for analysis to the laboratory in boxes containing ice. On arrival, the samples were first air-dried and sieved with a 2 mm mesh size to remove stones and other extraneous materials. Stock solutions of Zn [$ZnCl_2$], Cu [$CuSO_4$] and Pb [$PbCl_2$] (Lab grade) were used and prepared in deionized water and sterilized by filter membrane (0.22 μm) and stored at 4°C.

3) Isolation of microorganisms

3.1) Total heterotrophic bacterial count

Serial dilutions of each soil sample were carried out by weighing 10 grams of each soil sample into a sterile 250 ml conical flask containing 90 ml of normal saline water. The flask was agitated vigorously to homogenize the suspension. Thereafter, 1 ml aliquots from 10^{-1} dilution were transferred into another test tube containing 9 ml of normal saline water to obtain 10^{-2} dilution. Further dilutions were made until 10^{-8} dilution was obtained. From 10^{-8} dilution, 1 ml of the inoculum was placed on nutrient agar (NA) (Lab M, UK) plates using the pour plate method. Aseptically, the inoculum and the nutrient agar were thoroughly mixed together, allowed to set and invertedly incubated at 37 ºC for 24 hours. Colonies were counted and recorded after 24 hours as colony-forming units (CFU) per gram (Eq.1).

$$CFU = \frac{\text{Number of colonies per plate x dilution factor}}{\text{Volume of aliquot used}} \quad \text{Eq.1}$$

3.2) Fungal count

Fungal counts were determined from 1 ml of 10-4 dilutions transferred to sterile petri dishes containing potato dextrose agar (Lab M, UK) supplemented with 1% chloramphenicol and then incubated at 28°C for 72 hours. The 1% chloramphenicol was added to potato dextrose agar to inhibit bacteria growth. Colonies were counted and reported as cfu/g.

3.3) Purification and identification of bacterial and fungal isolates

Distinct colonies of bacterial and fungal isolates were purified on nutrient agar and potato dextrose agar by repeated sub-culturing. Pure cultures of each colony were maintained on their respective agar slant and stored at 4ºC in a refrigerator prior to use.

The bacterial isolates were identified based on their morphological and biochemical characteristics and examined according to the Bergey's Manual of Determinative Bacteriology. Biochemical tests including catalase, citrate utilization, capsule staining, Voges-Proskaeur, methyl red and sugar fermentation tests were carried out on the isolates [14]. The fungal isolates were identified based on cultural and morphological characterization with reference to de Hoog et al. [15] and Ellis et al. [16].

4) Isolation of metal-resistant bacteria and fungi

For the selective isolation of metal-resistant bacteria and fungi, 0.1ml aliquots of the diluted soil samples were spread-inoculated on sterile nutrient agar and potato dextrose agar plates amended with 100 mg/kg of metals (Pb, Cu and Zn), respectively. Control plates were set up without the metals. The plates were incubated at 37ºC for 48 hours. After the incubation period, the plates were observed for growth on the media. The isolated and distinct colonies on the media were subcultured repeatedly on the same media for purification [17].

5) Determination of bacterial and fungal tolerance by spectrophotometric method

Ten ml of nutrient broth of 50 mg/kg and 100 mg/kg of Pb, Cu and Zn were added in tubes

individually and sterilized in autoclave and sterilized for 15 minutes. The tubes were inoculated with freshly prepared 1 ml of 10^8 and 10^4 bacteria and fungi cells in duplicate. The 50 mg/kg and 100 mg/kg of the heavy metals without inoculation were used as controls. All inoculated test tubes were incubated at 37ºC for 72 hours and optical density (OD) was observed at 620 nm using a spectrophotometer against control [18].

6) Bioaccumulation studies

6.1) Accumulation of heavy metals by selected Bacterial and Fungal isolates

The isolates were checked for heavy metal bioaccumulation at varying concentrations (200 mg/kg, 400 mg/kg, 800 mg/kg and 1,600 mg/kg, 3,200 mg/kg) of heavy metal salts, made in sterile nutrient broth and potato dextrose broth. The bioaccumulation was studied at different temperatures (37°C and 27ºC for 24 hrs) at shaker conditions.

6.2) Further bioaccumulation studies

The most effective isolates were further checked for their bioaccumulation potential using the three heavy metal salts (lead chloride, copper sulphate, and zinc chloride). Heavy metal salt solutions (500 mg/kg) of lead chloride, copper sulphate and zinc chloride were made in sterile nutrient broth and potato dextrose broth, respectively. The solutions were inoculated with 24 and 72 hour old culture suspensions of bacterial and fungi isolates, respectively. The inoculated bacteria cultures were kept at 37°C for 24 hrs, while fungi at 28°C for 48 hours at shaker conditions. The broth was then centrifuged at 5,000 rpm for 15 min, the cells were harvested and the supernatant was analyzed using an atomic absorption spectrophotometer (BUCK 210 VGP) [19].

7) Ribosomal sequencing and phylogenetic relatedness of isolates

The most effective bacterial and fungal species were subjected to molecular identification. This was done to confirm the identity of the bacterial and fungal isolates and to obtain the closest strain from the sequences available online in the Gene Bank database (www.ncbi.nlm.nih.gov). This was done by extracting the genomic DNA of the isolates using PureLink Genomic DNA kits (Invitrogen Life Technologies, CA, USA) followed by amplification of 16S and 18S rRNA gene in 25 µL reaction premix using 27F (5'-AGAGTTTGATCMTGG CTCAG–3') and 1492R (5'–ACCTTGTTACG ACTT–3') primers [20]. The amplified 1500-bp fragments were resolved by electrophoresis on a 1% agarose gel. The PCR amplicons were purified and directly sequenced by using the ABI 3730 Genetic Analyzer at STAB VIDA Technologies, Portugal. To identify the genus, species and the closest strain of the isolates, the sequences were analyzed using the BLASTN program of the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA).

8) Statistical analysis

Data obtained were analysed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows (SPSS, Chicago IL, U.S.A.). The mean, standard deviation (SD), median and ranges were calculated for continuous variables, while proportions and frequency tables were used to summarize categorical variables. The level of significance was considered as $P< 0.05$.

Results

1) Enumeration of bacterial and fungal isolates

In the present study, the highest bacterial count (15.8 $\log_{10}$ CFU/g) was obtained from Alaba with the lowest (from Ogba) was 13.5 $\log_{10}$ CFU/g. For the fungal isolates, the highest count was recorded in Ikorodu (5.45 $\log_{10}$ CFU/g), with the lowest (3.21 $\log_{10}$ CFU/g) at Ogba (Table 1). Counts on nutrient agar and potato

dextrose agar supplemented with metals showed reduction in the number of organisms (Figure 1).

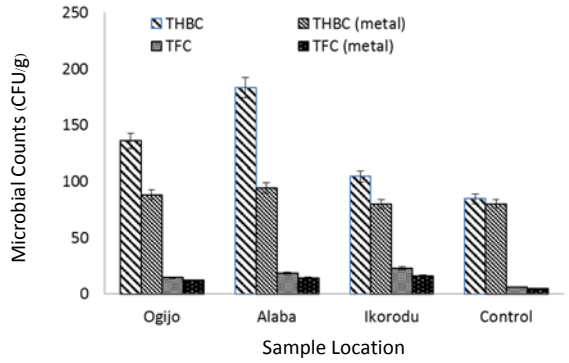

Figure 1 Isolation of metal resistant bacteria and fungi. KEY: THBC – Total Heterotrophic Bacterial Count, THBC (metal) – 100 mg/kg of metals added to media, TFC – Total Fungal Count, TFC (metal) – 100 mg/kg of metals added to media.

2) Bacterial and fungal count in soil samples when supplemented with metals

Figure 1 showed reductions in the total heterotrophic bacterial and fungal counts when the media was supplemented with 100 mg/kg of metals (Cu, Zn and Pb). Alaba had highest mean total heterotrophic bacterial count of 94.61±1.40 CFU/g followed by Ogijo (88.25±1.36 CFU/g). The lowest count (80.40±1.89 CFU/g) was recorded in Ogba (Control). For total fungal count, Ikorodu had the highest mean count of 16.36±2.30 CFU/g while the lowest count (4.62±0.78 CFU/g) was recorded in Ogba (Control). There was noticeable reduction in growth of bacteria and fungi when the media was supplemented with metal.

3) Heavy metals tolerance of microorganisms isolated from e-waste dumpsites

Tolerance of heavy metals with respect to control was determined spectrophotometrically for bacterial and fungal isolates. It was observed that growth declined with increasing concentration. A total of 22 isolates (bacteria and fungi) [*Alcaligenes faecalis*, *Aspergillus flavus*, *A. funmigatus, A. niger, Bacillus polymyxa, B. megaterium, B. alvei, B. sphaericus, B. licheniformis, B. pumilus, B. brevis, B. laterosporus, Corynebacterium equi, Micrococcus roseus, Penicillium notatum, Pseudomonas aeruginosa, P. cepacia, P.mallei, P. putida, P. stutzeri, Rhizopus stolonifera, Staphylococcus aureus*,] showed high tolerance to Zn, Cu and Pb (Table 2).

4) Accumulation of heavy metals by selected bacterial and fungal isolates

Table 3 showed the heavy metal bioaccumulation at varying concentrations of 200 mg/kg, 400 mg/kg, 800 mg/kg and 1,600 mg/kg and 3200 mg/kg in heavy metal salts. All bacterial and fungal isolates were able to accumulate the heavy metals at different rate. However, it was observed that Zn, Cu and Pb accumulated by the bacterial and fungal isolates decreased as the concentration of the metals was raised beyond 1,600 mg/kg. *Bacillus licheniformis* was found to have the highest cellular accumulation of the three metals, ranging from 7-38 mg/kg for Zn, 11-37 mg/kg for Cu and 5-27 mg/kg for Pb. The lowest bioaccumulation potential among the bacterial species was recorded in *Corynebacterium equi,* ranging from 8-18 mg/kg, 6-18 mg/kg, 4-20 mg/kg for Zn, Cu and Pb, respectively. For fungal isolates, *Aspergillus niger* was found to have the highest accumulation for Zn, Cu and Pb, ranging from 8-28 mg/kg, 6-27 mg/kg and 5-25 mg/kg, respectively, while *Rhizopus stolonifer* had the least bioaccumulation potentials of 10-19 mg/kg, 7-20 mg/kg and 7-21 mg/kg for Zn, Cu and Pb, respectively.

However, six isolates (*Bacillus polymyxa, Micrococcus roseus, Bacillus licheniformis, Pseudomonas aeruginosa, Aspergillus niger* and *Aspergillus flavus*) were observed to be the most effective of all the 22 bacterial and fungal isolates, and were therefore selected for further bioaccumaulation studies.

Table 1 Total heterotrophic bacterial and fungal counts at different locations

Sampling location	Total heterotrophic bacterial count (10^7 CFU/g)	Total fungal count (10^4 CFU/g)
Ikorodu	104.67±(4.055)[b]	23.00±(2.309)[c]
Alaba	183.33±(4.807)[d]	18.33±(1.453)[b, c]
Ogijo	136.00±(4.041)[c]	14.67±(2.333)[b]
Ogba (Control)	84.67±(1.76)[a]	6.33±(0.882)[a]

Note: Values are mean ± standard error of mean. Values followed by different letters within a column indicates significant differences according to the Duncan Multiple Range Test (DMRT), where $p<0.05$.

Table 2 Optical density values as an index for tolerance to heavy metals of isolates from the e-waste dumpsites

Isolates	Zn		Cu		Pb	
	50 mg/kg	100 mg/kg	50 mg/kg	100 mg/kg	50 mg/kg	100 mg/kg
Bacillus polymyxa	0.72 ± 0.23	0.60 ± 0.00	0.66 ± 0.00	0.50 ± 0.00	0.70 ± 0.00	0.46 ± 0.11
Staphylococcus aureus	0.48 ± 0.14	0.33 ± 0.11	0.39 ± 0.26	0.13 ± 0.11	0.41 ± 0.00	0.13 ± 0.00
Corynebacterium equi	0.71 ± 0.00	0.50 ± 0.26	0.37 ± 0.00	0.08 ± 0.00	0.65 ± 0.26	0.34 ± 0.00
Alcaligenes faecalis	0.69 ± 0.26	0.53 ± 0.00	0.73 ± 0.00	0.64 ± 0.26	0.69 ± 0.23	0.47 ± 0.17
Bacillus megaterium	0.68 ± 0.00	0.42 ± 0.00	0.59 ± 0.00	0.14 ± 0.00	0.65 ± 0.11	0.30 ± 0.00
Bacillus alvei	0.66 ± 0.00	0.39 ± 0.00	0.74 ± 0.26	0.24 ± 0.05	0.62 ± 0.00	0.38 ± 0.11
Pseudomonas putida	0.60 ± 0.26	0.52 ± 0.00	0.77 ± 0.00	0.56 ± 0.16	0.71 ± 0.00	0.40 ± 0.02
Pseudomonas aeruginosa	0.67 ± 0.00	0.50 ± 0.26	0.75 ± 0.11	0.66 ± 0.00	0.65 ± 0.00	0.53 ± 0.23
Pseudomonas cepacia	0.62 ± 0.17	0.39 ± 0.11	0.30 ± 0.26	0.14 ± 0.00	0.55 ± 0.11	0.30 ± 0.00
Bacillus sphaericus	0.65 ± 0.00	0.68 ± 0.00	0.61 ± 0.00	0.65 ± 0.17	0.64 ± 0.26	0.49 ± 0.00
Aspergillus niger	0.85 ± 0.00	0.76 ± 0.00	0.75 ± 0.00	0.72 ± 0.23	0.69 ± 0.23	0.50 ± 0.00
Aspergillus funmigatus	0.68 ± 0.00	0.64 ± 0.17	0.68 ± 0.20	0.53 ± 0.20	0.61 ± 0.00	0.61 ± 0.02
Penicillium notatum	0.62 ± 0.02	0.58 ± 0.00	0.55 ± 0.11	0.49 ± 0.11	0.58 ± 0.00	0.44 ± 0.00
Bacillus licheniformis	0.73 ± 0.05	0.62 ± 0.14	0.63 ± 0.00	0.58 ± 0.01	0.67 ± 0.21	0.55 ± 0.00
Bacillus pumilus	0.54 ± 0.00	0.32 ± 0.00	0.51 ± 0.02	0.24 ± 0.00	0..36 ± 0.00	0.24 ± 0.00
Micrococcus roseus	0.67 ± 0.00	0.38 ± 0.00	0.48 ± 0.00	0.16 ± 0.00	0.41 ± 0.00	0.32 ± 0.20
Pseudomonas mallei	0.72 ± 0.26	0.51 ± 0.00	0.66 ± 0.05	0.59 ± 0.0	0.68 ± 0.20	0.53 ± 0.00
Bacillus brevis	0.71 ± 0.00	0.59 ± 0.00	0.74 ± 0.26	0.65 ± 0.23	0.72 ± 0.02	0.48 ± 0.00
Bacillus laterosporus	0.78 ± 0.11	0.69 ± 0.11	0.72 ± 0.02	0.53 ± 0.11	0.64 ± 0.00	0.39 ± 0.00
Rhizopus stolonifer	0.69 ± 0.00	0.57 ± 0.00	0.66 ± 0.01	0.43 ± 0.01	0.63 ± 0.00	0.60 ± 0.17
Pseudomonas stutzeri	0.74 ± 0.01	0.68 ± 0.23	0.76 ± 0.20	0.63 ± 0.09	0.68 ± 0.20	0.49 ± 0.00
Aspergillus flavus	0.82 ± 0.11	0.75 ± 0.16	0.76 ± 0.11	0.73 ± 0.00	0.71 ± 0.01	0.63 ± 0.09

Note: Values are mean ± standard error of mean. Values followed by different letters within a column indicates significant differences according to the Duncan Multiple Range Test (DMRT), where $p<0.05$.

Table 3 Accumulation (dry weight of cells) of Zn, Cu and Pb by selected isolates

Isolate code	Zn (mg/kg)					Cu (mg/kg)					Pb (mg/kg)				
	200	400	800	1600	3200	200	400	800	1600	3200	200	400	800	1600	3200
**Bacillus polymyxa*	6	10	16	26	22	9	18	22	26	23	4	8	14	19	17
Staphylococcus aureus	5	12	19	23	19	8	17	21	25	22	3	9	16	20	16
Corynebacterium equi	8	13	15	18	15	6	11	14	18	15	4	10	19	20	14
Alcaligenes faecalis	8	17	24	28	24	4	12	19	25	20	4	9	17	19	16
Bacillus megaterium	3	9	14	23	19	7	16	19	29	24	2	6	12	16	14
Bacillus alvei	7	15	23	25	20	4	12	18	27	25	9	13	18	28	26
Pseudomonas putida	3	9	15	22	18	5	11	16	24	21	7	16	18	23	21
**Pseudomonas aeruginosa*	6	11	24	27	23	6	14	20	29	25	5	11	16	28	25
Pseudomonas cepacia	6	13	24	27	21	7	17	22	26	22	6	13	19	20	17
Bacillus sphaericus	5	16	29	24	17	9	15	19	25	23	7	15	22	25	18
**Aspergillus niger*	8	15	21	28	25	6	13	18	27	24	3	5	19	25	20
Aspergillus funmigatus	5	14	16	22	20	10	16	21	25	23	4	9	20	26	22
Penicillium notatum	8	16	21	25	17	6	11	15	19	15	5	8	14	28	23
**Bacillus licheniformis*	7	16	22	38	31	11	17	26	37	34	5	11	20	27	24
Bacillus pumilus	9	16	28	29	23	5	15	23	27	24	3	8	16	20	18
**Micrococcus roseus*	6	5	26	35	27	6	14	22	28	25	5	11	19	26	21
Pseudomonas mallei	5	11	19	29	24	8	16	24	29	25	4	10	21	23	20
Bacillus brevis	4	12	21	24	21	7	17	25	28	26	6	9	18	22	19
Bacillus laterosporus	6	10	16	27	24	8	12	17	23	20	8	16	18	21	20
Rhizopus stolonifer	10	13	19	17	15	7	11	14	20	19	7	10	16	21	17
Pseudomonas stutzeri	4	8	14	22	18	4	8	15	19	15	3	5	12	18	16
**Aspergillus flavus*	3	9	15	29	23	3	9	14	28	26	7	13	19	24	21

*Most effective isolates selected for further bioaccumulation studies

5) Bioaccumulation potential of selected bacteria and fungi

Only six isolates (four bacteria and two fungi) namely *Bacillus polymyxa, Micrococcus roseus, Bacillus licheniformis, Pseudomonas aeruginosa, Aspergillus niger* and *Aspergillus flavus*, all of which tolerated high concentration of heavy metals, were selected for bioaccumulation studies. Figure 2 showed the percentage accumulation of the heavy metals by the selected organisms. However, *Bacillus licheniformis, Pseudomonas aeruginosa* and *Aspergillus niger* showed higher bioaccumulation potentials than *Aspergillus flavus, Bacillus polymyxa* and *Micrococcus roseus*. *Bacillus licheniformis* was the most efficient in the removal of Cu (71.3%) and Pb (70.1%). Zn accumulation was higher for *Pseudomonas aeruginosa* and *Aspergillus niger* were 74.1% and 78.3% respectively. Nevertheless, Zn (61.80%) and Cu (68.4%) accumulation for *Aspergillus flavus* was also encouraging.

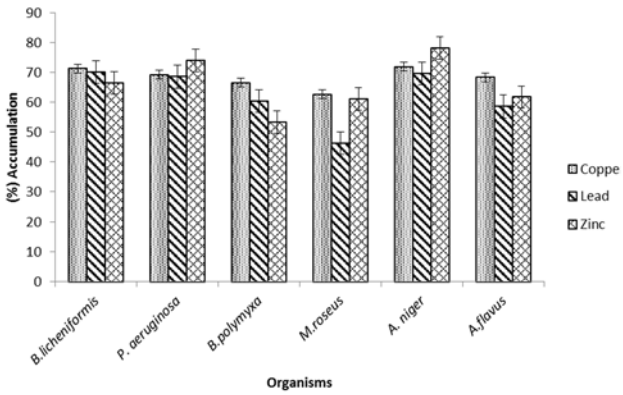

Figure 2 Accumulation percentage of heavy metals by the selected organisms from e-waste dumpsites

6) Molecular characterization

The four isolates (two bacterial and two fungal) showing higher bioaccumulation potentials were selected for PCR amplification, 16S ribosomal (Bacteria) and 18S ribosomal (Fungi) sequencing based on their bioaccumulation potential. On the basis of the 16S rRNA gene sequence analysis, the isolates were identified as *Bacillus licheniformis* BIBT VC, *Pseudomonas aeruginosa* HNYM41, *Aspergillus niger* strain ZRS14 and *Aspergillus flavus* strain MB38. The percentage sequence similarity ranged between 86% and 97% (Table 4). Figure 3 showed the gel electrophoresis of the organisms which revealed the location of the amplified DNA bands of the isolates.

Table 4 Similarity of screened isolate sequences with NCBI Gen-bank database sequences

Description of closest related taxa	Similarity (%)	Accession number
Bacillus licheniformis strain BIBT VC	92%	KM246409.1
Pseudomonas aeruginosa HNYM41	97%	JN999891.1
Aspergillus niger strain ZRS14	86%	KF414527.1
Aspergillus flavus strain MB38	91%	HQ844712.1

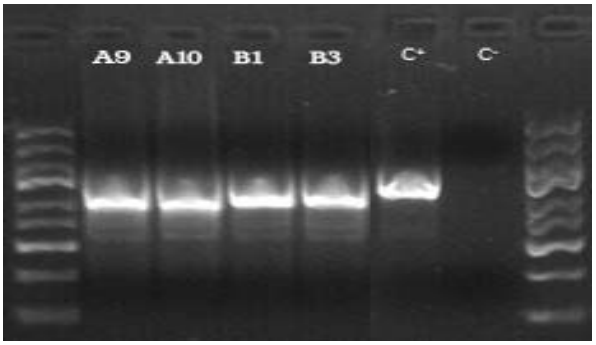

Figure 3 Gel electrophoresis of the amplified PCR profiles of bacterial and fungal isolates. A9 represents *Bacillus licheniformis*, A10 represents *Pseudomonas aeruginosa*, B1 represents *Aspergillus niger* and B3 represents *Aspergillus flavus*, C^+ represents positive control **(**Universal primer**)** and C^- represents negative control **(**Distilled water**)**.

Discussion

Rapid modernization has led to a soaring increase in e-waste from electrical and electronic gadgets. Unchecked dumping of this e-waste not only pollutes the environment but also impacts on human health. There is an urgent and fast-growing need for remediation strategies which are cost-effective and environmental friendly.

The present study found significant differences between the counts in all samples analysed. The highest bacterial count was obtained from a soil sample collected from Alaba, while the lowest was from Ogba. Alaba is home to the largest electronics market in West Africa, so the indiscriminate dumping of electronic waste in the locality is likely to be a major reason for the high count. However, the highest fungal count was recorded in Ikorodu, with the lowest count from Ogba, the control site. These results revealed higher counts of microorganisms in the dumpsite soils as compared with the control. These counts were similar to those reported by Wade and Dave [21], but much higher than those reported by Adebisi et al. [22]. However, the high bacterial counts in all the three e-waste dumpsites sampled in this study indicated that the soils were generally rich in

organic matter, which favours growth of micro-organisms.

When the nutrient agar and potato dextrose agar on which the isolates were grown were supplemented with metals, similar results were obtained with soil sample from Alaba recording the highest mean total heterotrophic bacterial count and Ikorodu, having the highest fungal count while the least count was recorded from Ogba. However, there was a significant reduction in the growth of bacteria and fungi when the media was supplemented with metal.

In this study, a total of 22 isolates comprising of 17 bacteria and 5 fungi were isolated. The isolates of *Bacillus sp*, *Pseudomonas sp*, *Staphylococcus sp*, *Aspergillus sp*, and *Micrococcus sp* from the e-waste soil samples in this work were similar to the work of Sanusi [23] who also reported the presence of these micro-organisms in soils from Alaba e-waste dump sites in Lagos State, Nigeria. However, similar microbial isolates as identified in this study had also been reported to be associated with wastes and waste biodegration in soil samples [24]. In this study, *Pseudomonas sp*, *Aspergillus sp*, *Bacillus sp* and *Micrococcus sp* showed high tolerance to Zn, Cu and Pb, in agreement with Li et al. [5] and Munees and Abdul [9]. On the contrary, Doku and Belford [25] reported *Aspergillus niger* to have a higher tolerance of heavy metals than was found in the current study.

The six selected isolates in this study were not only resistant to Zn, Cu and Pb but also had the capability to accumulate these heavy metals. These microorganisms had been previously reported to have an affinity for metals and are able accumulate them by various mechanisms [26]. Several principal sites of metal complex formation in biological systems have been reported by Vieira and Volesky [27], including accumulation in the cell wall, carbohydrate or protein polyphosphate complexes and complexation with carboxyl groups in the peptidoglycan cell wall, or by entering into the cell. Enhanced metal uptake by metal-resistant bacteria observed in medium in this study suggested that the metal uptake mechanism may involve diffusion. This study also found that the metal accumulating ability of isolates was reduced beyond the specific concentration of Zn, Cu and Pb. This might be because of the saturation of the isolates with metals or due to increase in toxicity of metals at high concentration [28]. This was similar to what was reported by Banerjee et al. [29] who studied accumulation of heavy metals ions by *Bacillus cereus* strain isolated from ash Dyke.

The highest adsorption in this study was observed for Cu. Zolgharnein [30] reported significant uptake of heavy metals ions by *Pseudomonas aeruginosa* strain MCCB 102 isolated from the Persian Gulf; however, in the current study, the highest adsorption was observed for Cu, Zn, and Pb.

In this study, *Bacillus Licheniformis* (71.3%, 70.1%, 66.5%), *Pseudomonas aeruginosa* (69.3%, 68.6%, 74.1%), *Aspergillus flavus* (68.4%, 58.6%, 61.8%) and *Aspergillus niger* (72%, 69.7%, 78.3%) were found to have a very high bioaccumulation capacity for Cu, Pb and Zn. The high accumulation ability of these organisms may be due to the presence of teichoic and teichuronic acids, which were the principal sites of metal binding in the microbial cell walls [30]. This ability of *Bacillus licheniformis* to have very high bioaccumulation ability in this study was similar to the result Sulaimon et al. (2014) [6] who also reported *Bacillus licheniformis* to have bioaccumulation percentage of 90.4% for both Cu and As. However, Doku and Belford (2015) [25] in their work had also reported *Aspergillus niger* to have a high accumulation of Zn and Pb ranging from 0.03 to 27.80 mg/l and 0.04 to 36.92mg/l.

Conclusions

From this study, the most promising Zn, Cu and Pb tolerant microorganisms were isolated from heavy metal contaminated soils and were used to assess their metal accumulating ability. The results revealed that soil samples obtained from Ikorodu e-waste dumpsite had higher microbial count than those from the other two sites; however, all showed reductions in the number of organisms when supplemented with metals. A total of 17 bacteria and 5 fungi showed high tolerance to Zn, Cu and Pb. Th. Although all bacterial and fungal isolates were able to accumulate the heavy metals at varying rates ranging from 200 – 3,200 mg/kg, only six isolates (*Bacillus polymyxa, Micrococcus roseus, Bacillus licheniformis, Pseudomonas aeruginosa, Aspergillus niger* and *Aspergillus flavus*) were observed to tolerate high heavy metal concentrations. The bioaccumulation studies revealed that *Bacillus licheniformis, Pseudomonas aeruginosa, Aspergillus niger* and *Aspergillus flavus* showed higher bioaccumulation potentials than other isolates in the removal of Cu, Pb and Zn. The molecular identification carried out on these four isolates confirmed their identity and relatedness to the strains in the NCBI Gene Bank database. The findings from this study revealed a positive indication for the use of bacterial and fungal isolates in the bioremediation of metal contaminated soils. There is a need for further in vitro and field studies to elucidate mechanisms and understand the potential for wide-scale applications of these isolates.

References

[1] Osborne, W.J., Subhashree, B., Jyotirmayee, K., Swarnkumar, B., Astha, S. 2014. Biodegradation of electronic waste using bacteria. World Journal of Pharmaceutical Research 3(10):753-760.

[2] MihaiIrimia-Vladua, B., Eric. D., Głowacki, B., GundulaVoss, B., Siegfried B., Niyazi, S. 2012. Green and biodegradable electronics materials today pp 340-346.

[3] Esakku, S., Palanivelu, K., Joseph, K. 2003. Assessment of heavy metals in a municipal solid waste dumpsite, Workshop on sustainable landfill management. 3-5 December, Chennai, India, pp 139-145.

[4] Amusan, A.A., Ige, D.V., Olawale, R. 2005. Characteristics of soils and crops' uptake of metals in municipal waste dump site in Nigeria, Journal of Human Ecology, 17(3), pp 167-171.

[5] Li, J., Duan, H., Shi, P. 2011a. Heavy metal contamination of surface soil in electronic waste dismantling area: site investigation and source apportionment analysis. Waste Management Research 29:727-738.

[6] Sulaimon, A.M., Odeyemi, A.T., Ogunjobi, A.A., Ibrahim, I.O. 2014. Bioaccumulation of heavy metals using selected heavy metal tolerant organisms isolated from dumpsite leachate. Nature and Science 12(10):101-106.

[7] Damrongsiri, S., Vassanadumrongdee, S., Tanwattana, P. 2016. Heavy metal contamination characteristic of soil in WEEE (waste electrical and electronic equipment) dismantling community: a case study of Bangkok, Thailand. Environmental Science and Pollution Research 23:17026-17034.

[8] Malik, A., Jaiswal. R. 2004. Pb accumulation by some resistant bacterial strains isolated from soil treated with industrial wastewater. World Journal of Microbiology and Biotechnology. 2004; 16: 111-122

[9] Munees, A., Abdul, M. 2012. Bioaccumulation of heavy metals by Zn resistant bacteria isolated from agricultural soils irrigated with waste water. Bacteriology Journal 2(1):12-21.

[10] Nies, D.H. 1999. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology 51:730-750.

[11] Rodriguez, M.L, de la Cruz R.L.C, Farias, R.N., Massa EM. 1993. Membrane-associated redox cycling of copper mediates hydroperoxide toxicity in Escherichia coli. Bio-chemistry Biophysical Acta 1144: 77-84.

[12] Schmidt, I., Sliekers, O. Schmid, M.C., Bock, E., Fuerst, J., Kuenen, J.G., Jetten, M.S., Strous, M. 2003. New concepts of microbial treatment processes for the nitrogen removal in wastewater FEMS Microbiological Reviews 27: 481-492.

[13] Jaafar, R., BioAl-Sulami, A., Al-Taee, A., Aldoghachi, F., Napes S. 2015. Biosorption and Bioaccumulation of some heavy metals by Deinococcus radiodurans isolated from soil in Basra Governorate-Iraq. Journal of Biotechnology and Biomaterial 5(2):1-5.

[14] Cappuccino, J.G., Sherman, N. 2002 Microbiology: A laboratory manual. Pearson Education Incorporated. USA. 193pp.

[15] de Hoog, G.S., Guarro, J., Gene, J., Figueras, M.J. 2000. Atlas of Clinical Fungi (Second Edition). Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. pp 1-150.

[16] Ellis, D., Davis, S., Alexiou, H., Hanke, R., Bartley, R. 2007. Description of Medical Fungi (Second Edition). University of Adelaide, Adelaide, Australia. pp 1-180.

[17] Banerjee, S., Gothalwal, R., Sahu, P.K., Sao, S. 2015. Microbial observation in bioaccumulation of heavy metals from the Ash Dyke of Thermal Power plants of Chattisgarh, India. Advances in Bioscience and Biotechnology 6:131-138.

[18] Vishnu, D.R., Joshi, P.K., Kumar, R., Namita, S. 2010. Isolation and screening of bacterial isolates for tolerance to heavy metals. pp 12-16.

[19] Ray, L., Paul, S., Bera, D., Chattopadhyay, P. 2006. Bioaccumulation of Pb (II) from aqueous solution by Bacillus cereus M1. Journal of Hazard Substance Research 5: 1-22.

[20] Lane, D. J., Weisburg, W. G., Barns, S. M., Pelletier, D. A. 1991. 16s ribosomal DNA amplification for phylogenetic Study. Journal of Bacteriology 173(3): 697-703.

[21] Wade, A., Dave G. 2006. Acute toxicity of leachates from waste dumpsites in Malaysia. Chemosphere. 64: 1777-1784.

[22] Adebisi, M.S., Adebowale, T.O., Adeniyi, A.O., Ismail, O.I. 2014. Bioaccumulation of heavy metals using selected heavy metal tolerant organisms isolated from dumpsite leachate. Nature and Science; 12(10).

[23] Sanusi, A.I. 2015. Impact of burning e-waste on soil physicochemical properties and soil microorganisms. British Microbiology Research Journal 8(2): 434-442.

[24] Obire, O., Nwabueta, O., Adue, S.B.N. 2002. Microbial community of a waste dump site. Journal of Applied Science and Environmental Management 6 (1): 78-83.

[25] Doku, T.E., Belford E.J.D. 2015. The capacities of *Aspergillus funmigatus* and *Aspergillus niger* in bioaccumulation of heavy metals from the Chemu lagoon, Ghana. Journal of Applied Biosciences 94: 8907-8914.

[26] Rani, A., Goel, R. 2009. Strategies for crop improvement in contaminated soils using metal-tolerant bioinoculants. In: Microbial Strategies for Crop Improvement Springer, Berlin, pp 105-132.

[27] Vieira, R.H., Volesky, B. 2000. Biosorption: A solution to pollution. International Microbiology 3: 17-24.

[28] Al-Garni, S.M. 2005. Biosorption of Pb by gram negative capsulated and non-capsulated bacteria. Water Science Technology 31: 345-349.

[29] Beveridge, T. J., Forsberg, C. W., Doyle, R. J. 1982. Major sites of metal binding in *Bacillus licheniformis* walls. Journal of Bacteriology, 150(3): 1438-1448.

[30] Zolgharnein, H., Karami, K., Assadi, M.M., Sohrab, A.D. 2010. Investigation of heavy metals biosorption on *Pseudomonas aeruginosa* strain MCCB 102 isolated from the Persian gulf. Asian Journal of Biotechnology 2: 99-109.