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Impacts of heavy metals and soil properties at a Nigerian e-waste site on soil microbial community

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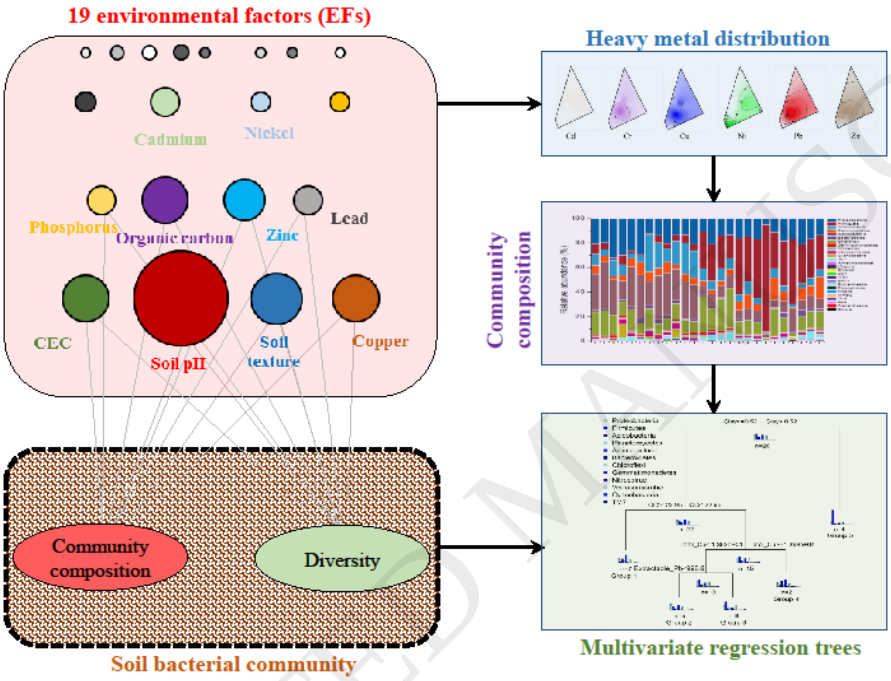
Graphical abstract



Highlights

- High level of heavy metal contamination at an e-waste site in Nigeria
- Heavy metal distribution is correlated to soil properties and their mobility
- Soil properties and heavy metal affect bacterial community structure and diversity
- Heavy metals affect bacterial abundance but vary across different taxa

Abstract

Heavy metal contamination is a serious problem worldwide threatening soil environment and human health. In the present study, concentrations of 6 heavy metals at an electronic waste (e-waste) site in Nigeria were correlated to their mobility, showing distinct distribution pattern between surface soils and subsoils. *Proteobacteria*, *Firmicutes*, *Acidobacteria* and *Planctomycetes* dominated the indigenous soil microbial communities, and there was significant discrimination of bacterial taxonomic composition between the heavy metal contaminated and uncontaminated areas. The abundance of most bacterial taxa changed with heavy metal contamination level to different extent. The multivariate regression tree (MRT) analyses illustrated that main environmental variables influencing bacterial taxonomic composition included soil texture (31%) and organic carbon (14%), whereas microbial diversity was affected by soil pH (32%) and soil texture (14). Our results surprisingly indicated that soil properties were more influential in determining soil bacterial composition and diversity than heavy metals even at the e-waste site which was seriously contaminated by heavy metals. The present study contributes to a deeper insight into the key environmental variables shaping the diversity and composition of soil microbes at heavy metal contaminated e-waste sites.

Keywords: electronic waste (e-waste); heavy metal; microbial community structure; high throughput sequencing; multivariate regression tree (MRT)

1. Introduction

With the development of the electronic industry, huge amounts of electronic wastes (e-wastes) are released into the environment at the rate of 20-50 million tons per year [1, 2]. E-wastes are chemically and physically distinct from other municipal or industrial wastes. Their chemical compositions vary depending on the age and type of the discarded items, mostly composed of a mixture of heavy metals attached to, covered with, or mixed with various types of plastics and ceramics [3]. Typical heavy metals found at e-waste sites are both valuable (*e.g.*, Cu, platinum group) and hazardous materials (*e.g.*, Pb, Sb, Hg, Cd, Ni) [4]. Investigations on the heavy metal contamination level in China [7] and Europe [4] at e-waste sites have identified Cu, Cr, Ni, Cd and Pb as the predominant contaminants (concentration as high as tens of

thousands of milligram per kilogram) and residents surrounding the e-waste recycling sites are facing a potential higher daily intake of heavy metals. Heavy metals are persistent in soils, leading to a serious problem in ecosystem and causing risks to human health through bioaccumulation in plants and animals or bioconcentration in the food chain [5-8]. Many studies on the e-waste contamination have been carried out and reveal the significant impacts of heavy metal contamination on environmental quality and public health [9, 10]. More importantly, the co-occurrence of heavy metals and organic pollutants at e-waste sites exhibits more complicated interactions in chemical processes, adsorption behaviors, and biological processes [11-15]. Co-existence of multiple pollutants results in competition for the binding sites of adsorbents and enzymes [16], inhibiting microbial metabolisms and thereby reducing the degradation efficiency of organic pollutants [17, 18]. Therefore, a deeper look into the ecological effects of e-waste disposal currently draws increasing attentions [19-21], which may greatly benefit land management and restoration in e-waste recycling regions.

Heavy metals are toxic to almost all the bacteria by affecting the growth, morphology and metabolism and inhibiting essential cellular functions such as protein synthesis and the integrity of cell membranes [22, 23], leading to the changes in function, activity and diversity of soil microbial community [24-28]. Due to the high sensitivity to environmental changes in their living habitats, the composition and diversity of soil microbial communities are increasingly studied. Significant negative correlations between soil enzyme activity, microbial abundance/diversity and heavy metal contamination gradients have been extensively discussed [27, 29-32]. For example, the elevated heavy metal concentration adversely affects the total population of bacteria and actinomycetes, and enzymatic activities in soil ecosystems [27]. A study on soil microbial taxonomic composition at an e-waste site illustrates significantly altered soil microbiotas between the contaminated and reference soils [33]. Recently, many studies are focusing on the ecological effects of heavy metals on microbial community structure and diversity with the development of high-throughput sequencing [28, 34-37]. Additionally, heavy metal exposure may also cause the bloom of metal-tolerant microbial populations [33], which has gained attentions in the past few decades [38, 39]. Some bacterial species tolerating heavy metals (*e.g.*, *Pseudomonas*, *Acinetobacter*, *Sphingomonas*) have been isolated and characterized

[38, 40]. Further investigations on soil microbial community can help in understanding microbial functions in ecosystem and tolerance to heavy metal contamination at e-waste sites.

In the present study, we focused on the contamination levels of heavy metals and their effects on bacterial community structure and diversity at an e-waste site in Nigeria. Besides analyzing the soil properties, heavy metal speciation and distribution, soil bacterial community composition and diversity were studied by high-throughput sequencing targeting bacterial 16S rRNA genes. The most important environmental variables influencing bacterial community composition and diversity were investigated via multivariate regression tree (MRT) analysis. This work revealed the response of soil microbiotas to soil properties in combination with different forms of heavy metals at an e-waste site.

2. Materials and Methods

2.1 Site description

The research area is an e-waste dumpsite at Alaba International Market (N 6°27'22", E 3°11'16.2"), Lagos State, Nigeria. This site has been used since 2006 for the disposal of e-wastes such as cathode ray tubes and television sets. Smouldering activities are still carried out to isolate heavy metals (mainly Cu) from e-wastes recently.

2.2 Sample collection and property analysis

The heavy metal contaminated soils were collected from 12 sites in the research area (S2, S4-S14), and their geographic locations were shown in Figure 1 and Table S1 (see Supporting Information). One site 1 km away from the e-waste site was sampled as the reference soil (S15). At each site, 100 g soils were taken from both surface (0-15 cm) and subsoils (15-30 cm) in triplicates. Parts of the soils were sent for DNA extraction directly, and the rest soils were blended and sieved through a 2-mm mesh to remove stones and plant debris for soil property analysis.

Soil pH was measured by a HQ411d pH meter (soil:water=1:1, m/m) after one hour shaking. Soil total carbon was determined by measuring the evolved CO_2 during dry combustion [41], and soil organic carbon was calculated by subtracting the inorganic carbon from calcium carbonate measured by colorimetric titration. Cation exchange capacity (CEC) and sodium saturation was measured by NaOAc saturation followed

by NH_4OAc extraction [42]. Soil phosphorous was determined by Olsen's method [43].

2.3 Heavy metal analysis

The total and HCl-extractable heavy metals in soils were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The total heavy metals were obtained by strong acid digestion (HNO_3:$HClO_4$=1:4, mole ratio). For extractable metals, HCl extraction was used as an effective approach for obtaining extractable heavy metals from soils, which has stronger extraction ability in comparison with EDTA and acetic acid [44]. Briefly, 1.0 g of soils were added with 40 mL HCl (0.1 M) and shaken at 22 °C for 16 h. After 3,000 rpm centrifugation for 20 min, the supernatant was collected and the pellets were washed by 5 mL deionized water. Followed by another 3,000 rpm centrifugation for 20 min, the supernatant was collected. The two supernatants were combined and added with deionized water to the final volume of 50 mL.

2.4 Bacterial community and diversity analysis

To determine the bacterial community structure and diversity in soils of different metal contamination level, the soil DNA was extracted with the PowerSoil kit (MO BIO Laboratories, USA) in accordance with manufacturer's instruction. The extracted DNA was quantified by spectrophotometry (Nanodrop 2000, Thermo Scientific, USA) and stored at -20 °C. Polymerase chain reaction (PCR) was carried out using the universal primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [45]. The 806R primer was labeled with a unique 12-bp barcode to distinguish among amplification products. PCR was performed to amplify 1 μL of template DNA in a 25-μL reaction system containing 12.5 μL rTaq premix buffer (TaKaRa) and 100 nM (0.5 μL) of each primer. Amplification was performed in triplicates as follows: 94 °C for 5 min; 28 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. The triplicate amplification products for each sample were pooled and purified using the MicroElute Cycle-Pure Kit (Bio-Tek). Sequencing was performed on Illumina HiSeq4000 after combining PCR products from different samples in approximately equimolar amounts. Raw sequence data were processed and analyzed with Mothur [46] and Quantitative Insights Into Microbial Ecology (QIIME) [47]. All

the reads passed the quality filtering, and those were discarded if the barcodes were uncorrectable, the bases with Phred Quality Score <19 covered above 30% of the read, or the ambiguous bases were over 5%. To reduce the error rate, singletons were removed. Representative sequences were chosen at a similarity level of 97% of operational taxonomic units (OTUs). Next, chimeric sequences identified by the UCHIME algorithm [48] were discarded. The taxonomic identification of representative sequences was determined based on the Greengenes 13.5 database using QIIME with its default settings. The relative abundance of each taxon and OTU was calculated by comparing its number of sequences with the number of total sequences.

2.5 Data analysis

The indices of diversity including Chao1, Shannon and Simpson were calculated by QIIME (v1.80) to assess the complexity of species diversity. Multivariate Regression Tree (MRT) analysis was carried out by using the package 'mvpart' within the software R (v3.0.3) to evaluate the correlation between environmental variables and bacterial taxonomic composition or standardized *a*-diversity estimates [49].

Contamination factor (*CF*) is defined as the ratio of the heavy metal concentration in the contaminated soil to the baseline concentration in reference soil [50], as shown in Equation (1). Pollution load index (*PLI*) is determined as the nth root of the *n CF* in Equation (2) [50]. The *CF* and *PLI* are empirical indices to evaluate the level of heavy metal contamination, and the higher values of *CF* and *PLI* indicate heavier contamination of individual and multiple heavy metals, respectively.

$$CF = \frac{[Heavy\ metal\ in\ sample\ soils]}{[Heavy\ metal\ in\ background\ soils]} \quad (1)$$

$$PLI = (CF_1 \times CF_2 \times \cdots \times CF_n)^{1/n} \quad (2)$$

All statistical analyses, *e.g.*, correlations, were performed using SPSS 17.0. The statistical significance of differences and variance analysis (p-value<0.05) was performed using a one-way ANOVA and least significant difference (LSD) test.

3. Results and discussion

3.1 Heavy metal contamination level

Physiochemical properties of surface soils (0-15 cm) and subsoils (15-30 cm) are

listed in Table S2 and Table S3 (Supporting Information), respectively. Most of the samples were sandy soils according to their texture (except S15_T and S14_B). The contaminated soils were neutral to slightly alkaline in both surface soils (pH 7.9±0.1) and subsoils (pH 8.0±0.1). Organic carbon contents (26.0±2.6 mg/g for surface soils and 18.5±2.2 mg/g for subsoils) were generally close to other e-waste sites ranging from 11-104 mg/g [51, 52]. CEC (8.3±1.7 cmol/kg for surface soils and 13.5±6.4 cmol/kg for subsoils) and phosphorus contents (102.8±13.4 mg/g for surface soils and 82.8±30.2 mg/g for subsoils) were also comparable to previous reports [33, 53]. Soil properties in surface soils and subsoils of the same sampling site were different, which might influence heavy metal speciation. It was therefore necessary to further evaluate the heavy metals as the total and HCl-extractable fractions in surface soils and subsoils [27, 32, 54, 55].

In terms of both total and HCl-extractable fractions, heavy metal contents in the study area were generally higher than the USEPA and European soil screening standards (Table 1 and Table 2). S15 was the reference soil and thereby had the lowest concentration of all the detected heavy metals. The predominant pollutants in surface soils were Cu (total, 8.65±1.52 $\times 10^3$ mg/kg; HCl-extractable, 3.81±0.50 $\times 10^3$ mg/kg), Pb (total, 6.80±1.18 $\times 10^3$ mg/kg; HCl-extractable, 2.31±0.37 $\times 10^3$ mg/kg) and Zn (total, 1.96±0.20 $\times 10^3$ mg/kg; HCl-extractable, 1.33±0.17 $\times 10^3$ mg/kg). All the sites were found with moderate concentrations of Ni but less Cd and Cr. Interestingly, the sites with high concentrations of total heavy metals also had high levels of HCl-extractable metals.

The heavy metal contamination levels in subsoils were similar to those in surface soils. Cu (total, 7.15±1.71 $\times 10^3$ mg/kg; HCl-extractable, 2.93±0.57 $\times 10^3$ mg/kg), Pb (total, 6.74±1.27 $\times 10^3$ mg/kg; HCl-extractable, 1.77±0.34 $\times 10^3$ mg/kg) and Zn (total, 2.01±0.24 $\times 10^3$ mg/kg; HCl-extractable, 1.23±0.17 $\times 10^3$ mg/kg) also had high concentrations in subsoils. Ni was of high concentrations only at some specific sites (S5), whilst Cd and Cr were relatively of low concentration. The differences of heavy metal concentrations between surface soils and subsoils possibly indicated the distinct vertical transportation abilities of different heavy metals, as easily-transportable heavy metals preferred to move from surface soils to subsoils, or otherwise stayed in surface soils [56].

Heavy metal contamination at e-waste sites is usually found, as they are widely used

in manufacturing a variety of electronic products, *e.g.*, Pb and Cd in circuit boards, Cd in computer batteries, and Cu in electrical wiring [7, 51, 57-59]. Heavy metals frequently found at e-waste sites include Cu, Pb, Sb, Hg, Cd and Ni [4]. A study on the heavy metal contamination at three e-waste sites in Nigeria has identified Cu, Pb, Cr and Mn as the predominant contaminants [60]. Tang *et al.* also reported that Cr, Pb and Zn are the most abundant heavy metals in an emerging e-waste recycling city in China [52]. Contamination levels of heavy metals in the present study are similar or slightly higher comparing to an e-waste site in China [33] and one or two orders of magnitude lower than those at e-waste sites in Switzerland [61]. Comparing to other heavy metal contaminated sites in Nigeria, *e.g.*, municipal dumpsites [62], arable soils around Pb-Zn mining localities [63, 64], cement factory [65], the heavy metal contamination level at this e-waste site is generally higher.

3.2 Distribution of heavy metals in soils

The *CF* was used in this study to evaluate the contamination level of each heavy metal (Table S4 and Table S5, Supporting Information). The two predominant heavy metals revealed by *CF*s were Cu (surface soil: total, 907.8±159.6; HCl-extractable, 585.4±102.1) and Pb (surface soil: total, 501.6±65.1; HCl-extractable, 238.6±37.8) in both surface soils and subsoils. The *CF*s of HCl-extractable Cd (73.5±12.2) were remarkably higher than those of total Cd (12.1±1.1), attributing to the low Cd concentrations in the reference soil (S15). The contamination of Cr was slight (the *CF*s of total and HCl-extractable fractions as 4.6±0.9 and 2.1±0.2, respectively). Unlike other heavy metals with homogeneously distributed *CF*s across the sites, the *CF*s of total Ni were much higher at some sampling sites (S6_T, S11_T and S14_T; S5_B) than others. The results suggested that Ni was relatively difficult to horizontally transport in the studied soils but stayed in the surface soils where it was originally disposed, because of the high Ni sorption capacity in soils [56].

As the concentrations of heavy metals generally decreased with depth, a positive log-linear correlation of *CF*s between surface soils and subsoils was observed (Figure 2A, R^2=0.7364, $p<0.05$). Since the e-wastes were originally disposed on the soil surface, the contamination in subsoils was mainly caused by the metal vertical transportation from the surface soils. Meanwhile, the heavy metal transportation ability depended upon its interaction with soil constituents.

Further investigation on the correlation of CFs between total and HCl-extractable fractions of heavy metals is illustrated in Figure 2B. Scatters of each heavy metal are clustered together and dependent on their concentrations. It is worth noting that *CF*s of total and HCl-extractable Cr, Cu, Ni and Zn are located on the central line, indicating that they have similar HCl-extractable fractions. However, *CF*s of total and HCl-extractable Cd and Pb are deviated from the central line, suggesting Cd tends to be extracted by HCl in comparison with other heavy metals, whilst Pb behaves opposite.

The spatial distribution of *CF*s for both total and HCl-extractable heavy metals in surface soils and subsoils was illustrated in Figure 3. The *CF*s of total heavy metals showed a gradual decrease from where they were originally disposed. Specifically, the *CF*s gradients of total Cr, Cu, Pb and Zn demonstrated a similar pattern that the most heavily contaminated sites were all located at the south-western corner of the study area (S7, S8 and S10). In contrast, the *CF*s of Ni in subsoils were different from those in surface soils, possibly caused by either a different contamination source or less mobility [56]. The *CF*s of the HCl-extractable metals did not vary as greatly as those of total metals, as their concentrations had relatively fewer variations (Table 1 and Table 2). Cd displayed distinct higher *CF*s of HCl-extractable fractions comparing to other metals. These results indicated that heavy metals could penetrate the soils and transport vertically towards subsurface layers, depending on their mobility.

Soil organic matters and pH are crucial factors influencing the mobility of heavy metals in soils. The high organic matters in surface soils have more sorption sites and reduce the metal mobility [66, 67], and the high soil pH reduces the solubility and hence mobility of heavy metals [68]. For example, the decrease in soil pH lowers the Cd adsorption and increases its mobility through the enhanced competition for negative surfaces between H^+ and dissolved metals [69]. Heavy metals speciation in environmental media also contributes to their mobility [6, 69], and it is related to the inherent nature of heavy metals, soil physiochemical properties and the interactions between heavy metals and soil particles [70, 71]. For instance, Cu and Pb are predominantly in the fixed fraction, whilst Cd is in the extractable form [6], resulting that most Cu and Pb retain in the surface soils whereas Cd has less decrease with depth [51].

The *PLI* is an indicator assessing the multiple contamination level of heavy metals. In

the present study, the highest *PLI* of total heavy metals was observed in surface soils of S8 (128.8), S14 (98.9) and S9 (96.1), and subsoils of S7 (90.6), S8 (90.1) and S6 (78.3), respectively. For the HCl-extractable metals, the surface soils of S14 (146.1), S11 (122.4) and S13 (75.7) were the most heavily contaminated, as well as the subsoils of S14 (161.1), S10 (112.0) and S6 (103.8). Such difference revealed by *PLI*s between total and HCl-extractable heavy metals was mainly caused by the high *CF*s of Cd, which were significantly higher for HCl-extractable fraction than total fraction. Our *PLI*s were remarkably higher than those at an e-waste site in China, where the *PLI*s ranged from 1.20 in deserted soils, 2.58 in vegetable gardens for e-waste recycling, 3.01 in paddy fields, 18.63 in pond areas, to 46.70 at open incineration sites [6]. The high *PLI*s in the present study were mainly caused by the long-term disposal of e-wastes (ever since 2006) and the relatively low heavy metal contents in reference soil S15.

3.3 Microbial community structure and diversity

The 16S rRNA gene sequencing generated a total of 470,976 quality sequences and 51,923 OTUs for 26 soil samples. Each of the communities contained 14,019 to 22,348 reads, with a range of OTUs from 928 to 2,664.

Taxonomic classification of all the OTUs identified 26 different phyla, and bacteria (99.86%) were predominant of 16S rRNA gene sequences whilst only a small proportion (0.14%) were assigned to archaea. The most frequently detected bacterial phyla among all the soils were *Proteobacteria* (4.8%-32.0%), followed by *Firmicutes* (0.9%-87.3%), *Actinobacteria* (3.1%-35.4%), *Chloroflexi* (2.5%-32.6%), *Acidobacteria* (1.6%-30.7%), *Planctomycetes* (0.6%-18.2%) and *Bacteroidetes* (0.1%-5.4%), as illustrated in Figure 4. The predominant bacterial phyla found in the present study were similar to those at other e-waste sites or heavy metal contaminated areas (*e.g.*, China and UK), where *Proteobacteria*, *Acidobacteria*, *Bacteroidetes* and *Firmicutes* are frequently observed [33, 72-74]. Additionally, our results were in accordance with previous studies reporting that *Deltaproteobacteria* [75-77] and *Firmicutes* [78] dominated the metal-tolerant cultures, since some metal-tolerant species are Fe(III)-reducing bacteria facilitating the release of soil-adsorbed metals and enhancing metal stress [79, 80].

Compared to the reference soils (S15_T and S15_B), other soil samples shared similar

compositions of predominant bacterial phyla regardless of the contamination levels. Nevertheless, heavy metal contents affected their relative abundance and the impacts varied among the taxa. For instance, *Proteobacteria* had the least abundance (4.8%) in S12_B and the highest abundance in S4_T (32.0%). The abundance of *Firmicutes* was significantly higher in S12_B (87.3%) than in S6_T (0.9%).

3.4 Environmental variables influencing bacterial composition and diversity

The MRT analysis revealed the relationship between bacterial composition and environmental variables in a visualized tree with 4 splits based on soil texture, organic carbon, total Cu and HCl-extractable Pb (Figure 5). The tree explained 63% of the variance of the bacterial composition (Table S6, Supporting Information). Bar plots at the four nodes of the tree effectively illustrated the overall profiles of bacterial community structure. Here, bacterial community compositions were first split by soil texture (clay percentage), which explained 31% of the variation. Group 5 with 4 soils having the clay percentage lower than 52% had the extremely high abundance of *Firmicutes* (54.4%), followed by *Proteobacteria* (13.2%) and *Chloroflexi* (8.0%). The other 22 soils in Groups 1, 2, 3 and 4 had the clay percentage higher than 52% and the predominant bacterial taxa were *Actinobacteria* (20.3%), *Proteobacteria* (18.6%) and *Firmicutes* (16.9%). Soil organic carbon further split the 22 soils into two branches and explained 14% of the variance. The first two splits including clay percentage and organic carbon can explain 45% of the variance. Group 1 contained 7 soils with organic carbon higher than 22.95 mg/kg, in which the abundance of *Actinobacteria* was 29.8%, followed by *Proteobacteria* (18.8%) and *Acidobacteria* (15.7%). Groups 2, 3 and 4 with organic carbon lower than 22.95 mg/kg were finally split by total Cu and HCl-extractable Pb content, which together explained 18% of the variance. The predominant bacterial taxa were *Proteobacteria* (23.2%), *Actinobacteria* (20.3%) and *Firmicutes* (14.1%) in Group 2, and *Firmicutes* (32.0%), *Proteobacteria* (23.2%) and *Chloroflexi* (19.1%) in Group 3. Of all the bacterial taxa, the abundance of *Proteobacteria*, *Firmicutes* and *Acidobacteria* were most altered by soil texture, organic carbon and heavy metal contents (Table S6). Other studies also reported that bacterial taxonomic composition was influenced by some environmental variables, such as soil K, NH_4^+-N, total Cu, available Zn and available Cu [74, 81], since they might affect the morphology, enzyme activities and metabolisms of soil microorganisms [82-84].

The relationship between community diversity and environmental variables was also illustrated by MRT analysis with 6 splits based on soil pH, texture (clay percentage), CEC, HCl-extractable Zn and total Cu (Figure 6, Table S7 in Supporting Information). The tree accounted for 79.1% of the variance of the standardized diversity indices. Soil pH split the 26 soils into two branches with different diversity patterns, 12 samples in Groups 1, 2, 3 and 4 with pH<7.94 and 14 samples in Groups 5, 6 and 7 with pH>7.94. Samples with lower pH had relatively lower diversity indices (Chao1, Shannon and Simpson). The two branches were further split by clay percentage (14%) and CEC (6.05 cmol/kg), and higher diversity indices were found in samples with lower clay percentage and higher CEC. Soil pH, as well as clay percentage and CEC, explained 59.3% of the variance. Heavy metal contamination levels including HCl-extractable Zn and total Cu further affected the bacterial diversity and explained 19.8% of the variance. Soils with higher contents of HCl-extractable Zn and total Cu had higher diversity indices.

The results in the present study showed that bacterial composition and diversity were affected by different environmental variables including soil properties (pH, texture, CEC and organic carbon) and heavy metal contamination level (total Cu, HCl-extractable Zn and HCl-extractable Pb). It is worth mentioning that, although many studies have demonstrated the correlations between heavy metal contents and bacterial community composition or diversity [29, 85-87], our results suggested that the key environmental variables influencing the composition and diversity of soil bacterial community at this e-waste site were soil properties, such as soil pH, CEC and clay percentage. Soil properties seemed to be more influential in determining soil bacterial community composition and diversity at heavy metal contaminated e-waste sites, possibly attributing to their influence on heavy metal mobility and speciation [53, 88]. Relationships between soil properties and heavy metal speciation have been previously reported in both field studies and experiments. The pH, $CaCO_3$ and organic matter contents of 15 agricultural soils played dominant roles in heavy metal speciation [69]. Sixteen soils from 13 provinces in China also indicated the influence of soil properties, *e.g.*, pH, clay content and CEC, on the speciation of heavy metals [89]. The addition of wine lees-derived biochars significantly increased soil pH and decreased the contents of soil exchangeable heavy metals, promoting the transformation heavy metal into residual fractions [90]. Similar findings were also

derived from an experimental study that the bioavailability and speciation of heavy metals altered significantly when amended with biochars [91].

The significant roles of soil properties on microbial composition and diversity were also observed in previous studies from other soils with different land use patterns. For example, Tang *et al.* reported that the contamination stress of heavy metals and PCBs had only a slight influence on microbial activities in paddy soils [52]. Fierer and Jackson found that soil pH explained 70% variation of the bacterial diversity in different terrestrial ecosystems and the bacterial diversity increased with soil pH within a proper range [92]. Soil microbial community and diversity were found mainly dependent on soil pH in a heavy metal-contaminated forest surrounding a zinc and lead industry region [93], e-waste contaminated soils [94] or the rhizosphere of Cu-tolerant plant *Elsholtzia splendens* [95]. More recently, Wu *et al.* also suggested the key roles of soil properties in determining microbial community structure in five soils with e-waste recycling activities, in which available phosphorus, soil moisture and mercury were identified as the major drivers [96].

4. Conclusion

In the present study, we investigated the heavy metal contamination level at an e-waste site in Nigeria. The analysis of *CFs* and *PLIs* identified Cu, Pb and Zn as the key metal contaminants in both surface soils and subsoils of the study area. Distribution of heavy metals in soils was related to heavy metal mobility and speciation. Bacterial taxonomic composition in the contaminated and uncontaminated areas varied significantly. Soil properties played a key role in influencing microbial composition and diversity, including soil pH, texture, CEC and organic carbon. Our results offer a better understanding of the crucial factors on microbial community structure at heavy metal contaminated e-waste sites.

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Figure Captions

Figure 1. Maps of research area and sampling sites.

Figure 2. (A) Correlation of contamination factors (*CF*s) between surface soils and subsoils. (B) Correlation of *CF*s between total and HCl-extractable fractions.

Figure 3. Spatial distribution of contamination factors (*CF*s) of each heavy metal in research area. (A) and (B) for total and HCl-extractable heavy metals in surface soils; (C) and (D) for total and HCl- extractable heavy metals in subsoils.

Figure 4. The taxonomic composition of soil bacteria on phylum level.

Figure 5. Multivariate regression tree (MRT) analysis of the correlation between environmental variables and soil bacterial composition. The bar plots illustrate the relative abundance of each phylum, and the patterns of bar plots represent the dynamics of community composition among each split. The numbers under the bar are the number of samples in each group. OC represents organic carbon.

Figure 6. Multivariate regression tree (MRT) analysis of the correlation between environmental variables and soil bacterial diversity. The figure shows the diversity indices as Chao 1, Shannon and Simpson, which were standardized for MRT. Bar plots show the multivariate means of diversity among each split. The numbers under the bar are the samples in each group. CEC represents cation exchange capacity.

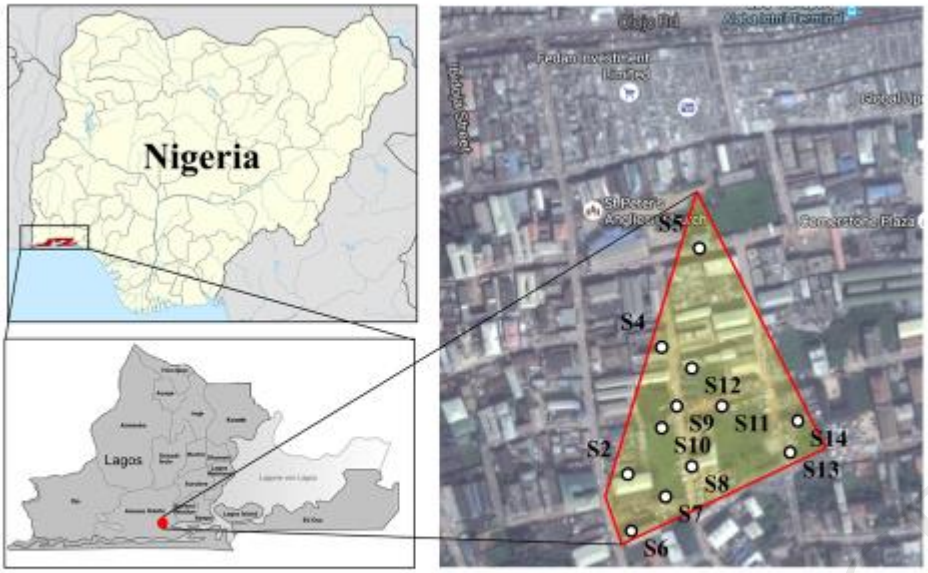
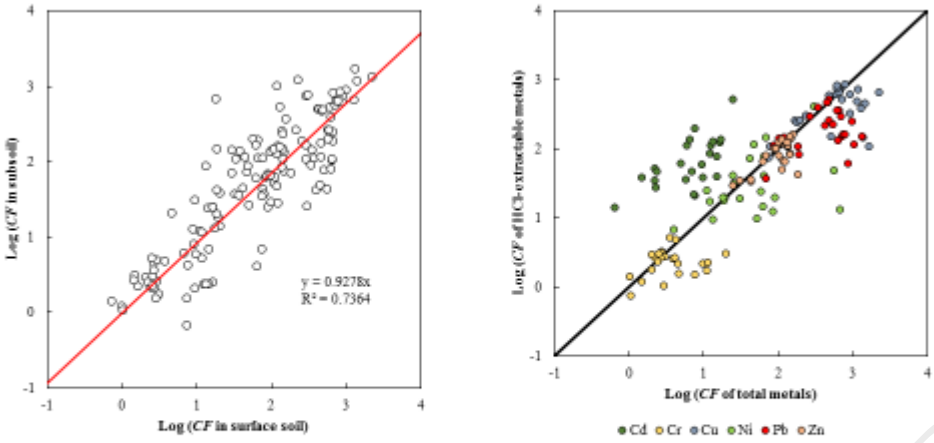


Figure 1



(A) (B)

Figure 2

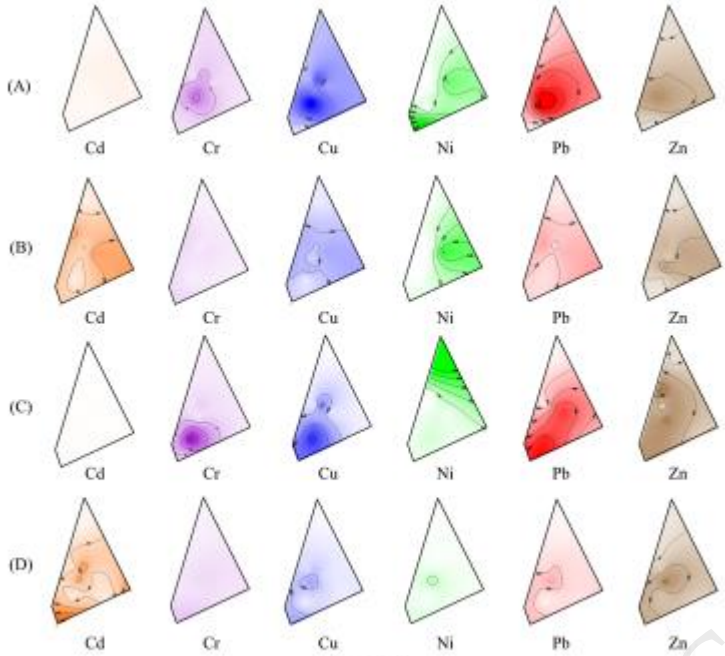


Figure 3

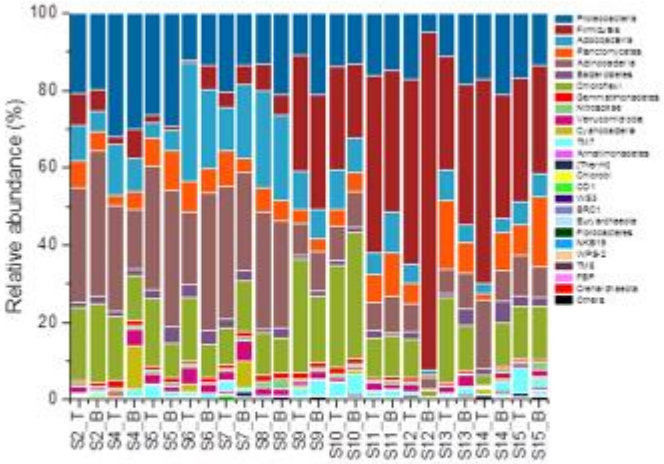


Figure 4

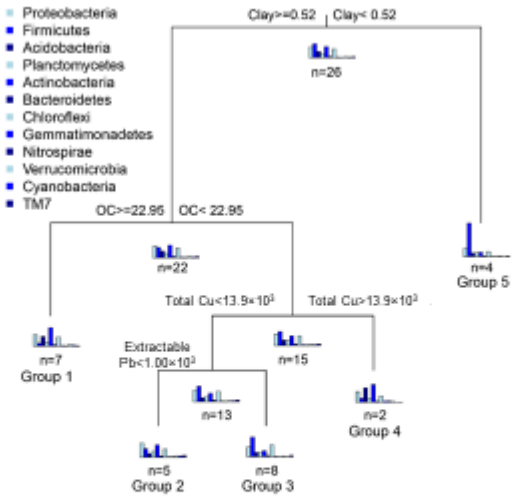


Figure 5

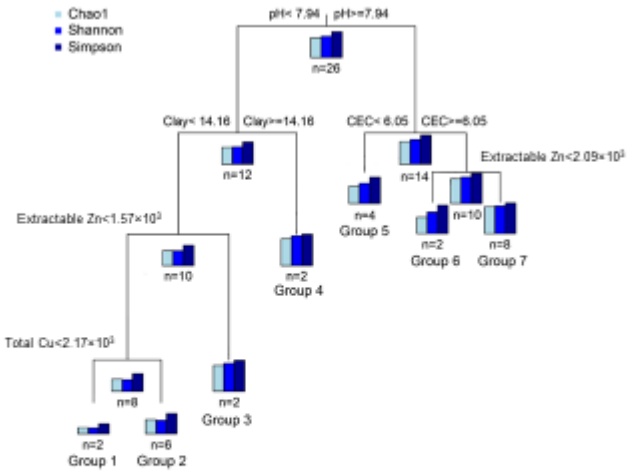


Figure 6

Table 1. Heavy metal concentration in surface soils (0-15 cm, mg/kg).

Soils	T-Cd	E-Cd	T-Cr	E-Cr	T-Cu	E-Cu	T-Ni	E-Ni	T-Pb	E-Pb	T-Zn	E-Zn
S2_T	14.8	8.6	64.6	31.0	8.85×10^3	4.70×10^3	0.35×10^3	0.05×10^3	4.95×10^3	2.10×10^3	2.41×10^3	1.07×10^3
S4_T	15.5	11.1	46.0	39.1	4.58×10^3	4.49×10^3	0.13×10^3	0.09×10^3	3.09×10^3	2.87×10^3	1.41×10^3	1.27×10^3
S5_T	9.2	2.1	48.7	12.5	4.95×10^3	1.14×10^3	0.13×10^3	0.08×10^3	2.21×10^3	0.79×10^3	0.88×10^3	0.52×10^3
S6_T	11.6	5.8	46.1	37.2	2.23×10^3	2.27×10^3	3.83×10^3	0.22×10^3	1.42×10^3	1.39×10^3	0.63×10^3	0.54×10^3
S7_T	9.2	2.1	79.3	18.5	13.0×10^3	1.11×10^3	0.01×10^3	0.04×10^3	12.1×10^3	1.11×10^3	2.30×10^3	0.79×10^3
S8_T	12.0	3.7	187	20.7	21.6×10^3	4.87×10^3	0.62×10^3	0.09×10^3	15.3×10^3	1.45×10^3	3.04×10^3	2.23×10^3
S9_T	18.2	3.9	129	18.1	13.8×10^3	3.40×10^3	0.43×10^3	0.07×10^3	8.42×10^3	1.44×10^3	2.09×10^3	1.26×10^3
S10_T	8.4	4.6	163	26.3	8.69×10^3	2.43×10^3	0.21×10^3	0.09×10^3	8.67×10^3	1.54×10^3	2.59×10^3	1.03×10^3
S11_T	20.5	12.3	50.2	32.5	6.54×10^3	5.29×10^3	2.07×10^3	1.93×10^3	8.02×10^3	2.88×10^3	2.09×10^3	1.91×10^3
S12_T	18.3	7.6	37.8	32.3	5.65×10^3	4.59×10^3	0.08×10^3	0.08×10^3	7.76×10^3	3.48×10^3	1.83×10^3	1.59×10^3
S13_T	16.5	12.7	17.7	9.1	6.23×10^3	5.01×10^3	0.44×10^3	0.36×10^3	4.02×10^3	3.76×10^3	1.91×10^3	1.61×10^3
S14_T	20.7	13.6	45.1	31.8	7.62×10^3	6.44×10^3	3.11×10^3	2.21×10^3	5.62×10^3	4.94×10^3	2.31×10^3	2.14×10^3
S15_T	1.2	<0.02	16.6	12.2	0.01×10^3	0.01×10^3	0.01×10^3	0.00×10^3	0.01×10^3	0.01×10^3	0.02×10^3	0.01×10^3

Note: T-Cd, T-Cr, T-Cu, T-Ni, T-Pb and T-Zn represent the concentration of total Cd, Cr, Cu, Ni, Pb and Zn, respectively; E-Cd, E-Cr, E-Cu, E-Ni, E-Pb and E-Zn represent the concentration of HCl-extractable Cd, Cr, Cu, Ni, Pb and Zn, respectively.

Table 2. Heavy metal concentration in subsoils (15-30 cm, mg/kg).

Soils	T-Cd	E-Cd	T-Cr	E-Cr	T-Cu	E-Cu	T-Ni	E-Ni	T-Pb	E-Pb	T-Zn	E-Zn
S2_B	13.9	6.1	85.5	23.9	7.79×10^3	3.87×10^3	0.18×10^3	0.08×10^3	6.67×10^3	2.38×10^3	2.18×10^3	1.48×10^3
S4_B	6.2	5.1	37.9	19.6	1.83×10^3	1.89×10^3	0.12×10^3	0.05×10^3	2.54×10^3	0.99×10^3	3.15×10^3	0.57×10^3
S5_B	1.8	1.4	28.3	13.3	0.82×10^3	0.55×10^3	4.56×10^3	0.03×10^3	0.94×10^3	0.34×10^3	0.72×10^3	0.48×10^3
S6_B	21.3	19.3	81.1	54.5	12.8×10^3	5.41×10^3	0.28×10^3	0.19×10^3	13.6×10^3	2.28×10^3	2.31×10^3	1.43×10^3
S7_B	4.1	3.8	377	34.4	17.9×10^3	0.80×10^3	0.59×10^3	0.03×10^3	12.1×10^3	0.56×10^3	2.43×10^3	1.13×10^3
S8_B	15.9	3.6	217	24.8	14.0×10^3	2.84×10^3	0.45×10^3	0.11×10^3	8.85×10^3	1.20×10^3	2.40×10^3	1.11×10^3
S9_B	18.4	13.6	77.3	29.5	12.4×10^3	3.59×10^3	0.40×10^3	0.06×10^3	7.63×10^3	2.09×10^3	2.27×10^3	1.47×10^3
S10_B	16.4	11.2	38.5	32.8	6.56×10^3	5.58×10^3	0.47×10^3	0.38×10^3	8.60×10^3	3.27×10^3	2.63×10^3	2.17×10^3
S11_B	6.6	4.6	47.4	33.5	1.95×10^3	1.80×10^3	0.17×10^3	0.11×10^3	11.0×10^3	1.48×10^3	2.18×10^3	1.66×10^3
S12_B	6.2	3.4	46.0	25.8	2.11×10^3	1.90×10^3	0.08×10^3	0.07×10^3	1.42×10^3	1.30×10^3	1.05×10^3	0.86×10^3
S13_B	6.4	2.7	19.5	15.6	0.83×10^3	0.79×10^3	0.03×10^3	0.02×10^3	1.22×10^3	1.01×10^3	0.41×10^3	0.39×10^3
S14_B	67.7	51.5	65.5	58.8	6.72×10^3	6.10×10^3	0.33×10^3	0.30×10^3	6.31×10^3	4.32×10^3	2.41×10^3	2.04×10^3
S15_B	2.7	<0.02	18.6	11.4	0.01×10^3	0.01×10^3	0.01×10^3	0.00×10^3	0.01×10^3	0.01×10^3	0.02×10^3	0.01×10^3

Note: T-Cd, T-Cr, T-Cu, T-Ni, T-Pb and T-Zn represent the concentration of total Cd, Cr, Cu, Ni, Pb and Zn, respectively; E-Cd, E-Cr, E-Cu, E-Ni, E-Pb and E-Zn represent the concentration of HCl-extractable Cd, Cr, Cu, Ni, Pb and Zn, respectively.