

SOIL MICROBIOMES FROM DEFUNCT BATTERY MANUFACTURING DUMP SITE AS INFLUENCED BY HEAVY METALS

Briki-Okolosi, E.O.¹, Nwokocha, A.¹ and Fagbola, O.²

 ^{1,2} Department of Agriculture and Industrial Technology, Babcock University, Ilisan Remo, Ogun State, Nigeria.
³Department of Soil Resources Management, University of Ibadan, Ibadan, Nigeria.
¹ebenezerbrikiokolosi@gmail.com
²nwokochaa@babcock.edu.ng
³fagbola1111@gmail.com

ABSTRACT

Purpose: Polluted soils have a negative impact on agricultural crop yield and food safety; hence, they are a global concern. A wet battery waste disposal area has been encroached due to farmers' attempts to increase productivity in the research area. The farmers' desire to expand productivity inspired this study, which assessed the impact of battery waste deposits at different spatial scales on the evenness and richness of beneficial microorganisms.

Design/Methodology/Approach: Twenty (20) points were randomly sampled from the non-contaminated site, the main battery dumpsite (MDS), and 20 and 40 m away from the MDS. The study adopted a factorial arrangement on a completely randomised design that was replicated three times. Analysis of variance was used to analyse the data, and microbiological analyses were conducted within the experiment. Where means were significant, Duncan's multiple range test separated them.

Findings: *Glomus* had the highest count of 144.3-fold in 20 MA, while Acaulospora and Funneliformis were significantly and predominantly abundant in 40 MA by 282.0 and 55-fold, respectively. The MDS had significantly the lowest or least numerical values in most AMF species across all the spatial distances.

Research Limitation: The work focused on an abandoned dumpsite by a defunct battery manufacturing company.

Practical Implication: For marginally contaminated areas, the findings can guide the selection of appropriate crops and associated beneficial microbes that minimise heavy metal uptake, enabling safer productive use of remediated lands.

Social Implication: This research can help identify whether harmful metals are becoming more bioavailable over time or being naturally immobilised, directly impacting health risk assessments for vulnerable populations.

Originality / **Value**: This research significantly advances our understanding of how complex microbial communities respond to and potentially mitigate the extreme environmental conditions created by battery waste.

Keywords: Battery defunct site. heavy metals. microbial growth. soil. spatial distance





INTRODUCTION

For terrestrial ecosystems to function, soil is necessary. Food production, water and climate regulation, energy provision, and habitat for various life forms are just a few of its many diverse roles (Rodrigues-Filho et al., 2023; Ponge, 2015). For the soil to perform its ecological functions, soil microbial populations are essential to the cycling of nutrients, soil fertility, and carbon sequestration (Fierer, 2017). The soil microbial community most likely has the highest amount of microbial diversity. The species diversity of these soil microbes is thought to be a critical component linked to soil resistance and resilience.

Heavy metals (HMs), insecticides, hydrocarbons, and their derivatives are just a few contaminants that soil can retain (Zhang *et al.*, 2020). The primary cause of heavy metal contamination in soils is anthropogenic activity, such as mining and industrial activities. Mining and processing plants can release heavy metals into the soil, which can then end up in agricultural land (Wang *et al.*, 2018). The high concentrations of HMs in agricultural soil can also be attributed to indiscriminate use of fertilizers and pesticides (Cui *et al.*, 2018). The mobility and distribution of metal pollutants can also be influenced by the pH and moisture content of the soil (Violante *et al.*, 2010). Soil particles carrying heavy metals can migrate over 20 kilometers from their original location due to natural processes including wind, rain, and erosion. This can indirectly impact the soil and water systems connected to the original site (Beattie *et al.*, 2018).

Due to the high toxicity of HMs', lack of biodegradability, and long-term cumulative trend, soil pollution with HMs has recently garnered attention globally (Fajardo *et al.*, 2019). HMs cause biodiversity loss, change soil fertility, and upsetting microbial ecology.

Therefore, the main objective of this study was to evaluate the effect of heavy metals from the defunct lead battery manufacturing company in Lagelu on soil microbial population and diversity.

LITERATURE REVIEW

Bacterial communities can vary in composition due to stress brought on by heavy metal pollution (Lin *et al.*, 2016). As resistant bacteria readily adapt and proliferate, the diversity and quantity of vulnerable soil bacteria might decline, forming a distinct bacterial community structure (*Liu et al.*, 2020). Biomass and bacterial activity are always impacted by HM contamination (Kavamura & Esposito, 2010). Mercury (Hg), lead (Pb), copper (Cu), cadmium (Cd), manganese (Mn), zinc (Zn), chromium (Cr), zinc (Zn), and arsenic (As, metalloid) are the most ubiquitous hazardous metals and metalloids. While the biological roles of Hg, Pb, Cd, Cr, and as have not been demonstrated, Zn, Mn, and Cu serve as microelements in plants (Beatie *et al.*, 2018; Cui *et al.*, 2018).





Accumulation of heavy metals has become an issue in Nigerian cities, causing environmental concerns and significant health problems. The need to study heavy metals in Nigeria is important as the country's exposure to these heavy metals in African markets grows, as well as the recording of blood lead levels in Nigerians of various ages, genders, and socioeconomic classes increases daily (Osakwe & Okolie, 2015). Heavy metal contamination is less evident in Nigeria than other types of pollution, but its impact on the environment and humans is significant (Galadima & Garba, 2012). Heavy metal analysis conducted in soil, vegetables, and fish in Kano State revealed that the concentration measured was above permitted limits, and its cause was attributable to industrial and agricultural operations (Edogbo *et al.*, 2019). Olatunde *et al.*, (2020) stated that cement manufacturing, which is a part of industrial activity, is one of the significant sources of pollution in Nigerian soils, and that the spread of alkaline dust and gases, which enter water bodies and plants, has a negative impact on the environment. Heavy metals found in cement dust, such as chromium, lead, nickel, mercury, and other organic pollutants, are harmful to human health and the environment.

There is a need to examine how heavy metals affect microbial density and diversity, with particular reference to the Nigerian situation, using the defunct lead battery manufacturing company in Lagelu as a case study. Previous studies have examined the chemistry components and plant diversity from this site; however, the effect of these contaminations on microbial diversity has not been studied.

Therefore, the main objective of this study was to evaluate the effect of heavy metals from the defunct lead battery manufacturing company in Lagelu on soil microbial population and diversity.

MATERIAL AND METHODS

Study Area

Soil samples for this study were collected from Lalupon and Egbeda, which are located in the Lagelu Local Government Area in Ibadan, Oyo State, Nigeria. Soils were sampled directly from three spatial distances, ranging from the main dump site of the defunct leadacid battery manufacturing company in Lalupon, whose co-ordinates lies between latitude 7°457358'N and longitude 4.068891E, and secondly from 20 metres apart from main waste site whose co-ordinates lies between latitude 7°457059'N and longitude 4.069613E, then thirdly from 40 metres away from main waste site whose co-ordinates lies between latitude 7°457074N and longitude 4.069598E using soil auger. Control for the experiment was sampled from the study area at a location far away from all the contamination within the dysfunctional battery company in Lagelu. Soils were sampled from a depth of 0-15 cm.





Sample Preparation

All soil samples were taken to Babcock University screenhouse where an experiment was set up while some parts of the soil were used in the laboratory for physical and chemical analyses.

Screenhouse experiment

Both contaminated and non-contaminated soil samples were used to set up an experiment in the screenhouse. Two kilogrammes (2 kg) portion of the soil was introduced into 2kg pots (2 litre bucket) perforated basally to facilitate excess water drainage. A 3 x 2 factorial was set up in a completely randomised design arrangement replicated three times. The factors were soil with four levels: non-contaminated, the main dump site of the dysfunctional battery company, 20 and 40 meters away from the main dump site. Phytoremediators: Cowpea and Maize. Heavy metals such as Lead, Cadmium, Cobalt, Chromium, Nickel, and Zinc were assayed on both contaminated and non-contaminated soil before phytoremediation investigations. Soil was sampled from the microcosm setup for heavy metal determination from 4, 8 and 12 weeks after planting phytoremediators, considering their life cycle, to determine the extent to which heavy metals were phytoremediated from the contaminated soils.

Laboratory Analysis

Physical and Chemical Analyses of Soil

The physical properties of the soil determined include: soil particle size, which was determined using the hydrometer method according to (Zhang et al., 2024; Stevenson et al., 2023; Bouyoucos, 1962; Agbenin, 1995). Other chemical soil properties determined include organic carbon content using the potassium dichromate method (Shamrikova et al., 2022; Walkey & Black, 1934). Soil pH was determined using the potentiometric method by using a pH meter in a soil solution ratio of 1:1 according to Brady and Weil (2005), while available phosphorus was determined using the Bray 1 extraction solution of Murphy and Riley (1972).

A given quantity of soil was extracted using neutral ammonium acetate (1N NH40Ac: pH=7) for Exchangeable bases determination (Kefas et al., 2023; Okalebo *et al.*, 1993). An exchangeable potassium and sodium content was estimated using a flame photometer. In contrast, the Versnate (0.1M EDTA) titration method was used to determine calcium and magnessium content of the soil. The exchangeable acidity was determined using 1N KCl extraction (Umoh et al., 2023; Mclean, 1965). The total N content of soil was determined after digestion of the samples with concentrated H₂SO₄ in the presence of Kjeldhl catalyst (Okeke et al., 2022; Bremner, 1965). The heavy metal content of soil was determined using the method described by AOAC (2010).





Microbial Assay

The soil samples collected from both contaminated and uncontaminated soil were bulked respectively to obtain a composite sample. Soil microbial population was determined using the dilution spread plate technique. Nutrient agar (NA) and Potato Dextrose agar (PDA) were the culture media for bacteria and fungi, respectively. Serial dilution to 10⁻⁶ level, 0.1 ml of the diluents from the fourth (10⁻⁴) to sixth (10⁻⁶) dilution factors was pipetted separately and aseptically into different sterile Petri dishes and 15 ml of the cool (45°C) sterile molten agar media was added under aseptic condition, swirled gently for even distribution of each inoculum, allowed to set and was incubated at 37 °C for 24 hours (for bacterial), and at 28 °C for 72 hours for fungi and colonies counted.

Isolation and purification of a bacterial strain.

The bacterial colonies were selectively isolated by the streak method to subculture the bacteria to obtain a pure isolate. Cultured media were incubated at 37°C in an inverted position for 24–48 hours. Individual bacteria colonies were identified by morphological and biochemical techniques using the taxonomy scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Isolation and Identification of fungal strain

The fungi isolate was stained with methylene blue and observed under the microscope to identify mycelia and spore structures. The fungi species were classified based on their cultural and microscopic spore characteristics.

Mycorrhizal Propagule Density and Diversity Analysis

Mycorrhizal propagule density and diversity were determined using wet sieving and decanting, as described by (Khémes et al., 2023; Gerdemann & Nilcoson, 1963).

Data Analysis

The results were subjected to analysis of variance (ANOVA) using Statistical Analyses and Reporting System (SARS) as applicable. Means were separated using Duncan Multiple Range Test at (p < 0.05).

RESULTS AND DISCUSSION

This section presents the results and discussions of the physical and chemical properties of the soil, including its heavy metal contamination levels, population counts of different AMF species across the four spatial distances, and the bacterial colony count from the four spatial distances.





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Table 1: Physical and Chemical Properties as well as heavy metals across the four spatial distances of contaminated and non-contaminated soil

Parameters	Control	20 MDS	40 MDS	MDS	SE
pH(H ₂ O)	5.34c	6.00b	5.99b	6.58a	0.326
Organic carbon (g/kg)	11.55b	21.12a	23.10a	6.60c	2.89
Total nitrogen (g/kg)	1.27b	2.32a	2.54a	0.13c	0.82
Av. P. (mg kg ⁻¹)	12.8a	10.6b	10.1b	11.0b	1.26
E.A. (Cmolkg ⁻¹)	0.41a	0.30b	0.30b	0.35ab	0.036
Ca(Cmol kg ⁻¹)	3.14c	6.99a	6.43a	4.46b	1.211
Mg(Cmol kg ⁻¹)	0.99a	0.91a	0.96a	0.76b	0.072
K(Cmol kg ⁻¹)	0.08c	0.21b	0.18b	0.65a	0.048
Na(Cmol kg ⁻¹)	0.43b	1.84b	0.76b	20.41a	0.388
Mn(mg/kg)	85.0c	89.0c	101.0b	123.0a	5.221
$Fe (mg kg^{-1})$	121.0c	141.0a	156.0a	148.0a	7.345
Cu (mg kg ⁻¹)	1.23b	1.19b	1.31b	6.0a	0.999
Zn (mg kg ⁻¹)	1.56b	1.74b	1.80b	3.25a	0.766
Sand (g/kg)	810a	820a	790a	470b	2.000
Silt (g/kg)	120b	130b	140b	220a	3.552
Clay (g/kg)	70b	50b	70b	310a	2.844
Pb (mg kg ⁻¹)	20.10d	11315.5b	3167.95c	35645.75a	12.33
Cr(mg kg ⁻¹)	10.85c	16.10b	18.65b	31.00a	2.56
Co(mg kg ⁻¹)	3.70c	16.45a	11.20b	5.25bc	1.466
As(mg kg ⁻¹)	1.25c	2.80bc	5.10ab	8.75a	1.18

Means with same letter (s) in a row are not significantly different at 5 % level of probability by Duncan's Multiple Range Test (DMRT), ns; not significant, 20 MDS = 20 meters away from the dump site, 40 MDS = 40 meters away from the dump site.





Table 1 shows the physical and chemical properties of the soil, including its heavy metal contamination levels. The pH values ranged from 5.34 to 6.58, with the control soil having a significantly lower value than other sites, and the main dumpsite (MDS) exhibiting a significantly higher value. All soils were moderately acidic, although the MDS was near neutral compared to the others. The sampling locations observed Significant differences in organic carbon and total nitrogen content. The highest organic carbon (23.10 g/kg was found at 40 meters away from the dumpsite(40MA), while the lowest (6.60g/kg) was found at the MDS. Similarly, the highest total nitrogen (2.54 g/kg was observed at 40MA, while the Main dumpsite (MDS) had the significantly lowest total nitrogen (1.3 g/kg). MDS treatment shows lower sand but higher clay and silt contents than others.

Using a textural triangle, these percentages show that the soil texture of Control, 20MA and 40MA are sandy loam and that of MDS is clay. Lead (Pb) concentration in the MDS was significantly higher than in the control soil by 99.94%. Levels at 20 and 40 meters away were also significantly higher than the control, by 99.82% and 99.36%, respectively. Lead concentrations differed significantly across all sampling locations. Chromium was significantly higher in the MDS compared to other treatment soils.

Cobalt concentration was significantly higher in the 20-meter-away site compared to the MDS, 40-meter-away, and the control soil. Arsenic concentration was significantly higher in the MDS than the control soil, but not significantly different from the 40-meter-away site. However, it was significantly higher than the 20-meter site. The higher concentration of heavy metals (HMs) in MDS could be because of the tiny particle sizes and high surface charges of clay particles, which are significant players in the binding of heavy metals. This result corroborates with constant emphasis paid to the immobilisation of HMs by clay mineral-microorganism complexes.

According to Fang *et al.* (2010), there is an increase in the adsorption capacity of mixes containing high clay mixtures and microbes. Significantly higher arsenic heavy metal levels were observed in 40MA compared to 20MA, possibly due to the higher organic carbon levels observed in this spatial distance compared to others. This result corroborates the findings of other researchers who reported that the interaction of microbes, organic materials, and complex heterogeneous soil ecosystems, which form organic matter as well as their sizes and properties, are the fundamental structural components of soil. These properties however, strongly correlates with the enrichment, mobility, and bioavailability of heavy metals (HMs) in soil (Gong *et al.*, 2014; Huang *et al.*, 2014; Wang *et al.*, 2015; Huang *et al.*, 2020).





Soil pH in MDS of this tended towards neutrality and was somewhat close to alkalinity, which could be part of the high accumulation of heavy metals observed in the main dumpsite. This is in line with some findings that high pH levels increase the solubility of HMs (Mani *et al.*, 2015). In alkaline soils, where the strength of the metal-humic substance complexes is largely pH-dependent, other studies have also noted similar effects (Evangelou *et al.*, 2004; Park *et al.*, 2013; Mani *et al.*, 2015).

Table 2 reveals population counts of different AMF species across the four spatial distances. There were significant variations in the population counts of different AMF species across the four spatial distances. Some AMF species show higher population counts in certain spatial distances than others. For instance, *Glomus* recorded the highest count of 144.3-fold in 20 MA.

Acaulospora and Funneliformis were significantly and predominantly abundant in 40 MA by 282.0 and 55-fold, respectively, compared to other soil treatments. Nevertheless, it was observed that MDS recorded either significantly lowest or least numerical values in most of the AMF species across all the spatial distances. Regarding the total spore count, the leading site also recorded the lowest count compared to the other three spatial distances. 20 MA and 40 MA indicated significantly higher counts for all the fungal species than MDS and control soil. Our result, which reported a significant reduction in Arbuscular mycorrhizal fungal count in MDS, is in disagreement with the findings of Krishnamoorthy et al. (2015), who reported that highly contaminated soil was found to have a considerably higher total average abundance (population) of AMF.

However, this result is in line with Xu *et al.* (2012), who documented the detrimental impact of high Pb concentrations on mycorrhizal colonisation, and Wu et al. (2010) reported that high levels of heavy metals like As and Pb had detrimental effects on the amount of AMF spores. At heavy metal-polluted areas, heavy metals lower, delay, or even eliminate AMF colonisation and spore density (Wei *et al.*, 2014). In the heavy metal mining, several researchers have also observed positive, negative, or neutral impacts on mycorrhizal colonisation and spore density (Khade & Adholeya, 2009; Wu *et al.*, 2010).

Table 3 represents the bacterial colony count from the four spatial distances. At the dilution level of $10^3 \times 10^6$, the lowest bacterial population was observed in the MDS (0.9) compared to other soil treatments. Control soil (1.6), however, recorded a higher numerical bacterial population compared to 20 MA (1.4) and 40 MA (1.2), but was not significantly different. At $10^4 \times 10^6$, a significantly higher bacterial population was observed in the Control soil (11.6), compared to 20 MA (8.0), 40 MA (6.3), and MDS (4.7), while MDS recorded the lowest bacterial population compared to others.





A similar trend was observed at a dilution level of $10-4 \ge 106$, which was also found at a dilution level of $105 \ge 107$, where the highest bacterial population was observed in the Control soil (8.6). In contrast, the least was found in MDS (3.1). Compared to other soil treatments. This is not in line with the findings of Song *et al.* (2018), who reported that metal-tolerant bacteria make up over 80% of all soil bacteria in laboratories and field studies. It also disagrees with the findings of Zarbad *et al.* (2016), who reported that microorganisms with the proper mechanisms to live in these harsh conditions are selected when HM soil contamination persists for an extended period.

The two *Bacillus cereus spp* occupying node 2.624 are related to the bacteria spp on branch 0.000, attached to node 1.965 however, *Bacillus cereus* strain 6c1 and *Bacillus cereus* strain BHUAS2 are more closely related and have similar genetic make up for adaptation in stressful environment such as heavy metal polluted soil like battery defunct site compared to the other bacteria spp. (Figure1)





Table 2: Different Arbuscular mycorrhizal fungi and their population count as found in the four spatial distances of contaminated and non-contaminated soil

	Arbuscular mycorrhizal fungi species							
Soil	Glomus	Acaulospora	Funneliformis	Gigaspora	Entrophospora	Rhizophagus	Scutelospora	Spores (Total)
Control	97.7c	183.0c	45.0b	17.7c	0.0	32.0	0.0	375.3b
Main Site	54.3d	89.3d	41.0bbc	10.0c	0.0	31.0	0.0	225.7c
20 MA	144.3a	250.3b	31.7c	64.7a	0.0	36.7	0.0	527.7a
40 MA	117.3b	282.0a	55.0a	35.3b	0.0	31.3	0.0	521.0a

ns

ns

Means with the same letter (s) in a column are not significantly different at a 5 % level of probability by Duncan Multiple Range Test (DMRT)

ns; not significant





Table 3: Bacterial population density in dilution levels for the four spatial distances of contaminated and non-contaminated soil

Dilution Levels

Soil	10 ⁻³ x 10 ⁶	10 ⁻⁴ x 10 ⁶	10 ⁻⁵ x 10 ⁷
Control	1.6a	11.6a	8.6a
Main Site	0.9b	4.7d	3.1c
20 MA	1.4a	8.0b	4.7b
40 MA	1.2ab	6.3c	3.6c

Means with same letter (s) in a column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT)

ns; not significant





Figure 1: Bacteria Phylogenetic tree

CONCLUSION AND RECOMMENDATION

The main battery dumpsite had significantly the lowest or least numerical values in most AMF species across all the spatial distances. Hence, wet battery waste deposition significantly increases soil contamination with heavy metals. This area is unsuitable for the development of arable crops because the concentrations of heavy metals have a detrimental influence on the microbiological community.

The findings can guide the selection of appropriate crops and associated beneficial microbes that minimise heavy metal uptake in marginally contaminated areas, enabling safer, more productive use of remediated lands.





This research significantly advances our understanding of how complex microbial communities respond to and potentially mitigate the extreme environmental conditions created by battery waste. It has implications for both fundamental microbial ecology and applied bioremediation of similarly contaminated sites worldwide.

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