International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB)
Volume 8, Issue 1,2023, PP 1-10
ISSN 2455-4316 (Online)
https://doi.org/10.20431/2454-9428.0801001
www.arcjournals.org

Tolerance of Bacterial Isolates from an Electronic Waste Dumpsite to Some Heavy Metals

Williams, Janet Olufunmilayo[1*], James, Onimim[1], Robinson, Victor Kasi[1] and Nengi-Benwari, Anita O[2]

[1]*Department of Microbiology, Faculty of Science, Rivers State University, Nigeria*

[2]*Department of Crop and Soil Science. University of Port Harcourt, Nigeria*

***Corresponding Author:** **Williams, Janet Olufunmilayo,** Department of Microbiology, Faculty of Science, Rivers State University, Nigeria*

Abstract: *The response of bacterial isolates from electronic waste (e-waste) dumpsite to some selected heavy metals was investigated. Soil samples from points A (20m before e-waste), B (e-waste) and C (20m away from e-waste) were collected from an e-waste dumpsite, Kaduna Street, Port Harcourt City Local Government Area, Rivers State. The total heterotrophic bacteria, coliform and Pseudomonas of sample was determined using standard microbiological method. The physicochemical and heavy metal were also analysed using the APHA method. The bacterial isolates were screened for their tolerance to e-waste and some selected heavy metals such as Cu, Zn and Pb. Tolerance of isolates to heavy metal was determined by inoculating 1ml of the standardized isolates into sterilized nutrient broth supplemented with 50 and 100mg/ml concentrations of the respective metals and incubated for 24hours. After incubation, aliquots from the respective metal concentrations were plated out on nutrient agar plates, incubated for 24 hours. Plates were observed for growth and % survival was calculated for each isolate. The total heterotrophic bacterial count of A, B and C was 7.63×105, 3.38×105 and 6.24×105 cfu/g, respectively. The Pseudomonas count of A, B and C was 1.06×104, 3.00×102 and 2.00×102 cfu/g. The coliform counts of A, B and C was 2.16×105, 9.00×103 and 1.08×105 cfu/g, respectively. Percentage survival of Bacillus sp, Bacillus sp, Escherichia sp, Leminorellasp and Staphylococcus sp to Zinc at 50mg/ml concentration was 66.94±0.38, 3.32±0.73, 57.23±1.17, 56.00±0.10 and 59.15±0.39 % while at 100mg/ml, percentage survival was 68.68±0.26, 70.51±0.78, 53.03±1.87, 59.77±0.34 and 56.75±2.67%. Percentage survivals of Bacillus sp, Bacillus sp, Escherichia sp, Leminorellasp and Staphylococcus sp to copper at 50mg/ml concentration was 64.24±0.42, 79.35±0.49, 53.92±0.62, 57.43±0.18 and 54.74±1.17% while 61.4±1.56, 75.49±0.78, 49.06±1.59, 60.27±0.64 and 44.06±3.42% was recorded at 100mg/ml concentration. Percentage survivals of Bacillus sp, Bacillus sp, Escherichia sp, Leminorellasp and Staphylococcus sp to Lead at 50mg/ml concentration was 66.65±1.61, 85.55±1.43, 57.75±1.91, 61.92±0.42 and 57.23±0.39 % while the percentage survival at 100mg/ml was 62.73±3.19, 76.63±0.63, 56.9±2.21, 68.15±0.26 and 55.30±0.36%. Statistically, there was a significant difference in the survival of bacterial isolates (P<0.05). Bacillus sp were the most tolerant bacterial isolates to the heavy metals.*

Keywords: *e-waste waste dumpsite, heavy metal contamination, tolerance of bacteria*

1. INTRODUCTION

The rapid increase in population and the increased demand for industrial establishments to meet human needs have created problems such as over utilization of accessible resources and increased pollution taking place in the land, air, and water environments. Heavy metal is of economic significance in industrial use and has currently become a significant environmental problem throughout the whole world (Igiri *et al.*, 2018; Siddiquee *et al.*, 2015). One of the major sources of heavy metals in soil is from electronic waste (e-waste) dumpsites which is the discarding of electronic devices (Williams *et al.*, 2020). When the compost from an e-waste dumpsite is used as manure, some heavy metals are being subjected to bioaccumulation and may cause risk to human health when transferred to the food chain. Exposure to heavy metals may cause blood and bone disorders, kidney damage, decreased mental capacity and neurological damage (Yilmax *et al.*, 2005). Soil pollution with heavy metals represents a threat to the environment and food security due to the fast growth of industry and agriculture and the disruption of natural ecosystems by anthropogenic pressure linked to the growth of human populations (Schalk *et al.*, 2017).

Heavy metals are of economic significance in industrial use and have currently become a significant environmental problem throughout the whole world (Igiri *et al.*, 2018; Siddiquee *et al.*, 2015). Environmental pollution by heavy metals has become a serious threat to living organisms in ecosystem and it depends on the bioavailability of heavy metals and the absorbed dose (Okolo *et al.*, 2016).

Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Sobha *et al*., 2007). Chronic level ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure. Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary odema and death. Pulmonary effects (emphysema, bronchiolitis and alveolitis) and renal effects may occur following subchronic inhalation exposure to cadmium and its compounds (Duruibe *et al*., 2007).Toxicity of heavy metals is the ability of a metal to cause detrimental effects on microorganisms and it depends on the bioavailability of heavy metal and the absorbed dose (Rasmussen *et al*., 2009). Heavy metal toxicity involves several mechanisms, that is, breaking fatal enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species (ROS), destructing ion regulation and directly affecting the formation of DNA as well as protein (Ashruta *et al.*, 2014). According to Williams and Dilosi (2019), the site of action of any toxicant depends on the nature of the toxicant. Thus, toxicants which are not utilized as carbon source or for synthesis of any useful substance or material or the microorganisms could inhibit certain functions depending on the specific area where the toxicants could bind. Thus, the aim of the study was to evaluate the tolerance of bacterial isolates from an electronic waste dumpsite to some heavy metals.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study area was Kaduna Street, Port Harcourt City Local Government Area, Rivers State, Nigeria. The area is an electronic waste dumpsite; it is characterized with huge commercial activities including disposal of electronic wastes. Wastes such as television sets, computer monitors, radio sets, spoilt laptop parts, laptop batteries, microphone batteries, spoilt microphones and other electronic wastes. The dumpsite is located along the rail way around a major fruit garden market, houses and shops. The coordinates of the area are $7^{o}0'1.55"$ E and $4^{o}47'57.7"$ N.

2.2. Collection of soil samples

Soil samples were collected from surface soil within (0-15cm depth) into black polyethylene bags using a hand auger from three sampling stations around the e-waste dumpsite designated as stations A, B and C. Station A is 20m before the dumpsite, station B is the dumpsite while station C is 20m away from the e-waste dumpsite. Soil samples were taken randomly to cover the entire location. Before collecting the samples of the waste, debris were removed in order to expose the soil. The three samples were put in clean sterile polythene bags.

3. MICROBIOLOGICAL ANALYSIS

3.1. Enumeration of Bacteria from Soil Samples

The standard plate count method as described by Prescott *et al.* (2011) was used in cultivating the soil samples so as to enumerate the bacterial loads including isolating the bacterial types in the samples. A 10-fold serial dilution was carried out as described by Cheesbrough (2006). In this method, stock solution of the soil sample was first prepared by transferring 1g of soil sample into test tube containing 10ml sterile normal saline. After which, 1ml of the sample was withdrawn from the stock with the aid of a sterile pipette to a test tube containing 9ml sterile normal saline. This was done serially until a dilution of 10^{-6} was obtained. Aliquot (0.1ml) of 10^{-2} was inoculated on pre-dried Eosin methylene blue agar and cetrimide agar plates while aliquot of 10^{-3} was inoculated on nutrient agar plates. Plates were inoculated in duplicates and were spread using sterile bent glass rod before they were incubated at 37℃ for 24-48 hours. After the incubation, plates were observed for growth and the colonies were counted for enumeration of bacterial populations in the soil samples.

3.2. Isolation of Bacteria

Pure cultures of bacteria were obtained by aseptically streaking representative discrete colonies of different morphological types which appeared on the cultured plates onto freshly prepared pre-dried Nutrient agar plates and were later incubated at 37^{o}C for 24hours. After pure cultures were obtained, the bacterial isolates were preserved frozen in 10% glycerol in bijou bottles for later use. The 10% glycerol was prepared by adding 90 ml of water into a 10 ml glycerol solution in a conical flask and thereafter 5 ml was dispensed into bijou bottles, which were sterilized and allowed to cool before the isolates were transferred using sterile wire loop.

3.3. Characterization and Identification of Bacterial Isolates

The bacterial isolates were characterized by observing them microscopically and subjecting them to series of biochemical tests such as; citrate, oxidase, coagulase, Methyl Red, Motility, indole, starch hydrolysis, Voges Proskauer and sugar fermentation tests. Further confirmation was done by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Systematic Bacteriology and advanced bacterial identification system (ABIS) online identification tool.

4. SCREENING OF THE ISOLATES FOR HEAVY METAL TOLERANCE

4.1. Standardization of Inoculum size

Standardization was done using the method of Odokunma and Akponah (2010) with slight modification. The colony of the investigated bacterial isolates were first grown in 9ml sterile peptone broth and incubated for 18 hours at 37°C. After incubation, 1ml of the cells were suspended in 9ml sterile physiological saline and were subjected to serial dilutions up to 10^{6} dilutions. After which, aliquot (0.1ml) from each dilution was inoculated by spread plate technique into freshly prepared nutrient agar plates, which were incubated at 37°C for 24 hours. The dilutions that produced between 30-300 colonies were chosen and served as inoculum for preliminary screening experiments.

4.2. Preparation of Stock Solution

The stock solution of the electronic waste was prepared using the method of Odokuma and Akponah (2010) and William and Dilosi(2018) with slight modification. In this method, about 4g of the electronic wastes was dissolved in sterile 100ml deionized water.

4.3. Preliminary Screening Test

This test was carried out to ascertain the bacterial isolates that are resistant or can tolerate the electronic wastes. The method of Odokuma and Akponah (2010) was adopted. In this method, 9ml of contents from the electronic waste stock solution was dispensed into sterile labelled test tubes. About one milliliter (1ml) of 10^{-6} standardized culture of 18 hours old cultures of the bacteria were transferred into respective labelled test tubes containing 9ml of the electronic waste content. Another set of test tubes which contained only 9ml of normal saline and 1ml of the respective inoculum served as the positive control while an uninoculated 9ml test tube containing the e-waste content served as the negative control. After inoculation, test tubes were incubated for 24 hours at 37°C. After incubation, aliquot of 0.1ml from the respective test tubes were withdrawn using sterile pipettes and inoculated on the surface of sterile nutrient agar plates using the spread plate method. Inoculated plates were incubated at 37°C for 24 hours. The resulting colonies after incubation were used to determine the % survival and those with very high % survival was further screened for individual metal tolerance. The % survival was calculated using the formula below as described by Odokuma and Akponah (2009).

$$\% \text{ Survival} = \frac{\text{Log } count \ \ of \ Toxicant}{Log \ count \ \ of \ control} * 100 \qquad \text{equation 1}$$

4.4. Determination of Bacterial Tolerance to Selected Heavy Metals

The method of Akintoku*et al.* (2017) was adopted with slight modifications. Ten ml of nutrient broth of 50 mg/kg and 100 mg/kg of Pb, Cu and Zn were dispensed in tubes individually and sterilized by autoclaving at 121°C for 15 minutes. The tubes on cooling were labelled accordingly and were inoculated with 1ml of the standardized bacteria cells in duplicate. The 50 mg/kg and 100 mg/kg of

the heavy metals without inoculation including normal saline containing the isolates were used as negative and positive controls. All inoculated test tubes were incubated at 37℃ for 24 hours. After incubation, aliquot from the respective tubes was withdrawn with sterile pipettes and inoculated on freshly prepared nutrient agar plates. Incubation at 37℃ for 24 hours followed. After which, plates were observed for growth and counts were made for plates with growth which was used in determining the % survival of the isolates.

4.5. Analysis of Physicochemical Properties of Soil

Parameters such as moisture content of soil, pH, Temperature, Organic Carbon, Organic Matter, Nitrogen and Phosphorus of the soil samples were determined using the APHA method (APHA, 2012). While heavy metals analysed were Arsenic (mg/kg), Cobalt (mg/kg), Lead (mg/kg), Nickel (mg/kg) and Zinc (mg/kg).

5. Data Analysis

Statistical Package for the Social Sciences (SPSS) version 25 was used to analyze the data obtained for microbial load of different Soil Samples from the e-waste dumpsite. The data were summarized using descriptive statistics for tabulation. Analysis of Variance (ANOVA) was used to test for significant difference while Duncan was used to separate means.

6. Results

Results of the total heterotrophic bacterial, total *Pseudomonas* and total coli form counts of the various soil samples is presented in Table 1. Results showed that the total heterotrophic bacterial count of samples A (20 m before e-waste dump site), B (Electronic waste Dump site) and C (20m away from dump soil) was 7.63×10^5, 3.38×10^5 and 6.24×10^5cfu/g, respectively. The *Pseudomonas* count of samples A, B and C was 1.06×10^4, 3.00×10^2 and 2.00×10^2cfu/g. While the mean coli form counts of sample A, B and C was 2.16×10^5, 9.00×10^3 and 1.08×10^5cfu/g, respectively.

Table1. *Bacterial Counts (CFU/g) of the Soil Samples*

Sample	THB	TPS	TCC
A (20 m before dump soil)	7.63×10^5	1.06×10^4	2.16×10^5
B (Electronic waste Dump soil)	3.38×10^5	3.00×10^2	9.00×10^3
C (20m away from dump soil)	6.24×10^5	2.00×10^2	1.08×10^5

Keys: THB= total heterotrophic bacteria; TPS = total *Pseudomonas*; TCC = total coliform counts

Results of the suspected identities of the bacterial isolates from the soil samples showed that the twenty-four bacterial isolates from the different soil samples belonged to six genera such as *Leminorella*sp, *Kluyvera*sp, *Proteus*sp, *Bacillus*sp, *Staphylococcus*sp and *Escherichia* sp. The percentage occurrence of the bacterial isolates is*Leminorella*sp (16.7%), *Kluyvera*sp (12.5%), *Proteus*sp (12.5%), *Bacillus*sp (25%), *Staphylococcus*sp (20.8%) and *Escherichia*sp (12.5%) (Fig. 1). Based on the reported percentage occurrence, results showed that *Bacillus* sp were the most dominant bacterial isolates. The distribution of bacterial isolates across the study sites is presented in Table 2. Results showed that *Bacillus* sp, *Kluyvera*sp and *Leminorella*sp were isolated from all samples and were more distributed.

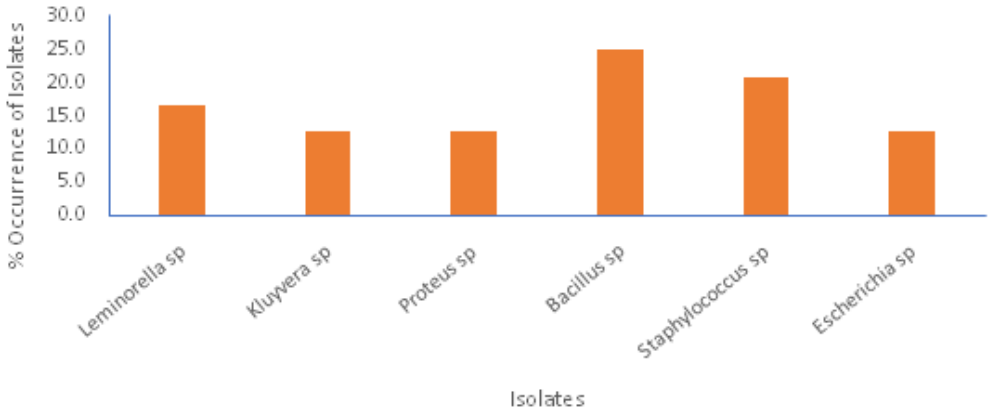

Fig1: *Percentage Occurrence of Bacterial Isolates*

Table2. *Distribution of Bacterial Isolates across the Samples*

Isolate	A	B	C
*Bacillus*sp	+	+	+
*Escherichia*sp	+	+	-
*Bacillus*sp	+	+	-
*Staphylococcus*sp	-	-	+
*Kluyvera*sp	+	+	+
*Leminorella*sp	+	+	+
*Proteus*sp	+	-	-

Keys: + = isolated; - = not isolated

6.1. Isolation and Screening of E-Waste Utilizing Bacterial Isolates

Results for the screened bacterial isolates are presented in Table 3. The bacterial isolates after being subjected to electronic waste substances from the dump sites, showed varied survival rates which was very high even though mortality was recorded in some isolates. Statistical analysis showed that despite the different values of survival recorded, there was no significant differences ($P > 0.05$) observed. Results also showed that *Leminorella*sp, *Bacillus* sp, *Escherichia* sp and *Staphylococcus* sp had higher survival rate including increasing in their population unlike others which recorded mortality. Although, higher tolerance to the electronic waste was mostly observed in *Bacillus* sp followed by *Staphylococcus* sp.

6.2. Tolerance of Bacterial Isolates to selected Metals

The bacterial isolates which exhibited high tolerance rate after screening were subjected to test on specific heavy metals associated with electronics. Results of their response to individual metal is presented in Table 4. Percentage survival of *Bacillus* sp, *Bacillus* sp, *Escherichia* sp, *Leminorella*sp and *Staphylococcus* sp to Zinc at 50mg/ml concentration was recorded as 66.94±0.38, 3.32±0.73, 57.23±1.17, 56.00±0.10 and 59.15±0.39 % while at 100mg/ml, percentage survival was 68.68±0.26, 70.51±0.78, 53.03±1.87, 59.77±0.34 and 56.75±2.67%. Percentage survivals of *Bacillus* sp, *Bacillus* sp, *Escherichia* sp, *Leminorella*sp and *Staphylococcus* sp to Copper (Cu) at 50mg/ml concentration was 64.24±0.42, 79.35±0.49, 53.92±0.62, 57.43±0.18 and 54.74±1.17% while 61.4±1.56, 75.49±0.78, 49.06±1.59, 60.27±0.64 and 44.06±3.42% was recorded at 100mg/ml concentration. Percentage survivals of *Bacillus* sp, *Bacillus* sp, *Escherichia* sp, *Leminorella*sp and *Staphylococcus* sp to Lead (Pb) at 50mg/ml concentration was 66.65±1.61, 85.55±1.43, 57.75±1.91, 61.92±0.42 and 57.23±0.39 % while the percentage survival at 100mg/ml was 62.73±3.19, 76.63±0.63, 56.9±2.21, 68.15±0.26 and 55.30±0.36%. Results showed that with the exception of *Leminorella*sp which increased in population with increased concentration of the metals, other isolates such as *Bacillus* sp, *Escherichia* sp and *Staphylococcus* sp declined in population with increase in concentration of heavy metals. Results also showed that despite the decline in population with respect to concentration observed, *Bacillus* sp were the most tolerant bacterial isolates to the heavy metals. Statistical analysis showed that at different metal concentrations, there were significant differences ($P<0.05$) in the percentage survival of the bacterial isolates with some being significantly higher than others.

Table3. *Response of Bacterial Isolates (cfu/ml) to Toxicants from Electronic Waste*

Isolate	% Survival
**Leminorella*sp	100.03±2.84[a]
*Kluyvera*sp	99.371±0.15[a]
*Proteus*sp	97.318±0.02[a]
**Bacillus*sp	102.25±0.75[a]
*Leminorella*sp	93.1±14.3[a]
**Escherichia*sp	101.84±0.15[a]
**Bacillus*sp	108.38±0.33[a]
*Staphylococcus*sp	97.38±0.11[a]
**Staphylococcus*sp	103.5±17.5[a]
*Bacillus*sp	94.98±0.00[a]

Means that do not share a letter are significantly different($P < 0.05$)

Table4. *Tolerance of Bacterial Isolates to Selected Heavy Metals*

Isolates	Tolerance (% survival of Isolates)					
	Zinc		Copper		Lead	
	50mg/ml	100mg/ml	50mg/ml	100mg/ml	50mg/ml	100mg/ml
Bacillus sp	66.94±0.38 [c]	68.68±0.26 [c]	64.24±0.42 [c]	61.4±1.56 [c]	66.65±1.61 [c]	62.73±3.19 [b]
Bacillus sp	73.32±0.73 [d]	70.51±0.78 [c]	79.35±0.49 [d]	75.49±0.78 [d]	85.55±1.43 [d]	76.63±0.63 [d]
*Escherichia*sp	57.23±1.17 [a]	53.03±1.87 [a]	53.92±0.62 [a]	49.06±1.59 [b]	57.75±1.91 [a]	56.9±2.21 [a]
*Leminorella*sp	56.00±0.10[a]	59.77±0.34 [b]	57.43±0.18 [b]	60.27±0.64 [c]	61.92±0.42 [b]	68.15±0.26 [c]
*Staphylococcus*sp	59.15±0.39 [b]	56.75±2.67 [ab]	54.74±1.17 [a]	44.06±3.42 [a]	57.23±0.39 [a]	55.30±0.36 [a]

Note: values are presented in Mean ± Standard Deviation

*Means with same alphabet down the group show no significant difference (P>0.05)

6.3. Physicochemical and Heavy Metals

Results of the physicochemical analysis is presented in Table 5. The mean ranges of the physicochemical parameters are given as; Nitrogen: 0.80±0.50 to 0.85±0.50%, organic matter: 6.50±0.50 to 6.90±0.60%, phosphorus: 38.0±0.00 to 40.0±1.00 mg/kg and temperature: 27.67±0.58 to 28.00±1.00 ℃. Values of the moisture content, organic carbon and pH remained the same across the respective sites.

Results of the heavy metal concentration of electronic waste is presented in Table 6. Results showed that the mean ranges of Arsenic, Lead, Nickel and Zinc were 2.89+00 to 4.00 +00, 13.00+00 to 15.00+00, 1.50+1.00 to 1.60+1.00 and 11.30+00 to 11.70±00, respectively. Results also showed that the e-waste site had the highest concentration of Arsenic, Lead and Zinc.

Table5. *Physicochemical parameters (Mean) of the Soil Samples from the e-waste Dump site*

Parameters	**A**	**B**	**C**	**WHO Limits**
Moisture (%)	4.00±0.00	4.00±0.00	4.00±0.00	-
Nitrogen (%)	0.81±0.10	0.85±0.50	0.80±0.50	≥ 40
Organic Carbon (%)	9.00±1.00	9.00±1.00	9.00±1.00	-
Organic Matter (mg/kg)	6.50±0.50	6.80±0.20	6.90±0.60	-
p H	6.33±0.58	6.33±0.58	6.33±0.58	6.5-8.5
Phosphorus (mg/kg)	40.0±1.00	39.0±0.00	38.0±0.00	≥ 40
Temperature (℃)	27.67±0.58	28.00±1.00	28.00±1.00	25 – 30

Table6. *Heavy Metal Concentrations of the Soil Samples from the e-waste Dump site*

Sample	**Arsenic (mg/kg)**	**Cobalt (mg/kg)**	**Lead (mg/kg)**	**Nickel (mg/kg)**	**Zinc (mg/kg)**
A	2.89±00	1.00±0.00	13.00±00	1.50±1.00	11.50±00
B	4.00 ±00	1.00±0.00	15.00±00	1.50±1.00	11.70±00
C	3.76 ±00	1.00±0.00	14.00±00	1.60±1.00	11.30±00
WHO Limits	0.9-10.0	1.0-10.0	0.3-10.0	0.1-0.5	1.20-6.00

Lead, cadmium, Nickel and Zinc exceeded the limits of heavy metals

7. DISCUSSION

The counts of total heterotrophic bacteria, total *Pseudomonas* and total coliform were higher in sample A (control) was higher than counts obtained in other two locations (B and C). The total heterotrophic bacterial and coliform counts were higher in sample C while total *Pseudomonas* counts in B was higher than those obtained in C. The high bacterial load recorded in the control sample could be attributed to high nutrient composition inherent in the soil especially since this area is rarely contaminated with electronic pollutants which could alter the microbial balance. Thus, lower counts in the dumpsite could be attributed to the wastes as well as other anthropogenic activities which could have altered the microbial composition. In agreement with this finding, a study by Ibrahim *et al.* (2021) attributed anthropogenic activity and deposition of metal wastes as the key factors which affected microbial community composition there by leading to low bacterial counts in their study. The finding also agreed with Pires (2010) who reported low bacterial counts from heavy metal contaminated site. In contrast to the findings in the current study, higher bacterial counts were reported from a heavy metal contaminated site in a previous study (Margesin *et al.*, 2011). One way analysis of variance showed that there was no significant difference ($P > 0.05$) in the total

heterotrophic bacterial counts of all three stations despite the disparity in the counts. Two-way ANOVA showed significant differences ($P< 0.05$) in the *Pseudomonas* and coli form counts of all three stations with stations A being significantly higher than stations B and C, respectively.

The twenty-four bacterial isolates in the current study were mostly the gram-negative isolates and two genera were gram positives. The dominance of gram-negative bacterial isolates in heavy metal contaminated site has been reported and their ability to thrive in the face of contamination has been attributed to the interaction between their cell wall and the metal ions on the surface and the interface of the bacteria (Ibrahim *et al.,* 2021). The bacterial isolates which include *Leminorella*sp, *Kluyvera*sp, *Proteus*sp, *Bacillus*sp, *Staphylococcus*sp and *Escherichia*sp were isolated from the respective samples. *Bacillus* sp, *Escherichia coli* and *Staphylococcus* sp which were isolated from the e-dumpsites in the current study have been reported in previous studies (Oluwafemi *et al.,* 2019; Akintokun*et al.,* 2017) except the presence of *Leminorella*sp, *Kluyvera*sp, and *Proteus*sp which were isolated in the current study. The disparity in the microbial types could also be due to the prevailing environmental factors as well as the different environment. The percentage occurrence of isolates showed that *Bacillus* sp was the most dominant and frequently isolated bacterial isolates across the location followed by *Kluyvera*sp and *Leminorella*sp as they were isolated in all locations. The least frequent bacterial isolate was *Staphylococcus* sp which was only isolated in one location (i.e., sample C). The distribution of bacterial isolates was not uniform as some genera which were isolated in one location were rarely present in other locations. The control sample had higher bacterial types compared to the e-waste dumpsite. This uneven distribution of the bacterial isolates could be attributed to the effect of heavy metals in the samples which while favouring the proliferation of isolates which could tolerate or bioaccumulate them eliminated or reduced the occurrence of none tolerant isolates. This agreed with Ibrahim *et al.* (2021) who reported that the presence of heavy metals in an environment selects the microbial types associated with such environment. Microbial survival in heavy metal polluted soils depends on intrinsic biochemical properties, physiological and/or genetic adaptation, as well as environmental modifications of metal speciation (Abou-Aly*et al.,* 2021).

The screening of bacterial isolates to electronic waste substances showed that out of the twenty-four bacterial isolates, only ten isolates such as *Leminorella*sp, *Kluyvera*sp, *Proteus*sp, *Bacillus* sp, *Escherichia*sp, *Bacillus* sp and *Staphylococcus*sp showed high e-waste tolerance. More so, *Leminorella*sp, *Bacillus* sp, *Escherichia* sp and *Staphylococcus* sp amongst other isolates showed higher survival rate but the most tolerant isolate was *Bacillus mycoides* followed by *Staphylococcus* sp. Despite the different values obtained in the percentage survival, there was no significant differences ($P > 0.05$) recorded in the survival rate of the different bacterial isolates.In this study, the two species of *Bacillus*, *Escherichia*sp, *Leminorella*sp and *Staphylococcus* sp showed high tolerance to different concentrations of Lead (Pb), Zinc (Zn) and Copper (Cu) despite the effect of the metals at higher concentration. Statistical analysis showed that at different metal concentrations, there were significant differences ($P<0.05$) in the percentage survival of the bacterial isolates with some being significantly higher than others. *Bacillus* sp were the most tolerant bacterial isolates to the various heavy metals. Tolerance of *Bacillus* sp to heavy metals have been reported and this agreed with findings in previous study (Akintokun *et al.,* 2017; Li *et al.,* 2011). The ability of these bacterial isolates to withstand the different heavy metal concentrations could be due to their bioaccumulation capacity or ability to degrade metals. It could also be that these isolates possess enzymes that has aided in the adaptation or ability to grow in the presence of the metals by having metal complex sites. This agreed with Vieira and Volesky (2000) who opined that different principal sites of metal complex formation in biological systems 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 could enhanced metal uptake by metal-resistant bacteria. Although, the bacterial isolates were highly resistant (tolerate) to the metals at low concentrations and declined at high concentrations. This observation could be attributed to the obstruction of metabolic pathways or other enzymatic functions of the bacterial isolates. Also, the intrinsic and extrinsic attributes of the microorganisms could also influence the metal utilization. This was observed in *Leminorella*sp which increased significantly with respect to increase in metal concentration where as other isolates decline in population with increased metal concentration. Metals can generally exert an inhibitory action on microorganisms by blocking essential functional groups, displacing essential metal ions or modifying

the active conformations of proteins and nucleic acids (Bruins *et al*., 2000). Although, the ability to grow at high metal concentration is found in many microorganisms and may be the result of intrinsic or induced mechanisms that may reduce metal toxicity (Pires, 2010). More so, bacteria that survive and flourish in such environments have developed or acquired genetic systems that counteract the effects of high metal ions concentrations (Pires, 2010). The tolerance to heavy metals could also be attributed to the fact that these bacterial isolates were isolated from a contaminated area containing high levels of heavy metals (Abou-Shanab*et al.,* 2007; Pal *et al*., 2005). Microorganisms are also of great importance in biotechnology, such as the use of bacteria in the bioremediation of pollutants and toxic wastes generated by the industry. Some bacteria are able to use pollutants as energy sources or to convert toxins into less harmful substances (Panizzon *et al.,* 2016). The bacterial isolates in the present study with the exception of *E. coli* which is mostly associated with faecal material are known to be widely distributed in the environment (i.e., soil and water). For instance, *Bacillus* sp is known to be widely distributed and can survive in any environment due to the presence of endospores (Prescott *et a.* 2011). Cosmopolitan microorganisms isolated from soil, water and vegetables such as bacteria of the Enterobacteriaceae family can also be found in the digestive tract of animals and humans, although it is also possible to find them in transient or normal microbiota (Cortés-sánchez*et al.,* 2015). Among them include: *Escherichia spp., Klebsiella* spp*., Salmonella spp., Enterobacter spp., Serratia spp., Hafnia spp., Citrobacter spp., Yersinia* spp., *Proteus* spp., *Rhanella ssp., Providencia spp., Morganella spp., Shigella spp., Edwarsiella*spp.,*Ewingella*ssp., *Budvicia*ssp.,*Tatumella spp., Erwinia spp., Koserella ssp., Kluyvera ssp., Hoganella ssp., Moellenella*ssp., *Leminorella sp., Buttiauxella spp. and Pantoea* spp. They can be pathogenic or opportunistic, occasionally forming components with physicochemical and biological properties beneficial to humans and the environment in which they operate (Apaydin *et al.,* 2005). Garbeva*et al*. (2003) points out that there is a wide variety of bacteria in the soil that, when associated with plant hosts, stimulate their growth, such as the rhizobacteria (Cortés-sánchez*et al.,* 2015). Taniguchi *et al.,* (2000) observed high level of passive biosorption of heavy metal ions for nonviable cells of *Pseudomonas putida*, *Brevibacterium* sp., and *Bacillus* sp. *Pseudomonas aeruginosa* biofilm cells show higher resistance to ions of copper, lead, and zinc than planktonic cells.

The physicochemical parameters of the soil samples were slightly acidic. It was documented in a previous study that African soil are slightly acidic (Akintokun*et al*., 2017). thus, this agreed with the current study which showed that the soils were all slightly acidic. The pH of these samples could have been influenced by the effect of anthropogenic activities and other natural causes (Ibrahim *et al.,* 2021). The pH values recorded in the current study do not corroborate with slight alkaline soils reported by Ibrahim *et al.* (2021). The pH influences microbial activity like solubility and ionization of inorganic and organic soil solution constituents which in turn affect soil enzyme activity (Ogbonna *et al.,* 2009).The presence of organic carbon, phosphorus, nitrogen and organic matter in the soil sample could be behind the proliferation of the respective bacterial isolates. It has been documented that macro and micro nutrient supports the growth of microorganisms as these organisms use these nutrients to build or synthesize materials required for metabolic processes (Prescott *et al.,* 2011).

The heavy metals concentrations of the soil samples showed that soil from e-waste dump soil had higher concentration of Arsenic, Lead, Zinc and Nickel. Furthermore, the concentrations of Lead Nickel and Zinc exceeded the WHO permissible limits of 0.3-10.0, 0.1-0.5 and 1.20-6.0 mg/kg for Lead Nickel and Zinc, respectively (WHO, 2008). Concentrations of heavy metals exceeding the permissible limits could alter the microbial diversity of the soil which in turn could also affect the fertility of that soil. Lead has been found to be the major cause of hypertension, impairment of central nervous system and other respiratory problems in adults (Kabiru *et al.,* 2015**)**. Zinc and lead are amongst the metals referred to as common urban pollutants (Demiguel *et al.,* 2007).

8. CONCLUSION

Microorganisms are generally found in all environments but their distribution could be influenced by different factors including the prevailing toxicant or nutrient in that environment. This study has shown that not all bacterial isolates from an electronic waste dump soil could utilize or bioaccumulate heavy metals in that environment. Although some which have become resistant to the metals may have synthesized certain enzymes or substances which might have aided their adaptation. The two species of *Bacillus, Escherichia* sp, *Leminorella*sp and *Staphylococcus* sp amongst other isolates were

the most preferred bacterial isolates with the ability to tolerate copper, zinc and lead. More so, the study has revealed that the increased dumping of electronic waste could be a great factor in the increased concentrations of some heavy metals.

REFERENCES

[1] Igiri, B.E., Okoduwa, S.I., Idoko, G.O., Akabuogu, E. P., Adeyi, A.O., and Ejiogu, I. K. (2018). Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater. A review. *Journal of Toxicology*; 1–16. 9

[2] Siddiquee, S., Rovina, K., Azad, S. A., Naher, L., Suryani, S., and Chaikaew, P. (2015). Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: *A* review. *Journal of Microbial and Biochemical Technology*; 7(6): 384–395.

[3] Williams, JO, Ugboma, CJ, Ibiene, F(2020). Bacteriological analysis of top soil from an electronic waste dumpsite in Port-Hacourt metropolis. *South Asian Joirnal of Research in Microbiology;* 29-30.

[4] Schalk, I.J., Hannauer, M., and Braud, A. (2017). New roles for bacterial siderophores in metal transport and tolerance. *Environ. Microbioly;* 13: (11).

[5] Okolo, N.V., Olowolafe, E.A., Akawu, I., and Okoduwa, S.I.R. (2016). Effects of industrial effluents on soil resources in Challawa industrial area, Kano, Nigeria. *Journal of Global Ecology and Environment*; 5: 1–10.

[6] Sobha, K., Poornima, A., Harini, P., and Veeraiah K., (2007). A study on biochemical changes in the fresh water fish, catlacatla (hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu University Journal of Science, Engineering and Technology*; 1(4): 1-4.

[7] Duruibe, J.O., Ogwuegbu, M.O.C., and Egwurugwu, J.N., (2007). Heavy metal pollution and human biotoxic effects. *International Journal of Physical Sciences;* 2 (5): 112-118.

[8] Rasmussen, L.D., Sørensen, S.J., Turner, R R., and Barkay, T., (2009). Application of a mer-lux biosensor for estimating bioavailable mercury in soil, *Soil Biology & Biochemistry*; 32(5): 639–646.

[9] Ashruta, G.A., Nanoty, V., and Bhalekar., U., (2014). Biosorption of heavy metals from aqueous solution using bacterial EPS. *International Journal of Life Science*; 2(3): 373–377.

[10] Williams, J. O. and Dilosi, L. B. (2018). Response of Chemolithotrophic Nitrobacter, Nitrosomonas to Toxicity of Organophoshphate and Pyrethroid Pesticides. *Asian Journal of Biology*, 7(1): 1-8.

[11] Prescott C, Sherwood LM, Woolverton CJ, Wiliiey JM, Harley F, Klen. (2011) Microbiology. 8th Ed. New York: Macgraw-Hill Higher Education;158-165.

[12] Odokuma, L. O. and Akponah, E. (2010). Effect of nutrient supplementation on biodegradation and metal uptake by three bacteria in crude oil impacted fresh and brackish waters of the Niger Delta. *Journal of Cell and Animal Biology*, 4 (1), 001-018

[13] American Public Health Association, (APHA), (2012). Standard methods for the examination of water and wastewater, 23rdEdition APHA, Washington D.C.

[14] Pires, C. (2010). Bacteria in Heavy Metal Contaminated Soil: Diversity, Tolerance and Use in Remediation Systems. (PhD Thesis) Cranfield Health Department, University of Cranfield. 1-229.

[15] Ibrahim, U. B., Kawo, A. H., Yusuf, I. and Yahaya, S. (2021). Physicochemical and molecular characterization of heavy metal–tolerant bacteria isolated from soil of mining sites in Nigeria. *Journal of Genetic Engineering and Biotechnology*, 19: 152

[16] Margesin, R., Plaza, G. and Kasenbacher, S. (2011). Characterization of bacterial communities at heavy-metal-contaminated sites. *Chemosphere,* 82(11):1583– 1588.

[17] Oyewole, O.A., Zobeashia, S.S.LT., Oladoja, E.O. et al. Correction to: Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil. SN Appl. Sci. 1, 985 (2019).

[18] Akintokun, A. K., Ezaka, E., Akintokun, P. O., Shittu, O. B., and Taiwo, L. B. (2019). Isolation, screening and response of maize to plant growth promoting. Sci.Agric. Bohem. 50, 181–190

[19] Abou-Aly H. E., Youssef A. M., Tewfike T. A., El-Alkshar E. A., El-Meihy R. M. (2021). Reduction of heavy metals bioaccumulation in sorghum and its rhizosphere by heavy metals-tolerant bacterial consortium. Biocatal. Agric. Biotechnol. 31:101911

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

[21] Vieira, R. H. and Volesky, B. (2000). Biosorption: A solution to pollution. *International Microbiology,* 3: 17-24.

[22] Abou-Shanab, R.A.I., van Berkum, P. & Angle, J.S. (2007). Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphre of Alyssum murale. Chemosphere 68, 360-367.

[23] Bruins, M.R., Kapil, S. & Oehme, F.W. (2000). Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety 45, 198–207.

[24] Panizzon J. P., Júnior, H. L. P., Knaak, N., Ziegler, D. R., Ramos, R. C. (2016) Bacteria-Soil-Plant Interaction: This Relationship to Generate can Inputs and New Products for the Food Industry. J Rice Res 4: 165.

[25] Cortés-sánchez, A. J., Diaz-ramirez, M., Hernández-álvarez, A. J., García-ochoa, F. and Villanueva-carvajal, A. (2015) Bio surfactants produced by enterobacterial. Glo Adv Res. J Microbiol 4: 103-112.

[26] Apaydin, Ö, Yenidünya, F., Harsa, Ş. and Güneş, H. (2005). Isolation and characterization of *Bacillus thuringiensis* strains from different grain habitats in Turkey. *World j microbbiot* 21, 285-292.

[27] Garbeva, P., Veen, J. D. and Elsas, J. D. (2003) Predominat Bacillus spp. in agricultural Soil Under Different Management Regimes Detected via PCR-DGGE. *Microbial Ecol* 45, 302-316.

[28] Taniguchi, J., Hemmi, H., Tanahashi, K., Amano, N., Nakayama, T. and Nishino, T. (2000). Zinc biosorption by a zinc-resistant bacterium, Brevibacterium sp. strain HZM-1, Applied Microbiology and Biotechnology, 54, no. 4, 581-588

[29] Ogbonna DN, Benjamin IKII and Patrick OY. (2009). Some physicochemical and heavy metal levels in soils of waste dumpsites in Port Harcourt Municipality and Environs. J. Appl. Sci. Environ. Manage. 2009; 13(4) 65 – 70

[30] Kabiru, S., Yakubu, R. Lukman, A., Akintola, T. and Alegbemi, M (2015). Heavy Metal Content in Soil in Garki Area Council of Federal Capital Territory, Abuja, Nigeria. *Analytical Biochemistry*, 4:197

[31] De Miguel, E., Irribaren, I., Chacon, E., Ordonez, A. and Charlesworth, S. (2007). Risk based evaluation of the exposure of children to trace elements in playgrounds in Madrid (Spain). *Chem*, 66: 505-513.

[32] Akintokun, A.K., Onatunde, O.O,. Shittu, O.B., Okeyode, I.C. and Taiwo, M.O. (2017). Bioaccumulation of Heavy Metals using Selected Organisms Isolated from Electronic Waste Dumpsite of two South Western States in Nigeria. *Applied Environmental Research*, 39 (2): 29-40.

[33] World Health Organisation (WHO), (2008). Air monitoring programme designed for urban and industrial area published for global environmental monitoring system by UNEP, WHO and WMO.

Citation: *Williams, Janet Olufunmilayo et al. "Tolerance of Bacterial Isolates from an Electronic Waste Dumpsite to Some Heavy Metals". International Journal of Research Studies in Microbiology and Biotechnology (IJRSMB). vol 8, no. 1, 2023, pp. 1-10. DOI: https://doi.org/10.20431/2454-9428.0801001*