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Isolation and identification of heavy metal-tolerant bacteria from e-waste-contaminated soils and their potential for bioleaching of waste printed circuit boards

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Abstract:

Background: Informal handling and disposal of electronic waste can release heavy metals into soil and select for metal-tolerant microorganisms. This study aimed to isolate and identify heavy metal-tolerant bacteria from soils around two informal e-waste handling sites in Ilorin, Nigeria, assess changes in dissolved metal concentrations during 21 days of sequential enrichment with waste printed circuit boards (WPCBs), and evaluate the isolates as potential candidates for future bioleaching studies.

Methodology: In this study, samples of soils in two metal-polluted markets (Sango Kulende and Ipata Oloje Spare Parts) and a non-market control zone at Ilorin, Nigeria, were collected at 5 sub-locations in each site. Powdered waste printed circuit boards (WPCBs) were added to the soil samples and cultured in Luria Bertani (LB) broth at successively growing pulp densities (1-5 per cent) for 21 days. Aliquots of the cultures were diluted in 0.85% NaCl solution and plated on nutrient agar plates, which were then incubated for 48-96 hours at 30°C. Pure isolates were identified by morphological and biochemical characteristics. Atomic absorption spectrophotometry (AAS) was used to confirm the presence of iron (Fe), copper (Cu), nickel (Ni), and zinc (Zn) in the WPCBs before and after soil enrichment.

Results: Four morphologically different isolates were obtained on nutrient agar following serial dilution of enriched cultures and morphological and biochemical characterisation identified them as *Lactobacillus plantarum*, *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus*. Monitoring AAS of culture supernatants in three replication weeks demonstrated the steady rise of dissolved metal concentration mainly during the first and third weeks with a significant rise of Cu and Ni concentrations, suggesting a continuous mobilization of metals by the bacterial isolates. After week three, the concentration of Cu in Ipata soil + powdered e-waste (IS +PEW) cultures was 14.429ppm, and the concentration of Ni in Sango soil + general e-waste (SS +GEW) cultures was 6.699ppm. The isolates, especially those of *B. subtilis* and *P. vulgaris*, exhibited strong tolerance to copper and nickel, which increased with increased exposure.

Conclusion: These results suggest that soil bacteria used in informal e-waste recycling locations can be utilized as a bioleaching source and should be more explored regarding their resistance and scalability.

Keywords: Electronic waste; bioleaching; heavy metal tolerance; printed circuit boards; bioremediation

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Isolement et identification de bactéries tolérantes aux métaux lourds dans des sols contaminés par des déchets électroniques et leur potentiel de biolixiviation des cartes de circuits imprimés usagées

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Résumé:

Contexte: La manipulation et l'élimination informelles des déchets électroniques peuvent libérer des métaux lourds dans le sol et favoriser la sélection de micro-organismes tolérants aux métaux. Cette étude visait à isoler et identifier des bactéries tolérantes aux métaux lourds dans les sols de deux sites informels de traitement des déchets électroniques à Ilorin, au Nigéria, à évaluer les variations de concentration des métaux dissous au cours de 21 jours d'enrichissement séquentiel avec des déchets de circuits imprimés (DCI), et à évaluer les isolats comme candidats potentiels pour de futures études de biolixiviation.

Méthodologie: Des échantillons de sol ont été prélevés dans deux marchés pollués par les métaux (Pièces détachées Sango Kulende et Ipata Oloje) et dans une zone témoin non marchande à Ilorin, au Nigéria, à cinq sous-emplacements de chaque site. Des déchets de circuits imprimés (DCI) réduits en poudre ont été ajoutés aux échantillons de sol et mis en culture dans un bouillon Luria-Bertani (LB) à des densités de pulpe croissantes (1 à 5%) pendant 21 jours. Des aliquotes des cultures ont été diluées dans une solution de NaCl à 0,85% et étalées sur des plaques de gélose nutritive, qui ont ensuite été incubées pendant 48 à 96 heures à 30°C. Des isolats purs ont été identifiés par leurs caractéristiques morphologiques et biochimiques. La spectrophotométrie d'absorption atomique (SAA) a été utilisée pour confirmer la présence de fer (Fe), de cuivre (Cu), de nickel (Ni) et de zinc (Zn) dans les WPCB avant et après enrichissement du sol.

Résultats: Quatre isolats morphologiquement différents ont été obtenus sur gélose nutritive après dilutions en série de cultures enrichies. Leur caractérisation morphologique et biochimique a permis de les identifier comme *Lactobacillus plantarum*, *Bacillus subtilis*, *Proteus vulgaris* et *Staphylococcus aureus*. Le suivi par spectrophotométrie d'absorption atomique (SAA) des surnageants de culture pendant trois semaines de répétition a démontré une augmentation progressive de la concentration en métaux dissous, principalement au cours des première et troisième semaines, avec une hausse significative des concentrations de cuivre (Cu) et de nickel (Ni), suggérant une mobilisation continue des métaux par les isolats bactériens. Après trois semaines, la concentration de Cu dans les cultures de sol d'Ipata + déchets électroniques en poudre (IS+PEW) était de 14,429 ppm, et celle de Ni dans les cultures de sol de Sango + déchets électroniques généraux (SS+GEW) était de 6,699 ppm. Les isolats, en particulier ceux de *B. subtilis* et *P. vulgaris*, ont présenté une forte tolérance au cuivre et au nickel, qui augmentait avec l'exposition.

Conclusion: Ces résultats suggèrent que les bactéries du sol utilisées dans les sites informels de recyclage des déchets électroniques peuvent servir de source de biolixiviation et méritent d'être étudiées plus en détail quant à leur résistance et leur potentiel d'application à grande échelle.

Mots-clés: Déchets électroniques; biolixiviation; tolérance aux métaux lourds; circuits imprimés; bioremédiation

Introduction:

Electronic waste (e-waste) is among the fastest growing waste in the world as its volume is affected by the shortening of product life cycles, the rapid pace at which technology takes over, and the rise in electronic device consumption per person (1). Existing estimates show that annual generation of e-waste in the world amounts to over 53 million metric tons of e-waste and hardly a small proportion of this e-waste is collected and recycled in a responsible manner (1).

Waste printed circuit boards (WPCBs) are the most problematic and complex items of e-waste. They are a heterogeneous mixture of polymers, ceramics, and metals including economically valuable copper, gold, and palladium and environmentally hazardous nickel, lead, and cadmium (2,3). Poor disposal through open burning, acid leaching, or landfill at informal recycling sites in the low and middle-income countries pollute the surrounding soils and water masses, with ripple effects on the microbial population, plant growth, and human health (4).

The traditional methods of metal recovery of e-waste are based on pyrometallurgical

smelting or hydro-metallurgical digestion in acid. The two systems are energy-easy, create secondary pollutants, and need lots of capital, and they are ill adapted to decentralised, low-resource environments (3). The biological solubilization of metals by microorganisms and their metabolites is called bioleaching and is a more sustainable alternative. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* chemolithotrophic acidophiles are the best-investigated bioleaching agents, but heterotrophic bacteria have been identified as viable alternatives, especially to alkaline or circumneutral-mixed waste matrices (5).

The repertoire of overlapping strategies used by microorganisms to tolerate heavy metals includes active efflux by active transport using RND, MFS, and P-type ATPase transporters, intracellular sequestration by metallothioneins, metal-binding peptides, enzymatic metal ion reduction and precipitation and extracellular chelation with biopolymers and siderophores (6,7). An example of this cation diffusion facilitator is CzcD, and the expression of this protein renders *Bacillus subtilis* resistant to Ni^{2+} , Cu^{2+} , Zn^{2+} , and Co^{2+} , and poly- γ -glutamic acid (γ -PGA) secretion denotes an extra cellular protection against zinc and copper toxicity (8). The cell

wall and exopolysaccharide matrix are the primary metal-binding surfaces in the lactic acid bacteria *Lactobacillus plantarum*, which facilitate passive biosorption of divalent cations in aqueous solution (9).

Informal electronics recycling in Nigeria, which operates centrally in urban markets in scrap yards, disposes of large amounts of e-waste on soils without containment (16). The soil surrounding these locations are unexploited source of native metal-tolerant bacteria that have evolved under long-term exposure to poly-metallic contamination. Analyses of analogous West African samples have also recorded high levels of Fe, Cu, Ni and Zn in market-area soils but has received little systematic microbiological characterization of the endangering metallo-tolerant communities (4,16).

This research aimed to isolate and identify heavy metal-tolerant bacteria of the soils surrounding two informal e-waste handling sites in Ilorin, Kwara State, Nigeria, to determine their profiles of metal tolerance over a 21-day sequential enrichment phase using the WPCBs as their metal substrate, and to determine their suitability as candidates in subsequent bio-leaching studies.

Materials and method:

Study area and sample collection:

The soil samples were taken at Sango Kulende Market and Ipata Oloje Spare Parts Market, Ilorin, Kwara State, Nigeria (around 8.5°N, 4.5°E). Both locations are described as having a high turnover of the trade in motor vehicle parts and second-hand electronic products and initial reconnaissance verified that there were observable metal littering, battery acid spills, and wiring waste lying on the soil surface. A third sample (control) was also collected directly in an adjacent non-market zone where no observed e-waste activity was observed.

Surface soil (depth 020 cm) at each site were sampled at 5 sub-locations with a sterile hand trowel, composite, and transferred in sterile and capped polypropylene containers. Products waste printed circuit boards (WPCBs) were obtained at an informal e-waste dismantling yard in the Challenge area of Ilorin.

Preparation of waste printed circuit boards:

The WPCBs were washed with distilled water, sheared, and ground to fine powder. PCB was immersed in a 10 M solution of NaOH (48 hours) to eliminate surface chemical coatings and washed using a running tap (to remove residual solder). The treated material was dried

in 60°C and sieved to obtain two size fractions of material: powdered ($\leq 120 \mu\text{m}$) and granulated ($\leq 250 \mu\text{m}$) (10).

Metal analysis of WPCBs by atomic absorption spectrophotometry (AAS):

A 1-gm finely ground WPCB sample was agitated in 48 h aqua regia ($\text{HCl}:\text{HNO}_3$, 3:1 v/v) at room temperature. The mixture was centrifuged at 5000xg/min and the product was filtered using Whatman No. 1 filter paper. Flame atomic absorption spectrophotometry (AAS) was used to determine the concentrations of iron (Fe), copper (Cu), nickel (Ni), and zinc (Zn). Measurements were conducted on untreated, NaOH-treated powdered and NaOH-treated granulated fractions (17).

Bacterial culture media preparation:

Nutrient agar (NA), prepared at 28 g L⁻¹ as per the specifications of its manufacturer, was inoculated in conical flasks, plugged with cotton wool and autoclaved at 121° C. Luria-Bertani (LB) broth was prepared using peptone (10g L⁻¹), yeast extract (5g L⁻¹) and NaCl (10g L⁻¹), and again autoclaved at 121°C.

Sequential enrichment and bacterial isolation:

Sequential enrichment followed variation of an outlined procedure described by Kumar et al., (11). One gram of composite soil of each location was inoculated in 250 mL of Erlenmeyer flasks containing 100 mL of LB broth with the addition of 1% of WPCBs (particle size $\leq 150 \mu\text{m}$). The flasks were incubated at 30°C during week 1 at 150 rpm for 7 days. Then, 5 mL of enriched culture was inoculated into fresh LB with 2.5% WPCBs (week 2), and then with 5% WPCBs (week 3) and incubated further in the fresh LB. After 21 days, 5 mL aliquots were diluted in sequence (10^{-1} to 10^{-7}) in 0.85% NaCl solution and plated on NA. The plates were incubated for 48-96 hours at 30°C. Pure isolates were obtained by morphological selection and subsequent sub-culture in NA.

AAS monitoring of metal dissolution during enrichment:

Cultures were filtered with Whatman No. 1 paper at the conclusion of every enrichment cycle per week and centrifuged at 7,000x g, for 10 min. The cell-free supernatant was re-filtered using 0.45 μm membrane filters to eliminate residual particulates and metal concentrations (Fe, Cu, Ni, Zn) in each supernatant were determined using the AAS and gave an indirect indication of the metal solubilization activity of the cell-free supernatant over a period.

Morphological and biochemical identification of bacterial isolates:

Colony morphology was documented in every isolate on NA (shape, margin, elevation, surface texture and pigmentation). Gram staining was done by performing the standard four-step procedure (12). Microscopy of the cell shape and arrangement was done under oil-immersion. Biochemical identification tests were performed with motility (triple sugar iron agar stab), catalase (3% H₂O₂), oxidase (Kovacs reagent on filter paper), coagulase (plasma slide test), indole (Kovacs reagent in tryptophan broth after 24 hours at 37°C), incubation with citric acid (citrate utilization agar), urease (plasma slide test), and gelatin agar flooded with mercuric chloride (gelatin hydrolysis). Comparison with standard keys was used to identify the isolates through biochemical enhancement of types (12).

Results:

Metal composition of WPCBs before and after NaOH treatment:

The analysis of the AAS showed that the top oil was the dominant Fe in untreated WPCBs (5.786 ppm), then Zn (2.775 ppm), Ni (1.549 ppm), and Cu (1.442 ppm) (Table 1). NaOH treatment significantly decreased the amount of Fe in the powdered (1.616 ppm) and granulated (1.429 ppm) fractions, which probably indicates the reagent washes away loosely bound impurity iron oxide phases.

On the contrary, it resulted in higher levels of Ni concentration following treatment in

both fractions (powder: 2.063 ppm; granulated: 2.539 ppm), which implies that the alkaline wash revealed the hidden or blocked surfaces with nickel. The concentrations of Zn and Cu were relatively constant regardless of treatment.

Bacterial isolation and identification:

After 21 days of sequential enrichment in NA plates, 5 morphologically distinct types of colonies were obtained. The morphological and biochemical identification revealed four bacterial species; *Lactobacillus plantarum* from SS+PEW samples, *Bacillus subtilis* from IS+PEW and CS+GEW samples, *Proteus vulgaris* from IS+GEW sample, and *Staphylococcus aureus* from SS+GEW sample (Table 2). *Bacillus subtilis* was obtained in the two independent sample combinations suggesting that the bacteria were uniformly enriched when subjected to varied combinations of sites and substrates.

Table 3 shows the morphological and biochemical profiles used to identify the bacterial species. *Staphylococcus aureus* was identified as Gram-positive, coagulase-positive, urease-positive, and β -haemolytic on blood agar. *Bacillus subtilis* was identified as Gram-positive, endospore forming, oxidase-positive, and growth on starch hydrolysate. *Proteus vulgaris* was identified as Gram-negative, motile, indole-positive, Voges-Proskauer and urease-positive. *Lactobacillus plantarum* was identified as Gram-positive, non-motile, catalase-negative, with most testing negative for degradation, in line with the characteristics of homofermentative lactic acid bacteria.

Table 1: AAS metal concentrations (ppm) in WPCBs before and after NaOH treatment

S/N	Metal	Untreated (ppm)	Treated Powder (ppm)	Treated Granulated (ppm)
1	Iron (Fe)	5.786	1.616	1.429
2	Copper (Cu)	1.442	1.481	1.511
3	Nickel (Ni)	1.549	2.063	2.539
4	Zinc (Zn)	2.775	1.673	2.159

Table 2: Bacterial isolates recovered from sequential enrichment cultures after 21 days

S/N	Sample	Identified bacteria
1	Sango soil + Powdered e-waste (SS+PEW)	<i>Lactobacillus plantarum</i>
2	Ipata soil + Powdered e-waste (IS+PEW)	<i>Bacillus subtilis</i>
3	Ipata soil + Granulated e-waste (IS+GEW)	<i>Proteus vulgaris</i>
4	Sango soil + Granulated e-waste (SS+GEW)	<i>Staphylococcus aureus</i>
5	Control soil + Granulated e-waste (CS+GEW)	<i>Bacillus subtilis</i>

Table 3: Morphological and biochemical characteristics of the four bacterial isolates

Test	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. vulgaris</i>	<i>L. plantarum</i>
Shape	Cocci	Rod	Rod	Rod
Gram reaction	+	+	-	+
Motility	-	+	+	-
Endospore	+	+	-	-
Catalase	+	+	+	-
Oxidase	-	+	-	-
Coagulase	+	-	-	-
Indole	-	-	+	-
Citrate utilization	+	+	+	-
Urease	+	-	+	-
Gelatin hydrolysis	+	+	-	-
Methyl Red (MR)	+	+	+	+
Voges-Proskauer (VP)	-	-	+	-
Glucose fermentation	+	+	-	+
Sucrose fermentation	+	+	+	+
Lactose fermentation	+	+	-	+
Starch hydrolysis	-	+	-	+
H ₂ S production	-	+	+	-
Gas production	-	-	+	-
Haemolysis	+	-	+	-

+, positive; -, negative; FA, facultative anaerobe

Metal dissolution during sequential enrichment:

The concentration of dissolved metal in culture supernatants varied significantly in three weeks of enrichment period. In week 1, the only significant metal mobilized was Ni and the anti-maxima values were obtained in the SS+PEW (mean 3.148 ± 0.007 ppm) and IS+GEW (mean 2.390 ± 0.007 ppm) cultures (Table 3). Fe registered the highest in IS+PEW (mean 3.055 ± 0.008 ppm) whereas Zn and Cu were relatively low in all the samples.

At week 2, there was a sharp rise in copper concentrations in IS+PEW (mean 8.489 ± 0.004 ppm), as well as IS+GEW (mean 7.941 ± 0.007 ppm), SS+GEW (mean 7.871 ± 0.006 ppm), and CS+GEW (mean 7.556 ± 0.006 ppm), suggesting active mobilization of copper in the second enrichment passage at WPCBs concentration of 2.5. Ni still increased in IS+GEW (mean 4.525 ± 0.003 ppm) and SS+GEW (mean 4.528 ± 0.008 ppm) cultures (Table 4).

Table 3: AAS mean concentrations (ppm) of metals in culture supernatants after week 1 (day 7)

S/N	Sample	Fe (ppm)	Cu (ppm)	Ni (ppm)	Zn (ppm)
1	SS+PEW	1.006 ± 0.002	1.404 ± 0.003	3.148 ± 0.007	1.325 ± 0.003
2	IS+PEW	3.055 ± 0.008	2.542 ± 0.006	0.758 ± 0.003	1.812 ± 0.007
3	IS+GEW	1.712 ± 0.007	1.465 ± 0.003	2.390 ± 0.007	1.850 ± 0.004
4	SS+GEW	1.790 ± 0.010	1.462 ± 0.002	2.357 ± 0.004	1.848 ± 0.006
5	CS+GEW	1.488 ± 0.110	1.451 ± 0.008	0.994 ± 0.006	0.272 ± 0.002

Values are presented as mean $\pm$ standard deviation of triplicate determinations ($n = 3$)

SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

Table 4: AAS mean concentrations (ppm) of metals in culture supernatants after week 2 (day 14)

S/N	Sample	Fe (ppm)	Cu (ppm)	Ni (ppm)	Zn (ppm)
1	SS+PEW	1.225 ± 0.002	0.927 ± 0.009	3.403 ± 0.005	1.616 ± 0.006
2	IS+PEW	2.347 ± 0.006	8.489 ± 0.004	3.302 ± 0.006	1.952 ± 0.003
3	IS+GEW	1.614 ± 0.009	7.941 ± 0.007	4.525 ± 0.003	1.987 ± 0.008
4	SS+GEW	1.541 ± 0.011	7.871 ± 0.006	4.528 ± 0.008	1.987 ± 0.004
5	CS+GEW	1.749 ± 0.140	7.556 ± 0.006	2.993 ± 0.004	1.374 ± 0.006

Values are presented as mean ± standard deviation of triplicate determinations ($n = 3$)
 SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

Table 5: AAS metal concentrations (ppm) in culture supernatants after week 3 (day 21)

S/N	Sample	Fe (ppm)	Cu (ppm)	Ni (ppm)	Zn (ppm)
1	SS+PEW	1.443 ± 0.004	0.449 ± 0.002	3.658 ± 0.008	1.906 ± 0.004
2	IS+PEW	1.638 ± 0.003	14.429 ± 0.008	5.846 ± 0.004	2.092 ± 0.006
3	IS+GEW	1.516 ± 0.004	14.416 ± 0.005	6.659 ± 0.006	2.123 ± 0.003
4	SS+GEW	1.292 ± 0.120	14.279 ± 0.004	6.699 ± 0.003	2.126 ± 0.007
5	CS+GEW	1.505 ± 0.043	13.661 ± 0.004	4.992 ± 0.007	2.476 ± 0.004

Values are presented as mean ± standard deviation of triplicate determinations ($n = 3$)
 SS = Sango soil; IS = Ipata soil; CS = control soil; PEW = powdered e-waste; GEW = granulated e-waste

The week 3 outcomes demonstrated an additional increase in dissolved Cu that reached its highest in the IS+PEW (mean 14.429±0.008 ppm), IS+GEW (14.416±0.005 ppm), SS+GEW (14.279±0.004 ppm), and CS+GEW (13.661±0.004 ppm) cultures to the tune of about 10 times the result of week 1. Ni levels also increased up to mean values of 6.699±0.003 ppm in SS+GEW and 6.659±0.006 ppm in IS+GEW (Table 5). However, in contrast, week 1 showed higher Fe concentrations compared to week 3 in all the samples, which could suggest the precipitation of Fe or biosorption onto bacterial biomass.

Discussion:

The abundance of Fe in untreated WPCBs (5.786 ppm) and the relatively low level of Cu (1.442 ppm) here is in contrast with high-copper profiles previously reported in newer generation consumer electronics PCBs, which can contain Cu up to or above 20% by weight (2). The difference is probably due to the variation in age and kind of electronic equipment obtained in informal markets in Ilorin and the rather small sample sizes on which the digestion process in this paper was carried out. However, the four metal in the analytes Fe, Zn, Ni, and Cu

are the most tenacious ones and microbiologically significant metals in the WPCB leachates, and the bacterial mobilization is extensively reported (5,13).

The significant, linear increase in dissolved Cu in all of the enrichment cultures between Week 1 and Week 3 is also worth noting and the approximate 10-fold rise in solubility of Cu between days 7 and 21 may indicate that the bacterial populations were experiencing adaptive enrichment as the WPCB pulp density increased (13). The trend resembles those shown in acidophilic cultures that oxidize iron, where serial sub-culturing in progressively higher PCB concentrations enhanced metal tolerance and have an accelerated rate of metal dissolution (13). The ability in the current research was demonstrated by heterotrophic mesophilic genera, as opposed to acidophilic chemolithotrophs that are widely reported in bioleaching literature, which expands the array of organisms with viable application to e-waste (13).

The most frequent isolation used in this study was *B. subtilis*, as it was isolated in two separate enrichment combinations. Cation diffusion facilitator CzcD supports Ni^{2+} , Cu^{2+} , and Zn^{2+} resistance in this species concurrently and poly-gamma-glutamic acid (g-PGA) secretion supplies further extracellular chelation of these

metals in excess conditions (8). At the same time, *B. subtilis* implements biosorption using cell wall peptidoglycans, extracellular polymeric substances (EPS) and bioprecipitation as supplementary mechanisms of coping with poly-metallic stress which together explain its dominion in metal-contaminated habitats (7).

In weeks 2 and 3, a significant level of tolerance to Cu and Ni was observed (Cu: 14.416 ppm; Ni: 6.659 ppm in IS+GEW). High-metal exposure of this Gram-negative organism triggers broad-spectrum stress response mechanisms and induces metal-resistance genes (5). Although *P. vulgaris* has not received as much attention on bioremediation as *Bacillus* or *Pseudomonas* species in bioremediation studies, its survival in this case under two independent soil enrichments with granulated WPCBs necessitates additional characterization of its tolerance machinery at a molecular level (5).

The fact that *L. plantarum* can survive in enrichment cultures is especially interesting, as lactic acid bacteria are not traditionally connected with metallic-polluted soil and the biosorption of copper through wine-relevant lactobacilli through cell wall binding mechanisms has been documented and more recent studies have verified the ability of *L. plantarum* to remove Zn and Cu in aqueous solution at up to 84% (9). *L. plantarum* provides extensive carboxyl, hydroxyl and phosphate groups on the cell wall-associated polar head of polysaccharides and protein to coordinate metal, thus allowing passive metal sequestration without spending metabolic energy which renders *L. plantarum* an object of biosorption-based metal capture as opposed to oxidative bioleaching itself (18).

In weeks 2 and 3, *S. aureus* exhibited tolerance of Cu and Ni. *S. aureus* metalliform resistance genes such as copper efflux operons (*copA/B*) and *czt* operon that regulates Zn/Co efflux, oftentimes overlap genomic loci that includes antibiotic resistance determinants on mobile genetic elements (MGEs) (14). Although the clinical importance of the organism provokes biosafety concerns when applied to industry, the fact that it grows in soils with a high concentration of metals supports the evidence that environmental *S. aureus* clonal forms that live in polluted niches develop metal resistance adaptations over time (14).

It is worth noting the observed decrease in Fe concentrations between Week 1 and Week 3 in all the enrichment cultures. In contrast to Cu and Ni, the dissolved Fe did not rise after two consecutive passages of enrichment though Fe was the most available metal within the original WPCB matrix. This trend is in line with microbio-

logically enhanced Fe²⁺ oxidation and subsequent circumneutral pH precipitation as iron oxyhydroxides, observed in heterotrophic biolagging systems (13). Alternatively, the reduction might be due to the sorption of Fe to bacterial biomass and EPS which are more heavily concentrated in mature cultures. Both interpretations are in line with the established tendency of the *Bacillus* to generate siderophores and EPS, which are highly coordinating in Fe³⁺ (7).

There are a few limitations to this study that should be considered. The identification of the bacteria was based only on phenotypic biomarkers; 16S rRNA gene sequencing a technique often considered the gold-standard of bacterial identification in the environment would give a more certain species-level name and demonstrate intraspecific variation in the *B. subtilis* population (19). Data in the AAS indicates total dissolved metal in bulk supernatants and makes no differentiation between biologically mobilized metals and metals liberated by abiotic dissolution. A more stringent evaluation of biological contribution would have been made possible by inclusion of abiotic WPCB-LB controls that lacked bacterial inoculation. In addition, the WPCB concentration employed in this case ($\leq 5\%$) is small compared with industrial-related densities of pulp, say 10-20%, and tolerance of the isolation to the increased metal loads must be determined (20).

Conclusion:

This study showed that the soils surrounding informal recycling e-waste sites in Ilorin, Nigeria, contain a culture of great metal tolerant bacteria, which can live and develop in complex poly-metallic WPCB structures. The most common tolerant species were identified as *B. subtilis*, *P. vulgaris*, *S. aureus* and *L. plantarum*, and mobilization of Cu and Ni increased significantly in three consecutive enrichment passages.

The progressive enrichment method used was effective in the selection of metal-adapted populations that have potential bioleaching and biosorption activity. The future research must integrate 16S rRNA gene sequencing to identify assured strain, abiotic dissolution in controlled conditions to stringently ascribe the metal solubilization to microbial activity, and molecular investigation of efflux gene complementation and the expression of metal-binding proteins. These measures are preliminaries to the assessment of scalability of Nigerian indigenous e-waste-tolerant bacteria as those suitable to be used as sustainable metal recovery agents.

Contributions of authors:

AAS conceived and designed the study, supervised the research, and contributed to data interpretation; BTT conducted the laboratory experiments, collected and analysed the data, and prepared the initial manuscript draft; BMA contributed to the methodology, data interpretation, and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript

Conflict of interests:

Authors declare no conflicts of interest.

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Artificial Intelligence (AI) use disclosure:

Authors declare that no generative AI tools were used in the original preparation, analysis, or writing of this manuscript. All experimental procedures, data analyses, interpretations, and written content were independently produced and verified by the authors.

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