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Microbial Recovery of Metals from Electronics Waste Using *Providencia* spp. and *Pseudomonas* spp.

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Abstract: Microbial recovery of metals from printed circuit boards of electronic waste using *Providencia manganoxydans* and *Pseudomonas fluorescens* was assessed. *Providencia manganoxydans* was isolated from coal mine drain while *Pseudomonas* spp. was isolated from soil. The setup for solubilization comprises of waste printed circuit boards of particle size 50 µm, suspended in nutrient broth in conical flasks. *P. fluorescens* and *P. manganoxydans* isolates were added to the respective flasks with (NH4)2SO4 and NPK added as sources of nitrogen. The setup was incubated at ambient temperature for 24 days. The total nitrogen, pH, ORP, available phosphorus, and solubilization of metals (copper, nickel and zinc), were determined at days 0, 8, 16 and 24. Results obtained showed that at the end of the 24th day, the percentage solubilization of metals by *P. manganoxydans* with (NH4)2SO4 as nitrogen source was 89% for copper, 74% for nickel and 75% for zinc, while the percentage metal solubilization of *P. fluorescens* with NPK was 77% for copper, 80% for nickel and 74% for zinc and with (NH4)2SO4 was 74% for copper, 66% for zinc and 70% for nickel. The recovery of the metals from the solution was carried out using precipitation methods with NH_3(aq), HCl(aq) and 0.5M $K_4Fe(CN)_6$ for zinc precipitation, $K_4Fe(CN)_6$ for copper precipitation, and with NH_3(aq) and dimethylglyoxime (DMG) reagent for solubilized nickel precipitation. The results show that the isolates can effectively solubilize copper, nickel and zinc from e-waste. As the surge in global electronic waste continues to pose a threat to the environment and ecosystem, microbial solubilization with *P. manganoxydans* and *P. fluorescens* for metals recovery represents an efficient approach to metal extraction from e-waste. It offers a greener, cost-effective, and efficient alternative to conventional metal recovery methods. It holds great promise for the future management of e-waste through metal resource bio-recovery.

Keywords: *Bio-Solubilization of Metals, E-Waste, Metal Resource Bio-Recovery.*

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I. INTRODUCTION

Electronic waste has become a global concern due to the rapid technological advancements and high consumption of electronic products. Majority of these gadgets that have reached their end of life are discarded indiscriminately, where the metals leach out and find their way into the ecosystem and food chain eliciting havoc. The role of microbes in solubilization of these e-waste metals offers an eco-friendlier and cost-effective alternative to traditional metal recovery methods (Dutta, Goel & Kumar, 2022).

Electric and electronic products continue to revolutionize communication, entertainment, transportation, education and health care around the world. There is no sign that this revolution will abate soon. The development of electronic and electrical industries and the widespread use of electronic technologies result in the production of more and new electronic devices (Wang, Faraji, Ramsay, & Ghahreman, 2021). When these products reach the end of their service life, a huge amount of electronic waste (e-waste) is accumulated (Wu, Liu, Zhang, Xhu, & Tan, 2018). Technical innovations will continue to be a cornerstone of social progress and advanced electronics are leading the way (Fisher, Frank, Tony, & Yamawaki, 2005). Wastes from electrical and electronic equipment are the fastest growing waste category.

The composition of e-waste is very heterogeneous and includes a variety of hazardous and/or non-hazardous substances including polymers, glass fiber, flame retardants, and ferrous and non-ferrous metals, which, if improperly managed, can be toxic to humans and the environment

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(Kudpeng, Bohu, Morris, Thiravetyan, & Kaksonen, 2020; Dutta, Goel & Kumar, 2022).

The electronic waste produced annually around the world would weigh as much as all the commercial aircraft's ever produced, or 5,000 Eiffel towers. This is a growing "tsunami" according to the UN, and it's fed by all the phones, tablets and other electronic devices that are thrown away each day (Brandl & Faramarzi, 2006).

It is estimated that about 20 - 50 million tons of electronic waste are manufactured in the world (Bas, Deveci & Yazici, 2013). E-waste's per capita was estimated to be 6.8 kg for every living person by the United Nations Environmental Program (UNEP). E-waste contains hazardous materials which are toxic to human health and non-hazardous materials which can be highly valuable (Widmer, Oswald-Krapf, & Sinha-Khetriwa, 2005). The metal contents of e-waste include larger quantities of precious metals such as Au, Ag, and Pt group metals, base metals e.g. Al, Co, Cu, Ni, Zn, and Fe, rare earth metals e.g., In, Nd, and Ta, and others e.g., Be, Cd, Cr, Hg, Pb, Sb, Sn, and Ti, than those found in some ores (Tan, Li & Zeng, 2015). One ton of mobile phone without batteries contains 340 g Au, 140 g Pd, 130 kg Cu, and 3.5 kg Ag (Hagelüken & Meskers, 2008).

Recycling electronic waste is an important subject not only from the point of waste treatment but also from the recovery aspect of valuable materials. However, recycling of electronic scrap is still quite limited due to the heterogeneity of the materials present in the products and the complexity of the production of this equipment (Needhidasan, et al., 2014). Precious metals have a wide application in the manufacture of electronic appliances, serving as contact materials due to their high chemical stability and their good conducting properties. It's a bitter irony that the e-waste mountains collecting in the world's poorest places contain a fortune.

Until now, different processes such as mechanical, pyrometallurgical, and hydrometallurgical methods has been used to recover precious metals from e- waste (Arshadi, Yaghmaei, & Mousavi, 2019). The mechanical process has a low recovery outcome and was used as a pre-treatment process. Other traditional methods produced atmospheric pollution and were not economically viable. In recent years researchers have tended towards biohydrometallurgy which is eco-friendlier. Bioleaching is an essential field in biohydrometallurgy, it is an environmentally friendly, low cost and an efficient process (Arshadi, Yaghmaei, & Mousavi, 2019).

In this study, the efficacy of microbial recovery of metals (Cu, Zn and Ni) from printed circuit boards of electronic wastes was assessed using *Providencia manganoxydans* and *Pseudomonas fluorescens. Providencia manganoxydans* used was isolated from coal mine drain, while *Pseudomonas* spp. was isolated from soil from an e-waste dump site.

II. MATERIALS AND METHODS

➢ *Sample Collection*

Soil samples were obtained from the waste metal dumpsite at the Nekede mechanic village Owerri falling between the geographical coordinates Lat. 5.454954ºN and Long. 7.039687ºE. A sterile spatula was used to obtain surface soil samples which were composited and transferred into a sterile bottle and was transported immediately to the laboratory for analysis. Water samples were obtained from the acid mine drainage water from Onyeama coal mine in Enugu, Enugu State (Geographical coordinates; Lat 6.4139ºN Long 7.457409ºE), using a sterile 1-liter bottle. The water sample was dipped in ice packs in an icebox and transported to the laboratory for analysis (Amiya, 2010). Discarded printed circuit boards were obtained from different electronics shop in Tetlow Owerri and Orlu international Market both in Imo state.

➢ *Sample Preparation*

- *Preparation of Printed Circuit Board*

Discarded PCBs obtained, were cleaned to remove dust and sand particles using iron brushes and crushed using a plastic crushing machine into 50 µm.

➢ *Isolation and Identification of Microorganisms*

- *Pseudomonas Fluorescens*

Aliquots of the soil's suspension in sterile distilled water were transferred into 20 ml test tubes containing nutrient broth in duplicate. This was followed by the incubation of the inoculated test tubes in an incubator at 28±2^{o}C for 24-48h. After incubation, the test tubes were observed for growth, indicated by the appearance of turbidity in the broth. Microorganisms were transferred from such turbid test tubes into a cetrimide agar (Oxoid UK) plate using a sterile wire loop and the plates incubated at 28±2°C for 24h in an incubator. Isolates from discrete colonies were purified by repeated sub-culturing and subsequently stored on a cetrimide agar slant in a refrigerator at 4^{o}C for subsequent identification and use.

- *Providencia Manganoxydans*

Metal solubilizing bacterial species from the coal mine water were isolated using the 9k medium, which is composed of solution A and B, prepared as stated below;

Solution A was prepared by mixing $(NH_4)_2SO_4$, 3 g; K_2HPO_4, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.1 g; $Ca(NO_3)_2$, 0.01 g and 700 ml of distilled water in a 1000 ml conical flask, and sterilized at 121°C for 15 mins at 15 psi.

Solution B was prepared by mixing 44.22 g of $FeSO_4.7H_20$ and 300 ml of distilled water. This was swirled and filtered using a sterile filter paper.

Solution A and B were mixed after cooling, and its pH adjusted to 2.0 by adding conc. H_2SO_4 and carefully distributed into sterile screw cap bottles for storage (Mustafa et al., 2023).

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The 9k medium was used for the isolation of metal solubilizing species from the coal mine drain. The coal mine drain sample (1 ml) was transferred into 20 ml test tubes containing 10 ml of the 9k medium in duplicate. The inoculated tubes were incubated at 28±2°C for 24 - 48h in a shaker incubator (Mustafa et al., 2023). After incubation, a loop full from turbid test tubes indicating culture with microbial growth were obtained using a sterile wire loop and inoculated on cysteine lactose electrolyte deficient (CLED) agar and nutrient agar plates for metal solubilizing bacterial isolation. Inoculated plates were incubated at 28±2°C for 24 h in an incubator. Discrete colonies obtained from inoculated plates were purified by sub-culturing and subsequently stored on a nutrient agar slant in a refrigerator at 4°C for subsequent identification and use.

- *Identification of Microbial Species*

The microbiological procedures involving Gram staining, the biochemical which include Oxidase test, catalase test, nitrate reduction test, citrate test, Glucose fermentation test and Urease test and the molecular identification procedures involving DNA extraction, quantification, 16S rRNA amplification and sequeencing were employed in the identification of isolated microorganisms.

- *Bioleaching Experiments*

The two steps bioleaching process was used in the bioleaching assay.

A sterile pipette was used to obtain 1 ml of the standardized microbial inoculum (*P. fluorescens* and *P. manganoxydans* respectively) (1.5 x 10^8 cfu/ml) was introduced into separate 250 ml conical flask containing 200 ml of nutrient broth with an adjusted pH. This was incubated for 24 h at 28±2°C in an incubator. Thereafter, five grams (5 g) of the individual nitrogen sources ($(NH_4)_2SO_4$ and NPK) and five grams (5 g) of the PCB (Ps 50 µm) were added. This was further incubated for 24 days at 28±2°C in an incubator.

- *Solubilization Assay*

After incubation, 10 ml of each test culture samples and control samples were collected on days 0, 8, 16 and 24, and assayed for pH, ORP, total N, available P and percentage metal solubilization. The control samples contained only the PCB and the nitrogen sources without any organism in the nutrient broth. The percentage solubilization was determined using the formula:

$$\frac{Ac - Ab}{Ab} x100$$

Where; Ac= metal concentration on day 24 and;

Ab = metal concentration on day 0.

- *Sample Analysis*

✓ *Determination of pH*

The pH of the experimental solution was determined using a pH meter.

✓ *Determination of Available Phosphorous*

Available phosphorus was determined using the modified Bray No.1 method (Olsen and Sommers, 1982).

✓ *Determination of Total Nitrogen*

Total nitrogen was determined using the macro and micro kjeldahl method of Walkley and Black (1934).

➢ *Recovery of Metals*

Metals were recovered using the precipitation method. A sterile pipette was used to obtain 10 ml of test culture from the test flask containing individual isolates, PCB and nitrogen source at the end of the 24 days, this was filtered into a test tube using a sterile filter paper. Specific anions for each of the metal of interest was added to the filtrate, to form precipitates with the metals of interest. These precipitates were further filtered using a filter paper and air dried using an aluminum foil.

III. RESULTS

The result of the agarose gel electrophoresis showing the amplified 16srRNA of the isolated test species are shown in Figure 1.

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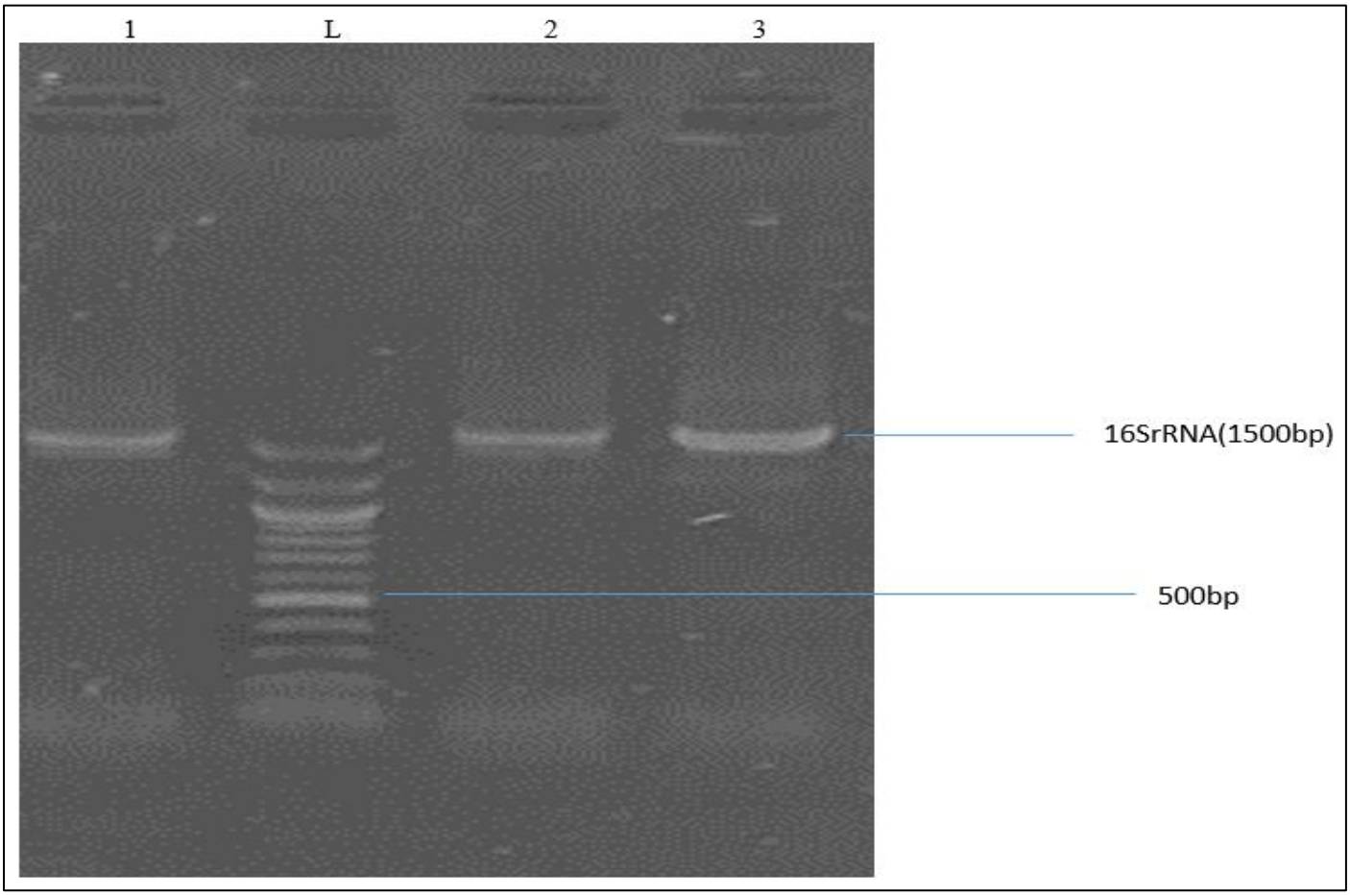

Fig 1 Agarose Gel Electrophoresis Showing the Amplified 16srRNA of the Test Isolates Lanes 1-3 Represent the Amplified 16srRNA at 1500bp while Lane L Represents the 100bp DNA Ladder.

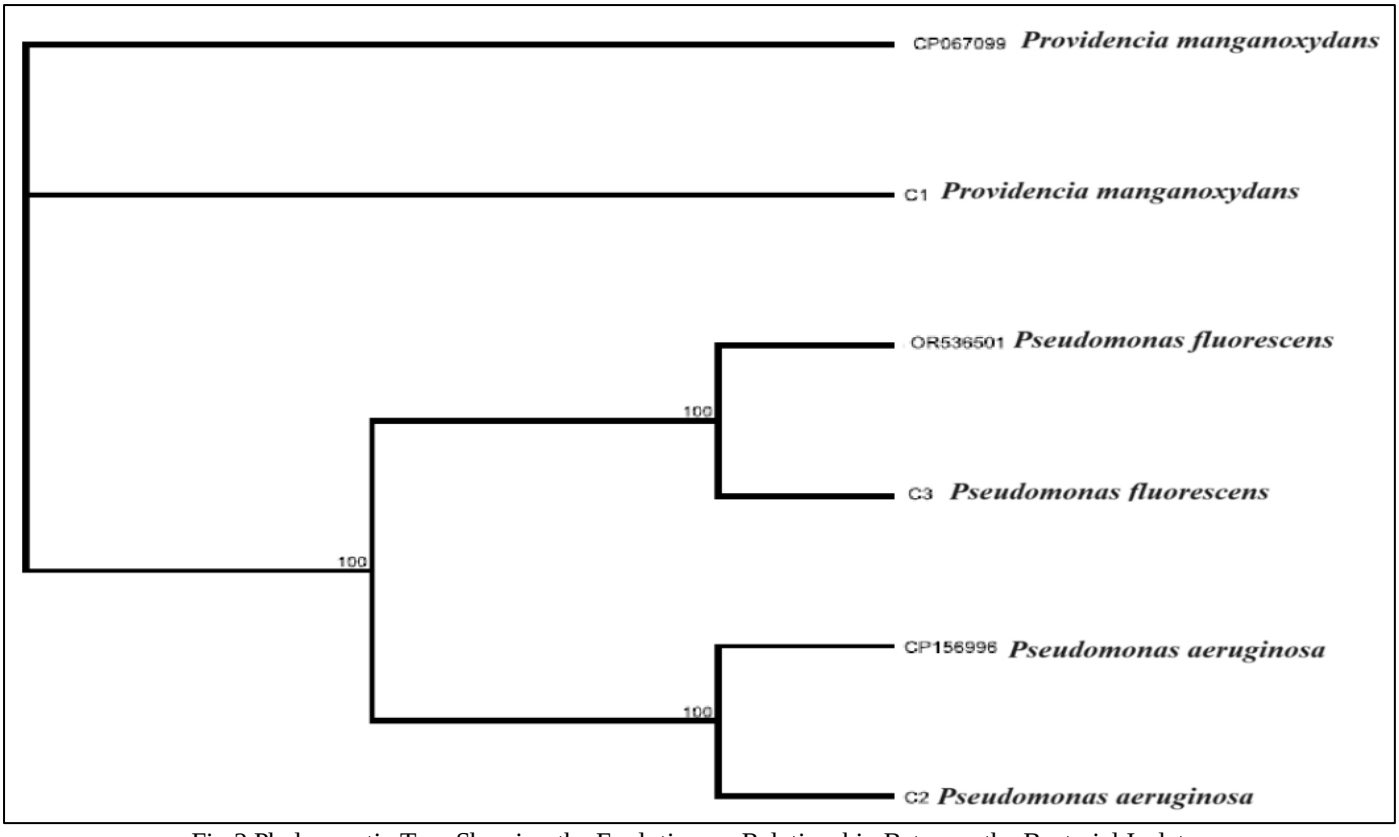

Fig 2 Phylogenetic Tree Showing the Evolutionary Relationship Between the Bacterial Isolates

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Table 1 Mean Percentage Solubilization of Copper, Nickel and Zinc in Culture with *P. fluorescens* and $(NH_4)_2SO_4$

Parameters	Control Day 0	Control Day 24	% mean increase	Day 0	Day 8	Day 16	Day 24	% mean increase
Cu (mg/l)	0.189	0.206	8%	0.274	0.337	0.417	0.478	74%
Ni (mg/l)	0.016	0.018	12%	0.121	0.146	0.178	0.206	70%
Zn (mg/l)	0.012	0.013	8%	0.015	0.017	0.022	0.025	66%

Table 2 Physiochemical Characteristics of Test Culture with *P. fluorescens* and $(NH_4)_2SO_4$

Parameters	Control Day 0	Control Day 24	Day 0	Day 8	Day 16	Day 24
pH	6.62	6.57	7.07	7.18	7.12	6.57
ORP (mV)	19	68	25	176	53	68
Total N (mg/l)	0.001	0.002	0.002	0.003	0.001	0.003
Avail P (mg/l)	0.04	0.04	0.19	0.23	0.25	0.29

Table 3 Mean Percentage Solubilization of Copper, Nickel and Zinc in Culture with P. *manganoxydans* and $(NH_4)_2SO_4$

Parameters	Control Day 0	Control Day 24	% mean increase	Day 0	Day 8	Day 16	Day 24	% mean increase
Cu (mg/l)	0.141	0.149	5%	0.163	0.219	0.278	0.301	84%
Ni (mg/l)	0.043	0.044	2%	0.042	0.053	0.61	0.073	74%
Zn (mg/l)	0.027	0.028	3%	0.034	0.042	0.051	0.060	75%

Table 4 Physiochemical Characteristics of Test Culture *P. manganoxydans* with $(NH_4)_2SO_4$

Parameters	Control Day 0	Control Day 24	Day 0	Day 8	Day 16	Day 24
pH	4.5	4.7	4.5	4	4.2	4.8
ORP (mV)	17	32	17	109	55	45
Total N (mg/l)	0.001	0.001	0.001	0.003	0.002	0.001
Avail P (mg/l)	0.06	0.06	0.13	0.11	0.09	0.09

Table 5 Experiment C: Mean Percentage Solubilization of Copper, Nickel and Zinc in Culture with P. *fluorescens* and NPK

Parameters	Control Day 0	Control Day 24	% mean increase	Day 0	Day 8	Day 16	Day 24	% mean increase
Cu (mg/l)	0.143	0.151	5%	0.162	0.392	0.645	0.288	77%
Ni (mg/l)	0.074	0.078	5%	0.076	0.096	0.119	0.136	78%
Zn (mg/l)	0.112	0.115	2%	0.128	0.187	0.205	0.223	74%

Table 6 Physiochemical Characteristics of Test Culture *P. fluorescens* with NPK

Parameters	Control Day 0	Control Day 24	Day 0	Day 8	Day 16	Day 24
pH	6.7	6.7	6.66	6.94	7.12	6.03
ORP (mV)	21	35	26	33	55	83
Total N (mg/l)	0.001	0.002	0.002	0.002	0.003	0.003
Avail P (mg/l)	0.31	0.24	0.23	0.33	0.14	0.09

Table 7 Experiment D: Metals Recovered from Different Treatments

Recovered Metals	*P. fluorescens* + $(NH_4)_2SO_4$	*P. manganoxydans* + $(NH_4)_2SO_4$	*P. fluorescens* + NPK
Zn^{2+}	+	+	+
Cu^{2+}	+	+	+
Ni^{2+}	+	+	+

IV. DISCUSSION

The rapid and improper disposal of electronic waste (e-waste) has become an issue of great concern, resulting in serious threats to the environment and public health. Microorganisms play a crucial role in the extraction of metals from e-waste, a technology known as bioleaching or metal solubilization (Nasiri et al., 2023). In this study, the isolate obtained from the coal mine drain was molecularly identified as *P. manganoxydans.* This provides insight on the possibility of isolating other metal solubilizing organisms from coal mines, away from the known traditional organisms associated with coal mine drains such as *Thiobacillus ferroxidans* and *Thiobacillus thioxidans* isolated by Chen et al., 2021 and Amiya, 2010, from a coal mine in China and India, used for the solubilization of Fe, Mn, Cr, Al metals. This finding is also distinct from other *P. manganoxydans* isolation reported based on site isolation, which was mainly from heavy metal contaminated soils and bioremediation sites as stated in the work by Li et al., (2022). Which was further employed in Manganese (Mn) solubilization. This would be the first time

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an isolation associated with coal mine drain is reported for the organism.

The solubilization analysis of the PCB by the bacteria isolates based on the particle size 50 µm, agrees with the findings of Rouchalova et al., (2020) on the effect of different particle sizes on bioleaching, with smaller particles having more bioleaching efficiencies when compared to bigger particles. It also had similar outcome with the study by Guven and Akinci, (2013), on the effect of particle size on metal bioleaching from bay sediments using fine (<45 µm), medium (45–300 µm), and coarse (300–2000 µm) size fractions of sediment samples contaminated with Cr, Cu, Pb, and Zn, who reported that the bioleaching efficiencies were more for lesser particle sizes when compared to bigger particle size particles. These similar outcomes could be attributed to the more surface area 50 µm particle size PCB in the medium, which may have increased the area of the PCB available for microbial activities by the test inoculum.

P. manganoxydans had a percentage solubilization outcome of 84%, 74% and 75% for copper, nickel and zinc in $(NH_4)_2SO_4$. This provides insight on the ability of *P. manganoxydans* to solubilize different metals and establishing its role as an efficient metal solubilization microbial species when placed in favourable environmental conditions. Although *P. manganoxydans* has been associated with the solubilization of Mn^{2+} from heavy metal contaminated soils (Li et al., 2022), no known report has been made of its solubilization of copper, nickel and zinc. This report is added information on the properties and potentials of the bacteria.

The solubilization assay showed that on day 1, 8, 16, and 24 for *P. fluorescens* with $(NH_4)_2SO_4$ as the nitrogen source solubilization rates were 74% for copper, 70% for nickel and 66% for zinc, at an average pH of 6.9 and ORP of 80 mV. This shows that *P. fluorescens* has the ability to solubilize multiple metals and proves its relevance as a good metal solubilizing bacteria. The solubilization outcome of *P. manganoxydans* with $(NH_4)_2SO_4$ as the nitrogen source at the end of the 24 days, showed an increase of 84% for copper, nickel, 74% and Zinc, 75%, at an average pH of 4.9 and ORP of 56.5 mV. The solubilization outcome of *P. fluorescens* with NPK as the nitrogen source showed an increase in copper, 77%; nickel, 78% and zinc, 74%, at an average pH of 6.6 and ORP of 49 mV. Indicating the metal solubilization potential of the two organisms in favorable environmental conditions. In comparison for the solubilization efficiencies across the nitrogen sources used, there was a significant difference at the solubilization outcome, with P value for *P. fluorescens* in $(NH_4)_2SO_4$ and NPK, also for *P. manganoxydans* in $(NH_4)_2SO_4$ and *P. fluorescens* in NPK. There was no significant difference with P value >0.05 for *P. fluorescens* in $(NH_4)_2SO_4$ and *P. manganoxydans* in $(NH_4)_2SO_4$.

The precipitation method applied for the metal recovery of copper, zinc and nickel in the solution containing *P. fluorescens* with $(NH_4)_2SO_4$, *P. manganoxydans* with $(NH_4)_2SO_4$ and *P. fluorescens* with NPK as their nitrogen source respectively, confirmed the presence of copper, nickel and zinc in the solution, which were precipitated from the reaction. This agrees with the results obtained by Wongnaree et al., (2025) on the recovery of valuable metals from leached solutions using precipitation methods. Therefore, affirming precipitation method as a valuable method in metal recovery, as the metals of interest were confirmed, recovered and obtained from the solution.

V. CONCLUSION

As the surge in global electronic waste continue to pose a threat to the environment and ecosystem, microbial solubilization presents a transformative approach to metal extraction that aligns with the growing global emphasis on sustainability and resource efficiency. By offering a greener, cost-effective, and efficient alternative to conventional methods, it holds great promise for the future of metal resource recovery.

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