Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Bioleaching assisted multiple metal recovery from printed circuit boards

Rajalakshmi Sridharan
Indian Institute of Technology
Shinjini Sen
Indian Institute of Technology
Piet. N. L. Lens
University of Galway
Mohanakrishnan Logan
mohanlogan@iitm.ac.in

Indian Institute of Technology

Research Article

Keywords: Bioleaching, Printed circuit boards (PCBs), E-waste, Metal recovery, Chemical precipitation

Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11593906/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Bioleaching assisted multiple metal recovery from printed circuit boards

Rajalakshmi Sridharan[1], Shinjini Sen[1], Piet. N. L. Lens[2], Mohanakrishnan Logan[1]*

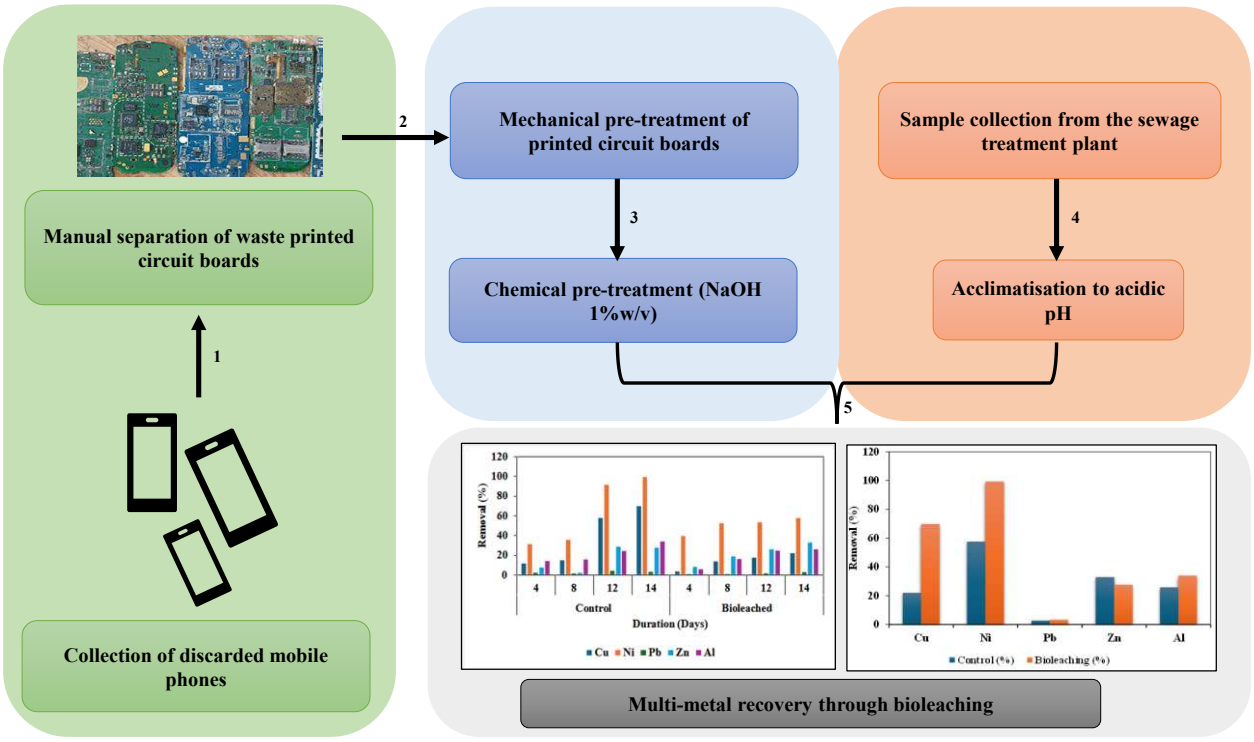
[1]Environmental Engineering Division, Department of Civil Engineering, Indian Institute of Technology, Madras, Chennai – 600 036, India

[2]University of Galway, College Road, Galway H91 TK33, Ireland

Corresponding author Email ID: mohanlogan@iitm.ac.in

Submitted to: Water, Air, & Soil Pollution

12 **Graphical abstract:**



13

14

Abstract

Electronic waste (e-waste) generation is increasing rapidly, highlighting the need for sustainable recovery of valuable metals. Printed circuit boards (PCBs) are particularly rich in both precious and hazardous metals, necessitating efficient extraction methods. In this study, we present a two-step bioleaching and chemical precipitation approach to recover multiple metals from alkali-pretreated mobile phone PCBs using an acidophilic bacterial strain isolated from sewage sludge. The strain was acclimated over six successive cycles to highly acidic conditions, demonstrated by pH reduction of the medium to pH 1, and Fe^{2+}oxidation to Fe^{3+}. Subsequent bioleaching achieved significant metal solubilisation from the PCBs: Ni 99.32 ± 0.09% > Cu 69.73 ± 0.43% > Al 33.88 ± 0.01% > Zn 27.56 ± 0.01% >Pb 3.27 ± 0.01 %. The resulting acidic bioleachate was neutralised with NaOH, precipitating metals non-selectively with an overall recovery efficiency exceeding 94%. This approach offers a sustainable and environmentally benign route for multi-metal recovery from e-waste, supporting circular economy and resource management initiatives.

Keywords: Bioleaching, Printed circuit boards (PCBs), E-waste, Metal recovery, Chemical precipitation

1. Introduction

The rapid expansion of electronic and electrical device consumption has significantly accelerated the generation of electronic waste (e-waste) worldwide. E-waste is now accumulating at a rate nearly three times faster than other waste streams. The global generation of e-waste rose from 44.7 million tonnes in 2016 to 53.6 million tonnes in 2019, and it is projected to reach 74.7 million tonnes by 2030 (Yaashikaa et al. 2022; Trivedi et al. 2023). This rapid accumulation poses significant environmental and resource management challenges, particularly due to the presence of valuable metals and hazardous substances in discarded electronic components.

Printed circuit boards (PCBs), a crucial component of electronic devices, comprise approximately 3–6% of the total e-waste (Vardanyan et al. 2024; Trivedi et al. 2023). They are widely used across various sectors, including telecommunication, automotive, aerospace, and consumer electronics. PCBs typically consist of approximately 28% metals and 72% non-metals (Van Yken et al. 2023; Priya et al. 2017). The metallic fraction primarily includes copper (Cu), nickel (Ni), aluminium (Al), silver (Ag), gold (Au), and cobalt (Co), whereas the non-metallic fraction mainly comprises polymers, resins, and ceramics. The economic value of raw materials contained in PCBs has been estimated to exceed USD 57 billion globally, and efficient metal recovery from these materials could potentially offset up to 60% of the annual global metal demand (Yazici et al. 2013; D'Adamo et al. 2025). Although the use of hazardous metals such as mercury (Hg), lead (Pb), and cadmium (Cd) has been restricted through regulations such as the Restriction of Hazardous Substances (RoHS) Directive (RoHS Directive, 2026), PCBs still present environmental and health risks due to their toxic metal composition (Santaolalla et al. 2021; Kaliyaraj et al. 2019).

Improper disposal of PCBs may result in metal leaching into the environment, leading to bioaccumulation, biomagnification, and long-term ecological imbalance (Kaliyaraj et al. 2019). Among the metals commonly found in PCBs, Cu and Ni are of particular concern due to their toxicity. Excess exposure to Cu has been linked to neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and can adversely affect aquatic organisms (Pohanka et al. 2019). Similarly, exposure to elevated levels of Ni has been associated with respiratory, cardiovascular, and urinary disorders as well as allergic reactions (Genchi et al. 2020).

Conventional metal recovery from PCBs is typically achieved through pyrometallurgical and hydrometallurgical processes (Zhu et al. 2023). Pyrometallurgical techniques, including smelting, incineration, and roasting, are

commonly used for the recovery of non-ferrous metals but require substantial energy inputs and often generate toxic emissions (Wan et al. 2021). On the other hand, hydrometallurgical processes rely on metal-specific chemical leaching using strong acids and oxidising agents. For example, nitric acid is used for the recovery of lead (Pb) and tin (Sn), while copper (Cu) can be extracted using sulfuric acid in combination with oxidants such as hydrogen peroxide (Jha et al. 2011; Hsu et al. 2019), supercritical methanol (Xiu et al. 2017) or cuprous chloride. Precious metals such as gold and silver are often leached using reagents including aqua regia, cyanide, thiosulfate, or halide solutions (Akcil et al. 2015). Despite their efficiency, these methods are often costly, energy-intensive, and environmentally hazardous due to the use of toxic chemicals and the generation of corrosive effluents (Liu et al. 2021; Becci et al. 2020).

In recent years, bioleaching has emerged as a promising sustainable alternative for metal recovery from e-waste. Bioleaching employs microorganisms capable of solubilising metals from solid matrices through biochemical reactions. Acidophilic bacteria such as *Acidithiobacillus ferrooxidans* and *A. thiooxidans* as well as fungi like *Aspergillus niger* have demonstrated the ability to leach metals including Cu, Zn, Ni, and even Au, from solid matrices through mechanisms such as acidolysis, redoxolysis, and complexolysis (Becci et al. 2020; Kaliyaraj et al. 2019). Compared with conventional methods, bioleaching offers several advantages: it operates at ambient temperatures, consumes less energy, and is generally more eco-friendly (Sanaolalla et al. 2021; Pakostova et al. 2024). Sewage sludge from wastewater treatment plants (WWTPs) is recognised as a reservoir of metal-tolerant microorganisms, including acidophilic bacteria capable of thriving under highly acidic conditions (pH < 3) (Vera et al. 2022; López-Martínez et al. 2024). These bacteria maintain intracellular pH homeostasis through proton gradient regulation and are thus well adapted for metal solubilization processes that occur at a pH of 2 to 5. *Acidithiobacillus* species derive energy by oxidising reduced sulfur and iron compounds. Sulfur oxidation generates sulfuric acid that lowers pH, while ferrous iron oxidation produces ferric ions (Fe^{3+}), which act as strong oxidising agents in indirect bioleaching. Furthermore, bacteria secrete extracellular polymeric substances (EPS) that enhance their adhesion to substrates, further improving metal dissolution and extraction efficiency (Núñez Ramírez et al. 2019).

Due to the heterogeneous composition of PCBs, an efficient recovery method should target the simultaneous extraction of multiple metals. However, many studies focus primarily on the recovery of individual metals, leaving the potential for multi-metal bioleaching less explored. In this context, the present study investigates the bioleaching of Cu, Ni, Al, Pb, and Zn from alkali-pretreated mobile phone PCBs using an indigenous acidophilic bacterium isolated from sewage

sludge, and the bioleached metals were subsequently recovered through alkaline precipitation to obtain metal salts. In addition, metal mass balances were evaluated for the integrated bioleaching–precipitation process, and a preliminary techno-economic assessment was performed to evaluate the feasibility of the proposed method. This approach demonstrates a circular-economy-oriented strategy for transforming hazardous e-waste into recoverable resources through a combined biotechnological and chemical recovery pathway.

2. Materials and Methods

2.1 Collection of PCBs

Printed circuit boards (PCBs) were manually extracted from twenty-five mobile phones obtained from a local scrap dealer in Chennai (India). Non-PCB components such as batteries, capacitors, and casings were removed before processing. Figure 1 shows representative PCBs recovered from the dismantled mobile phones.

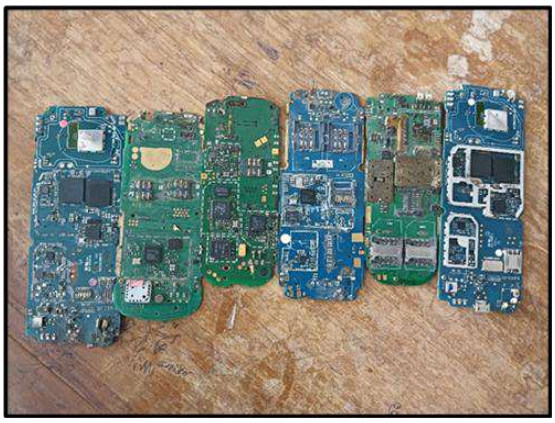


Fig 1: Manually separated PCBs from discarded mobile phones

2.2 PCB Pre-treatment Step

The separated PCBs underwent two distinct pre-treatment methods: mechanical and chemical. The mechanical pre-treatment involved shredding the PCBs into smaller fragments (<4.75 mm) to increase surface area and enhance metal bioavailability during the bioleaching process. The chemical pre-treatment was carried out on the mechanically shredded PCBs using sodium hydroxide (NaOH, 1% w/v). A total of 50 g of shredded PCBs was immersed in 500 mL of NaOH solution for three hours. This alkali treatment facilitates the removal of epoxy resin from the PCB surfaces. Following incubation, the supernatant was decanted, and the treated PCBs were rinsed three times with distilled water

and air-dried (Kang et al. 2021). The pre-treated PCBs were then analysed for Cu, Ni, Pb, Zn, and Al concentration using ICP-MS (NexION 300X, PerkinElmer Inc., Waltham, USA).

2.3 PCB Bioleaching Step

2.3.1 Inoculum source for acidophilic bacterial strain isolation

Activated sludge was collected from the sewage treatment plant at the Indian Institute of Technology Madras (Chennai, India). The sludge was enriched under acidic conditions (pH 2.5) in a 9K medium for 24 hours to promote the growth of acidophilic bacteria and suppress mesophilic populations. The 9K medium was prepared in two parts (Mora et al. 2025): Solution A contained $(NH_4)_2SO_4$ – 3 g/L, K_2HPO_4 – 0.5 g/L, $MgSO_4$ – 0.5 g/L, KCl – 0.1 g/L, and $Ca(NO_3)_2$ – 0.01 g/L and solution B consisted of $FeSO_4 \cdot 7H_2O$ – 44.7 g/L in distilled water at 37 ℃ under acidic conditions (pH 2.5).

2.3.2 Isolation of Acidophilic Bacteria for Bioleaching

Acidophilic bacterial strains were isolated from the enriched sludge using the spread plate technique on 9K medium (Mora et al. 2025). Serial dilutions of the enriched sludge were prepared, and 100 µL aliquots from the 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions were plated and incubated at 30 °C for 24 to 48 hours. Colony-forming units (CFU) were enumerated using the following equation:

$$CFU/mL = Number\ of\ colonies \times Dilution\ Factor\ /\ Volume\ plated\ (mL)$$

Individual colonies were streaked onto fresh 9K agar plates to obtain pure cultures. A 24-hour pre-culture was prepared from the isolated strain to assess its growth kinetics and biomass concentration. The culture at the logarithmic phase ($OD_{600} \approx 0.9$) was used as the inoculum for the 9K broth. Bacterial growth was monitored over six days by measuring optical density (OD_{600}) at 24-hour intervals. All growth experiments were conducted in triplicate, and growth curves were plotted accordingly (Wang et al. 2025). For biomass determination, the culture was centrifuged at 10,000 rpm for 20 min, and the resulting pellet was collected, air-dried overnight and weighed using an analytical balance (Labman Scientific Instruments, Chennai, India).

2.3.3 Bioleaching of PCBs

The pre-treated PCBs were subjected to bioleaching using the isolated acidophilic bacterial strain. Batch bioleaching experiments were conducted in 250 mL Erlenmeyer flasks, each containing 100 mL of 9K medium adjusted to pH 2.5. The medium was sterilized by autoclaving at 121 °C for 20 minutes and allowed to cool to room temperature before inoculation.

A pulp density of 2% (w/v) of pre-treated PCBs was added to each flask, followed by inoculation with the bacterial culture at its logarithmic growth phase ($OD_{600} \approx 0.9$). The flasks were incubated at 37 °C with shaking at 200 rpm in an orbital shaker incubator. Samples were collected at four-day intervals over 14 days. Each sample was centrifuged at 10,000 rpm for 10 min, and the supernatant was filtered. The filtrate was digested using aqua regia to ensure complete dissolution of the metals before analysis. The resulting digestate was analysed for its metal concentrations using ICP-MS.

All experiments were conducted in triplicate, along with two control setups: a positive control consisting of 9K medium with bacterial inoculum but without PCBs, and a negative control consisting of 9K medium with pre-treated PCBs but without bacterial inoculation. Both controls were maintained under identical experimental conditions (Khetwunchai et al. 2025).

2.4 Metal recovery from the bioleachate using chemical precipitation

Bioleached metals from PCBs were recovered through alkaline chemical precipitation. A 15 mL aliquot of bioleachate was treated with 1 N NaOH, added dropwise until the solution reached pH 10 to induce metal hydroxide precipitation. The mixture was then allowed to stand undisturbed at room temperature for 24 h to ensure complete settling of the precipitates. The clear supernatant was analysed using ICP-MS to determine the residual metal concentration (Meunier et al. 2006). The metal concentration measured in the bioleachate on the final day of the bioleaching experiment was considered the initial value for the precipitation process. Based on these measurements, the initial metal mass (mg), residual metal mass (mg), and recovered metal mass (mg) were calculated to establish the mass balance.

2.5 Analytical methods

2.5.1 Characterisation of the activity of the acidophilic bacteria

Bioleaching using the acidophilic bacterial strain is driven by energy generated through the microbial oxidation of ferrous iron (Fe^{2+}) present in the medium, producing ferric iron (Fe^{3+}) that acts as a strong oxidising agent, facilitating

153 metal solubilization (Tabrizi et al. 2010). Iron oxidation, a critical trait influencing the bioleaching efficiency, was
154 evaluated by determining ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron concentrations (Jones et al. 2023). The Fe^{2+} concentration
155 was determined by mixing 1 mL of culture sample with 1 mL of phenanthroline reagent and 3 mL of distilled water.
156 The resulting ferrous–phenanthroline complex formed a reddish-orange colour, and the absorbance was measured at
157 510 nm using a UV–Vis spectrophotometer (SpectraMax, Molecular Devices, LLC, San Jose, CA, USA).

158 Since Fe^{3+} does not directly form a chromophoric complex with phenanthroline, it was quantified indirectly. In a
159 parallel reaction mixture, 0.1 mL of ascorbic acid was added to reduce Fe^{3+} to Fe^{2+}, after which the absorbance was
160 measured again at 510 nm to determine total iron. The Fe^{3+} concentration was calculated by subtracting the initial Fe^{2+}
161 concentration from the total iron concentration. Fe^{2+} and Fe^{3+} concentrations were quantified using standard calibration
162 curves (Wang et al. 2025; Tabrizi et al. 2010).

163 **2.5.2 Metal analysis**

164 The bioleaching progress was monitored by analysing the metal concentration in the culture supernatant at four-day
165 intervals over 14 days. The bioleachate collected on day 14 was subsequently subjected to chemical precipitation and
166 analysed for metal concentrations using ICP-MS (PerkinElmer NexION 300X, PerkinElmer Inc., Waltham, USA).
167 The leachate was obtained by centrifuging the bioleaching culture (containing PCBs and bacterial cells) at 6000 rpm
168 for 20 min. The resulting supernatant was used for determining the concentration of metals released by the bioleaching
169 process (Khetwunchai et al. 2024).

170 The metal removal efficiency was calculated using the following equation:

171 $$Metal\ removal\ efficiency\ (\%) = \frac{(Cin - C)}{Cin} \times 100$$

172 where C denotes the metal concentration in the leachate (ppm), and C_{in} denotes the metal concentration in the control
173 (ppm).

174 The initial metal concentrations in the PCBs, the concentrations measured after bioleaching, and the total culture
175 volume (L) were used to determine the initial metal mass (mg), bioleached metal mass (mg), and recovery efficiency
176 (%). A comprehensive mass balance was subsequently established based on the metal inputs and outputs, including
177 contributions from the pre-treatment stage.

178 **2.6 Sustainability Assessments**

179 **2.6.1 Techno-Economic Assessment of Cu and Ni**

A preliminary techno-economic assessment (TEA) was conducted for copper (Cu) and nickel (Ni), the two metals exhibiting the highest recovery in the bioleaching–chemical precipitation process. Current market prices for Cu and Ni were obtained from recent commodity price reports (MCX Copper, 2026; MCX Nickel, 2026) and used to estimate the potential economic value of the recovered metals. This TEA considers only the theoretical value of the Cu and Ni, and does not include capital or operating costs such as labour, energy consumption, chemicals, or equipment. Therefore, it serves only as an initial indicator of the economic feasibility of the laboratory-scale integrated recovery process. The theoretical value of the recovered metal per litre of leachate was calculated using the following equation (Volkmann et al. 2018):

$$Value\ (Rs./\mathrm{L}) = \frac{M}{1000} \times \frac{P}{1000}$$

where M = Mass of metal recovered from the leachate (mg) and P = Market price (US \$/Kg) as per recent price listings.

2.6.2 Life Cycle Assessment (LCA)

The LCA was performed using SimaPro 10.1.0.5 software. LCA was used to quantify the resource inputs, energy consumption, emissions, and waste generation associated with each stage of the PCB treatment process. The ReCiPe 2016 Midpoint (H) V1.10 / World (2010) H method was employed, and eighteen midpoint impact categories were assessed. The impact assessment was performed to evaluate the environmental performance of the integrated bioleaching-based PCB treatment process (Rautela et al. 2025).

3. Results

3.1 Enrichment and growth of the acidophilic bacterial strain

3.1.1 Growth Kinetics

Subculturing the isolated acidophilic bacterium in 9K medium resulted in the enrichment of a dominant acidophilic bacterial strain capable of sustained growth at pH 2.5. The viable cell density of the isolate amounted to 3.12×10^8 CFU mL^{-1}. The OD_{600} values increased steadily from Day 1 to Day 5 (Figure 2), indicating continuous cell growth. In contrast, dry biomass reached a maximum on Day 2 and gradually declined thereafter. The discrepancy between the growth and biomass concentration suggests that the accumulation of intra- and extracellular substances at day 2 might have been redistributed for cell division or the low pH induced metabolic stress processes, such as the use of reserve materials or starvation. It is unlikely that cell aggregation was induced because the OD_{600} kept increasing.

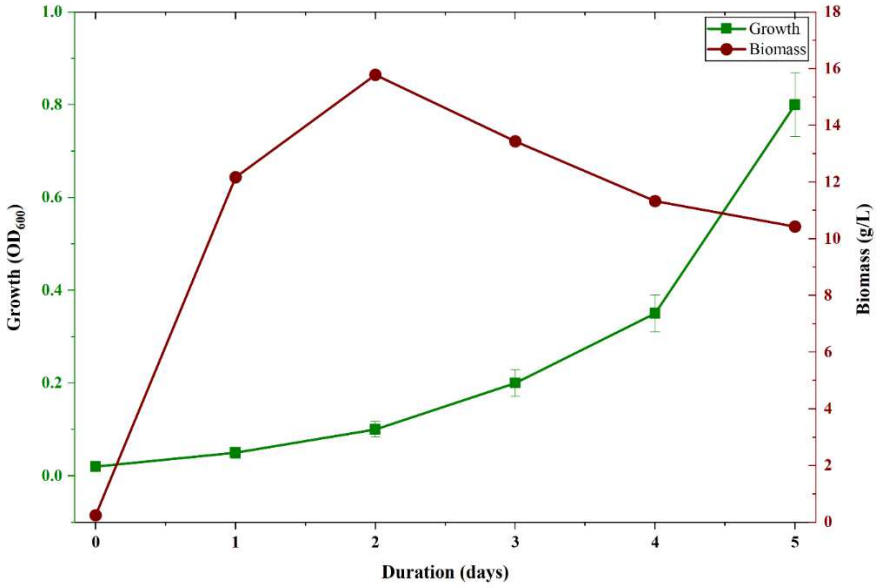
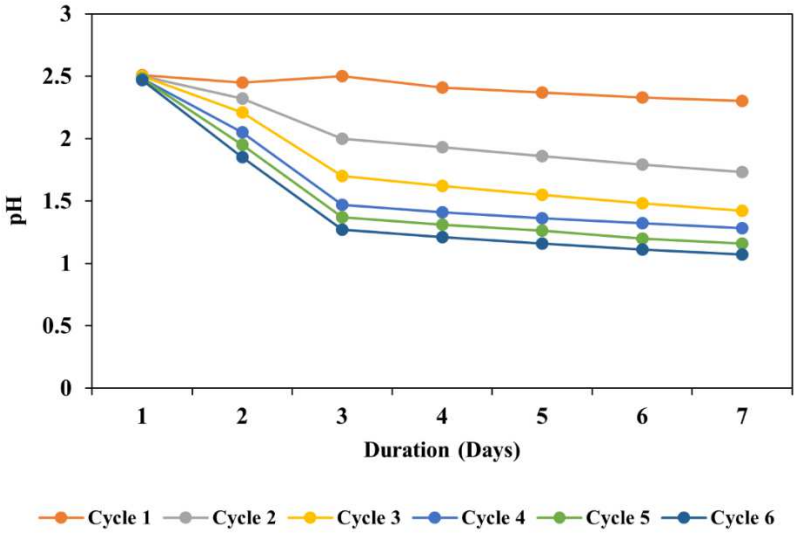


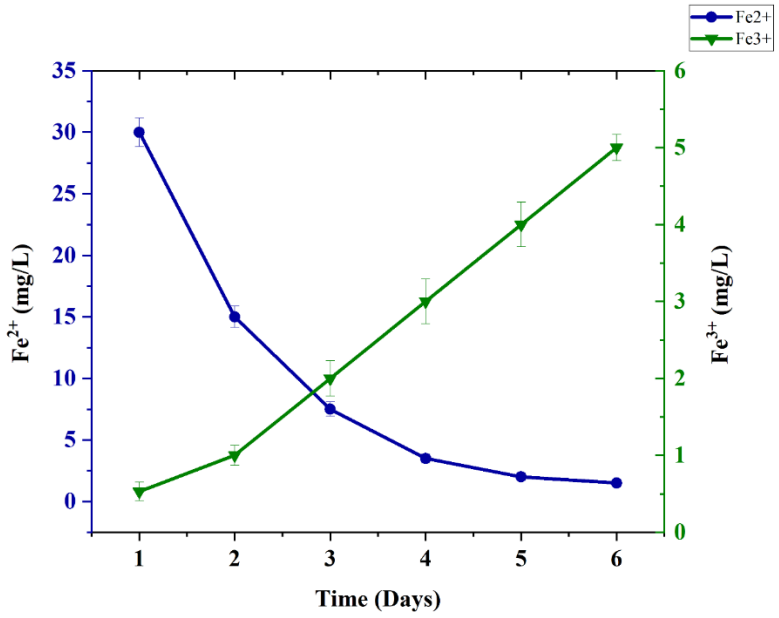
Fig 2: Growth and biomass concentration of acidophilic bacterial strain (37 °C and pH 2.5)

3.1.2 pH analysis and Fe^{2+} oxidation dynamics

The acidophilic bacterial strain was evaluated for its ability to modulate pH and catalyse ferrous iron oxidation, both of which are critical for the bioleaching performance. The pH of the 9K medium was monitored over six consecutive cycles (six days each). A consistent decline in pH from 2.5 to 1.05 was observed (Figure 3a), indicating progressive acidification of the system. The iron-oxidising capacity of the strain was assessed by quantifying the dissolved Fe^{2+} and Fe^{3+} concentrations over time (Figure 3b). The medium was initially supplemented with 30 mg/L Fe^{2+}, which showed a rapid decline, particularly within the first three days, indicating active microbial Fe^{2+} oxidation. However, the measured soluble Fe^{3+} concentration reached only ~5 mg/L by Day 6. The Fe oxidation profile (Figure 3b) is characterised by rapid initial Fe^{2+} oxidation followed by a plateau phase.



(a)



(b)

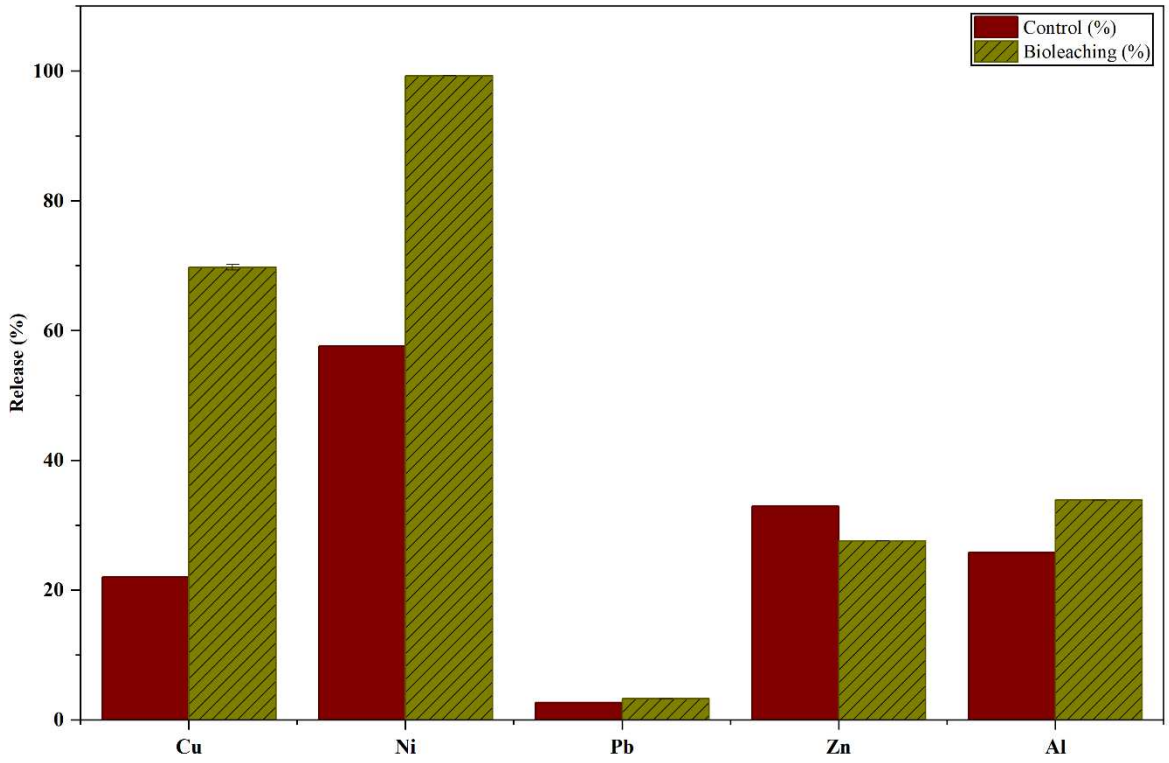
Fig 3: (a) Evolution of pH and (b) Fe^{2+} and Fe^{3+} concentrations during the incubation of the acidophilic bacterial strain with 9K medium at 37 °C during the first cycle

3.2 Bioleaching of pre-treated PCBs

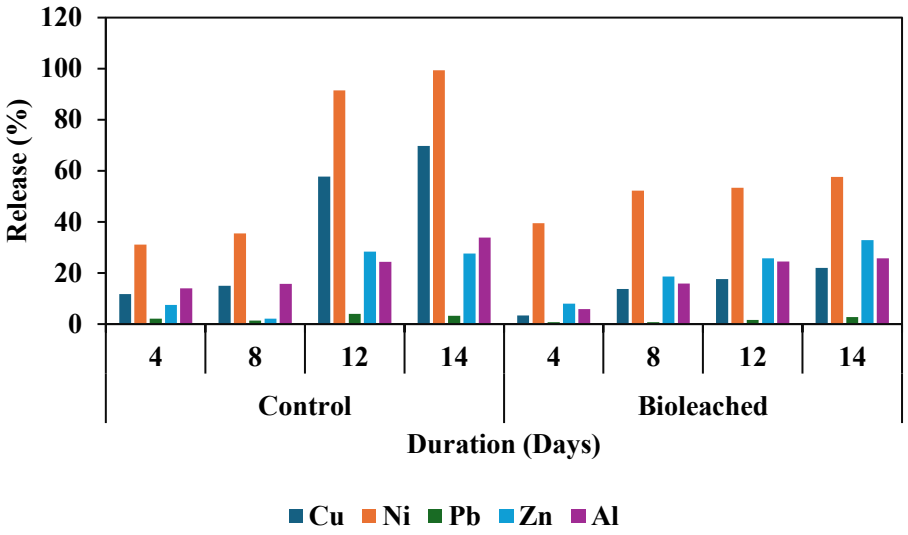
3.2.1 Metal Release

Bioleaching of chemically pre-treated PCBs was conducted in batch mode under continuous agitation (150 rpm at 37 °C) using the isolated acidophilic bacterial strain. Figure 4a shows the efficiency of metal solubilization—specifically for copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), and aluminium (Al). Metals released from the PCB were monitored at four-day intervals over 14 days (Figure 4b).

Abiotic controls consisting of pre-treated PCBs in acidified 9K medium (pH 2.5) without bacterial inoculation were used to distinguish biologically mediated leaching from purely chemical dissolution. The bioleaching using the bacterial strain resulted in higher solubilisation of Ni, Cu, Al, and Zn, confirming that the bacterial strain is efficient in metal solubilisation compared to abiotic control.



(a)



(b)

Fig 4: Bioleaching of metals from PCBs (a) after incubation 14 days (b) at regular 4-day intervals after incubation in 9K medium in the presence of the acidophilic bacterial strain

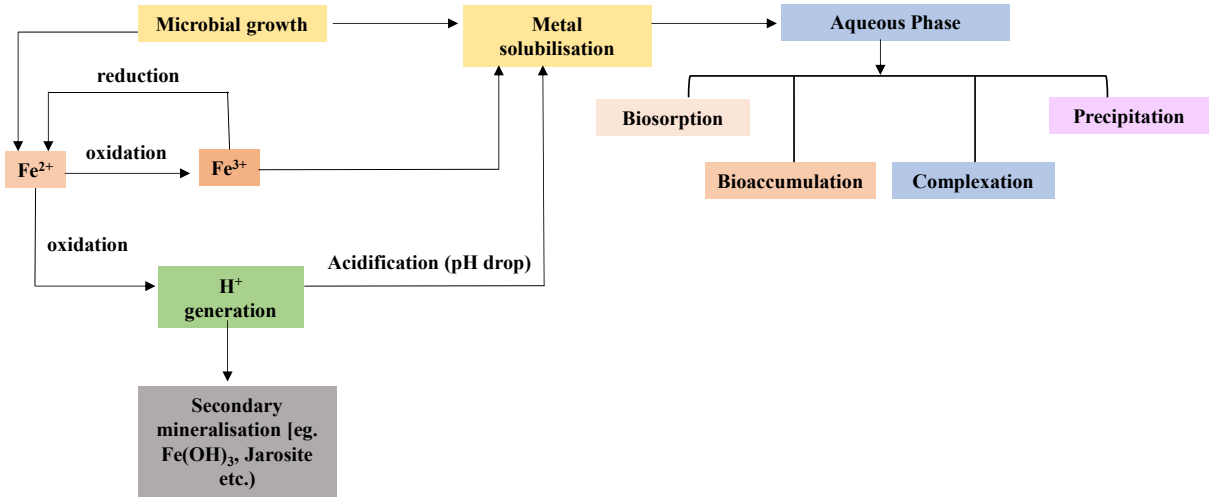


Fig 5: Schematic representation of the bioleaching mechanism for metal recovery from waste printed circuit boards

Figure 5 depicts the interrelationship between bacterial growth, pH decrease, and iron oxidation. The consistent decline in pH from 2.5 to 1.05 over six cycles (Figure 3a) correlated with the progressive oxidation of Fe^{2+} to Fe^{3+}, a characteristic of acidophilic, iron-oxidising bacteria. This pattern confirms the sustained metabolic activity of the

isolate obtained in this study. While the biological oxidation of Fe^{2+} itself does not directly generate protons, the subsequent hydrolysis of Fe^{3+} leads to H^+ release, thereby contributing to the observed acidification of the medium.

The mass-balance analysis (Table 1) revealed metal-specific differences after bioleaching (after 14 days): Ni showed near-quantitative bioleaching (99.32 ± 0.09 %), Cu exhibited substantial metal release (69.73 ± 0.43 %), whereas Zn (27.56 ± 0.01 %) and Al (33.88 ± 0.01%) were only moderately released from the PCBs, whereas Pb remained with minimal solubilisation (3.27 ± 0.01 %).

Table 1: Mass Balance of the metals bioleached from PCBs using the isolated acidophilic bacterial strain

Metal	Initial Conc. (mg/L)	Initial Mass (mg)	Bioleached Conc. (mg/L)	Bioleached Mass (mg)	% Recovery (Bioleached)	Control Conc. (mg/L)	Control Mass (mg)	% Recovery (Control)
Cu	6024	602.4	4202.67	420.27	69.8%	1325.64	132.56	22.0%
Ni	147.7	14.77	146.7	14.67	99.3%	85.13	8.51	57.6%
Pb	101.01	10.1	3.27	0.33	3.3%	2.73	0.27	2.7%
Zn	16.418	1.64	4.53	0.45	27.6%	5.394	0.54	32.9%
Al	23.311	2.33	7.89	0.79	33.9%	6.01	0.6	25.8%

3.2.2 Metal recovery through chemical precipitation

Metal recovery following bioleaching was quantified via mass balance analysis based on alkaline precipitation (1N NaOH) of the day 14 leachate. Table 2 provides a mass balance of the precipitation process. Recovery efficiencies were calculated by subtracting the initial dissolved metal concentrations from the residual concentrations after NaOH precipitation. The precipitation efficiencies for Cu, Ni, Pb, Zn, and Al were 99.9 ± 0.01%, 99.9 ± 0.01%, 96.8 ± 0.01%, 97.5 ± 0.03%, and 94.7 ± 0.01%, respectively.

Table 2: Mass balance of recovered bioleached metals through chemical precipitation using NaOH (1N)

Metal	Initial Conc. (mg/L)	Residual Conc. (mg/L)	Initial Mass (mg)	Residual Mass (mg)	Recovered Mass (mg)	% Recovery
Cu	4202.67	2.58	420.27	0.258	420.01	99.9%
Ni	147.7	0.1597	14.77	0.01597	14.75	99.9%

Pb	3.27	0.105	0.327	0.0105	0.3165	96.9%
Zn	4.53	0.113	0.453	0.0113	0.4417	97.5%
Al	7.89	0.418	0.789	0.0418	0.7472	94.7%

265

266 **3.3 Sustainability Assessment**

267 **3.3.1 Techno-Economic Assessment of recovered metals**

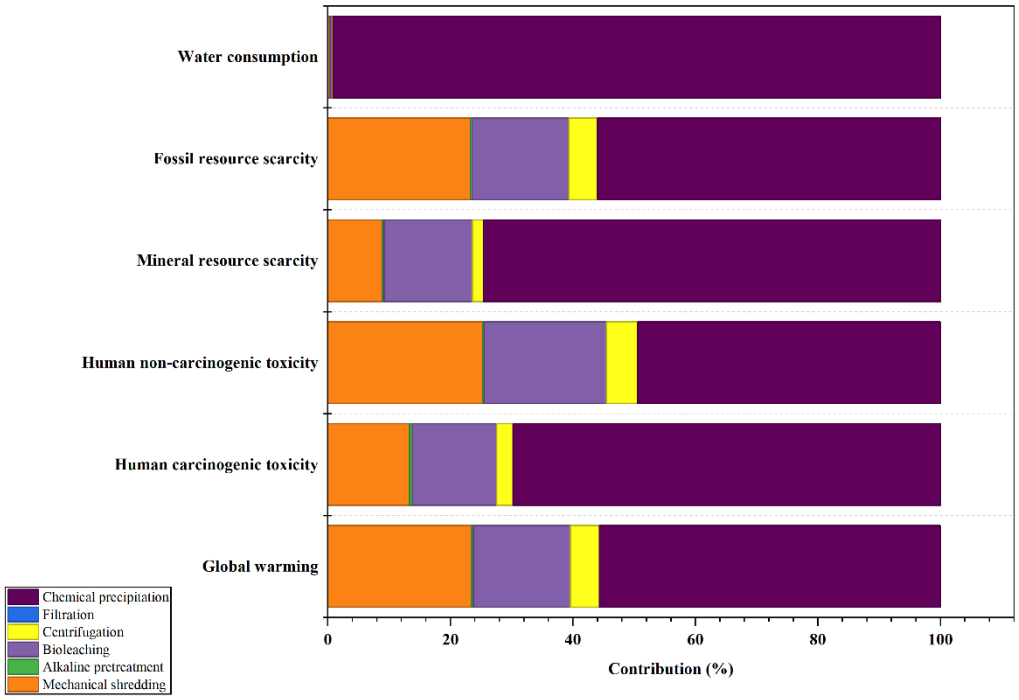
268 A preliminary techno-economic assessment (TEA) was conducted to estimate the theoretical economic value of Cu
269 and Ni recovered through the integrated bioleaching–chemical precipitation process. The recovered metal
270 concentrations in the leachate were 4.20 g/L for Cu and 0.15 g/L for Ni. Based on Indian market prices reported in
271 July 2025 (The Economic Times, 2025), Cu and Ni were valued at ₹901.35/kg and ₹1339.10/kg, respectively. As of
272 May 2026, the cost of Cu and Ni is 1312.5/Kg and 1812.5/Kg. The increase in the metal value emphasises the
273 importance of the recovery of Cu and Ni from PCBs. Accordingly, the gross theoretical value of the recovered metals
274 was estimated at ₹3.79 per litre for Cu and ₹0.20 per liter for Ni.

275 **3.3.2 Life Cycle Assessment**

276 The LCA results (Table 3 and Figure 6) indicate that bioleaching was not the major contributor to most of
277 the midpoint impact categories within the overall PCB treatment process. The global warming impact
278 associated with mechanical shredding and alkaline pretreatment was 0.020 and 0.0003 kg CO_2-eq,
279 respectively, whereas the contribution from bioleaching was 0.019 kg CO_2-eq. The human carcinogenic
280 toxicity and human non-carcinogenic toxicity associated with the bioleaching stage were 0.0001 and 0.005
281 kg 1,4-DCB-eq, respectively. The mineral resource scarcity, fossil resource scarcity, and water
282 consumption associated with bioleaching were 5.9×10^{-5} kg Cu-eq, 0.004 kg oil-eq, and 0.0002 m^3,
283 respectively. These results indicate that the environmental burden associated with the applied bioleaching
284 step is primarily influenced by electricity consumption and the upstream production of the 9K medium

285 rather than by the biological leaching mechanism itself. Overall, the LCA demonstrates the environmental

286 performance of the bioleaching process.



287

288 **Figure 6: Relative contribution of each process to the ReCiPe 2016 Midpoint (H) impact categories**

289 **Table 3 Impact categories contributed by each stage using 18 ReCiPe 2016 Midpoint (H) Categories**

Impact Category	Unit	Total	Mechanical Shredding	Alkaline Pretreatment	Bioleaching	Centrifugation	Filtration	Chemical Precipitation
Global Warming	Kg CO_2 Eq	0.12	0.02	0.0003	0.019	0.005	0	0.06
Human Carcinogenic Toxicity	Kg 1,4-DCB	0.0009	0.0001	4.5E-06	0.0001	2.5E-05	0	0.0006
Human Non-Carcinogenic Toxicity	Kg 1,4-DCB	0.02	0.007	6.9E-05	0.005	0.001	0	0.01
Mineral Resource Scarcity	Kg Cu Eq	0.0004	3.6E-05	1.1E-06	5.9E-05	7.3E-06	0	0.0003
Fossil Resource Scarcity	Kg Oil Eq	0.03	0.007	8.6E-05	0.004	0.001	0	0.01
Water Consumption	M3	0.05	0.0001	3.8E-06	0.0002	3.4E-05	0	0.04

290

4. Discussion

4.1 Metal recovery from PCBs

This study presented an integrated and sustainable approach for the bioleaching and recovery of the heavy metals from waste PCBs using an indigenous acidophilic bacterium and a simple alkaline precipitation method. The novelty lies in the simultaneous metal solubilisation and recovery of multiple metals through a biological process coupled with NaOH-mediated precipitation, thereby minimising the need for multiple recovery agents or complex downstream processing.

Table 4 overviews the bioleaching of different materials used and confirms that, among the reported literature, the current study shows that application of a single bacterial strain for multi-metal solubilisation from PCBs is as effective as the results obtained with bacterial consortia.

Table 4: Metal removal efficiency from different sources using various inoculum

Source of bioleached metals	Duration	Metal removal (%)	Inoculum	Reactor Type	Reference
PCBs	5 days	Cu – 6.68±0.67 Al – 1.34±0.13 Pb – 0.74±0.07 Zn – 0.74±0.009 Ni – 0.31±0.03	Individual bacterial strain (*Acidithiobacillus ferrooxidans*)	Batch study	(Hyra et al. 2022)
Mine tailing fractions	14 days	100% Co, 68% Zn, and 31% Cu	Co-culture (*Gluconobacter oxydans, Penicillium simplicissimum*)	2L Bioreactor	(Schueler et al. 2024)

Pyrite ores	21 days	3.6 mg/ml of Fe^{2+}, 0.21 mg/ml of Au	Individual bacterial strain (*Acidithiobacillus ferriphilus)*	Batch study	(Choinska et al. 2025)
PCBs	20 days	Cu recovery varied between 10.5% and 18%	Co-culture *(Pleurotus florida* and *Pseudomonas* sp)	Batch study	(Kaur et al. 2022)
Spent Lithium-Ion batteries	7 days	Co – 90.4% Li – 89.9% Mn – 91.8% Ni – 85.5%	Single bacterial strain	Batch study	Pinna et al. 2017)
Spent Lithium-Ion batteries	7 days	100% recovery of Li, Co, Ni, Mn and Cu	Co-culture (*Acidithiobacillus* and *Ali cyclobacillus* spp.)	Batch study	(Lalropuia et al. 2024)
PCBs	120 hours	90% Cu, 95% Ni, 96% Pb, and 99% Zn	Enzymatic bioleaching	Stirred Tank Reactor	(Trivedi et al. 2025)
PCBs	14 days	Cu - 69.73 ± 0.43%, Ni - 99.32 ± 0.09%, Zn - 27.56 ± 0.01%, Al - 33.88 ± 0.01%, and Pb - 3.27 ± 0.01	Individual bacterial strain	Batch study	This study

303

304 The system in this study exhibited strong metal-specific variability, with Ni (99.3 ± 0.09%) and Cu (69.8 ± 0.43%)
305 showing high solubilisation, while the solubilisation of Zn (27.6 ± 0.01%), Al (33.9 ± 0.01%), and Pb (3.2 ± 0.01%)
306 remained limited. These trends reflect differences in redox kinetics, solubility, and mineral associations of these
307 metals. High Ni and Cu recovery can be attributed to favourable dissolution under acidic and oxidising conditions.

The behaviour of Zn suggests partial association with acid-labile phases, as indicated by the comparable solubilisation in abiotic systems (Zeng et al. 2021). Zn also serves as a micronutrient for microbial metabolic activity. Zinc-binding sites may include negatively charged proteins or surfaces as well as intracellular enzymes. Moreover, metal accumulation or biosorption can occur through electrostatic interactions. The observed decrease in Zn solubilisation during bacterial bioleaching may also be associated with precipitation or immobilisation mediated by extracellular biomolecules or redox-active compounds (Yusof et al. 2019). Al solubilisation appears predominantly acid-driven and less dependent on iron cycling (Li et al. 2022). The immobility of Pb likely results from the formation of low-solubility secondary phases (e.g., $PbSO_4$) and preferential artitioning into non-aqueous fractions. Additionally, ferric precipitates may act as sinks for dissolved metals (Li et al. 2020) and create surface passivation layers that hinder further leaching (Jena et al. 2025).

The limited solubilisation of Zn, Al, and Pb may be governed by different mechanisms, which can vary depending on the microbial strain employed for bioleaching (Dong et al. 2023; Hu et al. 2024). For Pb, solubility constraints may be associated with the formation of sparingly soluble phases such as anglesite ($PbSO_4$), potentially favoured by the sulfate-containing components of the 9K medium which includes $(NH_4)_2SO_4$, $MgSO_4$, and $FeSO_4 \cdot 7H_2O$ (Jaiswal et al. 2025; Kumar et al. 2025). In contrast, Al is predominantly associated with oxide phases in PCBs, which may limit its dissolution under the applied bioleaching conditions (Hu et al. 2024). The relatively low solubility of Zn may potentially be associated with secondary-phase formation, co-precipitation, or adsorption onto bacterial biomass (Kumar et al. 2025). Although the present study did not quantitatively distinguish among these mechanisms, the observed metal solubilisation behaviour is consistent with that reported for acidophilic bacterial bioleaching systems (Dong et al. 2023).

Future studies incorporating residual-solid characterisation, biomass-associated metal analysis, phase identification, and comprehensive metal mass balances would help to quantitatively resolve the distribution and partitioning of the metals among the aqueous, solid, and biomass-associated phases.The two-step bioleaching of lithium-ion batteries using an acidophilic bacterial strain, resulting in 100% metal solubilisation (Pakostova et al. 2023). The application of an individual bacterial strain and a consortium containing ten bacterial strains in Cu bioleaching from waste electroformed components resulted in 94.7% (78 days) and 99.2% (59 days), respectively (Roger et al. 2012). The current study utilised bioleaching of multiple metals from PCBs using a bioleaching process of 14 days with metal

solubilisation of 99.3%, 69.8, 33.9%, and 27.6% for Ni, Cu, Al, and Zn, respectively, thus confirming the enhanced ability of the single bacterial strain in the bioleaching process.

4.2 Bioleaching and its mechanism

The acidophilic isolate demonstrated sustained activity under strongly acidic conditions (pH ≤ 2.5), confirming its suitability for bioleaching systems. Acidophilic bacteria possess bioleaching ability via mineral bio-oxidation through ferric iron, which includes sulfur/iron oxidising microorganisms, such as archaea or chemolithotrophs (Ilbert et al. 2013). These iron-oxidisers reduce oxygen via the electrons produced from Fe^{2+} and produce energy. The high redox potential of the $|Fe^{2+}/Fe^{3+}|$ redox couple and the acidic conditions make oxygen a viable and the only electron acceptor in this reaction (Bonnefoy et al. 2011). Acidophilic microbes maintain a transmembrane gradient by maintaining a high proton concentration outside the cell membrane with neutral pH inside the cell (Jones et al. 2023; Ilbert et al. 2013). Despite active oxidation, the limited soluble Fe^{3+} (~5 g/L) indicates substantial precipitation as ferric hydroxysulfates or association with biomass. These processes regulate from availability while potentially introducing diffusion limitations and localised toxicity.

The reactions of the iron-oxidation carried by the acidophilic bacteria are [57]:

$2Fe^{2+} + ½O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ ……. 1

$MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^0$ ……2

$MS + 2O_2 \rightarrow M^{2+} + SO_4^{2-}$ ……..3

$M + Fe_2(SO_4)_3 \rightarrow MSO_4 + 2FeSO_4$ ……4

These reactions highlight the central role of ferric iron as an oxidant and proton generation via Fe^{3+} hydrolysis. In addition to redoxolysis and acidolysis, metal mobilisation can be influenced by complexolysis and biomass interactions (Bonnefory et al. 2011; Desmarais et al. 2020). However, competing pathways can significantly affect system performance, as illustrated by the rather low Fe^{3+} concentration (~5 mg/L) despite active Fe^{2+} oxidation (Figure 3b). Formation of ferric precipitates (e.g., jarosite-type phases) can sequester the metals through co-precipitation or adsorption. Simultaneously, biosorption onto functional groups and intracellular bioaccumulation reduce dissolved metal concentrations (Sethurajan et al. 2021; Ji et al. 2022). These interactions collectively govern the iron oxidation/reduction kinetics as well as the metal speciation and the apparent metal solubilisation during bioleaching.

4.3 Metal recovery step

Metal extraction from the leachate is performed using techniques like solvent extraction, electrowinning, and ion-exchange (Dusengemungu et al. 2021). Solvent extraction is employed to extract metals from the aqueous leachate, which further requires a back extraction process to recover the reagent. The electrowinning process is used to recover metals from acidic and ammoniacal media (Wu et al. 2017). The current study shows that the alkaline precipitation achieved a recovery efficiency of greater than 94% for all metals, with near-complete removal of Cu and Ni, confirming its effectiveness as a downstream recovery step. However, hydroxide precipitation lacks selectivity and may produce mixed-metal phases, which can affect the purity and reuse potential of the metals (Pakostova et al. 2019). Lower recoveries for Pb, Zn, and Al reflect pH-dependent speciation and equilibrium constraints. In particular, amphoteric behaviour, notably for Al and Zn, may lead to partial resolubilisation at higher pH. The residual concentrations may also arise from incomplete nucleation, crystal growth limitations, or complexation effects (Chang et al. 2025). This highlights the need for controlled pH optimisation and post-precipitation characterisation (e.g. using XRD and FTIR) to assess product quality and downstream applicability.

Unlike research that focuses on the selective recovery of individual metals, the current study explores the collective leaching and recovery, highlighting the feasibility of treating complex electronic waste streams cost-effectively. The applied coupling of biohydrometallurgy with an environmentally friendly recovery strategy, therefore avoiding the use of highly toxic or energy-intensive chemical recovery systems and contributing to the sustainable circular economy for electronic waste by enabling both metal solubilisation and subsequent recovery within a single framework.

Following bioleaching, the dissolved metals from the bioleachate were precipitated chemically. The process is non-selective because of the simultaneous precipitation of multiple metals. Therefore, the recovered mixed-metal fraction will require additional downstream separation and purification steps for obtaining individual metal fractions. The present study primarily focused on the bioleaching and solubilisation of metals from PCBs rather than on selective metal recovery and purification. Further investigations are required to develop selective separation strategies for the recovery of individual metals from the bioleachate. The future scope for this study includes the enhancement of bioleaching using consortia and scale-up studies in laboratory-scale bioreactors, pilot-scale systems, optimised downstream processes, and evaluating the operational performance, economic viability, and stability of the bioleaching process.

4.4 Techno-economic and life cycle assessment of the bioleaching process

Figure 7 demonstrates that the bioleaching process offers superior techno-economic advantages over conventional leaching methods, such as heap leaching, tank leaching, and in-situ leaching using chemicals. Bioleaching, based on the results, is concluded as a sustainable, safe, and cost-efficient process. The relative score (1 to 4) given in the image compares the processes (Bioleaching and Conventional), indicating the techno-economic criteria as the least favourable, below average, average, and acceptable processes.

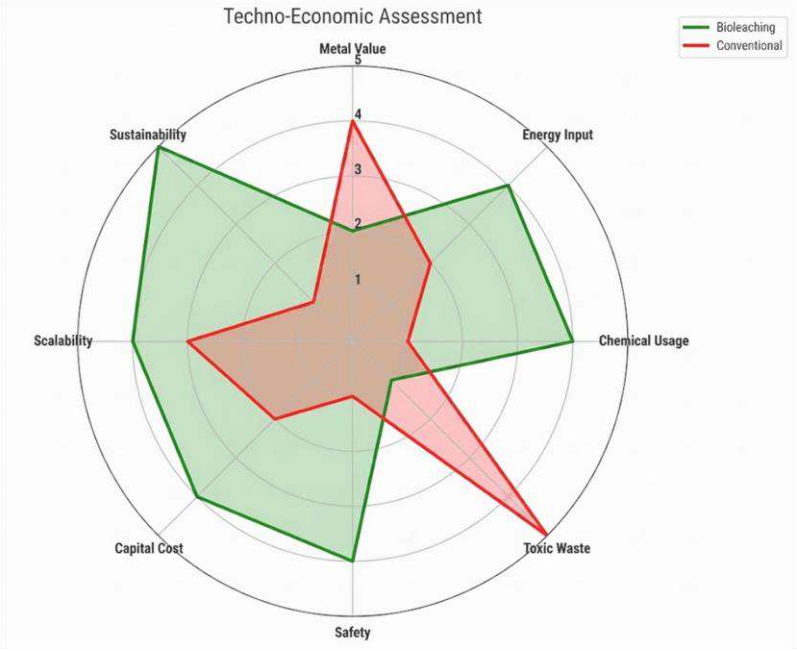


Fig 7: Radar chart showing techno-economic comparison between bioleaching and conventional methods

The high recovery of Cu and Ni translates into measurable economic potential, with estimated revenues (in the Indian scenario) of ₹1338.5/Kg and ₹1823/Kg, respectively, based on current market prices (29 May 2026). Cu clearly represents the dominant value stream. Metal recovery from secondary resources is generally more cost-effective than primary mining, as reported by (Zeng et al. 2021). However, the present estimates reflect only gross value and exclude process costs. Key factors such as chemical inputs, energy consumption, and solid–liquid separation will significantly influence the overall financial feasibility. Compared to conventional methods (Santaolalla et al. 2021; Król et al. 2020; Zhu et al. 2023; Oke et al. 2024), the bioleaching–precipitation system offers advantages in reduced energy demand, lower chemical usage, and minimised environmental impact, while maintaining competitive scalability (Becci et al. 2020; Van Yken et al. 2023). Identification of cost drivers and process bottlenecks will be critical for scale-up.

LCA provides a systematic approach for evaluating the environmental impacts associated with different stages of sustainable metal recovery processes and is widely applied in accordance with the principles of ISO 14040 and ISO 14044 (Jacquemin et al. 2012). In the present study, the global warming impact associated with the bioleaching stage was 0.019 kg CO_2-eq. This value is comparable to that reported for the bioleaching of waste PCBs using *Exiguobacterium himgiriensis* and *Enterobacter quasihormaechei*, followed by selective precipitation, solvent extraction, and metal refining, for which the bioleaching step resulted in a global warming impact of 0.035 kg CO_2-eq (Halder et al. 2026). The use of renewable electricity has been reported to reduce the environmental impact of bioleaching by approximately 60 % (Halder et al. 2026). Furthermore, Rautela et al. (2025) compared the environmental performance of bioleaching, glycine, nitric acid, and acetic acid leaching for lithium-ion battery recycling using LCA. Their results showed global warming impacts of 1194.48, 5349.23, 2668.46, and 4335.29 kg CO_2-eq for bioleaching, nitric acid, glycine, and acetic acid leaching, respectively. These findings indicate the potential environmental advantage of bioleaching over conventional chemical leaching approaches. However, the environmental performance of a metal-recovery process strongly depends on process configuration, energy sources, reagent consumption, and system boundaries. Overall, the integration of bioleaching with efficient downstream metal separation presents a promising pathway for sustainable metal recovery, consistent with circular economy principles by reducing dependence on primary mineral resources and potentially minimising waste generation.

5. Conclusion

This study demonstrates the effectiveness of an integrated bioleaching–chemical precipitation approach for the recovery of base and heavy metals from waste mobile phone printed circuit boards using an acidophilic, iron-oxidising bacterial isolate derived from sewage sludge. The isolate exhibited strong Fe^{2+}-oxidising activity and acid tolerance, enabling the mobilisation of Cu, Ni, Al, Zn, and Pb from pre-treated PCBs, with metal-specific trends indicating limited aqueous availability of Pb, Zn, and Al due to combined effects of solubility constraints, secondary phase formation, and biomass-associated partitioning (biosorption and bioaccumulation). Subsequent NaOH-induced alkaline precipitation achieved a recovery rate of greater than 94% across all target metals, confirming its efficiency as a downstream separation step. The coupled process effectively integrates biologically driven metal solubilization with chemical recovery, providing a resource-efficient and environmentally sustainable pathway for metal extraction from e-waste.

Declaration of competing interests

The authors declare that they have no conflict of interest.

Acknowledgement

This research received no external funding.

CRediT author statement

Rajalakshmi Sridharan – Conceptualisation, data curation, data visualisation, data validation, writing - original draft

Shinjini Sen - Conceptualisation, investigation, data curation, and writing - original draft

Piet Lens – Conceptualisation, validation, and writing - reviewing and editing

Mohanakrishnan Logan - Conceptualisation, supervision, project administration, validation, and writing - reviewing and editing

Data Availability

Data will be provided upon request.

References

1. Akcil A, Erust C, Gahan CS, Ozgun CS, Sahin M, Tuncuk M (2015) A.: Precious metal recovery from waste printed circuit boards using cyanide and non-cyanide lixiviants--A review, *Waste Manag.* **45**, 258–271.
2. Becci A, Kumar A, Dey A (2020) Bioleaching of metals from waste printed circuit boards: A review, *J. Hazard. Mater.* **397**, 122731.
3. Bonnefoy V, Holmes DS (2011) Genomic insights into microbial iron oxidation and iron uptake strategies in extremely acidic environments, *Environ. Microbiol*. 14, 1597–1611.
4. Chang HF, Lin JY, Cheng TM, Lai CH (2025) Advanced absolute chemical precipitation for high-purity metal recovery in all-types of lithium-ion battery recycling, *Sep. Purif. Technol. 361*, 131454.
5. Choińska-Pulit A, Sobolczyk-Bednarek J, Kania M (2025) Isolation, Testing, and Adaptation of Bacteria to Bioleach Metals from Pyrite, *Minerals*. *15*(9), 946.
6. D'Adamo I, Ferella F, Gastaldi M, Ippolito NM, Lunadei D (2025) Circular economy and profitability: A techno-economic analysis of precious metal recovery from waste printed circuit boards, *Sustain. Mater. Technol.* 10:e01812.
7. Desmarais M, Pirade F, Zhang J, Rene ER (2020) Biohydrometallurgical processes for the recovery of precious and base metals from waste electrical and electronic equipment: Current trends and perspectives, *Bioresour. Technol. Rep.* **11**, 100526.
8. Dong Y, Zan J, Lin H (2023) Recovery of precious metals from waste printed circuit boards though bioleaching route: A review of the recent progress and perspective, *Journal of Environmental Management*, *348*, 119354.
9. Dusengemungu L, Kasali G, Gwanama C, Mubemba B (2021) Overview of fungal bioleaching of metals, *Environ. Adv.* **5**, 100083.
10. Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A (2020) Nickel: human health and environmental toxicology, *Int. J. Environ. Res. Public Health.* **17**(3).
11. Halder B, Dutta R, Kumari N, Tanishka, Anuradha A, Nigam V K, Sampath M K (2026) Life cycle assessment of bioleaching for metal recovery from waste PCBs: A sustainable approach using openLCA, *Sci Rep*.

12. Hsu E, Barmak K, West A, Park AHA (2019) Advancements in the treatment and processing of electronic waste with sustainability: A review of metal extraction and recovery technologies, *Green Chem.* **21**(5) 919–936.
13. Hu S, Wang H, Li X, He W, Ma J, Xu Y, Ming W (2024). Recent advances in bioleaching and biosorption of metals from waste printed circuit boards: A review, *Journal of Environmental Management*, *371*, 123008.
14. Hyra K, Nuckowski PM, Willner J, Suponik T, Franke RD, Pawlyta M, Matus K, Kwaśny W (2022) Morphology, phase and chemical analysis of leachate after bioleaching metals from printed circuit boards. *Materials*. *15*(13), 4373.
15. Ilbert M, Bonnefoy V (2013) Insight into the evolution of the iron oxidation pathways, *Biochim. Biophys. Acta Bioenerg*. 1827, 161–175.
16. Jacquemin L, Pontalier P Y, Sablayrolles C (2012) Life cycle assessment (LCA) applied to the process industry: a review, *The International Journal of Life Cycle Assessment*. *17*(8), 1028-1041.
17. Jaiswal A, Raj SI, Isiaka Adetunji A, Negadi L, Singh S, Tumba K, Bahadur I, Uddin I (2025) Bioleaching as an Eco-Friendly Nano-Factory for Sustainable Inorganic Waste Management: Current Advancements, Challenges, and Opportunities, *ChemistryOpen*, *14*(9), p.e202500104.
18. Jena S, Swain BP (2025) Surface Passivation and Surface Treatment of Coating Materials, Advances in Mechanical Coating. Materials Horizons: From Nature to Nanomaterials.
19. Jha MK, Lee JC, Kumari A, Choubey PK, Kumar V, Jeong J (2011) Pressure leaching of metals from waste printed circuit boards using sulfuric acid, *JOM.* **63**(8), 29–32.
20. Ji X, Yang M, Wan A, Yu S, Yao Z (2022) Bioleaching of typical electronic waste-printed circuit boards (WPCBs): A short review, *Int. J. Environ. Res. Public Health* **19**(12) (2022).
21. Jones S, Santini JM (2023) Mechanisms of bioleaching: Iron and sulfur oxidation by acidophilic microorganisms, *Essays Biochem.* **67**(4), 685–699.
22. Kaliyaraj D, Rajendran M, Angamuthu V, Antony AR, Kaari M, Thangavel S, Venugopal G, Joseph J, Manikkam R (2019) Bioleaching of heavy metals from printed circuit board (PCB) by *Streptomyces albidoflavus* TN10 isolated from insect nest, *Bioresour. Bioprocess.* **6**, 1.

503 23. Kang DK, Ilankoon IMSK, Dushyantha N, Chong MN (2021) Assessment of pre-treatment techniques
504 for coarse printed circuit boards (PCBs) recycling, *Minerals.* **11**(10), 1134.
505 24. Kaur P, Sharma S, Albarakaty FM, Kalia A, Hassan MM, Abd-Elsalam KA (2022) Biosorption and
506 bioleaching of heavy metals from electronic waste varied with microbial genera, *Sustainability*. *14*(2),
507 935.
508 25. Khetwunchai N, Akeprathumchai S, Anusaraporn S, Krobthong S, Yingchutrakul Y, Thiravetyan P
509 (2025) Enhanced bioleaching of copper and gold from waste printed circuit boards: Stepwise process,
510 pretreatment strategies, metabolomics analysis, and the role of N8-acetylspermidine, *Process Saf.*
511 *Environ. Prot.* **194**, 289–305.
512 26. Khetwunchai N, Akeprathumchai S, Thiravetyan P (2024) Importance of soybean meal and ozone in the
513 extraction of gold from silicate ore, *Waste Biomass Valor.* **15**(7), 4247–4264.
514 27. Król A, Mizerna K, Bożym M (2020) An assessment of pH-dependent release and mobility of heavy
515 metals from metallurgical slag, *J. Hazard. Mater. 384*, 121502.
516 28. Kumar, Saxena P, Manna S (2025) Recovery of cadmium, lead, strontium and zinc from jarosite waste
517 by using a combinatory approach of bioleaching and acid leaching, *Canadian Metallurgical*
518 *Quarterly*, *64*(3), 1449-1457.
519 29. Lalropuia L, Kucera J, Rassy WY, Pakostova E, Schild D, Mandl M, Kremser K, Guebitz GM (2024)
520 Metal recovery from spent lithium-ion batteries via two-step bioleaching using adapted
521 chemolithotrophs from an acidic mine pit lake, *Front. Microbiol*, *15*, 1347072.
522 30. Li W, Johnson CE (2022) Relationships among pH, aluminium solubility and aluminium complexation
523 with organic matter in acid forest soils of the Northeastern United States, *Geoderma*, *271*, 234-242.
524 31. Li X, Zhang Q, Yang B (2020) Co-precipitation with CaCO to remove heavy metals and significantly
525 reduce the moisture content of filter residue, *Chemosphere* **239**, 124660.
526 32. Liu G, Pan D, Wu Y, Yuan H, Yu L, Wang W (2021) An integrated and sustainable hydrometallurgical
527 process for enrichment of precious metals and selective separation of copper, zinc, and lead from a
528 roasted sand, *Waste Manag.* **132**, 133–141.

33. López-Martínez A, Martínez-Prado MA, Núñez-Ramírez DM, Medina-Torres L, Rojas-Contreras JA, Anguiano-Vega GA, Soto-Cruz NO (2024) Acidophilic bacteria for metal extraction: Biotechnological characteristics and applications, *Braz. J. Chem. Eng.*
34. MCX Copper, The Economic Times. Accessed May 29, 2026. https://economictimes.indiatimes.com/commoditysummary/symbol-COPPER.cms
35. MCX Nickel, The Economic Times. Accessed May 29, 2026. https://economictimes.indiatimes.com/commoditysummary/symbol-NICKEL.cms
36. Meunier N, Drogui P, Montané C, Hausler R, Mercier G, Blais JF (2006) Comparison between electrocoagulation and chemical precipitation for metals removal from acidic soil leachate, *J. Hazard. Mater.* **137**(1), 581–590.
37. Mora CC, Rojas Contreras JA, Rosales Villarreal MC, Urban Martínez JL, Delgado E, Roldan HM, Hernández Rodarte FS, Reyes Jáquez D (2025) Identification of microorganisms at different times in a bioleaching process for the recovery of gold and silver from minerals in oxide form, *Heliyon* **11**(2), e41878.
38. Núñez Ramírez DM, Ramírez Torres LA, Medina-Torres L, Calderas F, González Lozano MA, Ponce Peña P, Fierros Romero G, Manero O (2019) A rheological study of the bioleaching process of an iron ore for the elimination of gangue minerals. *Miner. Eng.* **144**, 106023.
39. Oke EA, Potgieter H (2024) Recent chemical methods for metals recovery from printed circuit boards: A review, *J. Mater. Cycles Waste Manag.* **26**(3), 1349–1368.
40. Pakostova E, Graves J, Latvyte E, Maddalena G, Horsfall L (2024) A novel closed-loop biotechnology for the recovery of cobalt from a lithium-ion battery active cathode material, *Microbiology*. *170*(7), 001475 (2024).
41. Pakostova E, Herath A (2023) A bioleaching process for sustainable recycling of complex structures with multi-metal layers, *Sustainability*. *15*(19), 14068.
42. Pakostova E, Johnson DB (2019) Microbial generation of sulfuric acid from granular elemental sulfur in laboratory-scale bioreactors, *Hydrometallurgy*. *190*, 105152.

555 43. Pinna EG, Ruiz MC, Ojeda MW, Rodriguez MH (2017) Cathodes of spent Li-ion batteries: Dissolution
556 with phosphoric acid and recovery of lithium and cobalt from leach liquors, *Hydrometallurgy*. *167*, 66-
557 71.
558 44. Pohanka M (2019) Copper and copper nanoparticles toxicity and their impact on basic functions in the
559 body, *Bratisl. Lek. Listy.* **120**(6), 397–409.
560 45. Priya A, Hait S (2017) Comparative assessment of metallurgical recovery of metals from electronic
561 waste with special emphasis on bioleaching, *Environ. Sci. Pollut. Res.* **24**(8), 6989–7008.
562 46. Rautela R, Wandhre R, Bherwani H, Yadav B R, Kumar S (2025) Sustainability evaluation of leaching
563 processes for lithium-ion battery recycling using life cycle assessment, costing, and multi-criteria
564 decision analysis, *Chemical Engineering Journal*. 172336.
565 47. Roger M, Castelle C, Guiral M, Infossi P, Lojou E, Giudici-Orticoni MT, Ilbert M (2012) Mineral
566 respiration under extreme acidic conditions: From a supramolecular organisation to a molecular
567 adaptation in *Acidithiobacillus ferrooxidans*, *Biochem. Soc. Trans.* 40, 1324–1329.
568 48. Santaolalla A, Lens PNL, Barona A, Rojo N, Ocio A, Gallastegui G (2021) Metal extraction and
569 recovery from mobile phone PCBs by a combination of bioleaching and precipitation processes,
570 *Minerals.* **11**(9), 1004.
571 49. Schueler TA, Schippers A, Goldmann D (2024) Bioleaching for metals removal from mine tailings
572 flotation fractions, *Hydrometallurgy*. *225*, 106286.
573 50. Sethurajan M, Gaydardzhiev S (2021) Bioprocessing of spent lithium-ion batteries for critical metals
574 recovery – A review, *Resour. Conserv. Recycl.* **165**, 105225.
575 51. Tabrizi AB (2010) Development of a dispersive liquid-liquid microextraction method for iron speciation
576 and determination in different water samples, *J. Hazard. Mater.* **183**(1–3), 688–693.
577 52. Trivedi A, Hait S (2023) Metal bioleaching from printed circuit boards by bio-Fenton process:
578 Optimisation and prediction by response surface methodology and artificial intelligence models, *J.*
579 *Environ. Manag.* **326**(Pt B), 116797.
580 53. Trivedi A, Hait S (2025) Semi-scale stirred tank enzymatic bioleaching system for metal recovery from
581 PCBs of end-of-life mobile phones: Process parameter optimisation, predictive modelling, and economic
582 assessment, *Waste Management*. *204*, 114916.

54. Van Yken J, Cheng KY, Boxall NJ, Nikoloski AN, Moheimani N, Valix M, Kaksonen AH (2023) An integrated biohydrometallurgical approach for the extraction of base metals from printed circuit boards, *Hydrometallurgy.* **216**, 105998.
55. Vardanyan A, Vardanyan N, Abrahamyan N, Aatach M, Gaydardzhiev S (2024) Sequential biologically assisted extraction of Cu and Zn from printed circuit boards (PCB), *Int. J. Environ. Stud.* **81**(4), 1756–1771.
56. Vera M, Schippers A, Hedrich S, Sand W (2022) Progress in bioleaching: Fundamentals and mechanisms of microbial metal sulfide oxidation - Part A, *Appl. Microbiol. Biotechnol.* **106**(21), 6933–6952.
57. Volkmann SE, Kuhn T, Lehnen F (2018) A comprehensive approach for a techno-economic assessment of nodule mining in the deep sea, *Miner. Econ.* **31**(3), 319–336.
58. Wan X, Kleemola L, Klemettinen L, O'Brien H, Taskinen P, Jokilaakso A (2021) On the kinetic behaviour of recycling precious metals (Au, Ag, Pt, and Pd) through copper smelting process, *J. Sustain. Metall.* **7**(3), 920–931.
59. Wang K, Liu Y, Liu R, Belqadi W, Zeng W, Yu R, Wu X (2025) Isolation, sphalerite bioleaching, and whole genome sequencing of *Acidithiobacillus ferriphilus* QBS3 from zinc-rich sulfide mine drainage, *Life.* **15**(5), 792.
60. Wu Z, Yuan W, Li J, Wang X, Liu L, Wang J (2017) A critical review on the recycling of copper and precious metals from waste printed circuit boards using hydrometallurgy, *Front. Environ. Sci. Eng.* 11, 1–14.
61. Xiu FR, Weng H, Qi Y, Yu G, Zhang Z, Zhang FS, Chen M (2017) A novel recovery method of copper from waste printed circuit boards by supercritical methanol process: Preparation of ultrafine copper materials, *Waste Manag.* **60**, 643–651.
62. Yaashikaa PR, Priyanka B, Senthil Kumar P, Karishma S, Jeevanantham S, Indraganti S A (2022) Review on recent advancements in recovery of valuable and toxic metals from e-waste using bioleaching approach, *Chemosphere* **287**(Pt 2), 132230.
63. Yazici EY, Deveci H (2013) Extraction of metals from waste printed circuit boards (WPCBs) in H_2SO_4–$CuSO_4$–NaCl solutions, *Hydrometallurgy.* **139**, 30–38.

64. Yusof H M, Mohamad R, Zaidan UH, Abdul R N A (2019) Microbial synthesis of zinc oxide nanoparticles and their potentialapplication as an antimicrobial agent and a feed supplement in animal industry: a review, J Anim SciBiotechnol, 10:1–22.
65. Zeng X, Mathews JA, Li J (2021) Urban mining of e-waste is becoming more cost-effective than virgin mining, *J. Mater. Sci. Eng.* **9**, 100034.
66. Zhu P, Xu Y, Wang Y, Wang H (2023) Metal recovery technologies from waste printed circuit boards: A review, *Resour. Conserv. Recycl.* **189**, 106699.