Bioleaching of electronic waste

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Academic year: 2023/2024

ASSIGNMENT OF DIPLOMA THESIS
(project, art work, art performance)

Name and surname: **Eyoel Legesse Teklemariam**
Personal number: **T22996**
Study programme: **N0712A030002 Environmental Engineering**
Type of Study: **Full-time**
Work topic: **Bioleaching of electronic waste**

Theses guidelines

1. Study all literature and information sources related to the topic. Prepare theorethical part of thesis
2. Cultivate the microorganisms and test the microorganisms of capability to leach the elements from the electronic waste
3. Compare the bioleaching with classical extraction methods
4. Process the measured and recorded data and critically evaluate the results.

Form processing of diploma thesis: **printed/electronic**
Language of elaboration: **English**

Recommended resources:

1. RAWLINGS, Douglas E., ed. *Biomining* [online]. Berlin, Heidelberg: Springer Berlin Heidelberg, 1997 [cit. 2021-04-09]. ISBN 978-3-662-06113-8. Dostupné z: doi:10.1007/978-3-662-06111-4
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3. CSUROS, Maria a Csaba CSUROS. Environmental Sampling and Analysis for Metals. Boca Raton: Lewis Publishers, 2002. ISBN 1-56670-572-x.
4. SANZ-MEDEL, Alfredo a Rosario PEREIRO. Atomic Absorption Spectrometry: An introduction. 2nd edition. New York: Momentum Press, 2014. ISBN 978-1-60650-435-2
5. Scientific resources: such as Web of Science, ScienceDirect, SciFinder Scholar, Medline etc.

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Date of assignment of diploma thesis: **January 2, 2024**
Submission deadline of diploma thesis: **May 10, 2024**

L.S.

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In Zlín February 6, 2024

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ABSTRAKT

Tato práce se zaměřuje na možnosti bioloužení jako udržitelného řešení pro obnovu vzácných a drahých kovů z odpadních desek s plošnými spoji (WPCB). Mezi výzkumné cíle patří především zkoumání vlivu koncentrací síry na účinnost bioloužení. V této práci je i zahrnuto porovnání bioloužení s metodou chemického loužení mikrovlnným rozkladem. Metodika zahrnovala přípravu základních desek jako vzorky, které neměly velikost větší než (≤ 1 mm) a vzorky, které měly velikost cca (15 x 25 mm). Dále pak byl otestován vliv koncentrací síry (1 g/l, 2 g/l a 3 g/l) na růst mikroorganismů *Acidothiobacillus ferrooxidans*, což bylo ověřeno měřením parametrů jako pH, ORP a Fe^{3+} během 7 dnů. Nejlepších výsledků bylo dosaženo u vzorků, které obsahovaly 1 g/l a 2 g/l přídavku síry. Koncentrace sledovaných prvků byly poté měřeny na přístroji plamenové atomové absorpční spektroskopie (FAAS). Bylo zjištěno, že po 24 hodinách prokazují významnou účinnost regenerace mědi a niklu v odpadu WPCB. Zatímco vzorky prachu vykazovaly lepší účinnost pro mangan s nejvyšší účinností 29,3 %, míra regenerace niklu byla pozoruhodná ve větších vzorcích s hodnotami v rozmezí od 85 % do 102,4 %. Je zajímavé, že velikostní rozdíl mezi prachem a většími vzorky významně neovlivnil úrovně koncentrace kovů. Navíc prodloužená bioloužení až 55 dní vedla ke zvýšené účinnosti regenerace v testovaných vzorcích prachu, přičemž všechny vzorky prachu dosahovaly 100 % účinnosti niklu a 30 % účinnosti mědi, což zvýrazňuje potenciál biologického vyluhování pro lepší regeneraci kovů v průběhu času. Tato zjištění podtrhují selektivní povahu biologického loužení při získávání cílových kovů z odpadu WPCB a zdůrazňují důležitost optimalizace podmínek biologického loužení pro zlepšení účinnosti.

Klíčová slova: bioloužení, *Acidithiobacillus ferrooxidans*, elektronický odpad, mikrovlnný rozklad, kovy, odpadní desky s plošnými spoji (WPCB)

ABSTRACT

This thesis focuses on exploring the potential of bioleaching as a sustainable solution for the recovery of rare and precious metals from Waste Printed Circuit Boards (WPCBs). The research objectives mainly include investigating the influence of sulphur concentrations and size reduction on bioleaching efficiency and comparing bioleaching with the chemical leaching method, in this case, microwave-assisted acid digestion, for total recovery. The methodology involved preparing WPCBs in the dust ($\leq$ 1mm) and coarse forms (15 x 25 mm) and subjecting them to varying sulphur concentrations (1 g/L, 2 g/L and 3 g/L) biomediums containing *Acidothiobacillus ferrooxidans*. pH, ORP and Fe^{3+} were measured during a 7-day bacterial cultivation period in which 1 g/L and 2 g/L showed optimal bacterial growth and ORP potential. Leaching data of flame atomic absorption spectroscopy (FAAS) after 24 hours demonstrates significant recovery efficiencies for copper and nickel in WPCB waste. While dust samples exhibited better efficiency for manganese, with the highest 29.3 % efficiency, nickel recovery rates were remarkable in coarse samples, with values ranging from 85% to 102.4%. Interestingly, the size difference between dust and coarse samples did not significantly affect metal concentration levels. Moreover, an extended contact time of 55 days resulted in increased recovery efficiencies in the tested dust samples, with all Dust samples reaching 100% of Nickel and 30% of copper efficiency, highlighting the potential of bioleaching for enhanced metal recovery over time. These findings underscore the selective nature of bioleaching in recovering target metals from WPCB waste and emphasize the importance of optimizing bioleaching conditions for improved efficiency.

Keywords: bioleaching, *Acidithiobacillus ferrooxidans*, electronic waste, acid digestion, metal recovery, heavy metal, Waste Printed Circuit Boards (WPCB),

CONTENTS

INTRODUCTION

Electrical and electronic equipment (EEE) has become an integral part of modern society, revolutionizing communication, upgrading living standards, and making daily tasks easier. However, as it has its advantages, it also has a downside, with the widespread demand for EEE leading to a dramatic increase in electronic waste (e-waste) production, making it the fastest-growing waste stream globally. Both developed and developing nations are struggling with the challenges of managing and recycling e-waste, especially in developing countries, due to high domestic generation and also illegal imports from developed countries. When electronic waste is not properly discarded in landfills, hazardous materials are leached into the surrounding environment through processes like leaching and incineration. This contaminates the soil, water, and air, posing high risks to both human health and the ecosystem.

Waste Electrical and Electronic Equipment (WEEE) can serve as a valuable secondary source of metals, particularly rare and precious ones, despite the associated risks. Utilizing secondary raw materials for resource recovery not only safeguards primary ores but also markedly diminishes ecological and carbon footprints. Printed circuit boards (PCBs), the predominant component of electronic waste, emerge as the principal source of valuable metals.

Conventional methods used for e-waste recycling, like pyrometallurgy and hydrometallurgy, have been used for e-waste for a long time now, but they come with significant environmental impact and energy consumption. For instance, hydrometallurgy requires large amounts of chemicals and water, and the released waste contributes to environmental degradation. Pyrometallurgy recycling involves high temperatures, which leads to energy inefficiency and air pollution. In contrast, bioleaching is starting to show as a promising alternative for e-waste recycling. By utilizing naturally occurring microorganisms to dissolve metals from electronic waste, bioleaching operates at ambient temperatures and uses minimal chemicals, making it a greener and more sustainable approach to metal recovery.

I.   THEORY

1 ELECTRONIC WASTE

Waste Electrical and Electronic Equipment (WEEE), or e-waste, is a term used to describe undesired Electronic and electrical equipment (EEE) that are abandoned, outdated, or at the end of their useful lives (Alabi et al., 2021). Modern society has been completely transformed by electrical and electronic equipment (EEE), which has raised living conditions and produced new forms of communication (Cheshmeh et al., 2023). It is now an integral part of life as it allows people around the globe to live better because of its vast range of applications and availability. E-waste is created due to the inappropriate ways in which it is made, utilized, and discarded. Basic kitchen equipment, PCs, and smartphones all contribute to the generation of e-waste. It also originates from transportation, security, energy, and medical facilities (Bagwan, 2024).

The amount of e-waste produced worldwide is rising at a startling rate of roughly 2 Mt every year. As a result, e-waste is now the waste stream that is expanding most rapidly worldwide (Alabi et al., 2021). According to current United Nations (UN) projections, in 2021, each individual is predicted to generate approximately 7.6 kg of E-waste per capita, which will result in a massive quantity of 57.4 MMT globally (Murthy & Ramakrishna, 2022). Working from home and homeschooling after COVID-19 may also contribute to the increased global e-waste due to the increased demand for electronics (Maes & Preston-Whyte, 2022). Global e-waste generation is predicted to reach 74 MMT by 2030 as shown in **Figure 2** (Widanapathirana et al., 2023). A technology boom that has greatly benefited society by generating jobs, money, and generally raising living standards around the world has been sparked by the continuous and unparalleled consumer desire for the newest gadgets and devices. However, during the process, significant amounts of electronic and electrical waste were produced and are being produced. The issue with e-waste is that it is toxic, has an increasing volume, and contains valuable materials that are lost when it is disposed of (Hong & Valix, 2014). In 2019, only 17 % of electronic waste was formally gathered and recycled, this includes various valuable metals, such as gold, silver, copper, platinum, and other high-value elements which were estimated to be worth US $57 billion (Pathak & Sushil, 2022). The difficulty in managing e-waste will be in the development of environmentally conscious, cost-effective recycling technology that can handle its volume and complexity (Hong & Valix, 2014). E-waste management and recycling is a challenge for both developed and developing nations, with developing nations facing greater challenges in this area as a result

of domestic generation as well as illegal importation of e-waste from developed countries (Patil & Ramakrishna, 2020).

1.1 Generation and source of e-waste

E-waste occurs in a variety of places, such as the institutional and research sectors, the household sector, and the manufacturing sector (Ankit et al., 2021). Rapid technical advancements and the rise in demand for electronics are both linked to the production of e-waste. Additionally, a lack of international agreement on the management of e-waste and inadequate user knowledge contribute to the extraordinary rise in the production of e-waste (Işıldar et al., 2018). Over 82 % of electronic waste generated globally is still neither documented nor recycled (Moossa et al., 2023).

The products that make up the largest percentage of e-waste include vacuum cleaners, air conditioners, washing machines, dryers, refrigerators, computers, phones, printers, CDs, DVDs, fax machines, etc.; these items account for almost 30 % of the total; televisions make up 10 %; and monitors alone account for 10 %. **Figure 1** shows the percentage of small equipment in the total amount of e-waste produced in 2019, which was the highest (17.4 %), followed by large equipment (13.1 %) and temperature-exchange equipment (10.8 %) Mt (Ankit et al., 2021). These electronic devices quickly become outdated due to the rapidly growing electronic technology, and new device generations swiftly substitute the older ones, producing an alarming amount of e-waste. Apart from these domestic appliances and information and communication technologies (ICTs), toys, consumer and lighting equipment, electrical and electronic tools, medical gadgets, monitoring and control instruments, and automatic dispensers are also included in the category of e-waste (Patil & Ramakrishna, 2020).
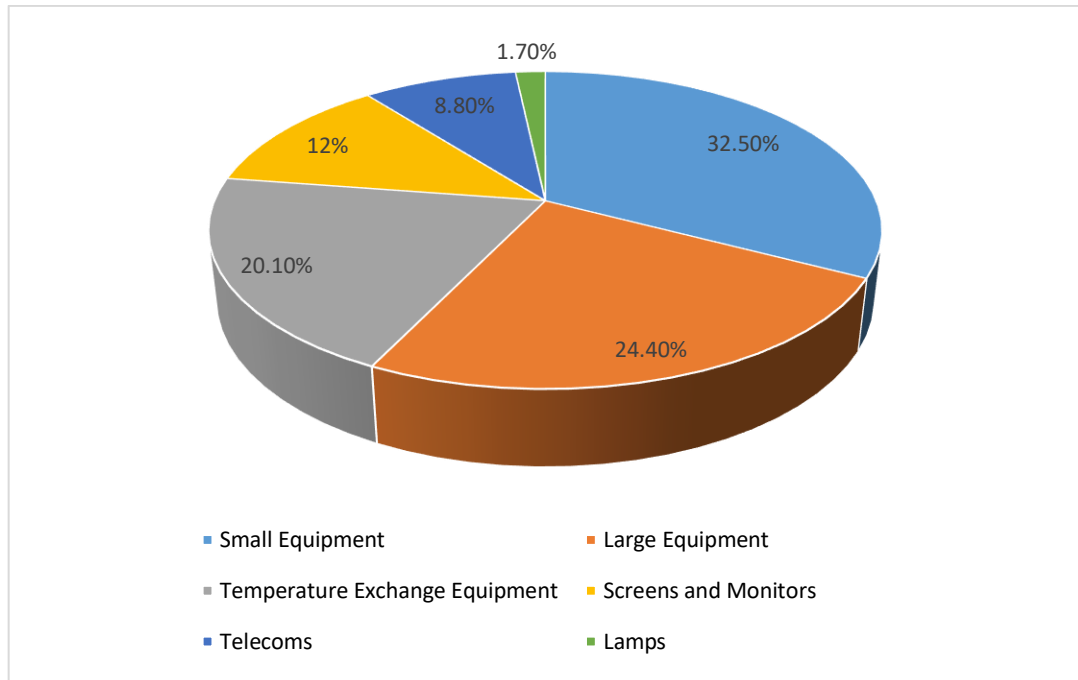
Figure 1 Components contributing to e-waste 2019 data (Ankit et al., 2021)

While large corporations and government agencies are, to some extent, required to dispose of their waste to the appropriate vendor, local rag pickers freely dispose of domestic waste, which is handled poorly and has several negative health impacts on the recyclers. (Ankit et al., 2021).
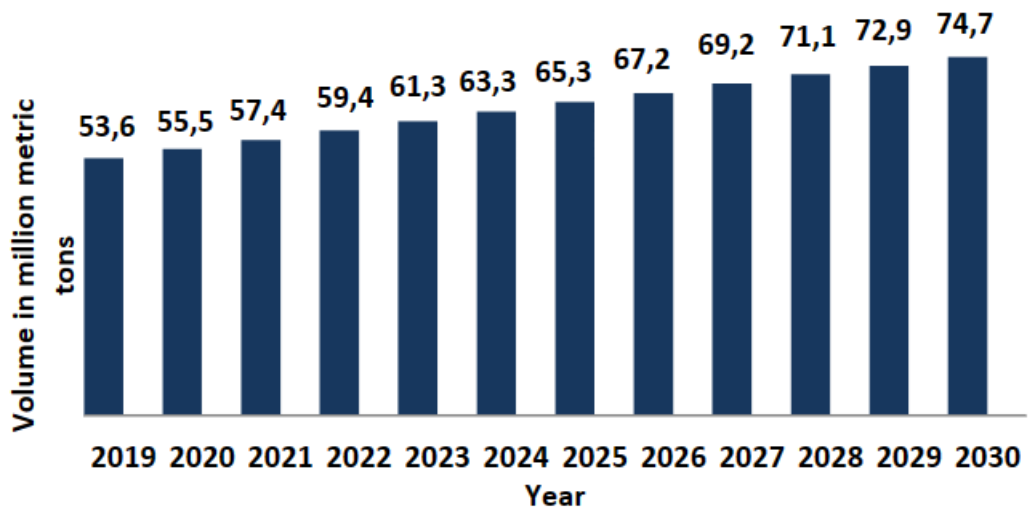
Figure 2 Projected electronic waste generation worldwide from 2019 to 2030 (in million metric tons) (Zamtinah et al., 2021)

1.2 Countries with the most production of E-waste

The current data shows that the largest producers of electronic waste are China, the US, and India. Table 1 lists the top 10 e-waste-producing nations in the world expressed in Kilotons Based on the overall volume produced (Ruiz Arabella, 2023).

According to the most recent statistics available (updated September 2023), the individual recycling rate for each of the top 10 nations (Ruiz Arabella, 2023).

Table 1 Top E-waste producing countries (Ruiz Arabella, 2023)

Rank	Country	E-Waste Produced (Kt)	Recycling Rate
1	China	10129	16%
2	USA	6918	15%
3	India	3230	1%
4	Japan	2569	22%
5	Brazil	2143	0%
6	Russia	1631	6%
7	Indonesia	1618	n/a
8	Germany	1607	52%
9	UK	1598	57%
10	France	1362	56%

1.3 Environmental and socio-economic impacts of E-waste

E-waste that is improperly disposed of in landfills poses a risk to the environment by releasing toxic substances that harm environmental resources like air, water, and soil. Toxic contaminants, including dioxins, are present in the dust particles generated by e-waste, and in soil samples from e-waste, persistent organic pollutants (POPs) such as PBDEs and PAHs are frequently detected (Lin et al., 2022). These toxic substances have been linked to major health effects. Hazardous heavy metals, flame retardants, and other toxic pollutants that

leach into the soil and damage soil biodiversity by biomagnifying and entering the food chain have a significant negative impact on the ecosystem and human health (Dixit et al., 2023).

1.3.1 Toxicity of E-waste

E-waste that is handled and processed improperly, can release toxicants that can enter the human food chain and endanger the ecosystem by contaminating the water, air, and soil (Ankit et al., 2021). Increased production volume, improper disposal, and informal recycling have turned electronic waste into a major global health and environmental concern. Many hazardous materials, including heavy metals and persistent organic pollutants, are present in e-waste and have the potential to pollute the environment if improperly treated or recycled (Alabi et al., 2021). The primary environmental implications of E-waste recycling plants vary depending on the type of processing activity. Non-thermal processes, such as hydrometallurgy, produce a substantial amount of wastewater due to the higher volumes of water used. whereas thermal methods (pyrometallurgy) produce numerous gaseous contaminants such as lead fumes and dioxins (Ackah, 2017).

1.3.2 Toxic effect on soil and soil microbial community

Both controlled and wild ecosystems depend on healthy soil systems to function. However, soil is prone to chemical pollutants. As a result, soil organisms and active processes are susceptible to numerous toxic and persistent contaminants (Nnaji et al., 2023). E-waste contains toxic chemicals and contaminants that harm soil bacteria and deteriorate the soil. It is well-established that hazardous metal and chemical concentrations have an impact on soil microbial communities and related activities (Ankit et al., 2021). Soils are also susceptible to contamination from the absorption of numerous gaseous and particulate air pollutants generated by e-waste recycling (Ackah, 2017). Significant concentrations of polychlorinated biphenyls (PCBs) were found in a soil sample from a Chinese e-waste recycling site (Akram et al., 2019). Fosu-Mensah et al. conducted a study around Korle Lagoon, Accra, Ghana, on soil from vegetable farmland polluted with e-waste burning, which showed that Pb (184.44 mg/kg), Cu (202.99 mg/kg), Cd (103.66 mg/kg), Ni (72.00 mg/kg) and Sn (705.32 mg/kg) concentrations were higher than the WHO/FAO limit for agricultural soils as in **Table 2** (Alabi et al., 2021). A study by (Wu et al., 2015) showed that due to the in situ washing of e-waste with sulphuric acid, severe soil acidification was discovered in the acid-leaching site; because the combustion site is close to the acid-leaching site, the soil was also severely acidified.

In addition to providing nutrients for plants, soil also serves as a sink for surface contaminants and provides natural habitats and energy sources for soil microorganisms. Microorganisms are an integral component of soil and serve as both indicators of soil quality and the effects of contaminants in the soil. Through the breakdown of organic matter and the cycling of nutrients, microorganisms are crucial for maintaining soil fertility and primary production (Ankit et al., 2021). Heavy metals in e-waste can affect microorganisms by changing the nucleic acid structure, causing functional disruption, altering cell membranes, oxidative phosphorylation, and limiting enzyme activity, and all have an impact on the metabolism, morphology, and development of microorganisms (Nnaji et al., 2023). The two main pollutants of concern for soil impacted by e-waste are lead (Pb) and cadmium (Cd) (Lin et al., 2022). According to research done on ashes from open burning of electronic waste at Agbogbloshie and Koforidua in Ghana polluted soil samples. Pb, Cd, and antimony were identified in concentrations greater than 100 times the usual background levels for soils (Alabi et al., 2021). High concentrations of V, Cr, Mn, Co, Cu, Zn, Mo, Ag, Cd, In, Sn, Sb, Pb, TI, and Bi were found in 10 surface soil samples from the backyard of a licensed E-waste recycling plant, and reference sites in Bangalore and Chennai, India (Ackah, 2017).

Table 2 WHO/FAO Soil Heavy Metal Limits (Nweze et al., 2021)

Heavy Metals	Maximum limits allowed in soil (mg/kg)	Maximum allowed limits in the plant (mg/kg)
Arsenic (As)	20	0.02 (FAO)
Chromium (Cr)	100	0.85 (WHO)
Nickel (Ni)	80	0.20 (EU)
Copper (Cu)	30	0.20 (WHO)
Lead (Pb)	100	0.01 (WHO)
Zinc (Zn)	300	5.0 (WHO)
Cadmium (Cd)	3	0.10 (WHO)

1.3.3 E-waste Surface and Groundwater Pollution

In underdeveloped countries like Africa, as well as, to some extent, in developed nations as well, a lack of proper e-waste disposal causes major environmental and health problems (Ankit et al., 2021). Leaching from disposed-of, processed or raw E-waste can introduce pollutants into aquatic systems. Contamination of aquatic systems can also occur from acid disposal after hydrometallurgical recycling of E-waste and dissolution of E-waste airborne particles (Ackah, 2017). A study done by (Wu et al., 2015) showed that Water in a nearby pond was highly acidic and polluted with heavy metals from a former recycling activity. Groundwater is a valuable resource that is becoming more quickly contaminated and polluted. One of the main contributors to this threat is thought to be e-waste (Ankit et al., 2021). Recycling locations for electronic waste, particularly those where waste undergoes combustion, are typically placed close to water sources, such as ponds and streams so that possible water sources for metal extraction operations can be easily accessed (Ackah, 2017). Due to nearby e-waste dumping sites, the quality of the groundwater is significantly impacted (Ankit et al., 2021). In Ghana, the Odaw River's sediments were more heavily contaminated with Ni, Cu, Cd, and Pb close to an e-waste burning site than they were farther away, such as in an area used for vegetable farming or where there was no E-waste recycling activity (Ackah, 2017). The health of children and adults may be at risk as a result of the toxic components in e-waste reacting with groundwater and altering its conductivity, total dissolved solids (TDS), turbidity, pH, and other parameters (Ankit et al., 2021). Heavy metals from E-waste can contaminate soil and eventually reach groundwater. Heavy metals enter groundwater and eventually end up in rivers, ponds, and lakes. This causes acidification and toxification in water, harming animals, plants, and communities (Jain et al., 2023).

1.3.4 Toxic effects on aquatic organisms

Leaching allows the toxins from e-waste to easily infiltrate a water body, which has a variety of effects on aquatic organisms, including growth and reproduction (Ankit et al., 2021). For instance, heavy metals (HM) can affect beneficial organisms like fish and other invertebrates (Zaynab et al., 2022). It is likely a very revealing relationship that establishes a one-to-one relationship between pollution and organisms that the number of fungi is inversely proportionate to the amount of e-waste pollution (Ankit et al., 2021). Fish and other aquatic species have been observed to be exposed to a variety of HMs. Heavy metals are extremely

dangerous environmental contaminants due to their chronic toxicity, non-biodegradability, and environmental bioaccumulation (Zaynab et al., 2022). As a result of biomagnification, it builds up in their tissues (Ankit et al., 2021). Moreover, informal e-waste processing methods such as hydrometallurgical procedures might result in the release of acid into soil and water sources (Alabi et al., 2021). An examination conducted on the level of heavy metal pollution in groundwater and water ponds at a closed Langtang, China, e-waste recycling facility, showed that the water was severely acidified and contaminated due to acid leaching and had mean metal concentrations of Cd 1.59–1.66, Cu 31.1–55.1, Pb 0.10–0.12, chromium (Cr) 1.74–2.28, manganese (Mn) 16.7–17.1, nickel (Ni) 7.31–9.04, and zinc (Zn) 21.0–24.4 mg/L (Lin et al., 2022).

1.3.5 Toxicity effects on plants

Numerous plant species, such as weeds, trees, grasses, and vegetable crops, are known to accumulate a variety of metals (Ackah, 2017). Plants (S. viridis and peanuts) in e-waste recycling locations had total metal concentrations that were twice as high as comparable plants from farms (Ackah, 2017). If plant foods accumulate significant quantities of metals, there could be consequences for public health; also, leaf fall and dissemination could be dangerous (Ackah, 2017). Although the toxicity of e-waste on the plant has not yet been well investigated, its contents, particularly the heavy metals present in the leachate, are known carcinogens including Cd, Mn, Ni, Cr, Zn, and Pd (Ankit et al., 2021). E-waste leachates can impair photosynthetic efficiency, chlorophyll synthesis, and the growth of primary producers like microalga (Pseudokirchneriella subcapitata) by reducing the number of active reaction centres in photosystem II (PSII) (Ankit et al., 2021). Ascorbate peroxidase (APX), chloramphenicol acetyltransferase (CAT), glutathione S-transferases (GST), and superoxide dismutase (SOD) are examples of anti-oxidative enzymes that can be hampered by chemical pollutants like polybrominated diphenyl ethers (PBDEs) and metals (Ankit et al., 2021). An investigation on the e-waste contamination of rice grown in Taizhou, an eastern Chinese town known for its e-waste processing, revealed that the amounts of Cd and Pb in food samples were two to four times higher than the maximum permissible limits (0.2 mg/kg) of these metals in China (Alabi et al., 2021). Leachates from e-waste frequently have high copper (Cu) concentrations, which have been shown to interfere with plant physiological functions. High concentrations of HMs have a negative impact on plants and accumulate inside them through their roots. These metals make it difficult for plants to absorb other crucial nutrients. The metals also replace the vital nutrients at the plant's cation exchange

site, which damages the plant cell's cytoplasmic enzymes and causes oxidative stress. The type of HMs involved in the process also affects how the plant reacts, as most HMs—such as Pb, Cd, Hg, Cr, As, Be, etc.—have no positive impact on plant development while others, like Cu, Fe, and Al, do so to a lesser level (Ankit et al., 2021).

1.3.6 E-waste Contribution to Air Pollution

Informal disposal of E-waste, such as disassembling, shredding, or melting components, releases dust particles and different toxins into the atmosphere, leading to air pollution. E-waste emits fine particles that can be difficult to manage and pose health risks to humans and animals (Jain et al., 2023). The WHO 24-hour PM2.5 ambient air quality criteria, set at 25 µg m^{-3}, were exceeded by the total concentrations of PM2.5 in an E-waste recycling region, compared to higher amounts in a reference location (Ackah, 2017). Heavy metal and flame retardant-containing dust particles are emitted into the atmosphere by e-waste disposal facilities (Rajesh et al., 2022). Higher concentrations of lead (Pb), polybrominated diphenyl ether (PBDE), dibenzofurans (PCDFs), and polychlorinated dibenzodioxins (PCDDs) have been found in the air and dust at e-waste recycling facilities in China (Guiyu) and India (Delhi). PCDD/Fs (64.9-2765 pg/m^3) and PBDD/Fs (14.3 ng/m^3) concentrations were found to be higher in the air in e-waste sites in Guiyu and Taizhou (Khan et al., 2019). Significant concentrations of Cu, Sb, and Pb, as well as trace metal(loid)s like Be, Cr, Mn, Co, As, Ag, Cd, and Ba, were found in the flue gas produced by burning personal computer motherboards and keyboards (Ackah, 2017). Elevated heavy metal concentrations (Cr, Cd, Mn, and Pb) in particles (PM < 2.5 µm) were detected at the Guiyu e-waste site (Khan et al., 2019). High concentrations of bromine (21%), lead (8%), copper (5%), and tin (1%) were found in printed circuit board fly ash in a simulated open burn facility (Ackah, 2017). People who handle this type of waste are particularly at significant risk (Jain et al., 2023).

1.3.7 E-waste toxicity on human health

The primary human exposure pathways to the hazardous components found in e-waste are ingestion, skin contact, and inhalation (Alabi et al., 2021). E-waste exposure can have a variety of negative effects on humans, such as altered thyroid function, altered cellular function and expression, altered psychological behaviour and temperament, and decreased lung function (Ankit et al., 2021). Food chains can act as a biomagnification and transfer pathway for heavy metals, which poses a major risk to human health (Zaynab et al., 2022).

High levels of Pb, Cd, and Cu were found in rice and dust samples near e-waste facilities, raising the risk of neurotoxicity and developmental problems in children through food consumption and dust ingestion (Khan et al., 2019). For instance, lead (Pb) has a negative impact on behaviour and cognitive capacities, copper impairs liver function, and high concentrations of cadmium induce lung cancer and kidney damage. Individuals who work in the production and recycling of e-waste are more vulnerable to negative impacts (Ankit et al., 2021). Due to structural similarities with both polychlorinated biphenyls and thyroid hormones, polybrominated diphenyl ethers (PBDEs), which are found in e-waste, have the potential to be carcinogens and affect thyroid hormones in humans (Tipre et al., 2021). All age groups of humans are impacted by exposure to such harmful chemicals, although the effects are different in children and adults (Ankit et al., 2021). Workers at an e-waste recycling site in Agbogbloshie, Accra, Ghana, had urine concentrations of Fe, Sb, and Pb that were significantly higher than those at reference sites (M. Premalatha Tabassum-Abbasi & Abbasi, 2014). Numerous studies on human biomonitoring state that women and children in developing countries are significantly exposed to a wide range of toxins from recycling facilities during the prenatal period (Ankit et al., 2021). Each metal has a distinct impact on human health as shown in **Table 6** (Khan et al., 2019). Pollutant exposure in children begins with breastfeeding from the mother and continues throughout life (Ankit et al., 2021). A fetus may potentially be exposed while a pregnant woman is carrying it (Ankit et al., 2021). High heavy metal levels in children's blood samples collected from an e-waste site have been linked to human health concerns such as asthma and other respiratory issues, as well as problems with immunoglobulin synthesis. Additionally, around 50 % of the children exposed to e-waste pollution were unable to build immunity to hepatitis (Khan et al., 2019). Metals are persistent, which means they last longer in the environment and do not disintegrate. When they enter the body through various routes, metals, including antimony, arsenic, chromium, cadmium, etc., may have negative consequences on people. Through the food chain, these metals gather in the plants, animals, and eventually people. When these metals come in contact with humans, they can impose various negative consequences (Ankit et al., 2021).

1.4   E-waste as a secondary source of metals

Over the past ten years, the approach to solid waste management has changed from semi-engineered landfill disposal to recovering materials and energy from secondary resources (Işıldar et al., 2018). Aside from all the risks associated with WEEE, it can be a good

secondary source of metals, especially rare and precious metals (Ankit et al., 2021). The protection of primary ores is made possible by using secondary raw materials for resource recovery, greatly decreasing the carbon and ecological footprints (Işıldar et al., 2018). The primary component found in all electronic waste and the primary source of valuable metals are printed circuit boards, or PCBs (Asha B. Sodha Monal B. Shah & Dave, 2019). The mass percentage of metals in e-waste is around 30 %; the remainder is composed of plastics, refractory oxides, and ceramics. In the PCB of e-waste, metal makes up about 30 % (Tipre et al., 2021). Waste PCBs have a higher concentration of metals than naturally occurring minerals, with 10 times higher purity, making them a possible secondary supply of these metals (Ankit et al., 2021). There are 282.31, 112.63, 221.0, 247.13, and 296.62 mg of total metals per gram of respective PCB in desktops, computer parts, desktops without components, laptops, and mobile phones (Işıldar et al., 2018). Metal concentrations in different waste PCBs range from 10 to 26.8 %; for example, Al can range from 0 to 4.78 %; Pb can range from 0 to 4.48 %; Zn can range from 0 to 2.17 %; Ni 0.28 to 3.32%; Fe 1.22 to 7.47 %; Sn can range from 5.28 % to 1.82 %; and precious metals like Au can range from 80 to 1000 ppm, Pt from 0 to 30 ppm, Ag 0 to 3300 ppm, and Pd from 0 to 294 ppm. Total metal contents range from about 21% to about 50% (Tipre et al., 2021).

In addition, WEEE currently is a main target in urban mining of end-of-life (EOL) gadgets due to its high proportion of valuable essential metals. It is a significant second source of metals in the shift to a circular economy (Işıldar et al., 2018). PCBs from various e-waste kinds, such as cell phones, computers, televisions, LX, and tube lights, comprise 411.69, 593.46, 432.89, 230.48, and 399.16 mg of total metals per gram of the corresponding PCB. Cell phone PCBs have the highest concentration of copper (360 mg/g), followed by television PCBs (154.80 mg/g), computer PCBs (37 mg/g), and tube light PCBs (69.75 mg/g) among the e-waste types researched (Tipre et al., 2021). Metal demand is expected to rise in future years (Ankit et al., 2021). Up to 60 elements can be found in modern electronics, which are made of different metal alloys. Many of which are technically recoverable (Işıldar et al., 2018). E-waste is a feasible alternative and more cost-effective source of gold than natural ores since it has a substantially higher gold content of roughly 10–1000 g/tonne than natural ores, which have a gold content of approximately 0.5–13.5 g per tonne (Tipre et al., 2021). The Tokyo 2020 medal project created medals for the Olympics and Paralympics using 78,985 tons of e-waste gathered throughout the nation. From the collected e-waste, about 32 kg of gold, 3500 kg of silver, and 2200 kg of bronze were recovered (Ankit et al.,

2021). Metal recovery from printed circuit boards (PCBs) is currently the primary utilization of e-waste (Tipre et al., 2021).

2 LEGISLATION AND REGULATION REGARDING E-WASTE IN DEVELOPED AND DEVELOPING COUNTRIES

International regulations and laws regarding e-waste are essential as they establish guidelines and directives that govern the conduct of government and private sector actors involved in e-waste, as seen in **Table 3** (Murthy & Ramakrishna, 2022). Apart from various remediation techniques, e-waste can also be resolved by legislative measures. Appropriate policies and laws can be a crucial component of the national strategy for legally and consistently tackling the issue. It is estimated that around two-thirds of the world's population is covered by e-waste laws (Ankit et al., 2021). An unofficial recycling sector is growing as a result of inadequate laws in developing countries and the rising cost of components recovered from abandoned e-waste items. A framework for e-waste legislation is needed to reduce the negative effects of e-waste pollution on the environment (Pathak & Sushil, 2022).

Throughout the past 10 years, many countries have established laws regarding the transportation and processing of electronic waste, including extended producer responsibility (EPR) and transboundary movement (TBM) restrictions (Pathak & Sushil, 2022). EPR regulations mandate that a product's manufacturer assumes full life cycle responsibilities, including collection, disassembly, and reuse at the end of the product's useful life (Moossa et al., 2023).

2.1 The Basel Convention

Illegal shipments of hazardous trash and ashes from developed countries were made to developing nations in Asia and Africa. At the Basel Convention, which took place in Basel, Switzerland, in 1989, 186 nations came together to solve this problem by signing the convention named "Transboundary Movements of Hazardous Wastes and Their Disposal."(Patil & Ramakrishna, 2020). The Basel Convention aims to protect the environment and human health from the harmful effects of managing, producing, transporting across national borders, and disposing of hazardous and other waste. E-waste issues were first addressed by the Basel Convention in 2002 (Moossa et al., 2023).

The EU was the first to implement these laws in its member states, however, the United States has not yet ratified the agreement (Murthy & Ramakrishna, 2022). Despite a decrease in cross-border hazardous waste movement following the Basel Ban, the treaty endeavour has not been able to eradicate it totally. There is still an illegal transfer of electronic waste from developed to developing nations (Patil & Ramakrishna, 2020). The majority of

countries around the world have ratified the Basel Convention, which limits the transboundary movement of WEEE (Işıldar et al., 2018).

2.2 Bamako Convention

An African convention known as the Bamako Convention was ratified in 1991 and went into effect in 1998. Similar to the Basel Convention, it imposes restrictions on the entry and transit of hazardous waste into and through Africa. In order to prevent toxic waste from crossing international borders and reaching African nations, the Bamako Convention is aimed at enhancing the Basel Convention (Shittu et al., 2021).

2.3 Stockholm convention on persistent organic pollutants 2001

A global agreement known as the Stockholm Convention seeks to protect human health and the environment from persistent organic pollutants (POPs), which are persistent substances that linger in the environment for extended periods of time. It also takes into account compounds that are environmentally harmful and spread geographically, as well as those that accumulate in the fatty tissues of humans and other animals. As may be observed, a lot of persistent organic pollutants are present in e-waste, and parties to the Stockholm Convention are obliged to take the appropriate steps to prevent the release of these pollutants from waste and stockpiles (Moossa et al., 2023). Although the Stockholm Convention has been successful in identifying new POPs, low- and middle-income Parties, in particular, continue to find it difficult to implement the convention at the national level. To help Parties carry out their responsibilities under the Convention, coordinated effort is required (Wang et al., 2022).

2.4 European legislation on e-waste

The cornerstone of the EU's waste strategy is still the waste hierarchy and achieving sustainable waste management and transitioning to a circular economy require advancing up the waste hierarchy (Cole et al., 2019). In Europe, the Waste Electrical and Electronic Equipment (WEEE) directive governs the management of electronic waste (Andersen, 2022). The WEEE Directive (2012/19/EU) was passed by the EU Commission in 2012 with the aim of establishing uniform regulations for the handling of e-waste across its member

states. established stricter guidelines for collecting aims than the previous directive (2002/96/EC), Along with the "Restriction of Hazardous Substances" (RoHS) regulation (2002/95/EC), which complements the regulations by making sure that any newly released EEE is free of mercury [Hg], lead [Pb], cadmium [Cd], polybrominated biphenyls [PBB], hexavalent chromium [Cr^{6+}], and polybrominated diphenyl ethers [PBDE] and on avoiding the landfilling and incineration of hazardous waste. Furthermore, the EU WEEE directive established the so-called "take-back system," which gives producers responsibility for collecting WEEE (Işıldar et al., 2018). According to the WEEE Directive, member states must promote the design and manufacture of EEE that is recyclable and that can be disassembled (Patil & Ramakrishna, 2020).

There are two main concepts found in the laws governing e-waste management in the European Union (EU). One is that producers must assume accountability for the end-of-life (EoL) phase of the products they manufacture; this is referred to as extended producer responsibility (EPR). The alternative, referred to as the polluter pays principle (PPP), stipulates that the person who generates the waste must cover the expense of disposing of it in an appropriate manner (Andersen, 2022). The goal of the EU waste directive is to prevent waste. Reuse preparation is the most popular waste management strategy, followed by recycling and recovery. For an EoL product, landfill disposal is the least desirable alternative (Andersen, 2022).

2.5 E-waste legislation in Asian countries

Most Asian nations have been struggling with the illegal import and processing of e-waste for many years. Asia has used the regulations from the European Union as a blueprint to create their own E-waste regulatory systems (Murthy & Ramakrishna, 2022).

India produces relatively low waste per capita because of its enormous population, but because informal recycling methods are accessible and affordable, a significant volume of e-waste is imported from developed countries (Widanapathirana et al., 2023). The "Hazardous Material Laws and Rules," a pertinent legislation, attempts to address India's significant WEEE problem. In India, where there is no distinct collection infrastructure, WEEE is classified as municipal waste (Işıldar et al., 2018). The "E-waste (Management & Handling) Rules, 2011" came before Hazardous Waste (Management and Handling) regulations from 2008 were used in India to manage its e-waste. India's first "E-waste (Management and Handling), Rule 2011", was passed in 2011, including the extended

producer responsibility (EPR) concept. Due to the drawbacks and difficulties associated with this rule, it was eventually repealed and replaced by the E-waste (Management) Rules, 2016, which expanded the producer's responsibilities to include dealers, re-retailers, manufacturers, consumers, bulk consumers, producers, and recyclers that were not covered by the E-waste Rule of 2011 (CPCB, 2011) (Ankit et al., 2021).

China is one of the world's top producers of e-waste, with an estimated 28.4 million tons of e-waste produced by 2030 (Borthakur, 2020). Regulations on the Administration of Recycling and Treatment of Waste Electrical and Electronic Equipment, a legislative regulation from China, went into effect in January 2011 (Işıldar et al., 2018). As of right now, China has two major e-waste rules in place: one addresses the management, recycling, and disposal of e-waste, while the other addresses the control of pollution produced by e-waste. Formal e-waste management in China is overseen by government agencies, and e-waste regulations in China are founded on the principles of PP, EPR, and the 3Rs (reduce, reuse, and recycle). Since China lacks effective legislation, e-waste recovery and reuse are frequently done informally (Andeobu et al., 2023).

2.6 American legislation on e-waste

The Americas' top producers of e-waste are the USA and Canada, with Mexico and Brazil following (Patil & Ramakrishna, 2020). E-waste laws have been passed in a few states, but there is an inconsistency. A few states have landfill bans and restrictions on producer take-back. Because of this, the bulk of e-waste is being shipped to countries like Mexico, China, and Africa, where it is illegally handled and pollutes the environment (Murthy & Ramakrishna, 2022). As of 2015, a patchwork of inconsistent laws governed the WEEE in around half of the US's federal states (Işıldar et al., 2018). To improve the collection and recycling of end-of-life electronics, as well as to simplify and standardize e-waste management in the United States, comprehensive electronic waste (e-waste) legislation is required (Schumacher & Agbemabiese, 2019).

Canada shares the same shortcoming in national e-waste management legislation as the USA. Nevertheless, local e-waste laws are in place in most Canadian states. In Latin America, only a few countries have enacted E-waste legislation since regulatory changes take time to take effect. Apart from Mexico, Costa Rica, Colombia, and Peru, the main actors in the region for environmentally responsible e-waste treatment are now working to enhance the current setups. The establishment of a clear legal framework for e-waste in 2020 was made possible by the efforts of Brazil and Chile (Murthy & Ramakrishna, 2022).

2.7 African legislation on e-waste

The majority of African countries are underdeveloped economically, and a large amount of electronic waste is imported directly from developed countries, such as the United States, Europe, and China. The lack of effective legislation for e-waste recycling causes a large volume of e-waste from various parts of the world to be illegally transported to numerous African countries, where they are recycled using inadequate informal methods. However, in recent times, the majority of African nations have been concerned about the risks associated with improper handling of electronic waste (Patil & Ramakrishna, 2020). E-waste regulations have only been passed by a few African countries, including Ghana, Egypt, Madagascar, Rwanda, Nigeria, Cameroon, Côte d'Ivoire, and South Africa. But putting the law into practice is complicated. To create a long-term E-waste management system in East Africa, Kenya, Tanzania, Burundi, South Sudan, Uganda, and Rwanda have joined forces to construct a regional E-waste plan. However, for an efficient E-waste management system, most African countries still lack the necessary E-waste legislation (Murthy & Ramakrishna, 2022).

As stated above, a significant amount of the world's electronic waste ends up in open disposal sites throughout many African nations. In 2019, it was estimated that the overall amount of e-waste in Africa (both imported and locally produced) was between 5.8 and 3.4 metric tons (Mt). It is thought that this is underestimated because there are significant gaps in the data that prevent more accurate assessments. Nigeria, Ghana, and Tanzania are the top three nations in Africa that get e-waste; other countries of concern are Senegal, Egypt, and Kenya (Maes & Preston-Whyte, 2022). For instance, about 215,000 tons of the approximately 10 million tons of commodities that Ghana imported in 2015 through the Tema port were used electronics, which included smartphones, televisions, and domestic appliances like ovens, washers, dryers, and refrigerators (Canavati et al., 2022).

According to assessments by the United Nations and the European Union, developed countries have turned to West African nations, in particular, as e-waste dumping sites. As of 2019, there were just 13 African nations with e-waste legislation, policies, or regulations. Egypt has no national e-waste policy or regulation despite being the country in Africa that produces the most e-waste. Insufficient enforcement and a lack of regulations have led to a growth in the importation and illegal operations of recycling e-waste, despite the fact that several African countries have signed international treaties pertaining to waste

transportation. Furthermore, a number of structural, administrative, and procedural shortcomings exist in the current legal frameworks (Cheshmeh et al., 2023).

In Ethiopia, more than 4300 tons of e-waste are stored in Ethiopian retailers, according to a 2011 assessment by PAN-Ethiopia and the Oko Institute. The nation's massive import of used electronics, low-quality equipment donated by other countries, and the import of rapidly obsoleting and planned devices all contribute to the country's e-waste generation. Additionally, data indicates that a sizable amount of antiquated electronics and electrical equipment is smuggled into the nation from Kenya (via Borena), Djibouti (via the Afar area), and Somalia (via Jigjiga). In the end, this is causing a spread and build-up of damaged, malfunctioning, and defective gadgets throughout cities and villages (Ali & Akalu, 2022). The Basel Convention and the Bamako Convention are the two agreements governing e-waste in Africa. In an effort to reduce and regulate the movement of e-waste both within and between African governments, a convention comprising these nations prohibits the importation of any hazardous waste. Theoretically, this guarantees that electronics are disposed of in line with green and clean environmental principles (Maes & Preston-Whyte, 2022).

Table 3 E-waste legislation and policy of selected countries (Akon-Yamga et al., 2021; Bimpong et al., 2023; Cheshmeh et al., 2023; Pathak & Sushil, 2022)

Country	Policy year	Policy title	Objective
The European Union (EU)	1993 (amended in 2007) (Jupille 1999; Pongrácz and Pohjola 2004)	Waste Shipment Regulation (WSR)	Emphasizes that no EU member state is allowed to export e-waste classified as hazardous to non-OECD countries.
	2002 (revised in 2006 and 2009) (European Parliament 2003; Cusack and Perrett 2006)	Restriction of Hazardous Substances (RoHS) Directive (2002/95/EC)	Restriction of the use of certain hazardous substances, changing product designs, and increasing recycling rates of WEEE.
	2006 (European Parliament 2006)	The Battery Directive	Regulates the manufacture, disposal, and trade of batteries in the EU.
	2012 (Sthiannopkao and Wong 2013; Koh et al. 2012; European Parliament 2012)	WEEE Directive (2012/19/EU)	To regulate the collection, recycling, and recovery of e-waste in the member nations from disposal to reuse.
The United Kingdom (UK)	2003 (The National Archives 2003), 2007 (The National Archives 2007)	E-waste Packaging Directive: (i) The Packaging (Essential Requirements) Regulations 2003 and (ii) The Producer Responsibility Obligations (Packaging Waste) Regulations 2007	(i) Minimized packaging requirement and restriction of dangerous substances (such as heavy metals). (ii) Obligates reduction in packaging for all UK companies with > 2 million GBP turnover or with a handling capacity of > 50 tons of packaging each year.
China	2000 (Chung and Zhang 2011; Hicks et al. 2005)	Ban on WEEE for managing waste import	Prohibits the import of second-hand electronic equipment and e-waste
	2006 (Chung and Zhang 2011)	The Law on the Prevention and Control of Environmental Pollution by Solid Waste (2004), Technical Policy on Control of WEEE	Aims to reduce the volume of e-waste, increase the reutilization rate for discarded EEE, and increase standards for e-waste recycling
	2007 (Chung and Zhang 2011)	The Cleaner Production Law (2002), The Ordinance on Management of Prevention and Control of Pollution from Electronic and Information Products	Reduction of use of hazardous and toxic substances in electronic appliances, reducing the pollution generated in the manufacturing, recycling and disposal of these products
	2008 (Chung and Zhang 2011)	The Circular Economy Promotion law (2008), Administrative Rules on Prevention of pollution by WEEE	To prevent pollution caused by the storage, transport, disassembly, recycling, and disposal of e-waste

Country	Year	Regulation	Description
China	2011 (Chung and Zhang 2011)	The Circular Economy Promotion law (2008), Collection and Treatment Decree on Waste Electrical and Electronic Equipment	Stipulates that e-waste should be collected through multiple channels and recycled by licensed recycling enterprises
	2012 (Chung and Zhang 2011)	Extended Producer Responsibility (EPR) system	Requires manufacturers to carry out environmentally safe management of their products after they are discarded
India	1986 (Ind 1986)	The Environmental Protection Act	Emphasizes prevention, control, and abatement of environmental pollution
	2000 (Ind 2000)	The Ozone Depleting Substances (Regulation and Control) Rules	Regulates the export and import of EEE-containing substances that can destroy the ozone layer
	2008 (Ind 2008)	The Hazardous Wastes Management, Handling and Transboundary Movement Rules	Requires companies/individuals receiving, treating, transporting, or storing hazardous waste to seek permission from the relevant State Pollution Control Board (SPCB) and ban the import of hazardous waste for disposal or dumping of e-waste
	2011 (Ind 2011; Bhaskar and Turaga 2018; Mehta 2018)	The E-Waste Management and Handling Rules	Regulates the e-waste management at every level of EEE life span from producers to recyclers
Nigeria	2011	National Environmental (Electrical/Electronic Sector) Regulations, Guide for importers of UEEE into Nigeria	Import of WEEE banned with a compulsory registration of imports
Ghana	2016	Hazardous and Electronic Waste Control and Management Act 917	Provides for the control, management, and disposal of hazardous waste, electrical and electronic waste, and related purposes.
		The Legislative Instrument on Hazardous and Electronic Waste Control and Management L.I. 2250	The regulation of the classification, control and management of waste or the requirements for the disposal of waste.
Ethiopia	2018	Electrical and Electronic Waste Management and Disposal Council of Ministers Regulation No. 425 /2018.	Reuse, refurbish and recycling of electrical and electronic waste; any electrical and electronic equipment producer, wholesaler, retailer, or importer is a producer's responsibility. Management responsibility and disposal are also recognized by the consumer. To collect, dismantle, refurbish, or reuse electrical and electronic waste, a certificate of competence is to be issued by the Minister.

1 BIOLEACHING

Due to the decline in ore grade, meeting the world's growing demand for metals has become difficult. Over the past ten years, end-of-life metal-bearing materials, also known as metal-bearing wastes, have gained widespread acceptance as a secondary source of essential raw materials since their metal concentration is similar to that of natural ores. In contrast to ores and concentrates, electronic wastes, for instance, have up to 26 times more Cu and 50 times more Au content (Tezyapar Kara et al., 2023). Bioleaching is a hopeful advance in technology that utilizes naturally occurring microorganisms and their metabolic products in extracting valuable and rare metals from waste, such as electronic waste and spent catalysts. The variety of waste that can be treated by bioleaching is a strong indicator of how simple it is to implement the process into practice. The growing interest in the utilization of the biohydrometallurgical pathway for reprocessing wastes is a result of the method's ability to significantly reduce operational costs and energy requirements while also being environmentally friendly (Hong & Valix, 2014b). Conventional methods of extracting metals from solid waste through chemical, pyrometallurgical, or hydrometallurgical processes can result in secondary contamination. For example, the acid generated during these processes may cause acidification of soil and water, and the combustion of these processes can release harmful gases into the environment (Zhang et al., 2018). Bioleaching is influenced by various process parameters, including pH, temperature, nutritional composition, concentration of carbon dioxide and oxygen, and mineral substrate. As a low-cost method of treating complicated and low-grade ores, bioleaching stabilizes sulphate toxicities, making it an environmentally acceptable procedure (Dixit et al., 2023). Bioleaching mechanisms that can be utilized to dissolve metals from waste can be divided into two, which are classified depending on their contact status as direct and indirect mechanisms (Tezyapar Kara et al., 2023).

- Direct Bioleaching: Microbes physically interact with the source of the metals in this process, using readily available minerals to oxidize the metals and enzymatic strike to remove them from the source (Dixit et al., 2023).

- Indirect Bioleaching: In this technique, microorganisms produce the leaching agent that is applied to the metal source in order to recover the metals without being physically attached to it. High demand exists for bioleaching in the commercial recovery of metals. There are three methods employed in the industry: slope, heap, and in-situ bioleaching (Dixit et al., 2023).
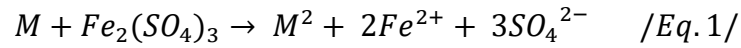
Members of the *Acidithiobacillus* (*Thiobacillus*) genus, namely *Acidithiobacillus ferrooxidans* and *A. thiooxidans*, are the bacterial microorganisms closely associated with bioleaching processes. These sulphur-oxidizing bacteria that are acidophilic generate sulphuric acid as a metabolic byproduct, which helps in the solubilization of metals found in many residue types, including sludges, solid residues, soils, and sediments (Rivas-Castillo et al., 2022). Due to their capacity to promote metal dissolution through a series of bio-oxidation and bioleaching reactions, chemolithoautotrophic bacteria, such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, have been the most widely considered group of microorganisms in terms of bioleaching applications. Other organisms have also been used to successfully dissolve different metallic fractions from e-wastes, such as the thermophiles *Sulfobacillus thermosulfidooxidans*, *Bacillus stearothermophilus*, and *Metallosphera sedula*, as well as the heterotrophic fungi *Aspergillus niger*, *Penicillium Simplicissimum*, and *Cyanobacterium violaceum* (Hong & Valix, 2014).

Acidithiobacillus aerobes are gram-negative, chemoautotrophic, acidophilic microorganisms that are well-known for their resistance to heavy metals and acidity (Chen et al., 2022). Such aerobes like *A. ferrooxidans* are able to survive in harsh conditions like coal mines, metal mines, and sewage treatment plant sludge primarily because they can withstand organic chemicals and metal ions up to a specific concentration (Zhang et al., 2018). *A. ferrooxidans*, *A. ferridurans*, *A. ferrivorans*, *A. ferrianus*, *A. ferriphilus*, *A. albertensis*, *A. caldus*, *A. thiooxidans*, *A. sulphuriphilus*, and *A. cuprithermicus* are by far the ten species that have been documented (Chen et al., 2022).

First identified as *Thiobacillus ferrooxidans* (T. ferrooxidans), *Acidithiobacillus ferrooxidans (A. ferrooxidans)* was initially isolated from acid mine drainage by Colmer and Hinkle in 1947 (Zhang et al., 2018) SEM image of *A. ferrooxidans* is shown in Figure 3. Belonging to the acidophilus chemoautotroph strain, the short rod bacterium is obligate aerobic, with a suitable growth pH value of 2.0-2.5 and a suitable growth temperature of 28-35°. It was widely used in the fields of bioleaching sulphide ore, coal biological desulphurization, and treating environmental pollution because it can obtain energy through the oxidation of Fe^{2+}, sulphur, and metal sulphides. It can also take oxygen as the final electron acceptor to create a strong acid environment (Yuan et al., 2016).

A. ferrooxidans uses the indirect cyclic mechanism outlined in Eqs. (1) and (2) to oxidize the elemental metals in E-waste to metal ions during the bioleaching processes of basic

metals. The oxidizing agents that liberate metals (M: Cu, Ni, Fe, Zn, etc.) from E-waste materials are ferric ions, according to the mechanism described (Arshadi et al., 2019).

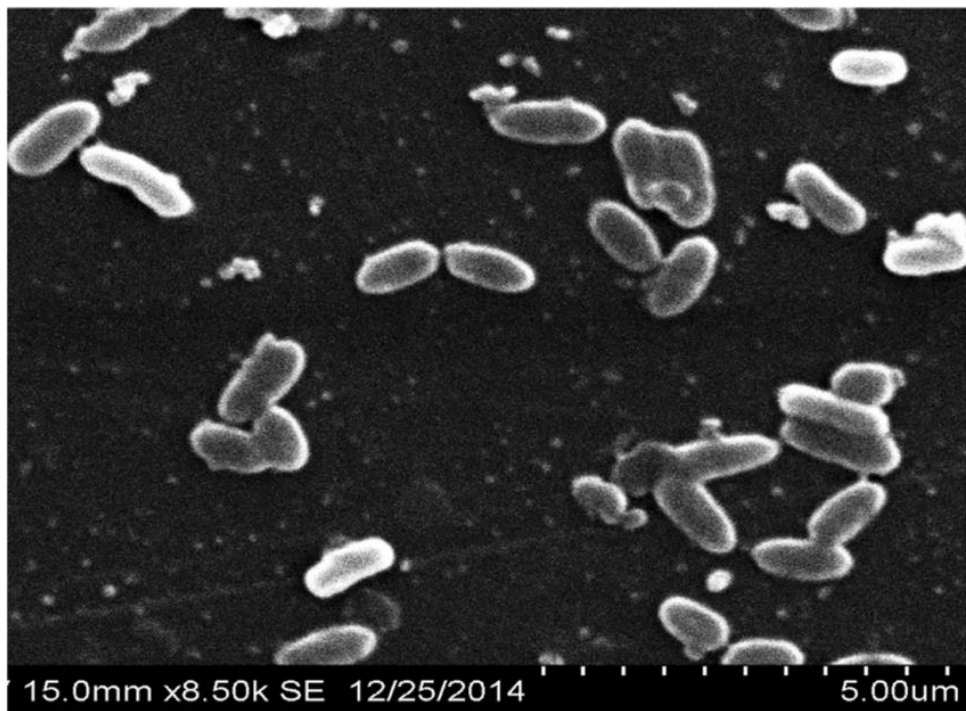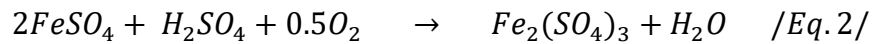$$M + Fe_2(SO_4)_3 \rightarrow M^2 + 2Fe^{2+} + 3SO_4{}^{2-} \quad /Eq.\,1/$$

$$2FeSO_4 + H_2SO_4 + 0.5O_2 \quad \overset{Bacteria}{\rightarrow} \quad Fe_2(SO_4)_3 + H_2O \quad /Eq.\,2/$$

Figure 3 SEM of *A. ferrooxidans* (Gao et al., 2018)

1.1 Bioleaching of electronic waste

Acidithiobacillus members are well-known and extensively researched organisms for metal bioleaching because of their exceptional resistance to heavy metal toxicity and low nutritional needs for metal solubilization (Adetunji et al., 2023). The ability of a pure culture of *Acidithiobacillus ferrooxidans* to mobilize metals from waste PCBs acquired from Pars Charkhesh Asia, a recycling centre in Tehran, Iran, was assessed by Arshadi and Yaghmaei. At their highest efficiency, copper (90 %) and nickel (88 %) recovered. An optimal condition with FeSO$_4$·7H$_2$O (60 g/L), an initial pH of 0.5, and a 6-day incubation period, Copper (76%), nickel (74%), and zinc (72%), obtained by bioleaching waste PCB sludge with *Acidithiobacillus ferrooxidans* (Adetunji et al., 2023). **Table 4** shows a study summary on pure culture e-waste leaching efficiency.

Table 4 Pure culture chemolithotrophic bacteria efficiency on different e-waste
(Adetunji et al., 2023).

Bacteria	Metal Source	Recovery Efficiency (%)	Bioleaching Conditions
Acidithiobacillus ferrooxidans	PCB	Cu (80 %)	pH 2.0; temperature 30 °C; incubation time 2d
Acidithiobacillus thiooxidans	Spent mobile phone PCB	Cu (98 %); Ni (82 %)	160 rpm; 30 °C; 72 h
Acidithiobacillus ferrooxidans	OLED touch screens of mobile phone	In (100 %); Sr (5 %)	Initial pH 1.1; 29 °C; 140 rpm; 15 d
Thiobacillus ferrooxidans	Nickel-cadmium batteries	Cd (100%); Ni (96.5 %); Fe (95 %)	pH 2.0; temperature 30 °C; incubation time 93 d; pulp density 0.2 g/L
Acidithiobacillus ferrooxidans	Nickel-cadmium batteries	Cd (100 %)	pH 1.5; temperature 30 °C; incubation time 28 d
Acidithiobacillus ferrooxidans	Mobile phone PCB	Cu (99 %); Ni (99 %)	170 rpm; temperature 30 °C; initial pH 1.0; pulp density 9.25 g/L; Fe^{3+} concentration 4.17 g/L; incubation time 55 d
Acidithiobacillus ferrooxidans	Nickel ion batteries	Co (65%)	pH 2.5; temperature 30 °C; incubation time 20 d; pulp density 5 g/L
Acidithiobacillus thiooxidans	Computer PCBs	Cu (75 %)	Pulp density 0.7% 9 d
Acidithiobacillus ferrooxidans	Nickel ion batteries	Co (99.9 %)	pH 3.0; temperature 35 °C; incubation time 6 d
Acidiphilium acidophilum	Computer PCBs	100 % (Cu)	Incubation time 10 d; H_2O_2 concentration 30%
Acidithiobacillus ferrooxidans	Mobile phone PCB	95 %-100 % (Cu)	Incubation temperature 30 °C; 130 rpm; pulp density 7.5 g/L; 48 h
Acidithiobacillus ferrooxidans	Computer PCBs	92% (Cu)	pH 1.8; pulp density 35 g/L; 30 °C; 170 rpm

The oxidation of Fe^{2+} ions by iron-oxidizing bacteria produces biogenic Fe^{3+}, a powerful oxidizing agent for metal leaching, which provides energy. Therefore, the rate at which

metals leach and the metabolism of microorganisms can both be impacted by the concentration of Fe^{2+} in the medium (Ji et al., 2022).

Crust et al. examined the impact of an initial Fe^{2+} concentration of 1-3 g/L on the dissolution of Cu, Al, Ni, and Zn. Concentrations of 7 to 9 g/L at first were found to increase by 20 % for Cu and 10 % for other metals. Operating at 9 g/L starting Fe^{2+}, the highest ORP values were achieved. Gu et al. investigated how the concentration of Fe^{2+} changed when *A. ferrooxidans* carried out their bioleaching process. 8.09 g/L was the starting concentration of Fe^{2+}, and it clearly decreased over the subsequent bioleaching phase. Numerous studies show that the group's bioleaching rate improves with decreasing Fe^{2+} concentration in the final medium (Ji et al., 2022).

The pH of the system plays a crucial role in acidophile bioleaching. The leaching medium's SOB contributes to the system's acidity, assisting in maintaining the pH of the system. Sulphur-oxidizing bacteria oxidize sulphur to accomplish these criteria (Ji et al., 2022). Crust and colleagues investigated how pH affected the solubility of metals. While the leaching of Al and Zn exhibited similar dissolving efficiency at pH 1.8 and 2.0, the leaching of Cu and Ni was shown to be most effective at an initial pH of 1.8. Unfavourable to the

leaching, jarosite and/or secondary passivation products were found to develop and precipitate at elevated pH values (>2 or 2.25). Gu et al.'s study looked at pH variation during bioleaching. After rising swiftly for five days and then gradually for seven days, the pH eventually reached a stable level. *A. ferrooxidans* metabolism involved the consumption of H^+, and the process by which zero-valent metal was converted into metal ions also involved the consumption of H^+ (Ji et al., 2022).
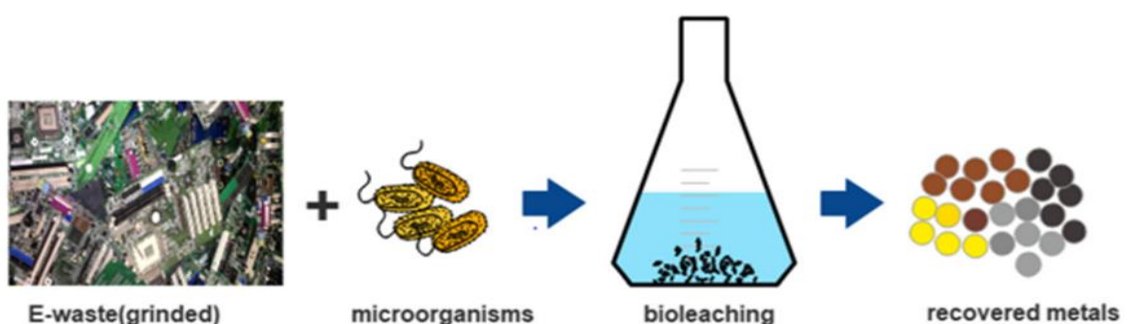

Figure 4 bioleaching of e-waste illustration (Abdelbasir & Kamel, 2018)

1.1.1 Waste printed circuit board

A Significant amount of waste from electrical and electronic equipment (WEEE) consists of waste from information technology (IT) and telecommunication equipment (WITTE), commonly referred to as Waste Printed Circuit Boards (WPCBs). Key components of PCBs, as depicted in **Figure 5** (Abbadi et al., 2024), include:

- The base: a thin, rigid, or flexible insulating board that accommodates conductors and components. Typical thickness varies from 0.2 to 3.2 mm, with prevalent applications falling within the range of 0.8–1.6 mm.

- Conductors: strips of high-purity copper adhered to the base.

- Components: various elements and materials affixed to the substrate, including chips (Ga, In, Ti, Si, Ge, As, Sb, Se, Te), connectors (Au, Ag), and capacitors (Ta, Al), among others.

Although WPCBs constitute only 5–10 % of the WITTE weight, they contribute significantly to the recovered value, accounting for up to 80% (Vermesan et al., 2019). Some PCB parts and their respective metal content are presented in **Table 5**. WPCBs typically consist of 40 % metals, 30 % ceramics, and 30 % polymers (Abbadi et al., 2024).

A study by Bai et al. employed *A. ferrooxidans* to extract heavy metals from WPCB sludge (Ji et al., 2022). The leaching rates of 76 %, 74 %, and 72 % were achieved for Cu, Ni, and Zn, respectively, under optimized conditions, including a $FeSO_4 \cdot 7H_2O$ concentration of 60 g/L, initial pH of 0.5, and a reaction period of 6 days.
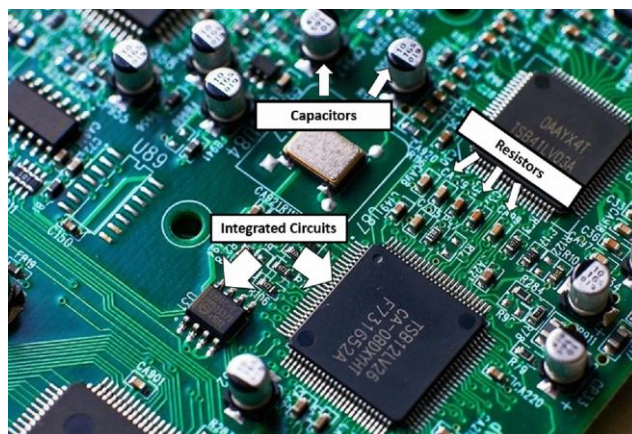

Figure 5 Typical PCB surface components (Abbadi et al., 2024)

Dalrymple (2007) reported that shrinking WPCBs could lower their recycling costs. Larger waste PCB processing, on the other hand, would remove the need for dust, making WPCB

manufacturing more environmentally friendly. WPCBs ground to 1 mm or less constitute the basis for the majority of conventional leaching techniques (Kumar et al., 2014).

Table 5 Printed circuit boards (PCBs) metal content in g/kg (Vermesan et al., 2019)

Electronic Parts of PCB	Weight/Piece (g)	Ag (g/Kg)	Au (g/Kg)	Pd (g/Kg)	Pt (g/Kg)	Cu (g/Kg)	Al (g/Kg)
Aluminum capacitor	5.551	0	0.001	1E-04	0.001	7.4	670
Tantalum capacitor	0.27	0	0.14	0	0	0.22	1.3
Ceramic capacitor	0.218	0.06	0.036	0	0	510	0.25
Smallest capacitor	0.006	100	0.008	35	5	2.4	1.3
Electronic resistance	1.625	57.6	3.601	2.509	0	380.4	34.94
Plastic IC chip	9.913	12.2	15,398	0.083	0.264	901	4.879
Central processing units (without metal film)	6.02	0.055	0.517	0.075	0	500	0
Integrated Graphics Processor (IGP)	4.13	0.032	0.501	0.041	0	171	0
Integrated Drive Electronics Connector	0.681	0.016	0.37	0.007	0	770	8.8
Small Computer System Interface Connector	0.882	0.002	0.7	0	0.052	0.21	110

2 METAL TOXICITY

As a result of growing urbanization and industrialization over the past few decades, the amount of heavy metals in our natural environment has increased significantly, creating significant concerns around the world (Naveed et al., 2023). Heavy metals are described as metals with a high atomic number (more than 20) and a high density (more than 5 g/cm3) (Moukadiri et al., 2023) and extreme toxicity even at extremely low concentrations, examples include iron (Fe), cobalt (Co), nickel (Ni), manganese (Mn), copper (Cu), zinc (Zn), chromium (Cr), lead (Pb), and so on (Naveed et al., 2023). Numerous man-made and natural activities release these metals into the environment. Volcanoes, weathering, erosion, and rock creation are natural pathways, while some of the anthropogenic activities are textile and paint industries, mining, smelting, pesticide and fertilizer discharges, biosolids, municipal sewage/sludge, and electroplating (Mishra et al., 2019). However, the vast majority of heavy metals come from man-made sources. Heavy metals can accumulate in the soil, groundwater, and in the atmosphere. Additionally, they have the ability to react with other elements in the environment and potentially grow more toxic (Moukadiri et al., 2023). The toxicity of heavy metals varies based on a number of variables, including exposure duration, metal species reactivity, metal concentration, and exposed individuals' health status. The three main pathways that heavy metals enter the human body are through food, water, and air (Arora & Chauhan, 2021). Some heavy metals have acute toxicity, whereas prolonged exposure to others can result in chronic toxicity (El-Degwy et al., 2022). While certain metals are necessary for biological processes, including hormone production and appropriate cell and enzyme function, humans only require these metals in trace levels (Naveed et al., 2023). Even in low concentrations, heavy metals have detrimental effects on humans, as seen in **Table 6**, because they can lead to a variety of negative conditions such as abnormal fetal development, infertility, immunodeficiency, cancer, organ dysfunction, physical, mental, and neurological disorders, nephritis, osteoporosis, elevated blood pressure linked to cardiovascular diseases, and, in rare cases, even death (El-Degwy et al., 2022). Heavy metal toxicity has been linked to numerous epidemics throughout history, including the Minamata outbreak in Japan in the 1950s, the Iraqi methylmercury (Me–Hg) epidemic, and the methyl- and ethylmercury outbreaks in Pakistan and Guatemala (Moukadiri et al., 2023).

Depending on the physio-chemical characteristics of the soil, heavy metals are bonded with multiple soil constituents and exist in a variety of chemical forms (Moukadiri et al., 2023).

Soil contamination by heavy metals leads to a decline in soil fertility, a significant decrease in agricultural productivity, and a fall in microbial diversity and activity (Arora & Chauhan, 2021), and this change in the soil's physiochemical characteristics can advance to contaminate the food chain (Naveed et al., 2023).

Heavy metals can accumulate in the tissues of aquatic organisms such as fish, crabs, and molluscs, and these pollutants have a tendency to magnify through the food chain. For example, fish may accumulate metals in their tissues by ingesting contaminated sediments and prey, as well as direct absorption through their gills (Chan et al., 2021). In addition to impairing fish reproduction, this results in behavioural, physiological, and morphological problems. As a result, consuming fish polluted with heavy metals increases the risk to human health (El-Degwy et al., 2022). These toxic metals interact with an organism's cellular structure to cause disruptions to its biological processes (Naveed et al., 2023). Heavy metal contamination is a serious problem with far-reaching and long-term repercussions for human health and the environment. As a result, effective heavy metal pollution removal and remediation are vital for protecting human and environmental protection (Nnaji et al., 2023).

Table 6 Toxicity of e-waste metals (Ramesh et al., 2023)

Source of e-wastes	Constituent	Health Effects
Soldering circuit boards, glass plates	Lead (Pb)	Harm to kidney and central nervous system. Affects the development of the child's brain.
Chip resistors semiconductors	Cadmium (Cd)	Affects liver and kidney. Causes nerve damage
Switches, relays & PCB's	Mercury (Hg)	Affects brain. Skin disorders and respiratory issues
Anti-corrosion of hardeners on unprocessed and galvanized steel, decorators, or steel housings.	Hexavalent Chromium (Cr) VI	Asthmatic bronchitis Harm of DNA

Source of e-wastes	Constituent	Health Effects
Wiring, computer case and motherboard	PVC and Beryllium (Be)	Lung Cancer Damage to the immune system. Causes skin diseases
Panels of cathode ray tubes.	Barium (Ba)	Causes of short-term exposure: Weakness of muscle. Heart, Liver and spleen damage

3   CHEMICAL LEACHING OF E-WASTE

The most common method of hydrometallurgy is acid leaching. Due to their low cost and very predictable and controllable behaviour, reagents such as H_2SO_4, HCl, and HNO_3 have been studied in detail in the past, as seen in the study summary in **Table 7**. Because of their greater electrochemical potentials, valuable metals (such as Ag and Au) and copper are difficult for weak dilute acids to leach. However, metals like Zn and Fe are successfully leached. A significant amount of these metals must be released by applying strong acids (like concentrated HNO_3) or weak acids combined with oxidizing agents (such as O_2, H_2O_2, Cl_2, Cu^{2+}, and Fe^{3+}) (Gulliani et al., 2023).

Various inorganic acids, such as sulphuric acid (H_2SO_4), hydrochloric acid (HCl), and solutions of H_2SO_4 and HNO_3, can be used to chemically leach metals from e-waste. Sodium hypochlorite, when combined with acid or alkali, can also be used to recover precious metals, such as gold (Needhidasan et al., 2014). Metal recovery from e-waste also involves organic acids such as citric acid, malic acid, ascorbic acid, and oxalic acid (Tipre et al., 2021).

Table 7 Summary of chemical leaching by acids (Gulliani et al., 2023)

Source	Reagent	Metal extracted (%)	Conditions	Particle size
Computer	PCBs $0.5H_2SO_4$ + 0.5 g per L Cu^{2+} + 25.6 g per L Cl + O_2	Cu: 100	80 °C, 2 h	< 0.25 mm
WPCBs	$0.5\ H_2SO_4$ + 35 % H_2O_2	Cu: 86	25 °C, 3 h	< 0.3 mm
WPCBs	HNO_3 + organic swelling	Pb: 99.9 Sn: 98.7	90 °C, 2 h (Sn), 45 min (Pb)	< 5 mm
WPCBs	2-5M HNO_3	Ag: 68 Cu: 99.9	30-70 °C, 2h	n.a.
WPCBs	4.5 M HCl (Sn), 0.1 M HNO_3 (Pb)	Pb: 99.9 Sn: 97.8	90°C 1h	< 5 mm

Examined WPCB leaching of Sn and Pb from solder using 0.2 M HNO_3, 99.99 % of the Pb was leached in the first step, but no Sn leaching was seen since Sn was converted to SnO_2. Step two involved treating the previously generated SnO_2 with 3.5 M HCl in order to extract the residual Sn. It was discovered that the best reagents for recovering Cu, Sn, and Pb were $H_2SO_4 + H_2O_2 + NH_3$, HNO_3, and HCl, respectively.

According to Kumar et al. (2014), with 100 g/L pulp density at 50 °C for 4 hours, copper leaching from waste PCBs increases as a sulphuric acid concentration of 1.2 M and hydrogen

peroxide concentration of 10 % are used. For the extraction of copper and other metals, nitric acid with 10 % hydrogen peroxide works better than sulphuric acid at 100 g/L pulp density (Tipre et al., 2021). Despite being a relatively fast procedure, acid washing is unsatisfactory from an environmental standpoint because, when done incorrectly, the massive amounts of chemical reagents utilized can pollute secondary soil and water sources (Awasthi et al., 2016).

3.1 Instrumentation and Analytical Methods

3.1.1 Atomic Absorption Spectroscopy (AAS)

The basic principle of atomic absorption techniques is the ground-phase atoms' absorption of atomic spectral lines in their ground electronic states. The atomic vapour, M(g), is often produced thermally in a graphite furnace tube (furnace AAS) or flame (flame AAS). However, in certain instances (most notably Hg), the atomic vapour is produced by chemical reduction to M(g). The apparatus is composed of an arrangement known as an "atom cell" that generates M(g), an optical spectrum analyzer that helps choose the proper spectral line and an atomic emission light source that is unique to each element that needs to be identified as seen in **Figure 6**. In the majority of implementations, AAS is limited to making decisions on individual elements at a time (Ketterer, 2017).
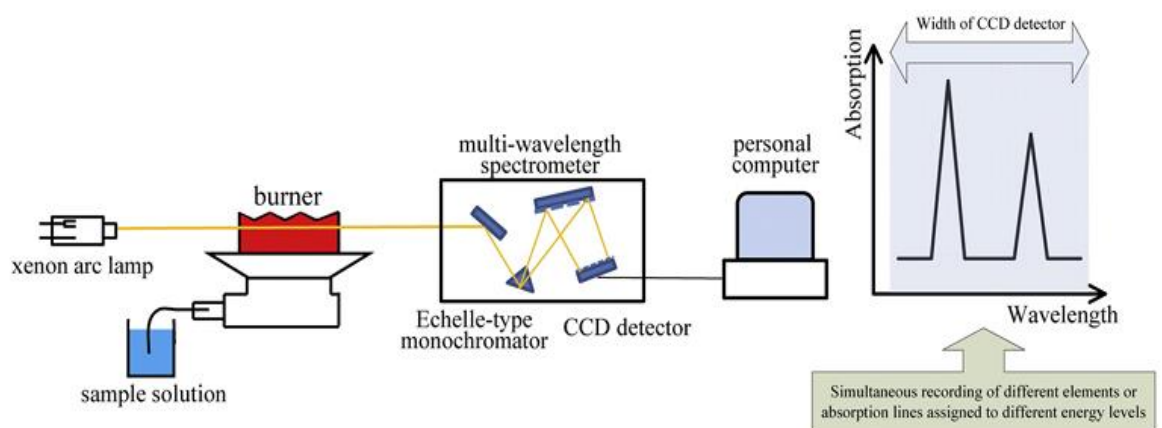

Figure 6 schematic diagram of FAAS (Toya et al., 2016)

3.1.2 X-ray Fluorescence Spectroscopy (XRF)

The electronic transitions of inner-shell electrons are the source of X-ray absorption and emission spectra, and the physical or chemical form of the element of interest has a negligible impact on these transition energies. Moseley found early in the 20th century that the atomic

number is closely related to the transition energies of K and L inner-shell electrons. A concentrated monochromatic X-ray beam can be used to create XRF spectra; monochromatic X-rays are created when a metal target in an X-ray tube is bombarded with electrons at energies between 10 and 100 keV. Inducing fluorescence in a sample and identifying and analyzing the distinctive X-rays it emits can be done in a number of ways (Kalnicky & Singhvi, 2001).

The encounter, in order to excite inner-shell electrons into higher energy states, X-rays contact with the sample atoms. The elements contained in the sample then cause X-rays with different energies to be released as a result, as seen in **Figure 7**. Electron microscopy in vacuo can produce comparable spectra by bombarding solid samples with electrons. Qualitative evidence of an element's presence is provided by the appearance of expected X-ray lines at particular energies. X-ray spectra produced by either method can provide both qualitative and quantitative information. Since the emission intensity and atomic concentration are proportionate, quantitative analysis is feasible (Ketterer, 2017)
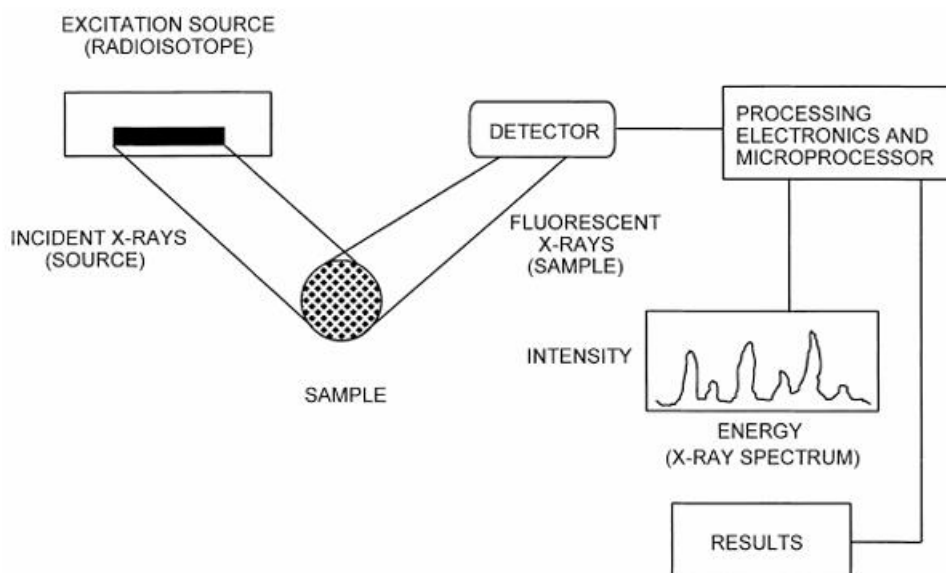

Figure 7 Block schematic of a standard EDXRF spectrometer (Kalnicky & Singhvi, 2001)

3.1.3 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICPMS is arguably the most significant elemental analysis technique available today, and its significance in geochemical analysis is even more apparent than in other applications. The Ar gas plasma is used by ICP-MS to produce gas-phase elemental ions. A differentially

pumped interface with two consecutive 1 mm openings is used to sample the ions from the atmospheric pressure plasma into a high vacuum environment. Mass spectrometry is used to evaluate the extracted ions; commercially available devices include quadrupole, magnetic sector, and time-of-flight mass analyzers. For general elemental analysis, the quadrupole system is the most straightforward, popular, affordable, and performs well (**Figure 8**). Applications requiring maximum sensitivity, immunity to interfering (polyatomic) ions, or high-precision isotope measurements are best served by magnetized sector ICP-MS. Though it is not commonly utilized, the time-of-flight ICP-MS is suitable for measurements of transitory multielement signals (Ketterer, 2017).
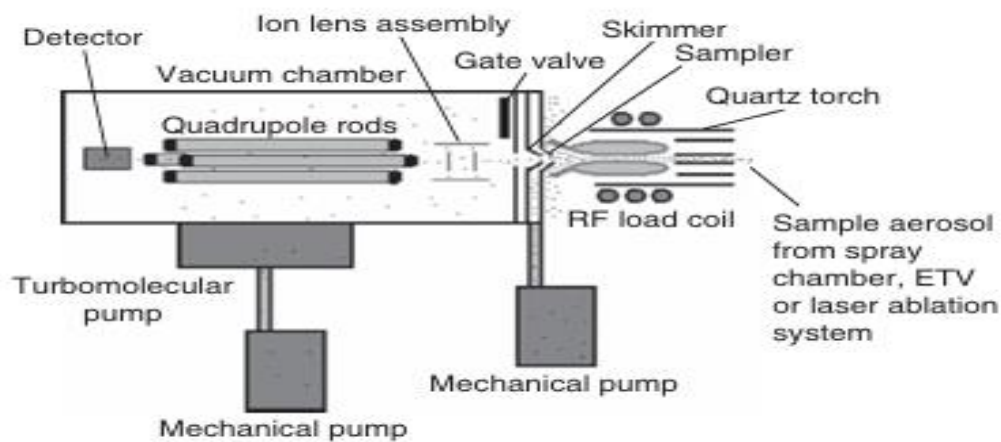
Figure 8 Schematic diagram of a quadrupole ICPMS system (Ketterer, 2017)

II.   ANALYSIS

4 EXPERIMENTAL PART

4.1 Materials

4.1.1 Laboratory Equipment

- ElvaX, X-Ray Fluorescence (XRF) Spectrometer, Ukraine

- Atomic Absorption Spectrometer ContrAA 800, Analytikjena, Germany

- Multimeter 720 WTW series InoLab - Combined pH electrode: pH electrode SenTix 41, WTW, pH range 0-14 /0-80 °C/ store in 3 mol/l KCl, Germany.

- Multimeter 720 WTW series InoLab – electrode SenTix ORP, store in 3 mol/l KCl, Germany

- Mineralizer MLS 1200, digestion/drying module, exhaust module EM-30, Milestone - microwave laboratory systems, Switzerland

- Analytical balances Kern ABJ 220-4NM, Germany

- Microscope OLYMPUS CX41, Japan.

- Thermostat (**Shaking Water Bath GFL 1092 with orbital motion**) Germany.

- UNICAM UV 500, Thermo Fisher Scientific.

- Retsch MM 301 vibratory mill, Germany.

- Sieve with mesh size 1 mm, STAS Ltd., Czechoslovakia

- Hemocytometer Cyrus II (Meopta, Czech Republic)

- Autoclave 5075ELV-D, Tuttnauer, USA

4.2 Chemicals

- Filter paper 50 mm, 0.45 μm, Papírna Perštejn, Czech Republic

- Single element AAS analytical standards, Astasol Plc, Czech Republic

- Multi element AAS analytical standard, Astasol Plc, Czech Republic

- Nitric acid, Penta Plc, Czech Republic

- Hydrochloric acid, Penta Plc, Czech Republic

- Hydrofluoric acid, Penta Plc, Czech Republic

- Sulphuric acid, Penta Plc, Czech Republic

- Ammonium sulphate, Penta Plc, Czech Republic

- Potassium chloride, Penta Plc, Czech Republic

- Dipotassium phosphate, Penta Plc, Czech Republic

- Magnesium sulphate heptahydrate, Penta Plc, Czech Republic

- Calcium nitrate, Penta Plc, Czech Republic

- Iron (II) sulphate heptahydrate, Penta Plc, Czech Republic

- Potassium hydroxide, Penta Plc, Czech Republic

- Buffer solutions (pH 4, 7, and 10), WTW, Germany

5 METHODOLOGY

5.1 Preparation of biomedium

The preparation of the bio medium for *Acidothiobacillus ferrooxidans* involved two separate sets of solutions: the first set comprised three bottles containing 2 g of $(NH_4)_2SO_4$, 0.1 g of KCL, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4$. $7H_2O$, 0.01g of Ca $(NO_3)_2$, and 700 ml of distilled water, while the second set contained 40 g of $FeSO_4$. $7H_2O$, 2 ml of 1 N H_2SO_4, and 300 ml of distilled water. pH adjustments were made within the range of 1.8 to 2.0 using dilute H_2SO_4 or KOH. After sterilization, the two sets of solutions were mixed to form the final biomedium, resulting in three bottles with 1 L of bio-medium each. Finally, 12 flasks were filled with 200 ml of the prepared biomedium and placed at a controlled temperature of 26°C.

5.2 Preparation of *Acidothiobacillus ferrooxidans* and sulphur addition for cultivation

In the experimental setup, the 12 flasks, each containing 200 ml of bio-medium, were prepared. After that, the sulphur supplementation in powder form was precisely applied to selected flasks, with samples 4, 5, and 6 receiving 1 g/L of sulphur, while samples 7, 8, and 9 were enriched with 2 g/L, and samples 10, 11, and 12 were added with 3 g/L of sulphur as seen in **Table 8.** After sulphur addition, 100 µL of *A. ferrooxidans* CCM 4253 (Collection of Czechoslovak Cultures of Microorganisms) were precisely added to each flask to ensure uniformity across all samples. Moreover, the sulphur addition nomenclature is shown in **Table 8**. Crucially, flasks 1, 2, and 3 served as blank controls, devoid of sulphur and *A. ferrooxidans* strain supplementation. The prepared flasks were then carefully placed into a thermostat set to maintain a constant temperature of 26 degrees Celsius, providing optimal incubation conditions.

Table 8 Biomedium sulphur addition

Flask	Sulphur addition	Nomenclature
1 to 3	No addition	blank
4 to 6	1 g/L	A1
7 to 9	2 g/L	A2
10 to 12	3 g/L	A3

5.3 pH, ORP and Temperature measurements (Basic parameters measurements)

Analytical measurements of pH, ORP and temperature levels of the 12 samples, each containing varying concentrations of sulphur (A1, A2, and A3) and *Acidothiobacillus ferrooxidans*, were monitored throughout a 7-day period. pH electrode was calibrated using WTW pH 4, 7, and 10 buffer solutions, and ORP monitoring was performed using an electrode containing a 3 M KCl electrolyte solution.

5.4 IRON (III) Monitoring

The concentration of iron (Fe) was assessed by preparing 12 solutions for each biomedium, each containing 250 μL of respective bio-medium and distilled water in a 25ml flask. To initiate the reaction, 1.25 ml of hydrochloric acid (1:1) and 2.5 ml of potassium thiocyanate were added to each solution, leading to the formation of an intense, red-coloured complex with iron, as seen in equation 3.

$$Fe^{3+} + 6\,KSCN \rightarrow Fe[Fe(SCN)_6] + 6\,K^+ \qquad \text{/Eq. 3/}$$
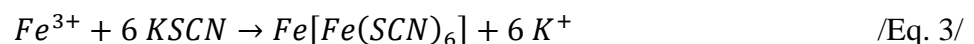
UV-visible spectroscopy was employed utilizing the fixed method at the specific wavelength of 500 nanometers. Each sample was measured three times to ensure accuracy, and an unknown concentration was calculated using linear regression from UV-VIS absorbance data. This process aimed to monitor the growth of *Acidothiobacillus ferrooxidans*, as the oxidation of Fe^{2+} to Fe^{3+} serves as an indicator of bacterial activity proliferation.

5.5 Microscopic cell counting of *acidothiobacillus ferrooxidans* using a Hemocytometer

Acidothiobacillus ferrooxidans monitoring and counting was done over 7 days using Olympus CX41 microscope. 20 μL was placed on hemocytometer Cyrus II, and by utilizing manual counting under a microscope, specifically at a magnification of 400x, *Acidothiobacillus ferrooxidans* population density estimates were derived across varying area sizes: 50 by 50 μm, 100 by 100 μm and 200 by 200 μm using a hemocytometer, which was described in the study (RŮŽIČKA, 2001). Average counts from each area were multiplied by their respective factors (8,000,000, 2,000,000, and 500,000) to extrapolate total populations within the sample and expressed in cells per millilitre. This method, known as manual cell counting or microscopic cell counting using a hemocytometer, enabled

precise quantification of *Acidothiobacillus ferrooxidans* populations, offering insights into their spatial distribution and abundance.

5.6 Metal content analysis using X-ray fluorescence

The motherboard coarse and dust sample was subjected to X-ray Fluorescence (XRF) analysis to have a comprehensive metal content analysis across the boards. This involved irradiating each component with high-energy X-rays to induce characteristic X-ray emission. The XRF spectrometer then detected and analyzed the emitted X-rays from each part, allowing for the identification and quantification of the metals present in each sample of the motherboard.

An energy dispersive spectrometer, paired with a rhodium-ray lamp, was utilized for measurements, with data processed using ElvaX 2.8.2 software on a computer. The experiment involved different conditions: no filter was used for light element spectra, employing an X-ray lamp with 64 A current and 10 kV voltage, while heavy element spectra were recorded with three filters, using a 45 kV voltage and 99.9 µA current. Samples underwent dual-mode analysis, capturing both light and heavy element spectra for 120 seconds. Additionally, the samples were housed in special polyethylene containers (30 x 22 mm), with one side sealed using a specialized polypropylene film (Prolene film, CAT. NO: 416, Chemplex INDUSTRIES, INC.; Palm City, USA, foil thickness 4 µm).

5.7 SAMPLE PREPARATION

5.7.1 Size reduction

The sample preparation process commenced with the utilization of used motherboard e-waste, from which a coarse sample weighing 130 grams was meticulously prepared. Manual crushing was employed to achieve a size of approximately 25mm by 15 mm for the coarse sample. Additionally, a fine sample weighing 130 grams, characterized by fine particles with a size ≤ 1 mm, was generated using a Mixer Mill 301. The grinding jar size utilized ranged from 1.2 to 25 ml, accompanied by a grinding steel ball with a diameter of 20 mm. The crushed waste underwent a dry grinding process with a vibration frequency of 30 Hz for a duration of 3 minutes. This dry grinding procedure was repeated iteratively to facilitate size reduction until the desired particle size of 100 nanometres was achieved, ensuring homogeneity throughout the sample.

5.7.2 Bioleaching

The leaching process started with the utilization of twelve pre-prepared leaching solutions, among which 3 served as blanks. Each of the remaining 8 flasks contained 200 ml media with *Acidothiobacillus ferrooxidans*, cultivated for seven days, with varying sulphur concentrations, A1, A2 and A3. Subsequently, 24 flasks were evenly filled with 100 ml of the leaching media, with 12 designated for coarse waste and twelve for fine waste. Within these, 3 were kept as blank then 5 grams of coarse and dust waste were measured and placed into the respective 12 flasks containing 100 ml of leaching media. The flasks were then subjected to agitation within a thermostat set at 26 degrees Celsius for 24 hours to facilitate the leaching process.

5.8 Determination of metals on Flame AAS

The metal content of both fine and coarse samples was analyzed using Flame Atomic Absorption Spectroscopy (AAS) by utilizing an air-acetylene flame with the ContrAA 800D atomic absorption spectrophotometer (Analytik Jena, Germany) following bioleaching., the resulting leach solutions were filtered to remove any solid residues using glass fiber filter paper with size 0.45 μm. Subsequently, aliquots of the filtrates were aspirated into the flame AAS instrument, where the samples were atomized and excited by a flame using 50 mm burner at high temperatures. The absorption of specific wavelengths of light by the metal atoms present in the sample was measured with respective flow rate as shown on table 7, enabling the quantification of metal concentrations. This approach provides an accurate assessment of metal content in both fine and coarse samples post-bioleaching, crucial for evaluating the efficiency of the bioleaching process in metal recovery.

To determine the concentrations of unknown elements in the samples, a multi-element standard solution was employed for calibration. This solution was diluted with 1% of HCl. 1 g/L of single element standard solution was used to prepare two calibration curves, each for 4 metals and blank with a concentration from 0 to 100 mg/L, and linear regression was used to calculate the concentration of the metals found in the samples. The measured values on FAAS were recalculated and expressed as mg per kg using following the equation 4.

$$c\left(\frac{mg}{kg}\right) = \frac{c\left(\frac{mg}{L}\right) \times V(mL)}{m(g)}$$ /Eq.4/

Where, c (mg/kg) – the concentration in the sample (mg/kg)

c (mg/L) – the concentration in the sample measured on AAS (mg/L)

V – the volume of the sample (mL)

m – the mass of the sample (g)

Table 9 The wavelength of the elements detected on Flame AAS.

Metals	Wavelength [nm]	C_2H_2-air [L/h]
Cu	327	50
Fe	371	60
Ni	232	55
Sn	224	75
Cd	228	50
Pb	217	65
Mn	279	80
Sb	217	90

5.9 Microwave-assisted acid digestion

The digestion method performed was US EPA Method 3052b, which involves a microwave-assisted acid digestion procedure with specific parameters (Bettinelli et al., 2000). Initially, a weighed sample (0.2 g) of the solid sample is placed in a fluoropolymer or quartz digestion vessel. A mixture of concentrated 2 mL nitric acid (HNO_3) and 6 mL hydrochloric acid (HCl) is then added to the sample, along with 2 mL hydrofluoric acid (HF), with the program of digestion as shown in **Table 10**. During this process, microwave energy facilitates the decomposition of organic and inorganic components present in the sample. After digestion, the vessels are cooled, and the resulting digestate is filtered to remove any insoluble residues. The filtered solution is then diluted in a 100 mL flask and analyzed for target analytes using appropriate analytical techniques, such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry.

Table 10 Operating conditions for microwave digestion systems: CEM MDS 2000 and Milestone MLS 1200

Step	Time (min)	Power (W)
1	2	250
2	2	400
3	10	500
4	8	250
5	4	400
6	6	600
7	2	0
8	3	300
9	2	0

5.10 Metal Leaching Efficiency

The metal leaching efficiency was determined by calculating the ratio of the metal concentration achieved through bioleaching to that obtained through acid digestion, expressed as a percentage, as seen in equation 5. This calculation was performed separately for both the dust and coarse samples (Jegan Roy et al., 2021).

$$Metal\ leaching\ efficiency\ (\%) = \frac{A}{B} \times 100 \qquad \text{/Eq. 5/}$$

Where, A – metal concentration in bioleaching

B – metal concentration in aqua regia leaching

Here, the metal concentration in bioleaching refers to the concentration of the target metal extracted from the sample using the bioleaching process. On the other hand, the metal concentration in aqua regia leaching represents the concentration of the target metal obtained by the microwave-assisted acid digestion method, which is used as a reference or control for comparison.

5.11 Statistical analysis

In this study, confidence intervals for all measured values were calculated in Excel using the standard deviation method as seen below with equation 6, with an alpha level of 0.05 and presented in error bars on the respective graph illustrations. These intervals represent a range

of values around the sample mean and, at a 95 % confidence level, provide insights on the precision and reliability of the estimates.

$$\sigma = \sqrt{\frac{\Sigma_{i=1}^{n}(x_i - \mu)^2}{n-1}} \qquad /Eq.6/$$

where,

σ – is a symbol that denotes the standard deviation

x_i – is the ith number of observations in the data set

n – is the number of observations in the data set

μ - is the mean of the sample

Σx – is the sum of all values in the data set

The values were calculated according to the Excel function of confidence as CONFIDENCE (A2, A3, A4), in which each symbol represents a different value: A2 – significance level (α=0.05), A3 – standard deviation in the population, which is also represented by equation 6, and A4 – number of samples.

6 RESULT AND DISCUSSION

6.1 Analysis of metal content using x-ray fluorescence waste characterization

The elemental composition of both motherboard dust and crushed samples was analyzed using X-ray fluorescence. Figures 6 and 7 illustrate the spectra obtained from these samples.
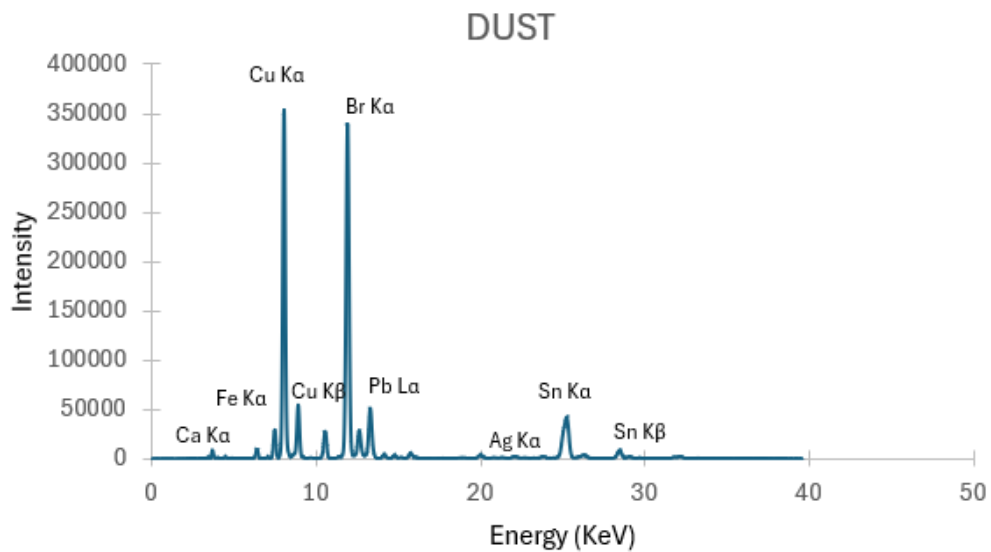
Figure 9 WPCB dust x-ray fluorescence metal analysis.

In both dust and coarse motherboard samples, the XRF analysis showed that sample compositions have copper content in abundance, with the highest in coarse samples at approximately 70.48 %, as seen in Figure 7. However, in the dust waste sample, other metals such as tin (Sn), iron (Fe), lead (Pb), and iron (Fe) were detected in smaller quantities, and coarse waste showed smaller concentrations of nickel (Ni), suggesting that size reduction may have an influence on homogeneity and surface area exposure on the samples when preparing to bioleaching process.
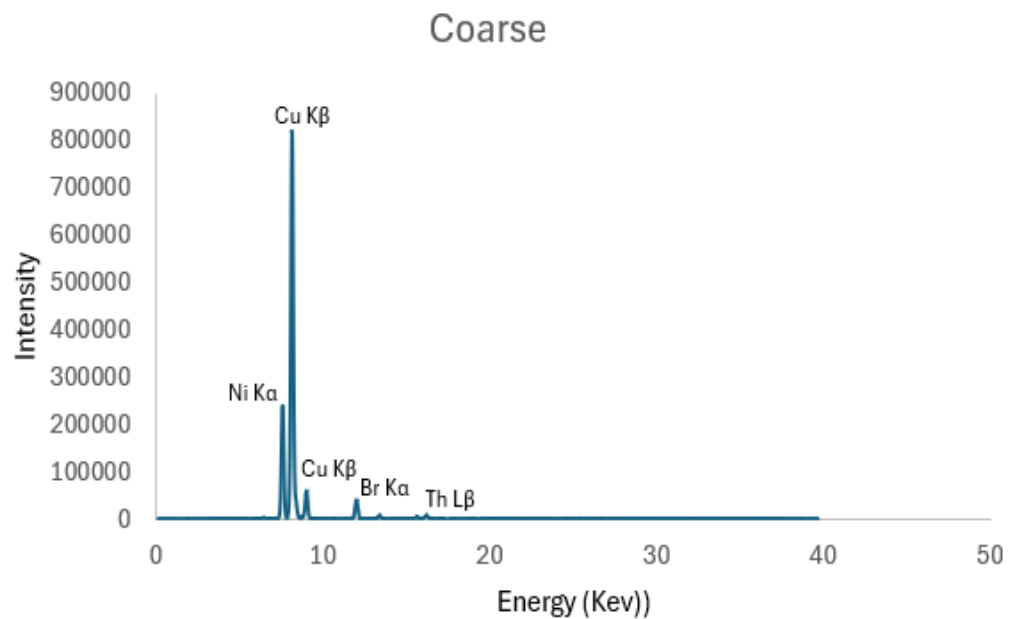
Figure 10 WPCB coarse x-ray fluorescence metal analysis.

6.2 pH and temperature analysis

The pH data measured over 7 days from biomedium containing *Acidothiobacillus ferrooxidans* (see **Figure 11**) and different sulphur concentrations reveals a clear sulphur influence on the pH levels of the mediums. Initially, on all biomediums, the pH remained relatively stable, around 1.96 to 1.98, during the first few days, indicating a low sulphuric acid production as a byproduct of the metabolic activity of the bacteria from sulphur oxidation. In the blank sample, pH remained relatively stable with minimal changes. However, starting from day 4, the biomedium with sulphur concentrations started a gradual increase in pH over time, reaching values of 2.26, 2.24 and 2.22 for biomediums with A1, A2 and A3 sulphur concentrations, respectively. However, the rate slowed on day 7, as seen in Figure 8. The measured temperature stayed consistent at 26 ºC throughout the 7-day period.

The observed variation in pH trends in the biomediums can be attributed to the metabolic activity of *Acidothiobacillus ferrooxidans* and their relative oxidation of sulphur compounds in the mediums. Higher sulphur concentrations provide more substrate for bacterial metabolism, leading to increased sulphur oxidation and subsequent acid production, and the observation showed the highest concentration of sulphur (A3) conserved a more acidic state compared to the A1 and A2 sulphur concentrations over the first 6 days and the trends were showing similar state on the 7th day. In bioleaching, pH fluctuations can impact bacterial

activity and metal solubility. These findings suggest sulphur content modulates biomedium acidity, potentially impacting bacterial activity and bioleaching efficiency. A study by Crust and colleagues investigated how pH affected the solubility of metals. While the leaching of Al and Zn exhibited similar dissolving efficiency at pH 1.8 and 2.0, the leaching of Cu and Ni was shown to be most effective at an initial pH of 1.8 (Ji et al., 2022).
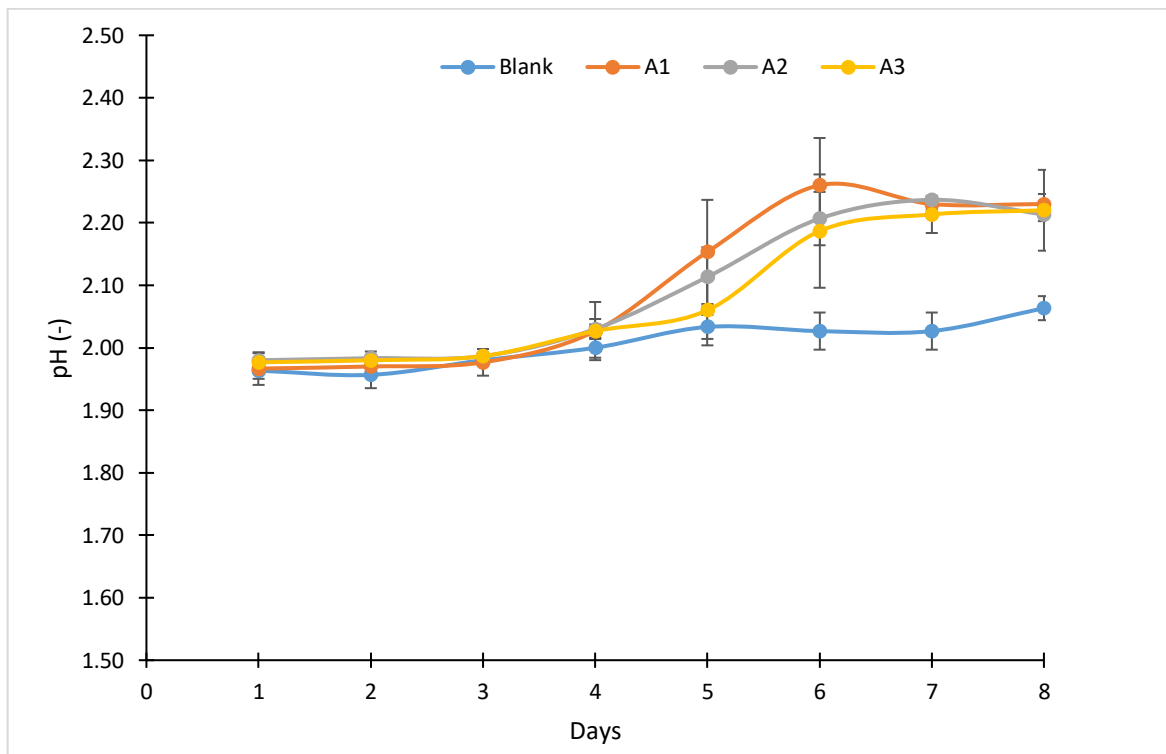

Figure 11 Sample pH measurements.

6.3 ORP analysis

Monitored ORP values showed a similar state for the first few days in all the biomediums, which indicated a relatively balanced state. However, starting from the third day, changes in the values are observed, especially in mediums with sulphur concentrations, which displayed a steady increase over the days, as seen in **Figure 12**. This observation suggests an increase in oxidative potential in all the biomediums. The blank sample shows relatively stable ORP values throughout the observation period, with an average value of 563.2 mV. In contrast, mediums with A1, A2, and A3 sulphur illustrated increasing ORP trends, with average final values of approximately 645.2 mV, 634.5 mV, and 634 mV, respectively. The biomedium with A1 sulphur showed the highest and most consistent increase in ORP. The findings indicate a more oxidizing environment conducive to microbial oxidation processes was

achieved by sulphur addition and suggests a direct relationship between sulphur concentration and oxidative conditions, likely through heightened microbial activity in sulphur-rich environments. Overall, the data suggests that moderate sulphur concentrations, particularly A1, have a relatively better impact on enhancing the oxidative potential compared to between the medium since such increases in ORP are crucial for promoting bacterial activity and optimizing metal extraction efficiency in bioleaching preparations. According to a study, an oxidation-reduction potential of 600 or more is a reliable sign of elevated Fe^{+3} in *A. ferrooxidans*. Oxidation-reduction potential above 400 mV during acidophilic bioleaching suggests a high Fe^{+3} concentration and high bacterial growth (Tezyapar Kara et al., 2023).
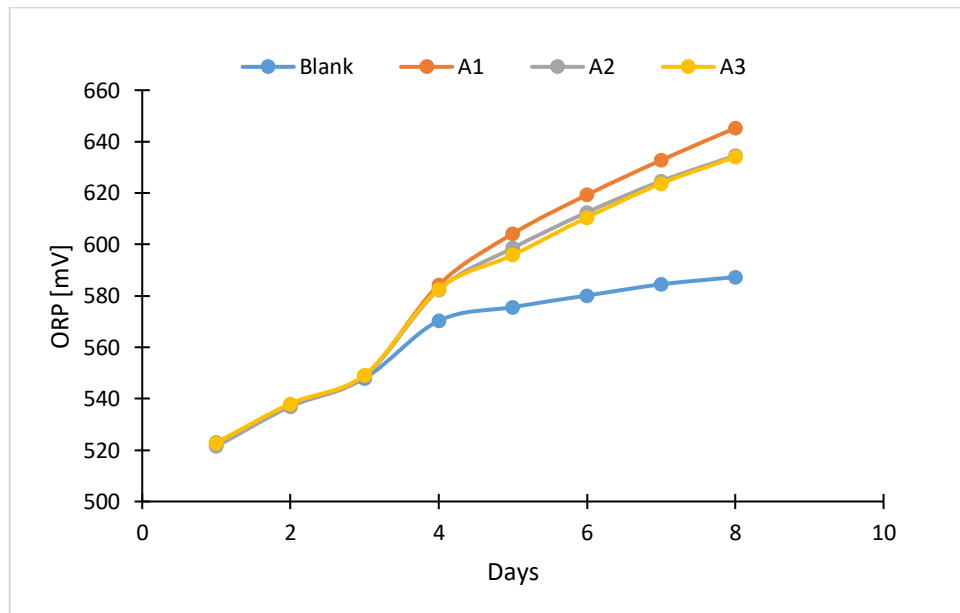
Figure 12 Sample ORP measurements.

6.4 UV-VIS IRON (III) measurement

The UV-VIS measurement of ferric ions data of the biomediums monitored over 7 days showed the clear sulphur influence on the microbial activity and oxidation of ferrous ions by the iron-oxidizing bacteria in this case *Acidothiobacillus ferrooxidans*. Except for the blank sample, all biomediums with different concentrations of sulphur displayed a similar increasing trend over time as seen on **Figure 13**. Baseline concentrations of Fe^{3+} were detected at the initial time of day 1. However, a steady increase of Fe^{3+} is shown starting from day 4 as shown in figure 10, indicating higher oxidation of ferrous iron (Fe^{2+}) by the

bacteria facilitated by the added sulphur. Even though all biomediums show similar trends, the lower concentration of A1 interestingly showed a relatively higher concentration over the monitored days. Sulphur serves as a key substrate for the metabolic activity of *Acidothiobacillus ferrooxidans*, fueling the oxidation reactions that result in the production of ferric ions (Fe^{3+}). However, these results suggest sulphur concentration alone does not determine Fe^{3+} production in the biomediums.
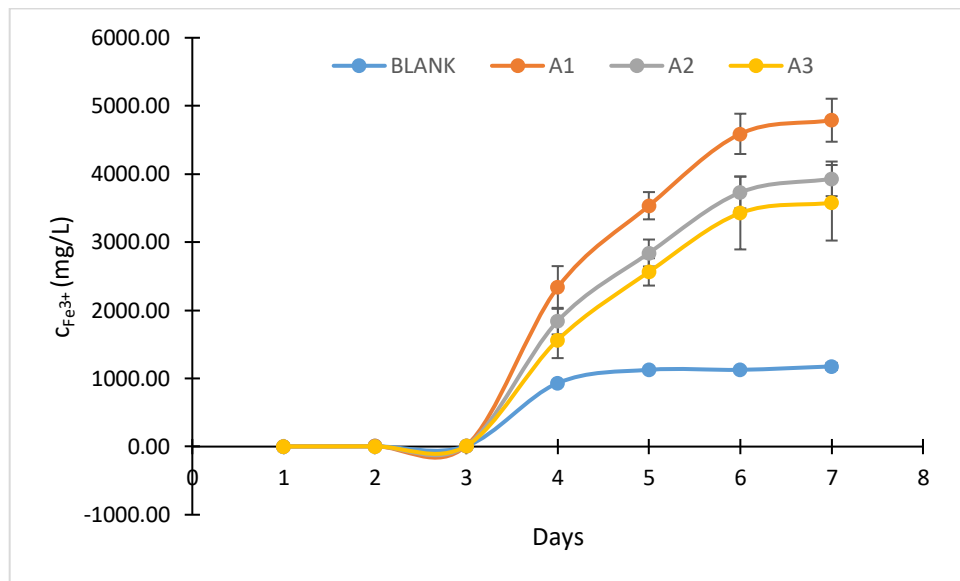
Figure 13 Fe^{3+} biomedium concentration over 7 days

6.5 Monitoring microbial growth

The growth patterns of *Acidothiobacillus ferrooxidans* in biomedium with varying sulphur concentrations monitored for 7 days showed the influence of the added sulphur in the proliferation and population dynamics of the bacteria. The growth pattern follows a similar bacterial growth curve for all mediums, especially for A1 and A2 sulphur concentrations, as seen in **Figure 14**. The illustration shows that there is minimal population growth in the lag phase (day 1) as bacteria adapt to the new environment except for the A3 sulphur medium. At A1 sulphur, there's a lag phase on day 1, followed by exponential growth from days 2 to 6. However, the rate of growth seems to slow down on the 7th day, suggesting an imminent stationary phase. Similarly, A2 sulphur showed initial growth on day 1, followed by a decline on day 2, then resuming to exponential growth until day 6. Similarly, the growth rate was slowed on day 7. In contrast, at A3 sulphur, substantial growth occurs on day 1, with a slight decrease on day 2, followed by relatively slower growth from days 3 to 7, again slowing on

day 7, indicating an approaching possible stationary phase. Despite these differences, all concentrations display exponential growth phases from days 2 to 6, highlighting a similar trend in their growth patterns. However, the major distinctions lie in the initial phases, with A1 sulphur showing a clear lag phase, A2 sulphur displaying an initial decline, and A3 sulphur exhibiting immediate substantial growth. Analyzing the data, the biomedium with A1 of sulphur exhibits the most consistent growth, with a steady increase from day 1 to day 7 and a slightly higher population on the 7th day, and A3 displayed unstable, and the trend was fluctuating over the monitored period. Overall, the sulphur content significantly influenced the *Acidothiobacillus ferrooxidans* growth rate, with an optimal concentration of A1 observed between the monitored mediums.
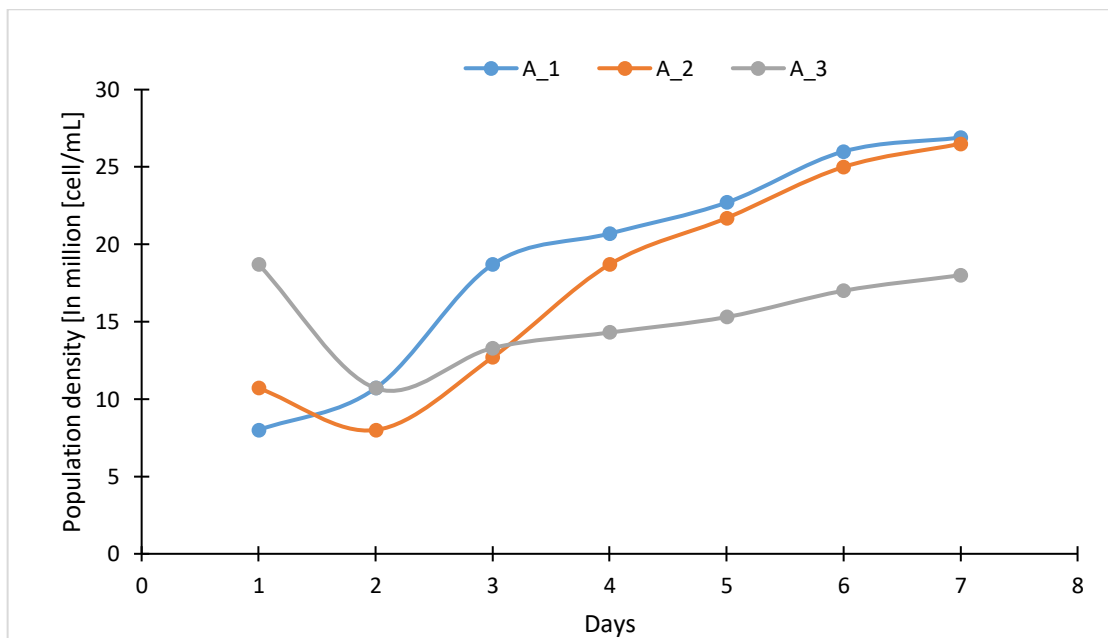
Figure 14 *Acidithiobacillus ferrooxidans* population growth over 7 days illustration

6.6 Sample microwave-assisted acid digestion

The total metal concentrations of waste samples after microwave-assisted acid digestion revealed copper content at a higher concentration in both samples, with 148.0 g/kg in the dust and an even higher concentration in the coarse sample with 422.2 g/kg. On the other hand, nickel and Iron show slightly higher concentrations in the dust sample, with 11.04 g/kg for nickel and 14.36 g/kg in the dust sample as seen on **Figure 15**.
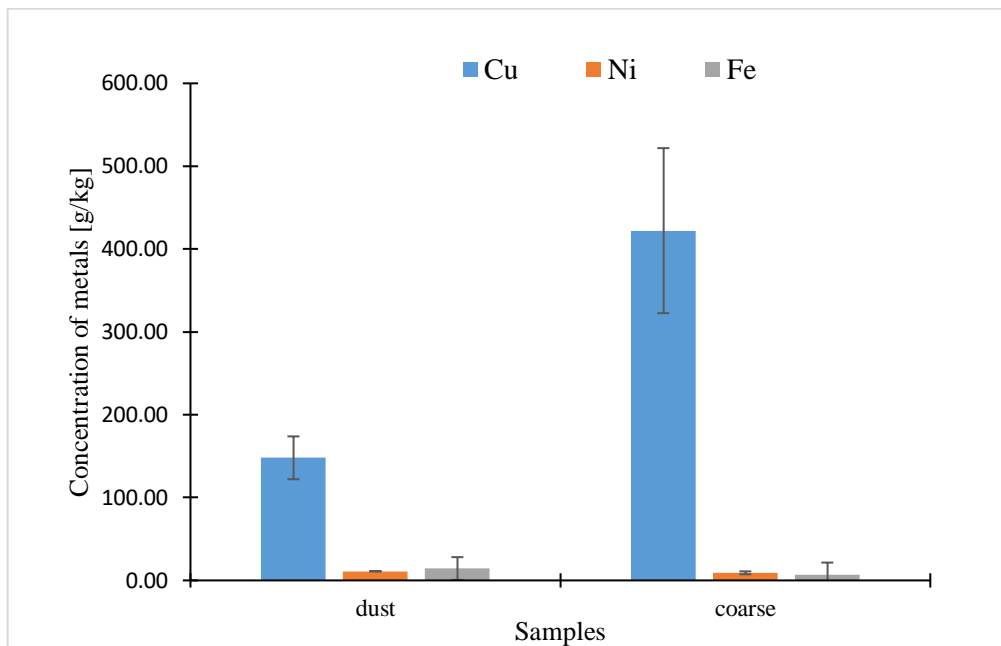
Figure 15 Metal concentrations of Copper, Nickel, and Iron in dust and coarse samples from WPCB microwave-assisted acid digestion.

Lead and tin are relatively at lower concentrations in both samples, showing higher values in the coarse sample with 16.8 g/kg and 74.47 g/kg, respectively as seen on **Figure 16**. However, antimony, cadmium and manganese showed very low or negligible values in both dust and coarse board samples. A similar study, Waste Printed Circuit Boards (WPCBs) processed in chemical leaching with 4.5 M HCl for tin (Sn) and 0.1 M HNO_3 for lead (Pb) at 90 °C for 1 hour, yielding extraction efficiencies of 99.9 % for Pb and 97.8 % for Sn for particles ≤ 5 mm. Another experiment involved treating WPCBs with 0.5 M H_2SO_4 and 35 % H_2O_2 at 25°C for 3 hours, resulting in an 86 % copper (Cu) extraction efficiency for particles ≤ 0.3 mm (Gulliani et al., 2023).
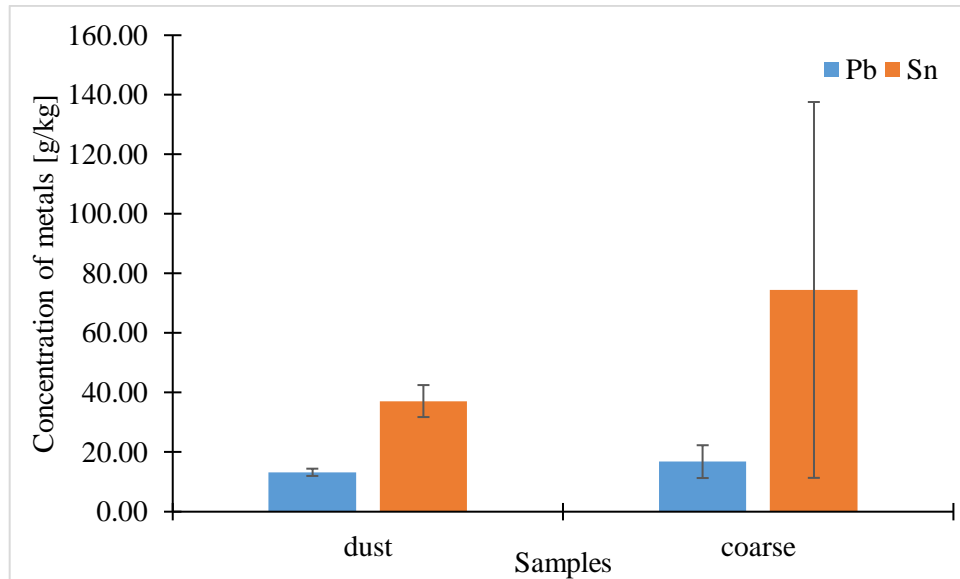
Figure 16 Metal Concentrations of Lead and Tin in Dust and Coarse Samples from WPCB Acid Digestion

6.7 Determination of metals on flame AAS after 24 hours of bioleaching

The AAS data after 24 hours of bioleaching shows visible changes in copper and nickel concentrations between dust and coarse waste WPCB samples placed in varying sulphur concentration biomediums, as illustrated in **Figure 17**. Dust_A1 has the highest copper concentration of (20.0 g/kg) and nickel (11.2 g/kg) concentrations among dust samples, whereas Coarse_A1 have the highest copper (50.3 g/kg) and Coarse_A2 leached the highest nickel concentrations of (13.4 g/kg) among coarse samples. Leaching in the blank samples for both dust and coarse waste is also observed with small concentrations.
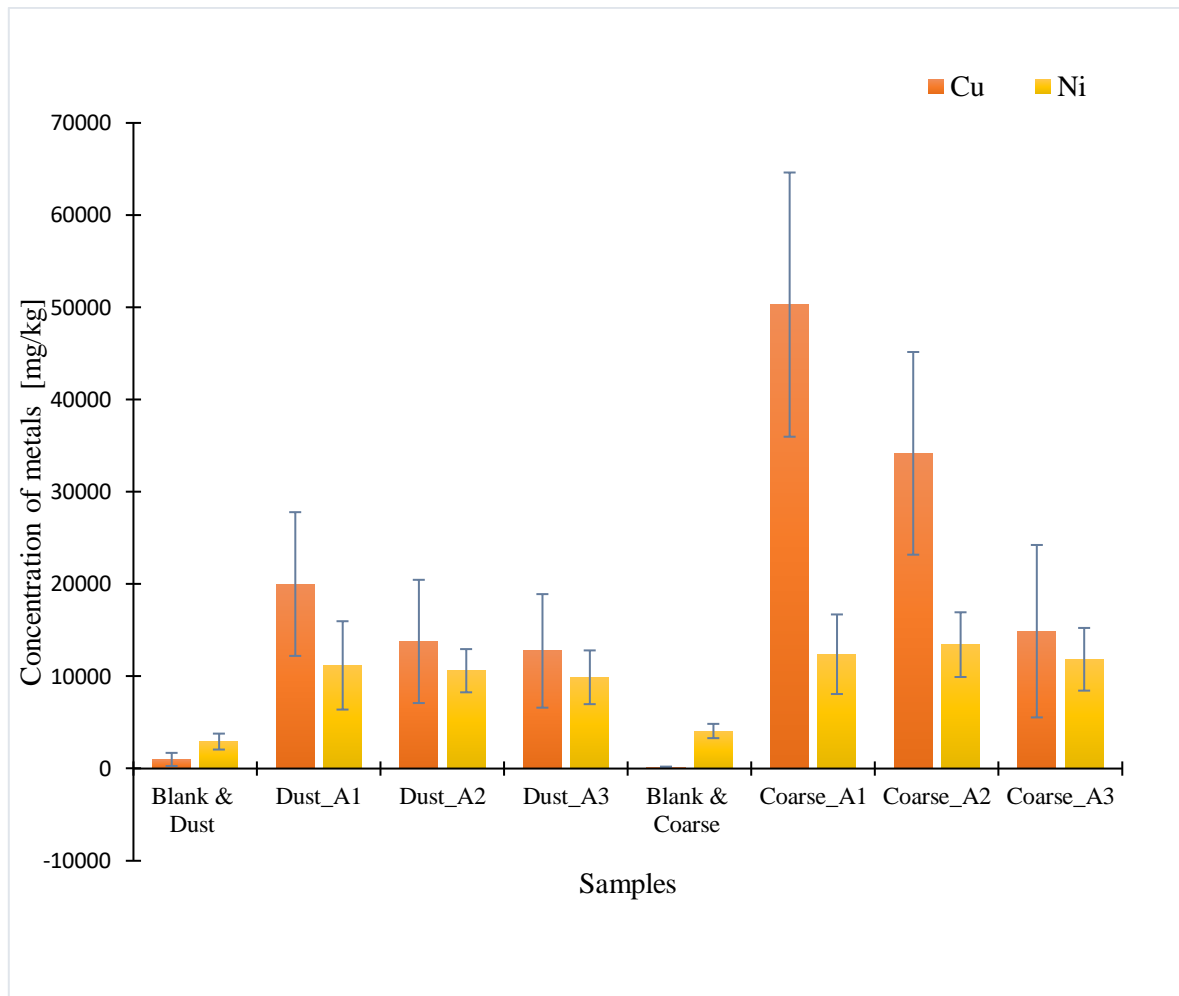
Figure 17 Copper and Nickel concentrations after 24 hours of bioleaching

On the other hand, lead (Pb), manganese (Mn), and tin (Sn) concentrations were leached with relatively low concentrations, as seen in **Figure 18**. Dust_A2 waste sample has the highest Pb at 125.1 mg/kg Mn at 163.5 mg/kg concentrations, and Sn at 3580.13 mg/kg concentration is the highest in Dust_A1. The highest Mn at 145.4 mg/kg and Sn at 582.8 mg/kg concentrations were leached in Coarse_A1 and Coarse_A2, respectively. It is observed that coarse waste samples, except blank, lacked detectable Pb concentrations.
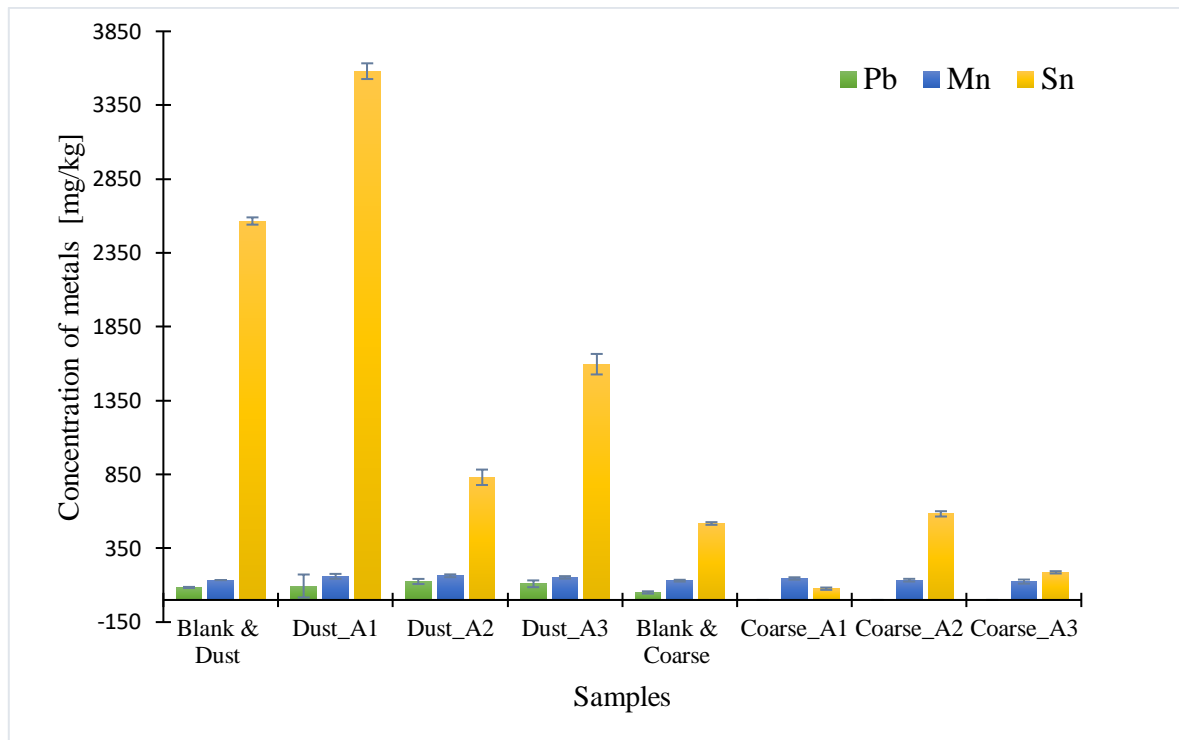
Figure 18 Lead, manganese, and Antimony concentrations after 24 hours of bioleaching.

6.8   Recovery efficiency of 24-hour bioleaching

The calculated recovery efficiency of bioleaching after 24 hours in the dust sample biomediums Nickel recovery shows the highest rates at 74.8 %, 69.6 %, and 63.1 % for sulphur concentrations of A1, A2, and A3, respectively, and Manganese recovery efficiency is shown only in the dust samples with the highest being 29.3 % in A2 dust samples while Copper recovered at 13 % for A1 sulphur which is also the highest as seen in **Table 11**. On the other hand, lead and tin show negligible recovery rates across all sulphur concentrations for both types of samples. In contrast, in the coarse sample biomediums, Nickel recovery efficiency was at high rates of 91 %, 102.4 %, and 85 % for A1, A2, and A3 sulphur concentrations, respectively, marking it as the highest recovery among all the metals that are analyzed. However, Pb, Mn, and Sn show negligible recovery rates across all coarse samples. A similar study on PCB on a 2-day incubation time with 2.0 pH and 30 °C had a recovery efficiency of 80 % copper recovery (Adetunji et al., 2023).

Table 11 Recovery efficiency after 24 hours of bioleaching

Samples	Cu %	Ni %	Mn %	Sn %
Dust_A1	8 ± 1.17	74.8 ± 0.09	25.7 ± 0.81	2.7 ± 0.4
Dust_A2	8.6 ± 0.63	69.6 ± 0.77	29.3 ± 0.82	*ND*
Dust_A3	8.0 ± 1.07	63.1 ± 0.6	18.5 ± 0.77	*ND*
Coarse_A1	12 ± 1.03	91.0 ± 0.93	*ND*	*ND*
Coarse_A2	8.1 ± 0.43	102.4 ± 1.3	*ND*	*ND*
Coarse_A3	3.5 ± 1.27	85.0 ± 0.67	*ND*	*ND*

6.9 Bioleaching after 55 days

Following a 55-day period, dust sample-specific bioleaching was carried out. This decision was based on the results of the X-ray fluorescence (XRF) analysis, which showed that the dust had a more range of metals than the coarse samples supported with bioleaching data after 24-hours that showed dust samples leaching wider range of metals. After 55 days, the concentration of copper reached a maximum of 44 g/kg in the dust_A1 sample, whereas the lowest concentration was 29.1 g/kg in dust_A2 sample, respectively. In the case of nickel, the highest concentration was measured for dust_A3, reaching 18.1 g/kg. Moreover, the lowest concentration was 13 g/kg, and it was found in the Dust_A1 sample, as seen in **Figure 19.** These results showed higher leaching efficiency compared to the 24 hours leaching, as seen in **Table 12**. However, other metals were not detected after 55 days of leaching. A study on mobile PCBs with the same temperature and lower pH of 1.0 with an incubation period of 55 days was able to reach 99 % Cu and 99 % Ni (Arshadi & Mousavi, 2015).
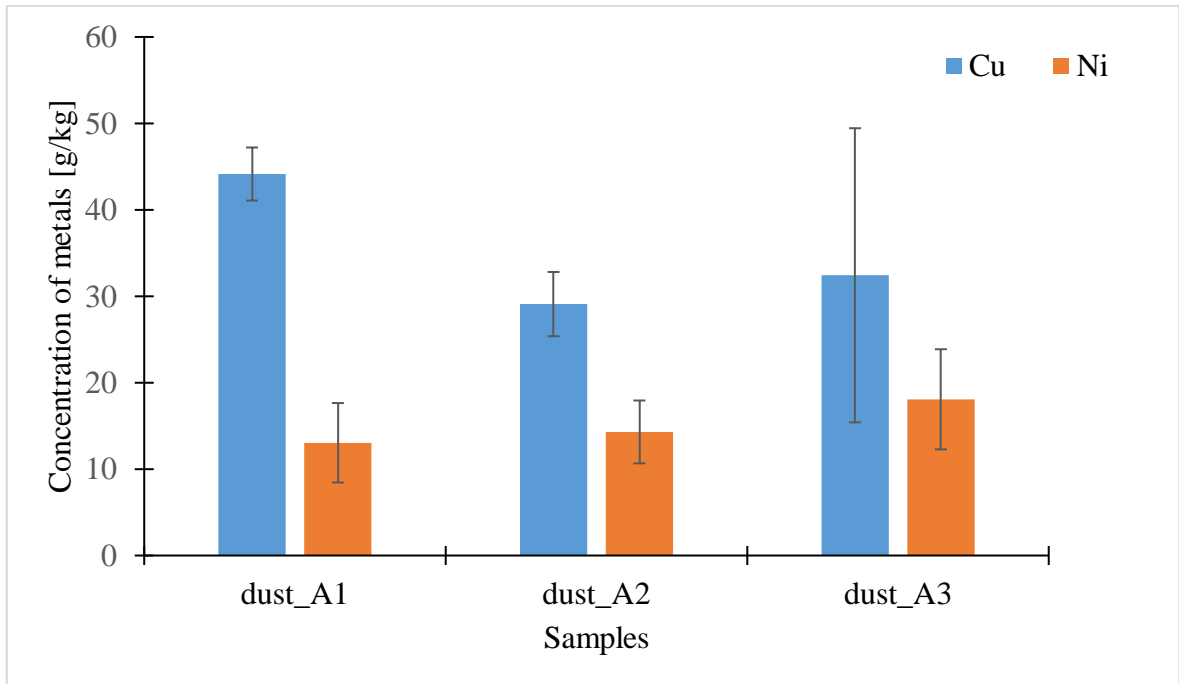
Figure 19 Copper and Nickel concentrations after 55 days of bioleaching

The leaching efficiency shown in **Table 12** indicates the increase in the recovery efficiency for Copper and Nickel after 55 days of bioleaching compared to the 24-hour bioleaching.

Table 12 Compared Copper and Nickel bioleaching efficiency after 24-hr and 55 days.

Samples	Ni %		Cu %	
	24-hr	55 days	24-hr	55 days
Dust_A1	74.8 ± 0.1	118.1 ± 0.5	8 ± 1.17	29.8 ± 1.2
Dust_A2	69.6 ± 0.8	122.5 ± 0.6	8.6 ± 0.63	19.7 ± 1.2
Dust_A3	63.1 ± 0.6	125.7 ± 0.7	8 ± 1.07	21.9 ± 0.9

CONCLUSION

The core of this study was to explore the efficiency of bioleaching in recovering metals from waste printed circuit boards (WPCB). The motherboard samples of different sizes (Dust and Coarse) were treated in biomediums inoculated with *Acidithiobacillus ferrooxidans*. As a result, experimental data showed an optimal sulphur addition of 1 g/L and 2 g/L. Moreover, these samples also demonstrated better metal recovery efficiency. This idea was also supported by the measured values of pH, ORP and iron (III) content.

Results from the AAS data obtained after 24 hours of bioleaching revealed promising concentrations and recovery efficiencies for various metals in WPCB waste. In 24 hours of bioleaching, Dust_A1 and Coarse_A2 samples revealed the highest concentrations of copper and nickel, with 74.8 and 100 %, respectively. On the other hand, Lead, manganese, and tin showed minimal to zero recovery efficiency across all coarse samples. Moreover, dust samples showed relatively better efficiency, especially for manganese, with the lowest 18.5 % and the highest 29.3 % for Dust_A2 and A3, respectively. Interestingly, the size difference between dust and coarse samples did not significantly affect the concentration levels of these metals. Nickel demonstrated remarkable recovery rates, particularly in coarse samples, with recovery efficiencies ranging from 85 % to 102.4 % across different sulphur concentrations. Conversely, lead, manganese, and tin exhibited negligible recovery rates in both dust and coarse samples, indicating challenges in their bioleaching.

The observation after 55 days showed that all dust samples showed an increase in recovery efficiency for nickel 100 % achieved in all samples, and copper recovery efficiency also greatly increased on the 55 days, with dust_A1 showing the highest increase from 8 to 30 %, highlighting no significant effect on impact of sulphur concentration and that longer period of contact increasing leaching capacity of the microorganisms and efficiency. These findings highlight the selective nature of bioleaching in recovering target metals from WPCB waste and emphasize the importance of optimizing bioleaching conditions for enhanced recovery efficiencies, particularly for metals with lower recovery rates.

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LIST OF ABBREVIATIONS

AAS - Atomic Absorption Spectroscopy

EEE - Electrical and Electronic Equipment

EOL - End of Life

EPR - Extended Producer Responsibility

EU - European Union

FAO - Food and Agriculture Organization

HM - Heavy Metals

ICTs - Information and Communication Technologies

LCD - Liquid Crystal Display

PCB - Printed Circuit Board

PM - Particulate Matter

POP - Persistent Organic Pollutants

RoHS - Restriction of Hazardous Substances Directive

SOB – Sulphur-oxidizing bacteria

SEM – Scanning Electron Microscope

UV-VIS - Ultraviolet–visible spectroscopy

WHO - World Health Organization

WEEE - Waste Electrical and Electronic Equipment

WITTE - Waste from Information, Technology, and Telecommunication Equipment

WPCBs - Waste Printed Circuit Boards

Mt - Metric Ton

MMT - Million Metric Tons

XRF - X-ray Fluorescence Spectroscopy

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Appendix P II: Appendix title