BIOLEACHING OF GOLD AND SILVER FROM MOBILE PHONE PRINTED CIRCUIT BOARDS (MPPCBS) USING *Pseudomonas balearica* SAE1

Project Thesis

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CERTIFICATE

This is to certify that the work submitted in this report entitled: “Bioleaching of precious metals gold and silver from mobile phone printed circuit boards (MPPCBs) using *Pseudomonas balearica* SAE1” submitted by Shivani Thakur (162551) in partial fulfilment of the requirements for the award of degree of Masters of Technology in Biotechnology, Jaypee University of Information Technology, Solan, has been carried out during a period from July 2017 to June 2018 under the supervision of Dr. Sudhir Kumar. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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LIST OF ABBREVIATIONS

Abbreviations	Full forms
MPPCBs	Mobile phone printed circuit board
MoEF	Ministry of Environment, Forests and Climate Change
PCBs	Printed Circuit Boards
EEE	Electrical and Electronic Equipment
RoHS	Restriction of the utilization of certain unsafe substances
Rpm	Rotations per minute
CPCB	Central Pollution Control Board
µm	Micro meters
%	Percentage
ºC	Degree Celsius
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyl
EC	Effective concentration
Mt	Million tones
OECD	Organization for Economic Cooperation and Development
NEPSI	National Electronics Product Stewardship Initiative
WSR	Waste Shipment Regulation
HARL	Home Appliance Recycling Law
MPPI	Mobile Phone Partnership Initiative
PACE	Partnership for Action on Computing Equipment
WEEE	Waste Electrical and Electronic Equipment

StEP	Solving the E-waste Problem
CI	Confidential interval

ABSTRACT

Electronic waste (e-waste) is known to be one of the major growing environmental issues around the globe. The global production of e-waste is increasing rapidly and is expected to accelerate in the near future. World has produced 5.9 kg/inhabitant (41.8 million tons) of e-waste and is expected to increase up to 6.7 kg/inhabitant (49.8 million tons) of e-waste by 2018. E-waste comprises of a multitude of components such as toxics metals, precious metals and persistent organic pollutants (PCBs and PBDEs).

These poisonous ingredients have adverse impact on human health and the surroundings if not controlled properly. On the other hand, e-waste is treated as secondary/artificial ore due to higher concentration of precious metals like Au, Ag and Pd. Among the e-waste, mobile phone printed circuit boards (MPPCBs) are the most important targets because of their rich metal content, high production volume and short life times. The total metallic content in a mobile phone is about 23%. Precious metals Au and Ag was in significant proportion i.e. 1800 ppm and 3589 ppm, respectively in MPPCBs. This makes MPPCBs recycling worth economic [1].The conventional hydrometallurgical and pyro metallurgical recycling of e waste consumes a large amount of energy and releases high volume of hazardous substance [24]. However, biohydrometallurgical technology which is based on microbial aided leaching of the precious metals (Au & Ag) from metals containing solid waste/ores/e-waste is an eco-friendly and cost effective technology. The MPPCBs waste in shredded dust and processed fine powder form of size <150um was procured from Exigo Recycling Pvt. Ltd. Haryana.

The present project highlights on the recovery of precious metals gold and silver from MPPCBs shredded dust and processed waste using *Pseudomonas balearica* SAE1,where two-step bioleaching was performed at an optimum condition i.e. at a pH of 9.0, a pulp density of 10 g/L, a temperature of 30°C, and a glycine concentration of 5 g/l.

Comparative toxicity assessment and its dose-response analysis showed higher tolerance of *Pseudomonas balearica* SAE1 to MPPCBs shredded dust i.e. EC50 = 15.6 g/l than for metals enriched waste MPPCBs i.e. EC50= 3.5g/l pulp density of the culture medium.

The results have shown that *Pseudomonas balearica* was able a mobilize 4.5% gold and 1.8% silver from processed MPPCBs e-waste and 4.01% Au and 4.06%Ag from shredded MPPCBs dust. Also pretreating the MPPCBs e-waste prior to bioleaching with nitric acid was able remove 79.9% and 90% Cu from both shredded dust and processed waste, respectively. This gave a positive effect on the recovery of precious metals gold and silver as 44% of Au was recovered from pretreated processed waste using *Pseudomonas balearica.*

CHAPTER 1.

INTRODUCTION

It has been observed that out of ten houses, every five house own obsolete electronic devices that might consist of outdated computers, mobile phones or laptops that apparently must have been covered in the dust laying on the surface of the desk and can be found in the trash can. These electronic waste otherwise end-of-life electronics are the main factors responsible for generating high toxic products and have become a possible danger to the environment.

As the change in an era with it has brought in various technological advances and innovations in the electronic items, has now massively improved the economic growth of the country and also has improved people’s lives in numerous ways. However, the increasing dependence of public on the short lifespan electronic machines both at the workplace and at home has led the way to the hazardous e-waste generation, which has been recognized as one of the major environmental challenges.

E-waste is now recognized as one fastest growing waste producing channel. Developing countries are now becoming the huge hazardous dump yard of e-waste as these electronic goods after a short period of time become unfit for their intended purpose. High economic growth, urbanization, industrialization and increased consumption have become the main reasons for massive generation and obsolesces of e-waste.

The global production of e-waste is increasing rapidly and is expected to accelerate in the near future. The world has produced 5.9 kg/inhabitant (41.8 million metric tons) of e-waste and is expected to increase up to 6.7 kg/inhabitant (49.8 million metric tons) of e-waste by 2018 [27].

E-waste comprises a multitude of components such as toxics metals, precious metals and persistent organic pollutants (PCBs and PBDEs). These toxic substances have an adverse impact on human health and the environment, if not recycled prior to disposal or handled carefully. On the other hand, e-waste is treated as secondary/artificial ore due to the higher concentration of precious metals like Au, Ag, and Pd. Among the e-waste, mobile phone printed circuit boards (MPPCBs) are the most important targets because of their rich metal content, high production volume and short lifespan [1]. Presence of copper and gold in e-waste are now becoming an important source in providing employment in the informal sector of upcoming industrialized or developing countries, but on the other side various safety issues arise as e-waste also generates toxic materials such as mercury, cadmium, lead, flame retardants and various toxic chemical present in the plastic. Various direct and indirect exposure to the amount and the type of e-waste are making children, who are more interactive to the environment more vulnerable to health risk in comparison to adults. Practicing primary recycling process such as the burning of e-waste to preserve the copper, has generated harmful by-products, which can further become the cause of spreading the risk of skin infection, respiratory disease, and cancer at a higher magnitude among the population. Thus, improper disposal of e-waste in landfills and other dumping sites, not only creates an adverse impact on the health of public but also affects the ecosystem at the utmost volume.

Many accumulated e-waste is not recycled as many countries do not implement proper rules and regulations and lack knowledge about e-waste management. Many poor countries cannot afford the expensive recycling of the e-waste and also lack proper technology to handle the harmful e-waste.

One of the major concerns in India is related to the workers at the recycling sites due to the low rate of literacy among them and the dearth of awareness about the hazardous potential of the e-waste. Many developed nations are sweeping their toxic e-waste pollutants to the poorest nations by selling and transporting large quantities of electronic products and dumping their e-waste in many countries like China, India for the recycling purpose. This has resulted in the persistent problems faced by many regions of these nations, demanding for environmentally sound options for the e-waste management.

Chemical leaching methods to recover gold and copper from e-waste and conventional recycling methods such as pyrometallurgy and hydrometallurgy consumes a large amount of energy, releases high volume of hazardous substances raising the concerns regarding possible formation of brominated and chlorinated di-benzo furans and dioxins in burning processes due to the presence of halogens in the plastic parts of electronic waste increasing cost of recycling and harming environment [15].

All these factors have resulted in shifting the focus toward bioleaching, a cheaper technology that has now become an alternate method that is replacing the traditional methods with the living organisms in extracting metals from their ore. Bioleaching is considered as one of the promising technology that uses cyanide producing bacteria to recover precious metals (Au and Ag) from our e-waste with economic and environmentally friendly advantages.

THE OBJECTIVES OF THE STUDY

1. Characterization of metal content of mobile phone printed circuit board (MPPCBs).
2. Toxicity assessment and dose-response analysis of the MPPCBs *on Pseudomonas balearica* SAEI.
3. Two step bioleaching of Cu, Au and Ag from MPPCBs shredded dust and processed e-waste using *Pseudomonas balearicia* SAE1 and *Chromobacterium violaceum*.
4. Pretreatment of MPPCBs e-waste and evaluating the metal leaching (Au Ag and Cu) capacity of *Pseudomonas balearicia* SAE1 and *Chromobacterium violaceum*.

CHAPTER 2.
REVIEW OF LITERATURE

2.1 Global e-waste generation

Improper and unsound disposal and treatment methods when applied to the increasing volume of electronic waste caused a remarkable risk to the human health as well to the environment. It has been calculated that approximately 75-90% of the population worldwide utilizes the electronic gadgets for their own motives and at the end, these electronic devices when of no use, are dumped by different countries based on the amount generated per inhabitant. In 2016, Asia was the area that was in charge of the generation of the biggest measure of e-waste i.e. around 18.2 Mt, trailed by Europe around 12.3 Mt, Americas (11.3 Mt), Africa (2.2 Mt), and Oceania (0.7 Mt) [22].

Among these nations, Oceania is viewed as the most noteworthy generator of 17.3 kg of e-waste per inhabitant, the second rank is involved by Europe that has created roughly of 16.6 kg/inhabitant. America, on the other hand, 11.6 kg/inhabitant. However, less e-waste (4.2 kg/inhabitant) was created by Asia and just 1.9 kg/inhabitant of e-waste was delivered by Africa.

As per National WEEE team, revealed that aggregate E-waste was around 146,000 tons/year in India in 2005 and it is estimated that around 90% of e-waste generated in India is managed by unauthorized sector and scrap dealers and they dispose of the products instead of recycling it [3]. On another hand, Central Pollution Control Board (CPCB) assessed that 1.347 lakh Mt of e-waste was created in the nation in the year 2005, which can be relied upon to achieve 8.0 lakh MT by 2012 **[**4]**.**

It was witnessed that the gross amount of e-waste reached 3.6 million tonnes in 2010 in China and had stretched to 5.5 million tonnes in 2013, and presumed to reach around 11.7 million tonnes by 2020 and 20 million tonnes by 2040.

There are a number of different modes that are causing the generation of e-waste. These involve developing different gadget, growth in cloud computing services, a growing number of data centers, and shorter replacement cycles. The overview of consumption rate of EEE in has also shown a speedy increase from 2000 to 2016. It has been evaluated that in 2017, the world e-waste generation shall cross 46 Mt. With an annual growth rate of 3 to 4%, it is estimated that growth of e-waste production may reach up to 52.2 Mt by 2021 as shown in Fig 2.1.

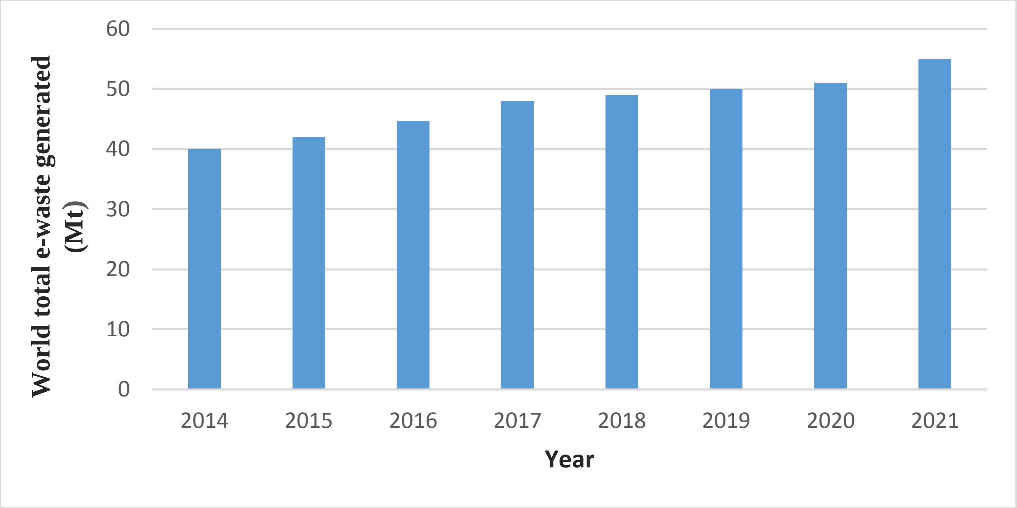

Fig 2.1: Global e-waste generated (Mt) [22]

2.2 E-waste management through regulations

The different nations and associations have created diverse methodologies for managing e-waste through various regulations.

According to European Union regulations, the Waste Shipment Regulation (WSR) was started in 1993 and corrected in 2007 was the main devoted e-waste control. It stresses that no EU part state is permitted to trade e-waste named unsafe to non-OECD (Organization for Economic Cooperation and Development) nations.

In 2003 the EU has passed e-waste Directive/enactment for changing item design and expanding reusing/recycling rates of disposed of WEEE and Restriction of the utilization of certain unsafe substances (RoHS). The RoHS directive tends to the start of the EEE life cycle by endeavoring to wipe out risky substances, for example, mercury, lead and fire retardants in locally delivered or imported electrical and electronic items [5].

The essential e-waste managing Packaging Directive was actualized into UK law through two segments of enactment:

1. The Packaging (Essential Requirements) Regulations 2003 (as amended) stated that packaging should be minimized so that it can be recycled and recovered and that dangerous substance (such as heavy metals) are restricted.

2. The Producer Responsibility Obligations (Packaging Waste) Regulations 2007 placed an obligation to reduce packaging on all UK companies on handling more than 50 tons of packaging each year.

In India, in 2006 The National Environment Policy distributed by the Ministry of Environment, Forests and Climate Change focused on the requirement for recuperation and reuse of any material that decreased the waste bound for conclusive transfer. Environmentally sound management instructions were discharged by CPCB in April 2008 which gave the directions to distinguish different origin of e-waste and a recommended systems for the treatment of e-waste [6].

India’s Ministry of Environment and Forest and climate change (MoEF) placed a legal liability for reducing and recycling electronic waste with producers for the first time under the E-waste (Management and Handling) Rules 2011 and came into effect 1st may 2012 that further restricted the usages of hazardous substances, which had become a major threat on dumping and causing hazardous effect on environment as well as human being.

In 1992 the Basal convention was drafted under United Nations Environment program to manage and direct the transboundary stream of dangerous electronic waste and their transfers [24].

Fig 2.2 : Percentages of obsolete EEE exported from USA

Different universal associations, for example, Mobile Phone Partnership Initiative (MPPI), solving the E-waste Problem (StEP), Partnership for Action on Computing Equipment (PACE), National Electronics Product Stewardship Initiative (NEPSI) WEEE Forum were propelled to control the e-squander issue [7].Japan propelled the Home Appliance Recycling Law (HARL) and Small Appliance Recycling Law to build the reusing rate because of the shortage of land mass for strong waste transfer.

2.3 Categories and hazards present in e-waste

Electronic waste, or e-waste, refers to all things of electrical and electronic hardware (EEE) and its parts that have been disposed of by its proprietor as waste without the goal of re-utilization. E-waste is also defined as WEEE (Waste Electrical and Electronic Equipment), electronic waste or e-scrap in various districts and under various conditions on the planet.

It incorporates an extensive variety of items - Hardware or electrical parts with power or battery supply. It covers six waste classes**:**

Table 2.1: Different categories of electronic waste [22]

CATEGORIES OF E-WASTE	**EXAMPLES**
Cooling And Freezing Equipments	Refrigerators, freezers, air conditioners, heat pumps.
Screens, Monitors	Televisions, monitors, laptops, notebooks, and tablets.
Lamps	fluorescent lamps, high intensity discharge lamps, and LED lamps
Small Equipments	vacuum cleaners, microwaves, ventilation equipment, toasters, electric kettles, electric shavers, scales, calculators, radio sets, video cameras, electrical and electronic toys, small electrical and electronic tools, small medical devices, small monitoring and control instruments.
Large Equipments	washing machines, clothes dryers, dish-washing machines, electric stoves, large printing machines
Small IT And Telecommunication Equipments	Mobile phones, Global Positioning Systems (GPS), pocket calculators, routers, personal computers, printers, telephones.

EEEs are made of a large number of segments, some containing dangerous substances that adversely affect human well-being and nature if not took care of appropriately.

Regularly, these risks emerge because of the despicable reusing and transfer forms used. Improper recycling of e-waste and disposal methods used can causes a high magnitude of damage to environment and people working in this sector.

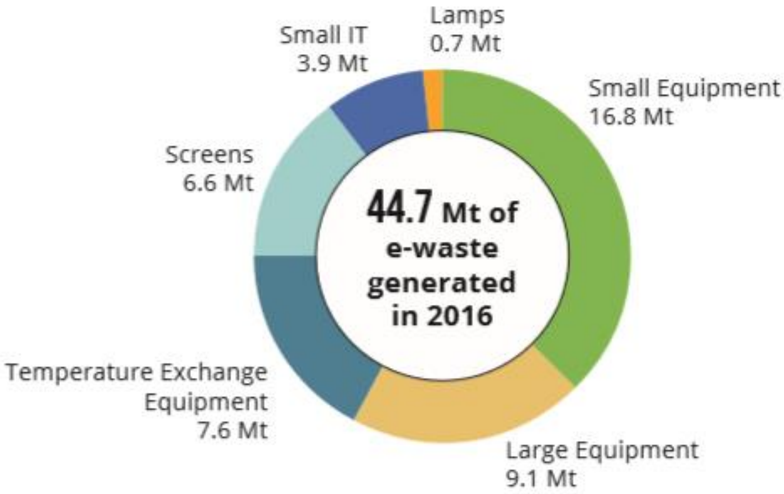

Fig 2.3 Estimates of e-waste totals per category in 2016 [22]

Table 2.2: Human and Environment Hazards from electronic waste [3]

E-WASTE COMPONENT	DISPOSAL PROCESS	HUMAN HEALTH HAZARD	ENVIRONMENTAL HAZARD
Printed circuit boards	De-soldering and removing computer chips	Tin and lead inhalation, brominated dioxin, beryllium, cadmium and mercury inhalation	Air emission of same substances
Chips and other gold plated components	Chemical stripping using nitric and hydrochloric acid along riverbanks	Acid contact causing damage to skin and eyes	Hydrocarbons, heavy metals, discharged directly into river and banks.
Cathode ray tubes (CRTs)	Breaking, removal of copper	Cuts from CRT glass, Inhalation or contact with phosphor containing cadmium or other metals	Lead, barium and other heavy metals leaching into groundwater, release of toxic phosphor

Plastics from mobile phones ,computer and peripherals, e.g. printers, keyboards,	Shredding and low temperature melting to be reutilized in poor grade plastics	Hydrocarbon, brominated dioxin, and heavy metal exposures	Emissions of brominated dioxins and heavy metals and hydrocarbons

Various other metals present in the electronic waste such as Lead has caused poisonous impacts on the different frames of the body by damaging all the parts of nephrons, causing anemia and had become another root cause in damaging male and female reproductive organ. Mercury and cadmium are another harmful and carcinogenic compounds that are present in many parts of electronic waste and on improper disposal and on reaching contact with these elements leads to damage of peripheral and nervous system, causing tubular dysfunctioning and gets accumulated in the body through the food chain [7].

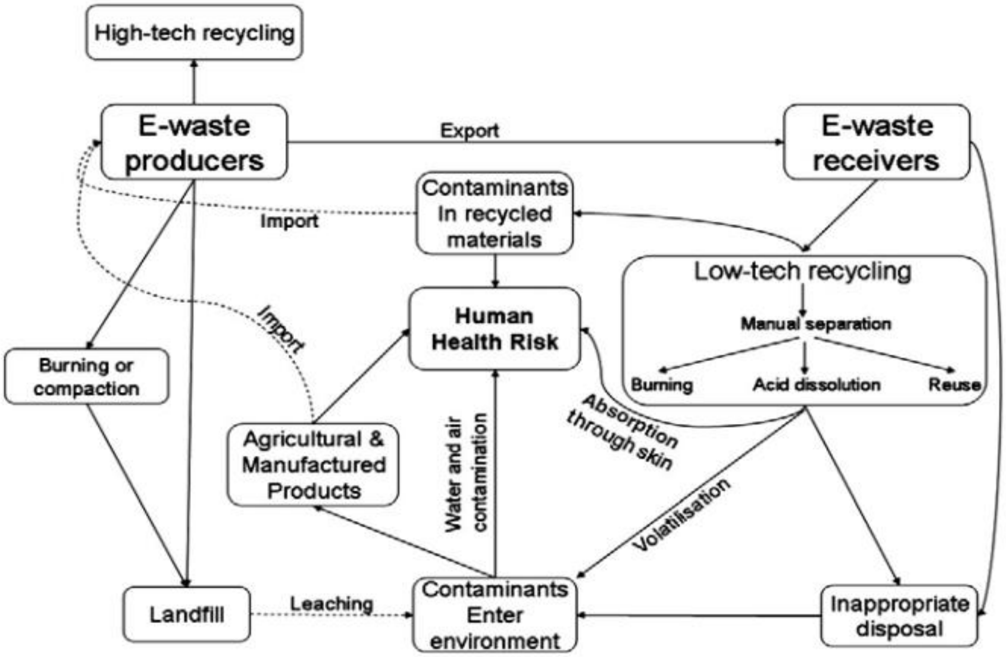

Fig 2.4: Roots of contaminants associated with E-waste from producers to receivers [7]

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2.4 Economic importance of electronic waste

Most of the metals, such as precious metals (gold and silver) are present in the electronic waste. But the conventional recycling of valuable metal mining and recuperation is expensive because of the high utilization of energy and utilization of chemicals for metal mobilization and the process can be hazardous. Mining practices are nowadays constrained by limited high-grade ores due to the exploitation of the natural resources joined with restrictions on the import of profitable metals to different countries.

Following dumps gathered at gold mine locals, electronic waste and other eccentric assets can be utilized to recover valuable metals [19]. Electronic waste thus can be considered as “artificial” or “secondary ore”[10] higher consisting higher concentration of precious metals than the natural ores thus making the recycling of e-waste an important subject in an environment and economic context.

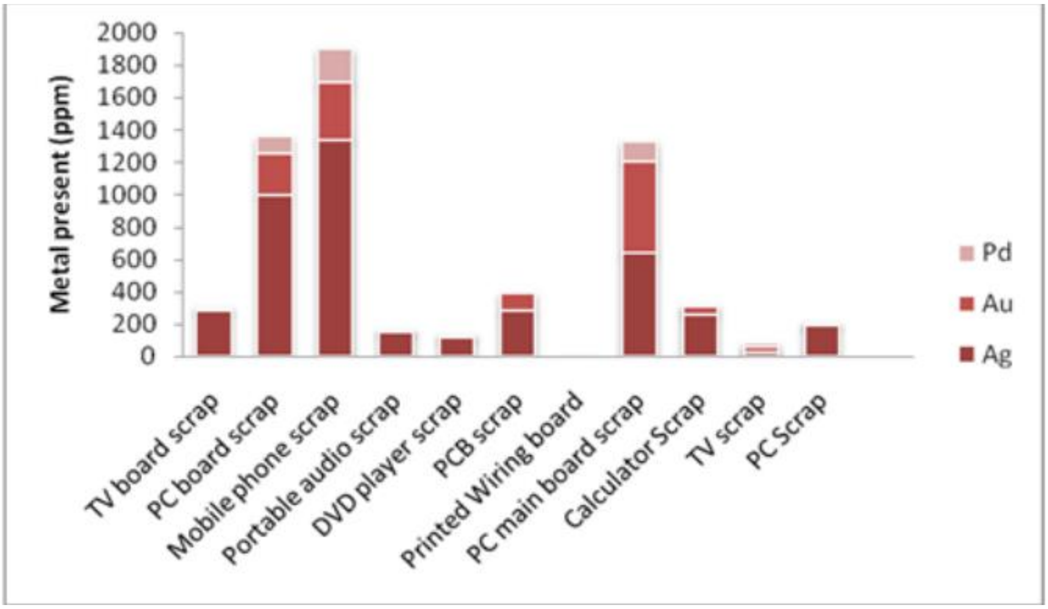

Fig 2.5: Precious metals present in various WEEE [8]

For every 1 million discarded mobile phone that are recycled around 35,274 lbs of copper, 772 lbs of silver, 75 lbs of gold, and 33 lbs of palladium can be recuperated. PCBs contain high measures of valuable metals; around 20 wt% copper, 0.04 wt% gold, 0.15 wt% silver, and 0.01 wt% palladium [30]. The extraction of these metals from PCBs is both productive and earth beneficial.Due to this characteristics e-waste are now considered as an "urban mine" as they are fundamentally wealthier in gold than the wellsprings of the essential metals. 200-250 g/t of gold are known to be found in PC circuit sheets.The metal substance of waste PCBs can be as high as 40% and such metals regularly incorporate Cu, Sn, Pb, Cd, Cr, Zn, Ni, and Mn. In this manner, PCBs are considered as an important tool during the recovery of electronic scrap. These waste PCBs are considered as the rich optional wellspring of important precious metals [30].

Considering the depletion rate of high concentration gold ores and the high demand and value of gold, gold recovery has become a high priority in the recycling of ESM. Electrical and electronic equipment constitute the largest contribution in the industrial demand for gold which is about 350-400 tons annually.

Gold is widely used in electrical and electronic products because of its numerous unique properties such as excellent corrosion and oxidation resistance, high electrical conductivity and low electrical resistivity. It is commonly used as coatings on electrical connectors and contacts, printed circuit boards and semiconductors. Compared with the gold content in natural gold ores which is around 0.5 to 13.5 grams gold per ton, the gold content in ESM is significantly higher at around 10 to 10000 grams gold per ton [9].

2.5 Conventional methods of recycling e-waste

There are various benefits that can be obtained from recycling and recovery of valuable metals from electronic waste. Electronic waste recycling can primarily be divided into 3 main steps, consisting of disassembly, upgrading and refining [9]. During selective disassembly, hazardous or valuable components are separated for special treatment. This is an essential step in the electronic waste recycling process. The upgrading step involves mechanical processing to increase desirable metals content and hence, prepares materials for the refining process. Lastly, in the refining stage, metal extraction takes place either through pyrometallurgical and hydrometallurgical processing where impurities are removed. Purification and pretreatment such as precipitation are then carried out to convert the metal solution to solid state Pyrometallurgical processing includes incineration, smelting in blast furnaces, sintering, melting and gas phase reactions at high temperatures. Cui and Zhang (2008) have discussed typical pyrometallurgical methods used for the recovery of metals from electronic waste. At elevated temperatures, metal compounds become relatively unstable, hence facilitating the release of metals. This method is commonly used to recover non-ferrous metals such as copper and precious metals such as silver from electronic waste in the past two decade [9]. Limitations to pyrometallurgical methods include the formation of toxic dioxins from the presence of halogenated flame retardants in the smelter feed, limited separation of metals that needs further hydrometallurgical or electrochemical processing and requirement of high grade feed and high energy costs.

Hydrometallurgical methods involve the use of aqueous chemistry and chemical properties of materials to extract metals from electronic scrap. The solutions containing the metals of interest are then subjected to separation procedures such as solvent extraction, precipitation, cementation, ion exchange, filtration and distillation to isolate and concentrate the metals of interest.

This method, while cheaper (e.g. lower power consumption and recycling of chemical agents is usually not efficient in that precious metals encapsulated within the ceramic part will not be recovered by acid leaching (Sum, 1991). Various reagents have been used in the chemical leaching of base and precious metals from electronic scrap. Extensive research has been reported on the recovery of both precious metals such as gold, silver, platinum and base metals such as copper, nickel, lead and zinc from electronic scrap [9] [32].

2.6 Bioleaching: a microbial process for metal recovery

Utilization of biotechnology in mining has been explored in different research associations and ventures inside an expansive number of nations for as far back as 40 years.

Utilization of microbial innovation in the mining business has demonstrated a dynamic improvement in metal extraction.Bioleaching, on the other hand, refers to the abilities of microorganisms such as bacteria and fungi to convert insoluble solid minerals into soluble form, which can be extracted. Here the metal of interest is recovered in the solution [10] [33].

Most bioleaching studies have focused on two types of microorganisms, namely mesophilic bacteria, and thermophilic archaea. Mesophilic bacteria are commonly used in bioleaching as ambient operating temperatures are suitable, while large amounts of energy must be supplied to systems using thermophilic archaea to provide a higher temperature for optimum growth[17].Examples of mesophilic bacteria include *Chromobacterium violaceum, Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Bacillus megaterium* and *Pseudomonas aeruginosa. Examples of thermophilic archaea include Acidianus spp. and Sulfolobus spp* [2].

2.6.1 Principles of microbial metal leaching

Microbially metal-extraction forms are normally more eco-accommodating than physicochemical procedures. They don't utilize a lot of energy when contrasted with smelting and roasting and don't create sulfur dioxide or other unsafe gases [33].

Microorganisms can mobilizes metals through the accompanying procedure:

1. Formation of organic and inorganic acids (proton formation).

2. Excretion of complexing agents (ligand formation).

3. Oxidation and reduction reactions.

2.6.2 Mechanisms of bioleaching

At first, a model including two components was proposed namely direct and indirect methods to clarify the solubilization of sulfide minerals using microbes.

Oxidization of metal sulfides are carried out by microorganism by the means of a direct mechanism diminished minerals provide electrons directly and the process works along with various enzymatically catalyzed reactions. For this situation, cells must be joined to the mineral surface and a nearby contact is required. The adsorption of cells to suspended mineral particles happens inside a few minutes or hours.

Another method called the indirect mechanism, where the oxidation of diminished metals are interceded by ferric (III) particle and this ferric is generated by microbial oxidation of ferrous which are oxidized in the minerals. Basically, the microbes release the lixiviants, which further chemically oxidized the sulfur [33] [10].

Due to the heterogeneity of the metals present in the e-waste, reusing of these waste are now becoming difficult. Conventionally, metal recovery was carried out using various chemicals but due to their toxicity and ability to cause detrimental effects on the health of human and leading to environmental damage, bioleaching using cyanide producing microorganism was sound leaching mechanism for the mobilization of the precious metals from electronic waste.

2.6.3 Benefits of bioleaching

•Economical: microbial leaching are typically easy to achieve and manage in contrast with conventional methods used, and fewer professionals are required and do not require any kind of sophisticated instruments when performed [33].

•Environmental: Bioleaching is a green technology in comparison with the ordinary mobilization methods used. Since the bacterias indulged in the recovery of metals grow naturally, causing less damage to the landscape.

•Microbial leaching is a low-cost approach in the obtaining metals from the low-grade ores or from the electronic waste when compared to the chemical leaching process which faces the major challenge in maintaining the cost when operated on depleted or limited ores.

•Microorganisms utilized in bioleaching are less expensive in comparison to regular procedures, making the bio-recuperation process a perfect, low utilization of energy and economical one.

2.7 Cyanide production by bacteria

Cyanidation is broadly utilized as a part of gold metallurgy to extract gold from minerals because of a blend of its accessibility, adequacy, financial aspects, despite the way that cyanides are profoundly lethal. Around 90% of the critical gold generating mechanism around the globe at present uses cyanide for extraction of gold [34].

Cyanide is produced as a secondary metabolite by many bacteria and fungi and these microorganisms are used in the mobilization of metals from the electronic scrap and are known as cyanogenic microorganisms [45]. In the cyanide-producing mechanism of cyanogenic bacteria, cyanide is produced by oxidative decarboxylation of glycine, a reaction which is mobilized by the enzyme hydrogen cyanide (HCN) synthase in C. violaceum [40]. There is numerous literature reporting on the different intermediates formed during the conversion of glycine to cyanide. In one of the widely reported mechanisms by Wissing (1974) and Knowles et al. (1986), glycine is primarily oxidized to iminoacetic acid and cyan formic acid. This is then followed by a dehydrogenase reaction which produces hydrogen cyanide and carbon dioxide by splitting the C-C bond .Figure 2.6 summarizes the cyanide-producing mechanism.

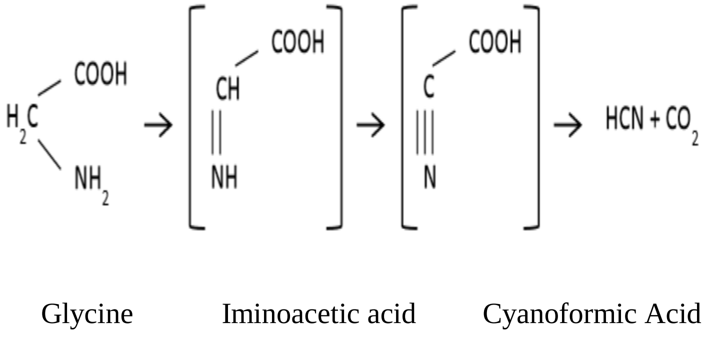

Fig 2.6: Cyanide production mechanism in cyanogenic bacteria [34].

Table 2.3: **Some of the reported cyanide producing microorganisms [38]**

BACTERIA	**FUNGI**
Chromobacterium violaceum	*Marasmius oreades*
Pseudomonas fluorescens	*Clitocybe sp.*
Pseudomonas plecoglossicida	*Stemphylium loti*
Pseudomonas aureofaciens	*Gloeocercospora sorghi*
Bacillus megaterium	
Pseudomonas putida	

Chromobacterium violaceum:

For *Chromobacterium* violaceum, cyanide production typically occurs for short period at the early stationary phase and can exist as free cyanide in solution which includes the form of cyanide ion (CN-) and hydrocyanic acid (HCN) [40].

Towards the late stationary and death phase, *C. violaceum* is able to detoxify itself of the cyanide by the bacteria producing an enzyme, β- cyanoalanine synthase which degrades cyanide into β-cyanoalanine [34]. HcnA, HcnB, and HcnC are the operon present in HCN synthase (in the *C. violaceum genome*, encoding a formate dehydrogenase and two amino corrosive oxidases, individually, which are engaged with the cyanic corrosive combination In the cyanide combination process, the four electrons created by HCN synthase are exchanged to oxygen, likely all through the respiratory chain. These responses happen at low levels of oxygen. So HCN is for the most part produced in vigorous conditions.Therefore aerobic conditions are the responsible for producing HCN [38].

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Pseudomonas: *Pseudomonas* are gram-negative bacteria and some of the reported species of *Pseudomonas* that produce cyanide and are used in bioleaching consists of *Pseudomonas plecoglossicida*, *Pseudomonas fluorescens* (*P. fluorescens)* and *Pseudomonas aeruginosa*.It was reported that due to the oxidative carboxylation of glycine these *Pseudomonas species* discharge around 300 µM of cyanide during the solubilization of metals from e-waste.

P. fluorescens:

They are known to produce a higher amount of toxic cyanide when compared to other cyanide producing microorganism. Despite the fact that P. fluorescens delivered more cyanide than *C.* violaceum without e-waste, it was observed to be more proficient in the bioleaching of gold from scrap electronics , on the grounds that *P. fluorescens* showed a higher development rate, a highermetal protection, and a more positive kinetics compared to *C. violaceum* within the sight of e-waste.

Pseudomonas aeruginosa:

P. aeruginosa, opportunistic pathogens and has been accounted for causing infections in people, creatures, and plants. The capacity of *P. aeruginosa* to harm people and plants has been attributed to its creation of different emitted and surface-related destructiveness factors. Aside from different protein poisons released, *P. aeruginosa* additionally delivers little toxic substance, for example, cyanide that encourages the general harmfulness of this bacteria against various host Cyanide in this bacteria is produced at the at the beginning of the stationary phase at an optimal temperature 34℃ to 37℃.

2.8 Reported studies on cyanogenic microorganisms in gold leaching efficiency from electronic waste.

“Metals bioleaching from electronic waste by *Chromobacterium violaceum* and *Pseudomonads* sp” : Jatindra Kumar Pradhan and Sudhir Kumar (2012) [12]

In this paper *Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* which are the cyanide-producing bacteria were examined for their efficiency in bioleaching of metals from electronic waste.

At an electronic waste concentration of 1% w/v, *Chromobacterium violaceum* was capable of solubilizing more than 79, 69, 46, 9 and 7% of Cu, Au, Zn, Fe, and Ag, respectively. When combinations of mixed cultures of cyanogenic bacterial strain i.e. *C.violaceum* and *P. aeruginosa* were used in bioleaching mechanism, it resulted in higher solubilization of copper, gold, silver zinc and iron from the total metals present in the electronic scrap.

“Gold Bioleaching of Electronic Waste by Cyanogenic Bacteria”: V.A. Pham and Y.P. Ting (2009)[46]

This work demonstrated about gold bioleaching from e-waste containing gold and copper by *Chromobacterium violaceum* and *Pseudomonas fluorescens*.

P. fluorescens demonstrated a higher gold leaching capability than *C. violaceum* at every pulp density of e-waste applied and a greater concentration of gold at 2.5 mg/l at 0.5%v/w was achieved.

“Bioleaching of Gold and Silver from Waste Printed Circuit Boards by *Pseudomonas balearica* SAE1 Isolated from an e-Waste Recycling Facility”:Anil Kumar · Harvinder Singh Saini and Sudhir Kumar (2018) [12]

In this paper, *Pseudomonas balearica* SAE1 which was isolated from the recycling is and was further investigated for the mobilization of gold and silver from the waste printed circuit board and it showed that the bacteria was able to leach out 33.8 % of silver and 68.5% of gold from the waste.It showed that *Pseudomonas balearica* was an efficient cyanide producing microorganism in bioleaching of precious metals from electronic waste.

“Two-step bioleaching and spent medium leaching of gold from electronic scrap material using *Chromobacterium violaceum*”:Gayathri Natarajan and Yen-Peng Ting (2013) [47]

In this paper, the authors used *Chromobacterium violaceum* for the recovery of the gold from the electronic scrap using two-step and spent media leaching and it was found that c*hromobacterium violaceum* was successful in leaching out gold from both leaching process from the e-waste.

2.9 Factors influencing the bioleaching of gold

Bioleaching of gold from electronic waste using microorganisms can be enhanced by optimizing some of the parameters such as pH, pulp density, temperature, oxygen supplied and composition of the media used for the growth of an organism.

1. pH:-

At physiological pH 7.0, cyanide is available chiefly as HCN and is unstable. As the expansion in pH, the instability is decreased. Thus, the presence of CN-in the mixture is exceptionally reliant on pH. It was seen that $Cu(CN)_2$ was more steady at pH under 9.0 while $Cu(CN)_3$ and $Cu(CN)_4$ was present in a large amount at pH over 9.0 in the nearness of high cyanide.In this way, high er stability of metal cyanide complexes and expanded steadiness of HCN at pH > 10 enhanced the metal mobilization at higher pH [37] .

It was reported by Chi et al. the cell population started to diminish with an expansion in the pH of the medium. At pH 7.4, the cell population achieved a most extreme after 5 d of culture, while at pH 11.0, the cell population was reduced to a lower number. Gold extraction elevated from 7.78% to 10.9% with an expansion in pH from 8.0 to 11.0 out of 8 days, and mobilization of the gold was found to be ideal at 10.0.This was credited to the necessity of a higher pH to maintain the stability of the metal cyanide mixture.

2.Oxygen concentration :-

Oxygen is not just an essential factor for the growth of microorganisms yet, in addition, the receptor of the electron as the response. *C. violaceum* uses the oxygen in the media at the stationary and growth phase during the gold leaching from the e-waste.

Kita et al (2006) inquired that, the dissolved oxygen was decreased steeply during the growth phase (0 to 48 hours) and stayed low (0.05 mmol/L) in the stationary stage. This decline represses the cathode response and reduced the Au mobilization rate. Air circulation elevated the oxygen level and enhanced the bioleaching of the gold [48].

Introduction of H_2O_2 is another strategy to supplement the oxygen. It was seen that the expansion of 0.004 % (v/v) H_2O_2 increased the bioleaching of gold.

3. Pulp density:-

It was noted that an elevation in the pulp density in the media brought about an abatement in the removal of gold and an increment in copper expulsion]. The decline in gold mobilization emerged from the toxicity of the waste that hindrance the growth of bacteria and cyanide production [40].

Y.P. Ting et al (2013) examined that the resilience differs with the kinds of microorganisms. *P. fluorescens* showed a higher growth rate (from OD estimations), a higher metal protection and an ideal kinetics during leaching than *C. violaceum* within the sight of electronic scrap.

Pseudomonas fluorescens demonstrated higher gold removal effectiveness than *C. violaceum* at a metal concentration running from 0.5% to 8% [26].

4. Nutrients supplied:-

The measure of cyanide created by *C. violaceum* firmly relies upon the medium in which it is developed. In past examinations, media with various nutrients in the media such as tryptone (10 g/L) and yeast extricate (5 g/L) [11, 21], or polypeptide (10 g/L) and yeast separate (2 g/L) [23-25] have been utilized. Other than these, some metal particles/ions, for example, $Na^{+,}$ $Mg2^{+}$, $Fe2^{+}$ added to the medium in low focuses can catalyze and improve the cyanide production capability of *C.violaceum*.

Studies have demonstrated that cyanide creation by bacteria was supported by the introduction of glycine to the growth media, that further enhanced the gold leaching efficiency of bacteria.

According to literature, when glycine (8 to 10 g/l) was added to cultures of *C. violaceum*, cyanide concentrations of up to 200 ppm were observed [32]. The same work also shows that when glycine addition was increased (>10 g/l), cell growth was observed to be inhibited. The time of addition of glycine is equally important as the concentration of glycine added; when glycine addition is delayed till the start of the stationary phase, higher cyanide production was attained whereas early glycine addition resulted in bacterial growth inhibition

5. Temperature:-

Temperature has a significant effect on bacterial growth, enzyme-catalyzed reactions and the secondary and tertiary folding of proteins. Each bacteria type has a narrow range of temperature that is optimal for growth. Beyond a certain temperature, the death of bacteria sets in as vital enzymes is denatured and loses their functions. Besides bacterial growth, temperature also affects cyanide production rate, volatilization and loss of cyanide, gold cyanidation rate and the equilibrium of the cyanide metal complexes formed, all of which affect the gold bioleaching efficiency.

2.10 Pretreatment of electronic waste

Contrasted with ordinary recycling strategies, the removal of gold from electronic waste utilizing bioleaching process is eco-friendly and less hazardous. The obsolete printed circuit board contains a large amount of copper in comparison with other metals. During the bioleaching of precious metals from the PCBs, copper starts competing with gold as Copper cyanide is produced in the form of various complexes, such as $Cu(CN)_2$, $Cu(CN)_3$and $Cu(CN)_4$, and other conditions, whereas gold mostly forms dicyanide anions ($Au(CN)_2$). and forms a stable copper- cyanide complex in the solution, thus reducing the efficiency of gold leaching.

In order to enhance the gold leaching and overcome this situation, the electronic waste is pretreated with Nitric acid or sulphuric acid assisted hydrogen peroxide to eliminate a large amount of copper from the e-waste [40].

CHAPTER 3.

MATERIALS AND METHODS

3.1 Chemicals and instrumentation involved

Perkin Elmer AAnalyst 400 Atomic Absorption Spectrophotometer, pH meter, Vacuum pump, 0.45µm Optical fibre filter Centrifuge Air pump, Spectrophotometer, Refluxing instrument, Nitric acid Hydrochloric acid, Luria broth and Nutrient Agar

3.2 Source of e-waste

Shredded dust and processed waste of obsolete MPPCBs I the form of fine dust powder were obtained in zipper storage bags from the Exigo Recycling Pvt. Ltd., Panipat, and Haryana, India.

The two black colored processed and dust MPPCBs e-waste were generated by the company by collecting the MPPCBS from various areas of the country and were further subjected to various physical and mechanical separation processes like shredding, dismantling and pulverizing during the recycling process .

The particle size of both the waste of MPPCBs were less than 150 µm determined using standard test sieves as per IS 460:1962 were used in the present work as shown in the Figure 3.1.

a) Processed MPPCs e-waste **b) Shredded MPPCBs e-waste**

Fig 3.1: Two type of MPPCBs e-waste

3.3 Microorganisms and culture conditions

Pseudomonas balearica SAE1 and *Chromobacterium violaceum* (MTCC 2656) were procured from the environment biotechnology Lab., of JUIT, Sloan, and HP.

Both the bacteria were inoculated in100 mL of Luria broth (LB) in 250-mL Erlenmeyer flasks and the flasks were incubated in an incubator shaker at 30 °C and 150 rpm for 24 hrs.

OD of both the organisms was taken using spectroscopy at 660 nm to check the growth. Further, the microorganisms were preserved on the Nutrient agar (NA) and all stock bacterial cultures were stored in a deep freezer at -80^{0}C, supplemented with 30% glycerol.

3.4 Metal content analysis

Both shredded dust and processed MPPCBs e-waste were put through the acid digestion using aqua regia (HCl: HNO_3 = 3:1) to determine the metals present in them [36].

One gram of both MPPCBs e-waste was dissolved in 100 ml of aqua regia in a round bottom flask. Refluxing was carried out in the round bottom flask for 1 hour at 100°C. The solution as shown in fig 3.3 was then cooled and using de-ionized water the volume was made up to 100ml.

The solutions were filtered through .45µm glass fiber filter to ensure the free suspension. The samples further received were then diluted 10x times with the respective solvents of the metals to be detected. The concentration of dissolved metal ions was determined using Perkin Elmer A Analyst 400 Atomic Absorption Spectrophotometer and was later stored in the bottle at 4°C.

3.5 MPPCBs e-Waste Toxicity Tolerance Levels and Dose–Response Analysis

Bacteria *Pseudomonas balearica* SAE1 was examined for its toxicity tolerance at at 1, 5, 10, 15 and 20% g/l pulp density of both dust and enriched form MPPCBs e -waste.

Bacterial growth was the parameter to determine the toxicity of MPPCBs e-waste at respective pulp density in terms of the colony-forming unit (CFU) count method. The experiments were performed in a 250-mL Erlenmeyer flask containing 100 ml LB medium. The flasks were inoculated with 1% (v/v) of inoculum containing 8 × 10^7 CFU/mL, along with the respective concentration of sterile MPPCBs e-waste. Erlenmeyer flasks containing LB medium and bacterial cells without MPPCBs shredded dust and processed e-waste were kept as controls.

All flasks (in duplicates sets) were incubated at 30°C at 150 rpm for 24 h. After 24 h, samples from each flask were taken following enumeration of bacteria by serial dilution and spread plate methods. For the dose–response curve, percent inhibition response was calculated as shown in Eq. (3.1):

$$\%\ \mathrm{IR} = \frac{\text{Control} - \text{Test Control}}{\text{Control}} \times 100 \qquad \ldots\ldots \quad \textbf{Eq.3.1}$$

Where IR is the inhibition response, Control is bacterial growth in the absence of e-waste, and Test is bacterial growth in the presence of e-waste.

Nonlinear regression was performed using Graph Pad Prism 6 (Graph Pad Software, Inc., La Jolla, California). The dose–response curve was determined using log (agonist) versus normalized response-variable slope procedure in Graph Pad Prism 6 [25]. EC50 value (statistically derived estimate about the concentration of a substance resulting in 50% reduction of growth in a specified time period) was estimated based on dose–response data.

3.6 Pretreatment of MPPCBs e-waste

Nitric acid was used to remove copper from both form i.e. Shredded dust and processed MPPCBs e-waste because its abundance exerted a significant interference to the cyanide-gold ion complex formation. Preferential copper dissolution over gold may be attributed to two reasons.

I. High grouping of copper frames complex with the cyanide which would have been accessible to tie with the gold.

II. Gold is more noble (E0 Au3+/Au: 1:52 V) than copper (E0 Cu2+/Cu: 0.34 V)

For conducting acid pre-treatment of both the forms of MPPCBs e-waste, the 6M nitric acid solution was prepared by diluting 193 ml of 68% of concentrated nitric acid with 307 ml of distilled water and to this solution, 7g of MPPCBs e-waste was added to 21 ml of 6M of nitric acid in 250 ml Erlenmeyer flask.

In order to avoid excessive frothing that occurred due to the production of nitric oxide gas, MPPCBs e-waste was incrementally added to the nitric acid solution using Atomic Absorption Spectroscopy (Perkin Elmer AAanalyst 400).

The remaining supernatant was discarded and the residue was washed with ample amount of deionized water and re-centrifuged. The cycle was repeated until traces of blue-green copper nitrate were no longer visible as shown in fig4.9. The pretreated e-waste pellets were finally removed, dried and weighed to constant mass, which was further subjected to determine the metal content using acid digestion method [20].

3.7 Two-step bioleaching process

Direct growth of micro-organisms in the presence of electronic waste is not recommended as e-wastes are highly toxic to the organisms hindering their growth and reducing leaching of metals. (Brandl et al., 2001).

A maximum concentration of metals can be solubilized in the solution by using two-step bioleaching that helps in increasing the efficiency of the microorganism to leach out precious metal from the MPPCBs e-waste [11].

In the process, *Pseudomonas balearica* SAE1 and *Chromobacterium violaceum* were inoculated into sterile 100 ml LB medium in the absence of MPPCBs e-waste in different 250 ml flask and was incubated at 30 °C and 150rpm for 48 h. 0.5 g of glycine was added to the LB media and pH 9 was maintained by adding sodium hydroxide pellets to the media. In this process, 5% (v/v) inoculum was added to the LB media. After 48 h both shredded dust and processed MPPCBS e-waste was added to culture flasks and incubated for a time period of 7 days. A control flask containing LB media and MPPCBs e-waste was kept running simultaneously. Two-step bioleaching was performed for both pretreated and untreated MPPCBs e-waste.

After a period of 7 days, the sample was centrifuged (Eppendorf Centrifuge 5804 R) at 7000 rpm for 15 min to remove solid particles (e-waste).The supernatant/leachate was collected and analyzed for the presence of metal ions using atomic absorption spectrometry (PerkinElmer AAanalyst 400; PerkinElmer) on respective wavelengths and the final pH of the leachate was calculated by portable digital pH meter (Eutech pH Testr30; Thermo Fisher Scientific, Inc., Massachusetts, USA.

Before analyzing the metals present, the supernatant was passed through a 0.45-µm glass fiber filter (PALL-GF-A/E-I) to ensure particle-free suspension.

CHAPTER 4.
RESULTS AND DISCUSSIONS

4.1 Revival of *Pseudomonas balearica* and *Chromobacterium violaceum*

Chromobacterium violaceum as shown in Fig 4.1 is a gram-negative bacterium (facultative anaerobe) is rod-shaped and often appears as small rods or coccobacilli that are 0.6-0.9 µm by 1.5-3 µm in size. This bacterium is mesophilic and grows in between 15 to 40º C with best in the range of 30 to 35^0 C. *Pseudomonas balaerica* (Fig 4.2) is also gram negative and are also found in soil, marshes, coastal marine habitats, and plant and animal tissue; generally, these bacteria can tolerate a variety of physical conditions and grows best at an optimum consitions 30^0 C. Both the organisms were transferred to sterile nutrient agar plate (streaked) and strored at 4ºC for further experiment.

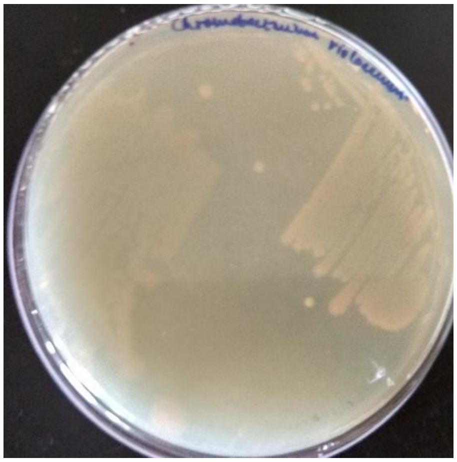


Fig 4.1: ***Chromobacterium violaceum***

Fig 4.2: ***Pseudomonas balaerica***

4.2 Metal content study of MPPCBs e-waste

The MPPCBS e-waste i.e. shredded dust and the processed waste of particle size less than 150um were analyzed for the metal analysis content by acid leaching i.e. aqua regia method. One gram of both the form of MPPCBs e-waste was dissolved in 100ml of aqua regia (HNO_3: HCl = 1:3) and was refluxed in round bottom flask at 100 c for 1 h.

The concentration of base metal copper was highest in both MMPCBs shredded dust 220mg/g (22%) and processed e-waste MPPCBs 690mg (69%) (Table 4.1). It was due the presence of larger amount of metals present inside the enriched e-waste of mobile phone printed circuit board when compared to the dust form of MPPCBs that had lower amount of metals present in them. This present work depicted that the metal concentration was higher than the other metal concentration determined by other researchers Natarajan et al. (2014); Pradhan and Kumar (2012).The concentration of precious metals (Table 4.1) obtained in this study for MPPCBs dust was Ag 3mg/g (0.3%) and Au 0.5mg/g (0.05%) and that of enriched e-waste MPPCBs was Ag 5mg/g (0.5%) and Au 0.06%.

However, it was found that the concentration of precious metals obtained in the present study was lower when compared to (Arshadi, et al. 2016), but was higher and was higher when compared to Xiang et al. (2010) and Liang et al. (2014) who reported 0.0144 mg/g and 0.014 mg/g of Au and 0.22 mg/g and 0.03 mg/g of Ag from waste PCBs, respectively.

Table 4.1: Metal composition of MPPCBs shredded dust and processed e-waste

METALS	SHREDDED MPPCB DUST (mg/g)	PROCESSED MPPCBS (m/g)
Cu	220	690
Fe	3.02	36.8
Zn	9.4	4.63
Au	0.56	0.62
Ag	2.58	5.04
Cr	2.51	20.1
Co	1.48	0.37

This heterogeneity present was observed in comparison with metals content obtained by various researcher and may be due to nature, origin of e-waste used, analytical method involved, various physical and mechanical treatment applied to obtain e-waste.

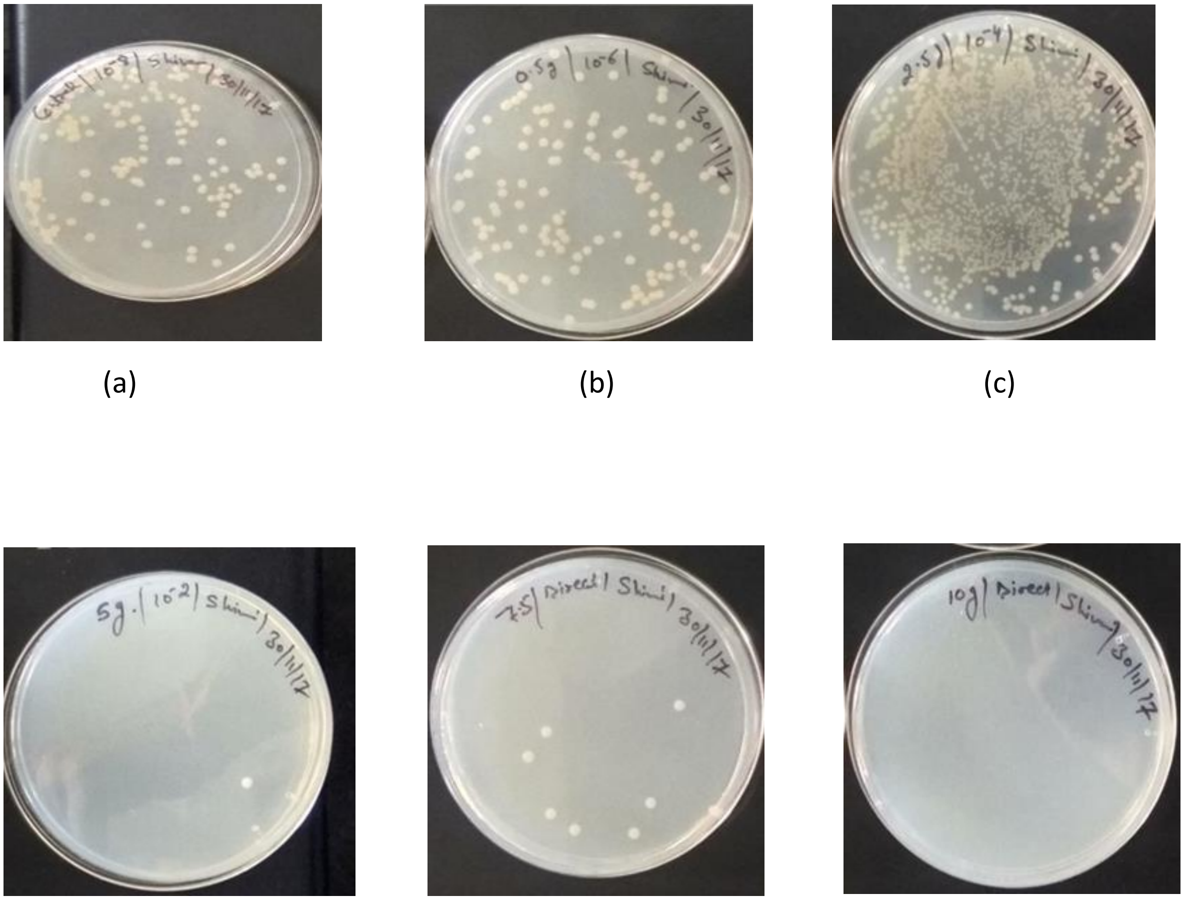
Fig 4.3: MPPCBs e-waste in solution form after performing aqua regia digestion

4.3 MPPCBs e-waste toxicity tolerant capability of *Pseudomonas balearica* SAE1

Toxicity assessment and dose-response analysis was performed to evaluate the half maximum effective concentration (EC50) of MPPCBs dusts and MPPCBs e-waste on *Pseudomonas balearica* SAE1. The cells were exposed to different concentration of e-waste i.e. 1, 5, 10, 15, 20% respectively.

Table 4.2: CFU/ml of dust MPPCBs and enriched MPPCBs e-waste

SNO:	MPPCBs shredded Dust	MPPCBs processed waste
Control	3 x10$^{\wedge 12}$	3 x10$^{\wedge 10}$
1%	3.16 x10$^{\wedge 10}$	1.9x 10$^{\wedge 10}$
5%	1.1x 10$^{\wedge 9}$	1.7x10$^{\wedge 5}$
10%	2.8 x10$^{\wedge 10}$	8.6x10$^{\wedge 3}$
15%	7x 10 $^{\wedge 6}$	2 x 10$^{\wedge 3}$
20%	2x10$^{\wedge 4}$	-



(a) (b) (c)

(d) (e) (f)

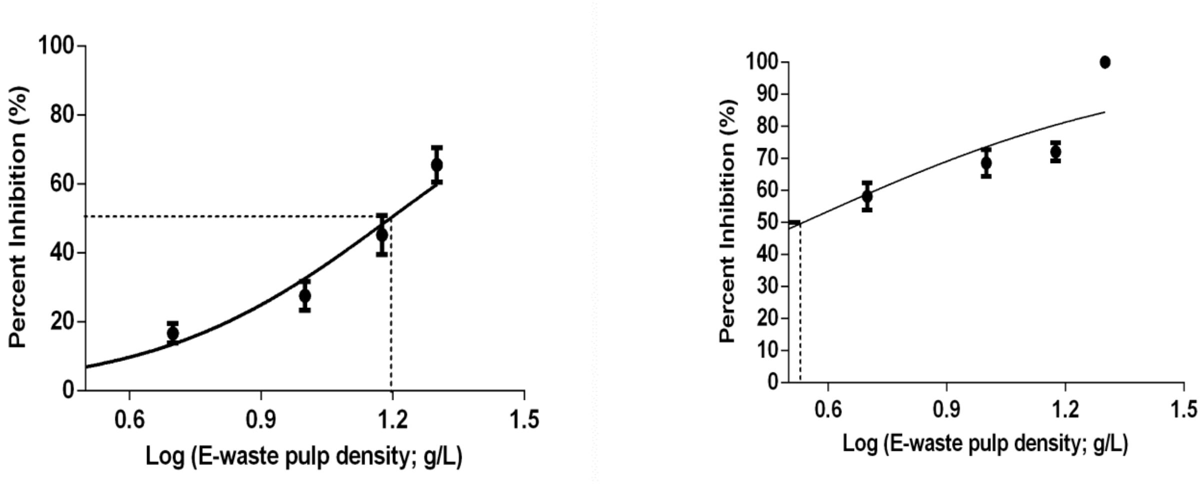
Fig 4.4: NA plates representing colonies of *Pseudomonas balearica* SAE1 obtained after performing toxicity test using shredded MPPCB dust at different pulp densities (a) control (b) 1 % (w/v) (c) 5 % (w/v) (d) 10%(w/v) (e) 15 % (w/v) (f) 20%(w/v)

Reduction in CFU/mL was used to access the toxicity as shown in Table 4.2 of *Pseudomonas balearica* SAE1 along control. In both MPPCBs shredded dust and processed MPPCBs e-waste the highest CFU/ml was seen at pulp density 10g/L (1%).

As the concentration of the metal was increased in the media the toxicity of metals increased, decreasing the tolerance of *Pseudomonas balearica* SAE1. Due to very low content of metal present in enriched MPPCBs dust, *Pseudomonas balearica* SAE1 could grow up to a pulp density of 200g/L (15%) but strain SAE1 was completely inhibited at enriched e-waste

MPPCBs pulp density of 200g/L and growth was seen up to pulp density of 150g/L(15%) as shown in Table 4.2

Comparative toxicity assessment and its dose-response analysis showed higher tolerance of *pseudomonas balearica* SAE1 to MPPCBs shredded dust i.e. EC50 = 15.6 g/l than for processed MPPCBs e-waste which was highly enriched in metals i.e. EC50= 3.5g/l pulp density of the culture medium



(a) (b)

Fig 4.5: Represents the dose– response curve of *P. balearica* SAE1 for (a) shredded MPPCBs e-waste (b) processed MPPCBs e-waste

The EC50 value of *Pseudomonas balearica a*nd *C. violaceum* received was 325.7 g/L (LogEC50 = 2.5) and 83.70 g/L (LogEC50 = 1.9), respectively. The 95% confidence intervals obtained were Log 2.5 to 2.6 (315.1–346.7 g/L) for *Pseudomonas balearica* SAE1 and Log 1.8 to 2.0 (68.7–102.0 g/L) for *C. violaceum* [12]. Results of the dose-response analysis suggested that there were technological feasibility and possible operation range of metals bioleaching from e-waste using *P. balearica* SAE1.

4.4 Two-step bioleaching of MPPCbs e-waste with *Pseudomonas balearica* SAE1 and *Chromobacterium violaceum*.

In two step bioleaching experiment initially biomass was produced in the absence of MPPCBs e-waste for 48h in order to reduce the impact of the toxicity on the microorganism.

Both shredded dust and processed MPPCBs waste of size <150um of pulp density with 1%(w/w) was added to 100ml of sterile LB media having *Pseudomonas balearica* and also media with *Chromobacterium violaceum*. Control flasks containing media and MPPCBs e-waste were run in parallel. All the flasks were incubated at 30^{o}C at 150 rpm for a period of 7 days. Glycine a metabolic precursor i.e.5g/l was added to the LB media and pH of all flasks was kept 9.0 as shown in Fig 4.6.

After 7 days of experiment it was noted that *Pseudomonas balearica* leached out 33.9% and 7.1 % of Cu from MPPCbs shredded dust and processed waste respectively.

Fig 4.6: Flasks containing bacteria + MPCCBs e-waste kept at incubation at 30^{o}C at 150rpm for 7 days for bioleaching

In case of precious metals, *Pseudomonas balearica* could solubilize Au (4.01%) and Ag (4.06%) from shredded MPPCBs and 1.8% Ag and 4.5% Au was recovered from processed MPPCBs e-waste. But in case of Kumar e.t al (2017), the study showed that *Pseudomonas balearica* was able to leach out higher amount of Au (68.5%) and Ag (33.8%) from Computer PCBs.

This tells that MPPCBs e-waste were highly toxic to *Pseudomonas balearica* when compared to computer PCBs due to which the bacteria could leach out less amount of precious metals Ag and Au from both shredded and processed MPPCBS e-waste.

A comparison bioleaching analysis was carried out with *Chromobacterium violaceum,* where 41 % Cu, 6.25 % Au and 13% Ag was leached out from shredded MPPCBs waste and only 15.8% Cu was recovered from processed waste whereas 11.03%Ag and 5.05% Au was leached out from processed MPPCbs e-waste by *C.violaceum.*

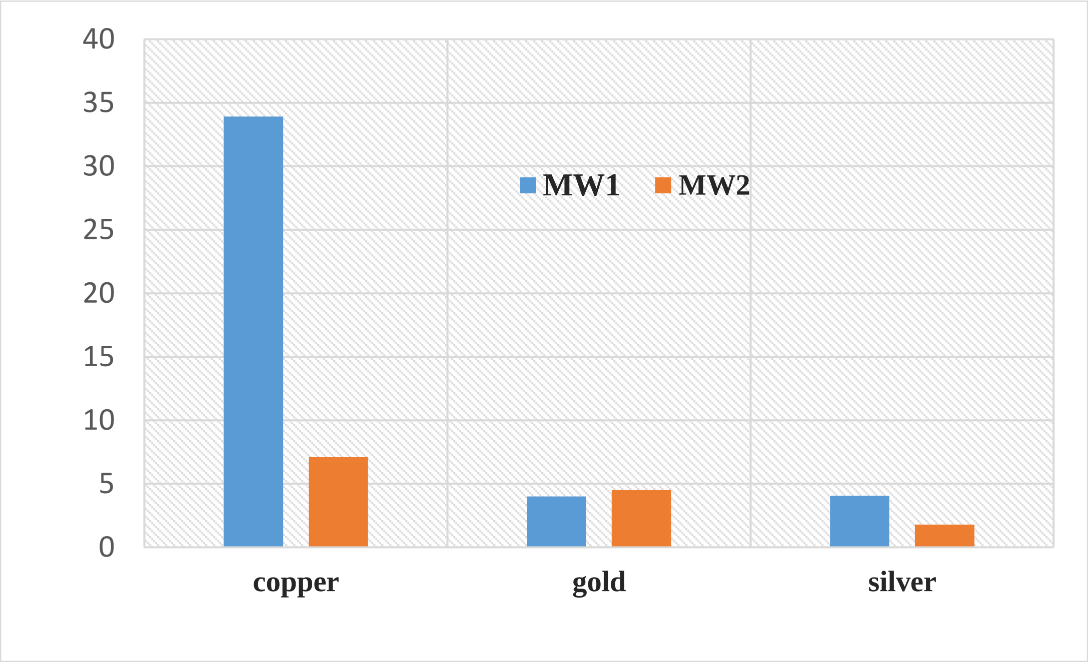


Fig 4.7: Two step Bioleaching of metals from MW1 and MW2 using *Pseudomonas balearica* SAE1.

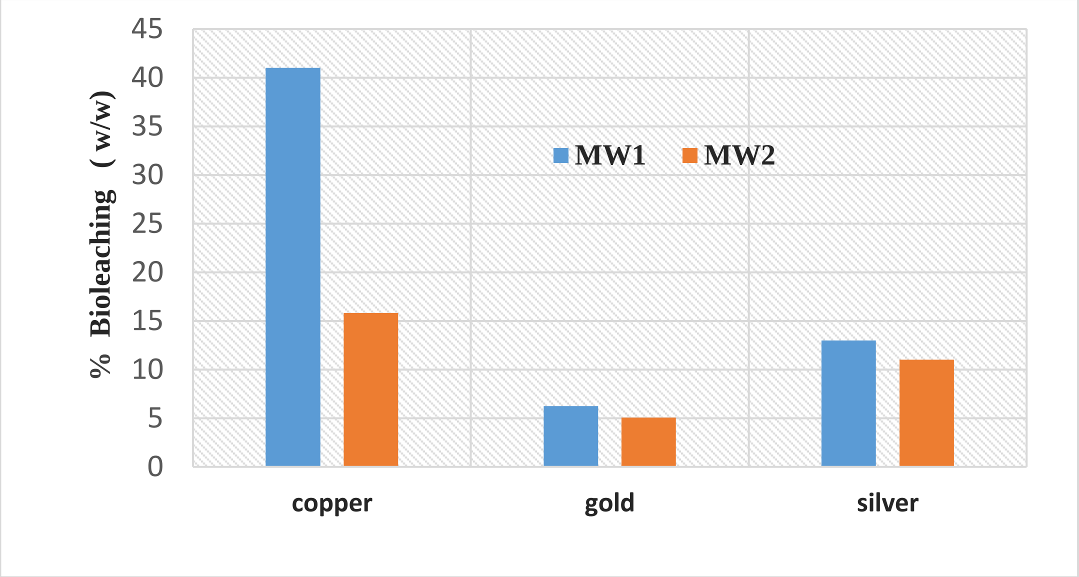


Fig 4.8: Two step Bioleaching of metals from MW1 and MW2 using *Chromobacterium violaceum.*

Where, MW1= Shredded MPPCBs

MW2= Processed MPPCBs

4.5 Metal content analysis of pretreated of MPPCBs e-waste

Copper being abundant in MPPCBS e-waste, it has always interfered in gold-cyanide complex formed during bioleaching process, thus reducing the removal of gold from the e-waste and therefore there was a need to remove the copper from e-waste to reduce the toxicity of the media containing MPPCBs e-waste by pretreating the e-waste with nitric acid.

Both shredded and processed MPPCBs dust after pretreating with 6M nitric acid was subjected to refluxing using aqua regia method at 100^{o}C for 1h. Metals ions of the leachate was determined using AAS which showed that 90% of copper was removed from the processed MPPCBs e-waste and 79.9% Cu was removed from shredded MPPCbs e-waste as shown in Table 4.4 and Table 4.5. Whereas 17% and 28% of Au and also 33% and 22.09% Ag were removed from processed and shredded MPPCBs e-waste respectively .Similar results have earlier been reported in Saini and Kumar 2016, Natarajan et al 2014, Ilyas et al. 2007; Liang et al. 2010; Xiang et al. 2010.

Thus, a higher concentration of copper removal from both MPPCBs e-waste with the pulp density of 1 % (w/w) stated that nitric acid was highly reactive and could solubilize copper when compared to other metals and could be a helpful tool in enhancing the gold cyanidation in subsequent bioleaching.

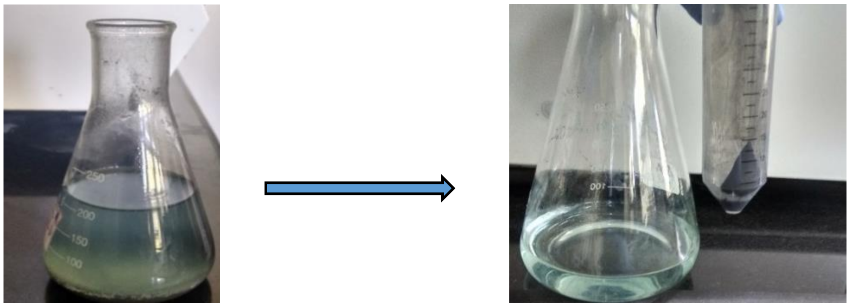


Fig 4.9: Pretreated MPPCB e-waste pellets after centrifugation at 1000 rpm for 10 mins.

Table 4.3:% removal of Cu, Au and Ag of untreated and pretreated shredded dust MPPCBs e-waste.

Metals	**Un-treated MW1 composition(mg/g)**	**Pre-treated MW1 composition (mg/g)**	**% Removal**
Cu	220 ± 2.4	45.5 ± 2.1	79.9
Au	0.56 ± 0.03	0.401± 0.04	20
Ag	2.58 ± 0.09	2.01 ± 0.02	22.09

Table 4.4: % removal of Cu, Au and Ag of untreated and pretreated processed MPPCBs e-waste.

Metals	Un-treated MW1 composition(mg/g)	Pre-treated MW1 composition (mg/g)	% Removal
Cu	690 ±0.23	67.7±0.14	90
Au	0.62 ±0.003	0.522± 0.003	17
Ag	5.04 ±.004	3.37±0.003	33

Where, MW1=Shredded MPPCBs

MW2= Processed MPPCBs

4.6 Comparison analysis of two-step bioleaching of pre-treated MPPCBs e-waste using *Pseudomonas balearica* SAE1and *Chromobacterium violaceum*

Pre-treatment was performed using nitric acid to remove the excess proportion of copper from both untreated shredded and processed MPPCBs e-waste so that copper during bioleaching process does not interfere with gold and silver in forming stable complexes with cyanide and thus enhance the mobilization of gold and silver from MPPCBs e-waste. Comparison of both *Pseudomonas balearica* SAE1 and *Chromobacterium violaceum* was performed for dissolution or recovery of gold, silver and copper from both pre-treated shredded and processed e-waste at pulp density of 1 %(w/v) under optimized condition. Percentage recovery of gold, silver and copper were examined on 2nd, 4th and 7th day of process.

4.6.1 Gold bioleaching profile after pre-treatment of shredded and processed MPPCBs e-waste using *Pseudomonas balearica* SAE1and *Chromobacterium violaceum*.

After addition of pre-treated shredded and processed MPPCB e-waste to the 24hr inoculated flasks with *Pseudomonas balearica* and *Chromobacterium violaceum* it was analysed that on the 2nd day of bioleaching process Au recovered was 7.09 %whereas 14.18 % and 20.09% on 4thand 7thday of bioleaching process as shown in Fig 4.10 and from Fig 4.11 *Chromobacterium violaceum* could leach out 12.24 % and 14.18% on 4th and 7th day which was low in comparison to *Pseudomonas balearica* from pre-treated shredded waste.

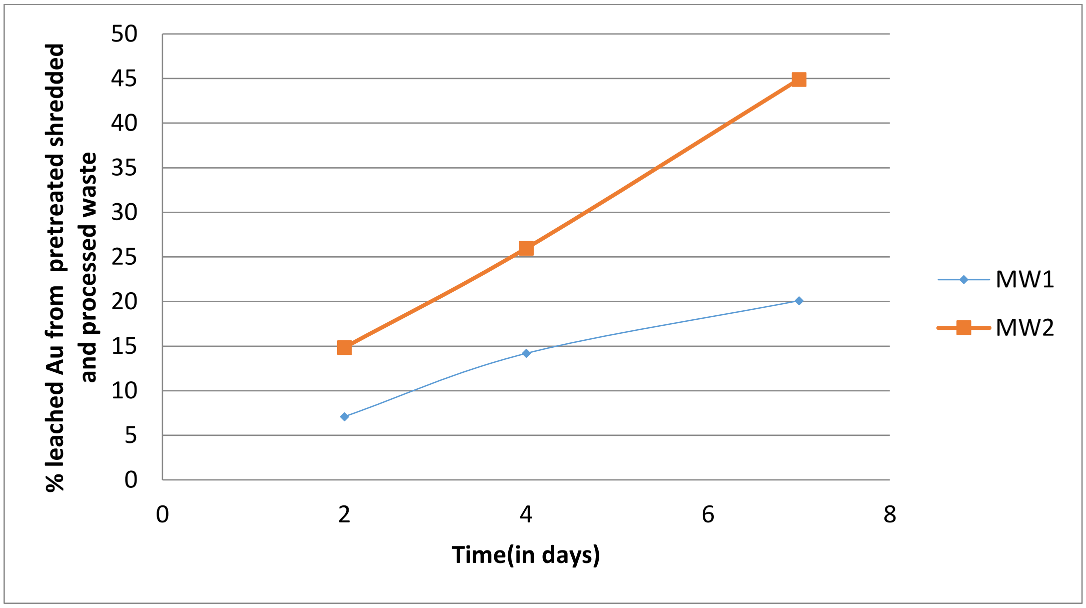


Fig 4.10: Percentage bioleaching of gold after pre-treatment of shredded (MW1) and processed (MW2) MPPCBs e-waste at 1 %(w/v) pulp density using *Pseudomonas balearica* SAE1.

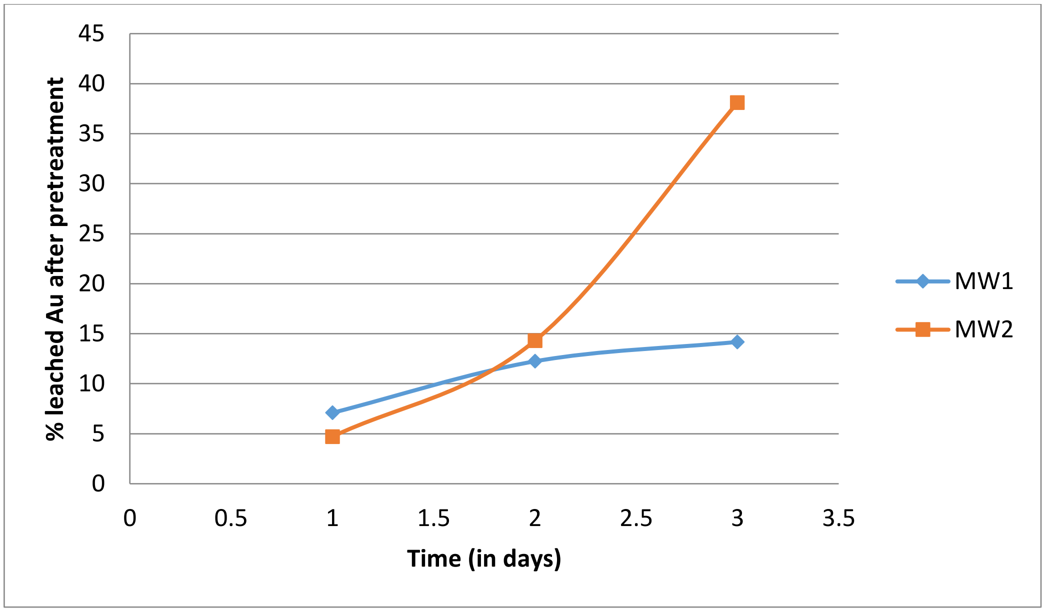


Fig 4.11: Percentage bioleaching of gold after pre-treatment of shredded (MW1) and processed (MW2) MPPCBs e-waste at 1 %(w/v) pulp density using *Chromobacterium violaceum.*

From Fig 4.10 and Fig 4.11 it was observed that from pretreated processed e-waste *Pseudomonas balearica* was able to leach out a maximum amount of Au from processed waste on 7th day i.e.44.9% whereas *Chromobacterium violaceum* leached 38.1% of Au on the 7th day. Similarly higher proportion of gold was leached on 2nd and 4th day i.e. 14.83% and 25.96% respectively by Pseudomonas balearica in comparison with *C.violaceum* where 4.76% and 14.3% Au was leached on 2nd and 4th day respectively.

A higher amount of gold was leached out from both the organisms from the treated waste in comparison to untreated waste as it was due to the removal of excess copper from the waste that otherwise would have become toxic to the microorganisms reducing metal mobilization.

A higher amount of cyanide ions were available for the gold to form the complex in the solution at there may be a maximum growth of bacteria at an alkaline conditions which can be considered another key factor responsible for the recovery of gold. A control sample was run in the parallel i.e. e-waste was added to the LB media and it was noted that no dissolution of Au occurred during the entire period of bioleaching process. This stated that cyanide was the only lixiviant that was responsible for the leaching of gold.

4.6.2 Copper bioleaching profile pre-treatment of shredded and processed MPPCBs e-waste using *Pseudomonas balearica* SAE1and *Chromobacterium violaceum*.

When interpreted from Fig 4.12 and 4.13 though mobilization of copper occurred after pre-treating the shredded MPPCBs e-waste but a very less proportion of copper was leached out using both microorganisms from pre-treated MPPCBs e-waste, compared to untreated MPPCBs e-waste. Only 0.09% of Cu was leached out on 7th day using *C. violaceum* and 0.12% by *P. balearica* on the 7th day of bioleaching experiment. In case of processed waste on the 7th day, only 0.05% and 0.03% copper was leached out by *P. balearica* and *C. violaceum.*

From Natarajan et.al (2013) it was noted that copper recovered from the bioleaching of untreated ESM were also lower (ranging from 18% to 71%), compared to the bioleaching of pre-treated ESM using *Chromobacterium violaceum.*

In the present study, it was confirmed that maximum amount of copper was dissolved in the solution while pre-treating the waste with nitric acid, therefore, leaving a very lower amount of copper for bioleaching.

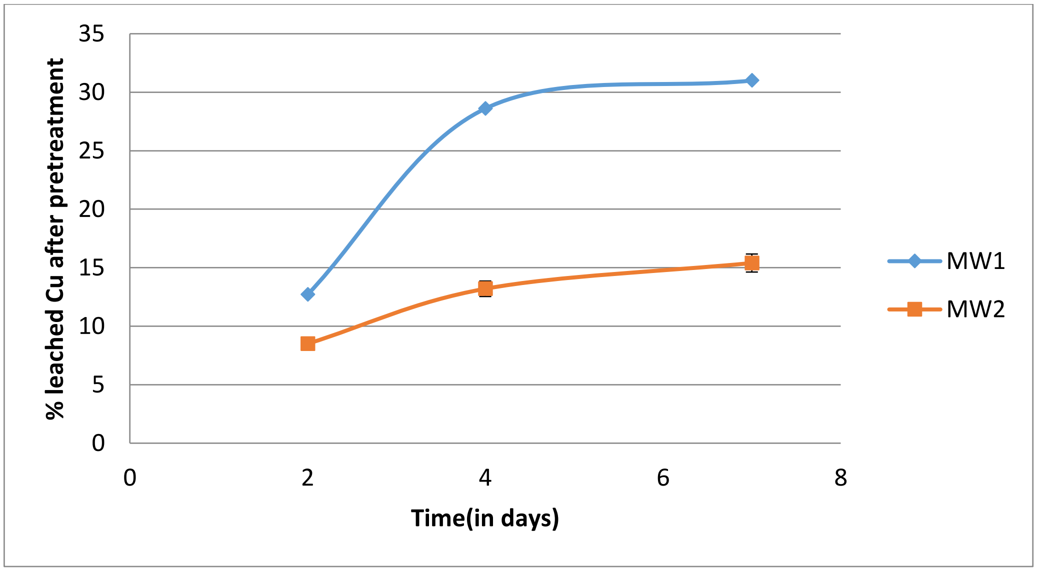


Fig 4.12: Percentage bioleaching of copper after pre-treatment of shredded and processed MPPCBs e-waste at 1 %(w/v) pulp density using *Pseudomonas balearica* SAE1.

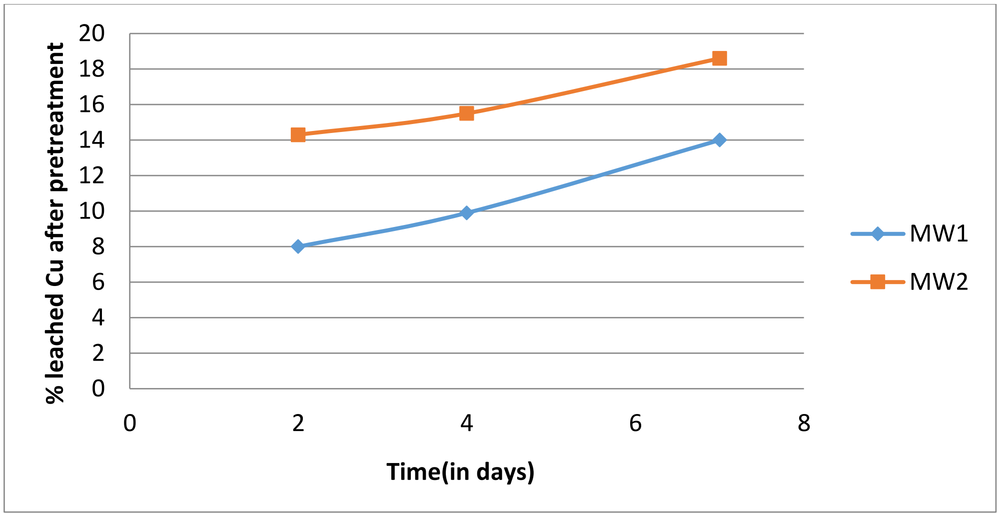


Fig 4.13: Percentage bioleaching of copper after pre-treatment of shredded and Processed MPPCBs e-waste at 1 %(w/v) pulp density using *Chromobacterium violaceum*.

4.6.3 Silver bioleaching profile after pre-treatment of shredded and processed MPPCBs e-waste using *Pseudomonas balearica* SAE1and *Chromobacterium violaceum*.

In case of silver leached from pre-treated shredded MPPCBs e-waste it was seen from Fig 4.14 and 4.15 *Pseudomonas balearica was* highly efficient in leaching silver than *Chromobacterium violaceum. P. balearica leached* 12.7%, 28.6% and 31% silver on 2nd, 4th and 7th day of the experiment. Whereas on the other hand 8%, 9.9% and 14% silver was leached out on 2nd, 4th and 7th day by *C. violaceum*. A comparative analysis was carried out in case of pretreated processed e-waste where *P. balearica* leached only 15.5% silver on 7th day whereas 18.6% was leached out by *C. violaceum.*

Less proportion of silver was leached out after pretreatment from both shredded and processed waste when compared to the recovery of gold. This might be due to the consumption and complexation of cyanide with gold that would have reduced the net free cyanide concentration to decrease with time. The decrease in free cyanide concentration could also be due to higher cyanide complexation with other metals present in untreated MPPCBs e-waste.

A possible reason for the reduction of the concentration of silver in bioleached solution after pretreating the processed MPPCs e-waste using *P.balearica* could be due to biosorption or bioaccumulation of silver (perhaps other metals) by bacteria during bioleaching process.

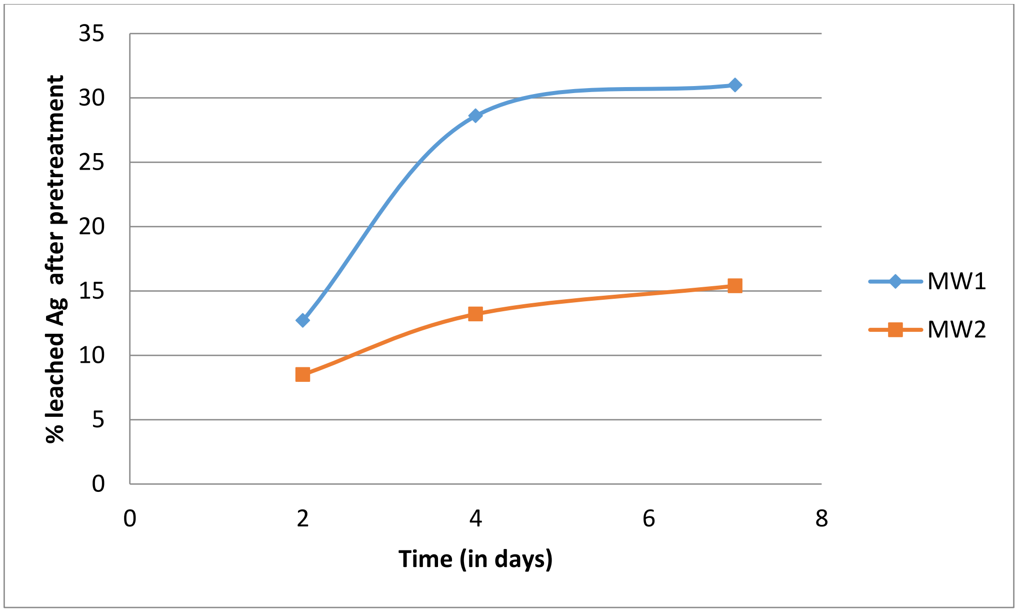


Fig 4.14: Percentage bioleaching of silver after pre-treatment of shredded and processed MPPCBs e-waste at 1 %(w/v) pulp density using *Pseudomonas balearica* SAE1.

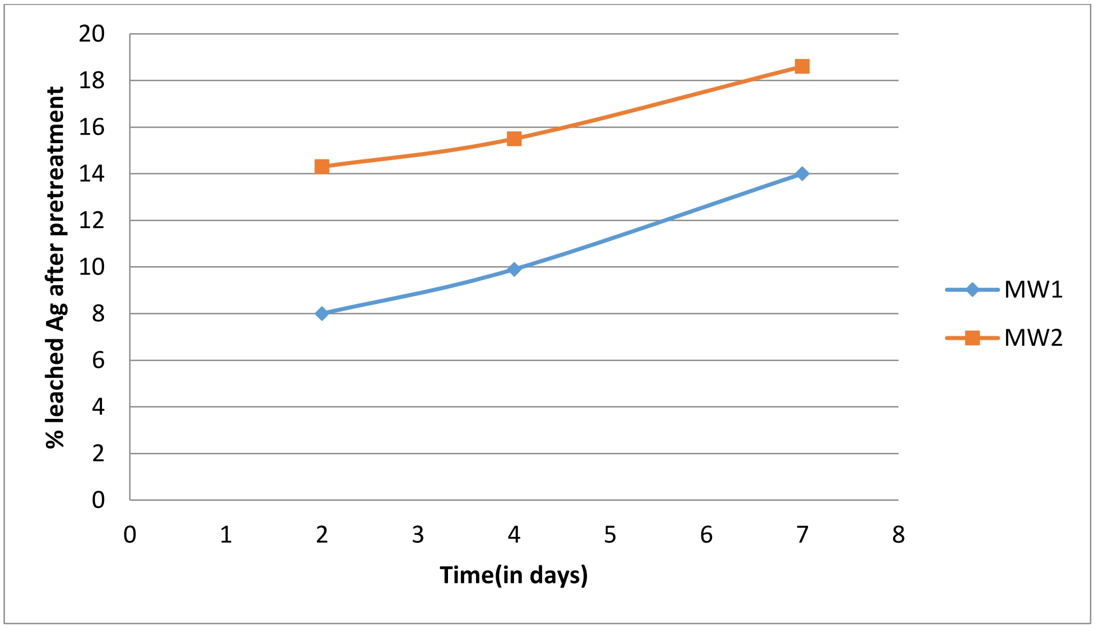


Fig 4.15: Percentage bioleaching of silver after pre-treatment of shredded and processed MPPCBs e-waste at 1 %(w/v) pulp density using *Chromobacterium violaceum*.

CHAPTER 5.
CONCLUSION

The present electronic time has seen the enormous expansion of electrical and electronic hardware particularly in the recent two decades. These devices have turned out to be key parts of human life and when these gadgets become unused and reach their end of life they are discarded by their users that give rise to electronic waste and these waste are one of the quickest developing waste around the globe. These disposed of electronic gadgets contain harmful components like plastics, heavy metals and on dumping these e-waste or incinerating them can cause a negative effect on human as well to the environment. E-waste has now become the one of the major concern growing worldwide and thus demands the need of proper recycling in an eco- friendly manner to reduce the toxic effects caused by these gadgets and to contribute in economic development. Mobile phones waste being a section of WEE contains various hazardous materials, but on the other side contains valuable metals and studies have revealed that 1000kg of mobile phone waste could generate 250-300g of gold making them secondary ore metals. On account of this fact, MPPCBs e-waste recycling in an environmentally friendly manner has now become a major driving factor to recover gold and other precious metals. The traditional recycling of electronic waste i.e. pyrometallurgy and hydrometallurgy is dangerous, costlier process and generates high amount of energy and toxic compounds and thus there is need to develop a process that is ecologically sound and thus helps in recovering the precious metals.

Microbial leaching or bioleaching is one of the promising technology that is used as alternate technology in recovering gold and silver from e-waste by mobilizing the metals in the solution with the help of microorganisms.

In the present project naturally cyanide producing microbes such as *Pseudomonas balearica* SAE1 and *Chromobacterium violaceum* which have previously been reported to recover a higher proportion of gold and silver were used to leach out precious metals from shredded and processed forms of MPPCBs waste. From the present study it was revealed that the metal composition of MPPCbs waste were different from those of computer PCBs this showed the presence of heterogeneity, but like other PCBs copper were present in larger amount in both shredded (220 mg/g) and processed(690 mg/g) MPPCBs waste in comparison to other metals. Due to this reason it enhanced the toxicity on the microorganisms by inhibiting their growth, thus lowering the recovery of gold and silver and from the present work results indicated that *P. balearica* leached 4.01 % and 4.5 % Au whereas *C. violaceum* could leach out 6.25 % and 5.05% Au at the end of bioleaching experiment from shredded and processed MPPCs e-waste. This revealed that though the presence of higher concentration of copper *P. balearica* was able to tolerate the toxic effecti.e. EC50 = 15.6 g/l for shredded waste and EC50= 3.5g/l for processed MPPCbs e-waste at pulp density of 1 % (w/w) and was equivalent efficient to *C. violaceum* in leaching gold and silver.

Pretreating with nitric acid gave a positive result in removal of massive amount of copper from both MPPCs e-waste. Though the level of cyanide production by *Chromobacterium violaceum* was higher, from the current study results revealed that *Pseudomonas balearica* was able to leach out more gold and silver from both pretreated shredded and processed MPPCBs e-waste on 2nd, 4th and 7th day of bioleaching experiment, thus making it competitive and highly efficient bacteria in mobilizing precious metals and also suitable for leaching at an industrial level. Bioleaching of shredded and processed MPPCBs e-waste was the best method chosen not only in terms of recovering gold and silver but also in terms of achieving economic goals. By providing suitable conditions during bioleaching process, it can increase the growth of bacteria thus making higher amount of cyanide ions available for the mobilization of precious metals.

REFERENCES

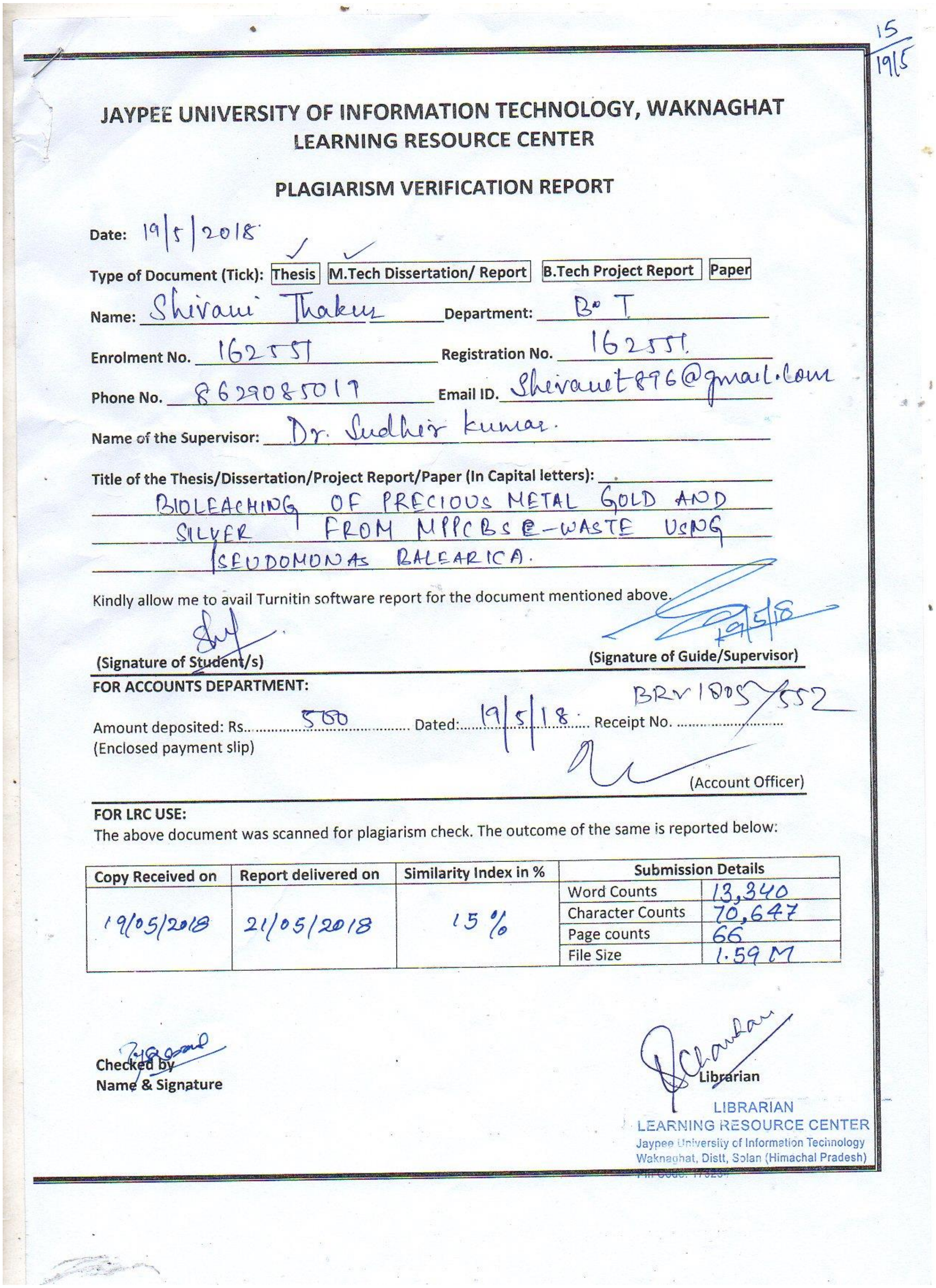
1. Sarath P, Bonda S, Mohanty S, Nayak SK. Mobile phone waste management and recycling: views and trends. Waste management. 2015 Dec 1; 46:536-45.
2. Brandl H, Bosshard R, Wegmann M. Computer-munching microbes: metal leaching from electronic scrap by bacteria and fungi. In Process Metallurgy 1999 Jan 1; 9, pp. 569-576.
3. Wath SB, Dutt PS, Chakrabarti T. E-waste scenario in India, its management and implications. Environmental monitoring and assessment. 2011 Jan 1; 172(1-4):249-62.
4. Awasthi AK, Zeng X, Li J. Comparative examining and analysis of e-waste recycling in typical developing and developed countries. Procedia Environmental Sciences. 2016 Jan 1; 35:676-80.
5. Kumar U, Singh DN. E-waste management through regulations. vol. 2013; 3:6-14.
6. Kaur H, Goel S. E-waste Legislations in India—A Critical Review. Management and Labour Studies. 2016 Feb; 41(1):63-9.
7. Widmer R, Oswald-Krapf H, Sinha-Khetriwal D, Schnellmann M, Böni H. Global perspectives on e-waste. Environmental impact assessment review. 2005 Jul 1; 25(5):436-58.
8. Cayumil R, Khanna R, Rajarao R, Mukherjee PS, Sahajwalla V. Concentration of precious metals during their recovery from electronic waste. Waste management. 2016 Nov 1; 57:121-30.
9. Cui J, Zhang L. Metallurgical recovery of metals from electronic waste: a review. Journal of hazardous materials. 2008 Oct 30; 158(2-3):228-56.
10. Ilyas S, Anwar MA, Niazi SB, Ghauri MA. Bioleaching of metals from electronic scrap by moderately thermophilic acidophilic bacteria. Hydrometallurgy. 2007 Aug 1; 88(1-4):180-8.
11. Pradhan JK, Kumar S. Metals bioleaching from electronic waste by Chromobacterium violaceum and Pseudomonads sp. Waste management & research. 2012 Nov; 30(11):1151-9.

12. Kumar A, Saini HS, Kumar S. Bioleaching of gold and silver from waste printed circuit boards by Pseudomonas balearica SAE1 isolated from an e-waste recycling facility. Current microbiology. 2018 Feb 1; 75(2):194-201.
13. Xiang Y, Wu P, Zhu N, Zhang T, Liu W, Wu J, Li P. Bioleaching of copper from waste printed circuit boards by bacterial consortium enriched from acid mine drainage. Journal of hazardous materials. 2010 Dec 15; 184(1-3):812-8.
14. Shin D, Jeong J, Lee S, Pandey BD, Lee JC. Evaluation of bioleaching factors on gold recovery from ore by cyanide-producing bacteria. Minerals Engineering. 2013 Jul 1; 48:20-4.
15. Priya A, Hait S. Comparative assessment of metallurgical recovery of metals from electronic waste with special emphasis on bioleaching. Environmental Science and Pollution Research. 2017 Mar 1; 24(8):6989-7008.
16. Borthakur A, Singh P. Electronic waste in India: Problems and policies. International Journal of Environmental Sciences. 2012; 3(1):353-62.
17. Olson GJ, Brierley JA, Brierley CL. Bioleaching review part B. Applied microbiology and biotechnology. 2003 Dec 1;63(3):249-57.
18. Babu BR, Parande AK, Basha CA. Electrical and electronic waste: a global environmental problem. Waste Management & Research. 2007 Aug; 25(4):307-18.
19. Mishra D, Rhee YH. Current research trends of microbiological leaching for metal recovery from industrial wastes. Curr Res Technol Educ Topics Appl Microbiol Microb Biotechnol. 2010; 2:1289-92.
20. Natarajan G, Ting YP. Pretreatment of e-waste and mutation of alkali-tolerant cyanogenic bacteria promote gold bio recovery. Bioresource technology. 2014 Jan 1; 152:80-5.
21. Chi TD, Lee JC, Pandey BD, Yoo K, Jeong J. Bioleaching of gold and copper from waste mobile phone PCBs by using a cyanogenic bacterium. Minerals Engineering. 2011 Sep 1; 24(11):1219-22.
22. Faramarzi MA, Stagars M, Pensini E, Krebs W, Brandl H. Metal solubilization from metal-containing solid materials by cyanogenic Chromobacterium violaceum. Journal of biotechnology. 2004 Sep 30; 113(1-3):321-6.

23. Brandl H, Lehmann S, Faramarzi MA, Martinelli D. Biomobilization of silver, gold, and platinum from solid waste materials by HCN-forming microorganisms. Hydrometallurgy. 2008 Nov 1; 94(1-4):14-7.
24. Kaya M. Recovery of metals and nonmetals from electronic waste by physical and chemical recycling processes. Waste management. 2016 Nov 1; 57:64-90.
25. Khanna R, Cayumil R, Mukherjee PS, Sahajwalla V. A novel recycling approach for transforming waste printed circuit boards into a material resource. Procedia Environmental Sciences. 2014 Jan 1; 21:42-54.
26. Kumar A, Holuszko M, Espinosa DC. E-waste: an overview on generation, collection, legislation and recycling practices. Resources, Conservation and Recycling. 2017 Jul 1; 122:32-42.
27. Balde CP, Wang F, Kuehr R, Huisman J. The global e-waste monitor 2014: Quantities, flows and resources.
28. Sahni A, Kumar A, Kumar S. Chemo-bio hydrometallurgy—a hybrid technology to recover metals from obsolete mobile SIM cards. Environmental Nanotechnology, Monitoring & Management. 2016 Dec 1; 6:130-3.
29. Arshadi M, Mousavi SM. Multi-objective optimization of heavy metals bioleaching from discarded mobile phone PCBs: Simultaneous Cu and Ni recovery using Acidithiobacillus ferrooxidans. Separation and Purification Technology. 2015 Jun 16; 147:210-9.
30. Sivaramanan S. E-Waste Management, Disposal and Its Impacts on the Environment. Universal Journal of Environmental Research & Technology. 2013 Oct 1; 3(5).
31. Willner J, Fornalczyk A. Extraction of metals from electronic waste by bacterial leaching. Environment Protection Engineering. 2013; 39(1):197-208.
32. Brandl H, Faramarzi MA. Microbe-metal-interactions for the biotechnological treatment of metal-containing solid waste. China Particuology. 2006 Apr 1; 4(2):93-7.
33. Mishra D, Kim DJ, Ahn JG, Rhee YH. Bioleaching: a microbial process of metal recovery; a review. Metals and Materials International. 2005 Jun 1; 11(3):249-56.
34. Knowles CJ, Bunch AW. Microbial cyanide metabolism. In Advances in microbial physiology 1986 Jan 1 (Vol. 27, pp. 73-111). Academic Press.

35. Akcil A, Erust C, Gahan CS, Ozgun M, Sahin M, Tuncuk A. Precious metal recovery from waste printed circuit boards using cyanide and non-cyanide lixiviants–a review. Waste management. 2015 Nov 1; 45:258-71.
36. Liang CJ, Li JY, Ma CJ. Review on cyanogenic bacteria for gold recovery from E-Waste. In Advanced Materials Research 2014 (Vol. 878, pp. 355-367). Trans Tech Publications.
37. Smith AD, Hunt RJ. Solubilisation of gold by Chromobacterium violaceum. Journal of Chemical Technology and Biotechnology. 1985 Jun 1; 35(2):110-6.
38. KL, Van Deventer JS. The role of metal-cyanide species in leaching gold from a copper concentrate. Minerals Engineering. 1999 Aug 1; 12(8):877-92.
39. Liu R, Li J, Ge Z. Review on Chromobacterium violaceum for Gold Bioleaching from E-Waste. Procedia Environmental Sciences. 2016 Jan 1; 31:947-53.
40. Awasthi AK, Zeng X, Li J. Comparative examining and analysis of e-waste recycling in typical developing and developed countries. Procedia Environmental Sciences. 2016 Jan 1; 35:676-80.
41. Kumar, U. and Singh, D.N. E-waste management through regulations.2013 *3;* .6-14.
42. Das S, Ting YP. Improving gold (bio) leaching efficiency through pretreatment using hydrogen peroxide assisted sulfuric acid. Clean–Soil, Air, Water. 2017 Jun 1; 45(6).
43. Gramatyka P, Nowosielski R, Sakiewicz P. Recycling of waste electrical and electronic equipment. Journal of Achievements in Materials and Manufacturing Engineering. 2007 Jan; 20(1-2):535-8.
44. Kita Y, Nishikawa H, Takemoto T. Effects of cyanide and dissolved oxygen concentration on biological Au recovery. Journal of biotechnology. 2006 Jul 25; 124(3):545-51.
45. Vijayaraghavan K, Yun YS. Bacterial biosorbents and biosorption. Biotechnology advances. 2008 May 1; 26(3):266-91.
46. Pham VA, Ting YP. Gold bioleaching of electronic waste by cyanogenic bacteria and its enhancement with bio-oxidation. In Advanced materials research 2009 (Vol. 71, pp. 661-664). Trans Tech Publications.
47. Natrajan, G. and Ting, Y.P., 2013. Two-step bioleaching and spent medium leaching of gold from electronic scrap material using Chromobacterium violaceum. In *Advanced Materials Research*, 825, pp. 270-273.

48. Kita Y, Nishikawa H, Takemoto T. Effects of cyanide and dissolved oxygen concentration on biological Au recovery. Journal of biotechnology. 2006 Jul 25; 124(3):545-51.



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