Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 21 | No. 2 | pp. 599-606 | 2022

Original Research Paper

https://doi.org/10.46488/NEPT.2022.v21i02.018

Open Access Journal

Bioleaching of Metals from Printed Circuit Boards

Pavithira V.*, Anchana Devi C.*† and Pushpa N.**
*PG and Research Department of Biotechnology, Women's Christian College, An Autonomous Institution, Chennai-600 006, Tamil Nadu, India
**PG & Research Department of Microbiology, Cauvery College for Women, Tiruchirapalli-620 018, T.N., India
†Corresponding author: Anchana Devi C.; dr.anchanadevi@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 01-07-2021
Revised: 30-08-2021
Accepted: 09-09-2021

Key Words:
Bioleaching, Electronic wastes, Printed circuit boards, *Stenotrophomonas maltophilia*, *Bacillus* sp., *Candida tropicalis*

ABSTRACT

Electronic waste or E-waste refers to the discarded electrical or electronic devices which have neared their useful life. Because of the toxicity and carcinogenicity of some compounds, the proper management and safe disposal of these electronic wastes have become serious challenges in recent years. Printed Circuit Boards (PCBs) are found almost in every other electronics these days, hence the present study focuses on bioleaching of metals from Printed Circuit Boards (PCBs) using bacterial and yeast strains (*Stenotrophomonas maltophilia, Bacillus* sp. and *Candida tropicalis*) isolated from heavily contaminated soil samples. A two-step bioleaching procedure was followed for maximum mobilization of metals. The isolated strains were able to mobilize metals from PCBs with different efficiencies depending on their ability to utilize the E-waste a carbon source when cultivated in minimal media. Bioleaching potential of isolated microbes on eight heavy metals (Cu, Ni, Mn, Pb, Fe, Cr, Zn & Co) in the sample were studied using AAS and SEM analysis before and after the two-step bioleaching process and found to be efficient. The study concludes that isolated bacterial and fungal species from the study can be further standardized with regard to the growth parameters and used on large scale to carry out the efficient recovery of metals that can help in recycling E-waste in the digital world.

INTRODUCTION

The use of electronics has become an essential commodity since the pandemic and the dependence on electronic gadgets has increased many folds. The discarded electronic and electrical instruments are called electronic waste (E-waste). E-waste is growing three times faster than other solid waste streams (Brandl & Faramarzi 2006). According to the UN's Global E-waste Monitor, 53.6 million tonnes (MT) of e-waste were produced globally in 2019. The report states that Asia produced the most E-waste in 2019-24.9 Mt, followed by America (13.1 Mt) and Europe (12 Mt), while Africa and Oceania produced 2.9 Mt and 0.7 Mt, respectively. Every year millions of tons of electronic waste are generated across the world. These E-wastes may contain more than 1000 different substances, which may be hazardous or non-hazardous (Widmer 2005). E-waste recycling is becoming more important not just in terms of waste management, but also in terms of recovering valuable metals like gold and silver, as well as toxic metals (like arsenic and mercury) and base metals (like copper and nickel) (Needhidasan et al. 2014). The type of electronic item, model manufacturer, and manufacturing date all influence the makeup of E-waste. Scarp from IT and Telecom system contains a comparatively higher quantity of precious metals than the scrap from the household equipment. For instance, one ton of mobile phone without batteries contains 340 g Au, 140 g Pd, 130 kg Cu, and 3.5 kg Ag (Hagelüken & Meskers 2008). It is estimated that more than 70% of globally produced waste electronics and electrical equipment (WEEE) are often disposed or recycled using crude, hazardous and inefficient processes, mostly by dumping and incinerating which causes severe impact on human life and the environment due to raw release of toxic metals directly into landfills or water bodies (Kaya 2016). Recycling of E-waste is limited due to the presence of heterogeneity in materials and difficulty in processing and segregation. Pyrometallurgy and Hydrometallurgy were initially used as traditional methods to recover metals from electronics. Since these processes raised concerns regarding risks of environmental impacts due to the release of toxic substances, Bioleaching or Biometallurgy is emerging as a promising method of metal recovery from electronic wastes.

Printed Circuit Boards (PCBs) are used in almost all electronic devices which may contain up to 60 different chemical elements and have a metal content as high as 40% by weight (Greenfield & Graedel 2013). The obsolete or used cell phones are components of waste electrical equipment because of their new technological improvements. Its period of use may be approximately one to two years and thus millions of cell phones are discarded every year,

generating large amounts of electronic waste. Cell phones are complex electronic devices that contain printed circuit boards that act as their brain. These PCBs are a complex mixture of materials such as plastics (13%), glass ceramics (24%), and metals (64%) including precious metals such as silver (Ag), gold (Au), and palladium (Pd), base metals such as copper (Cu), aluminium (Al), nickel (Ni), tin (Sn), silicon (Si), indium (In) and toxic metals such as cadmium (Cd), lead (Pb), arsenic (As), mercury (Hg), and bromine (Br) (Cui & Forssberg 2003, Kaya 2016). Henceforth, recovery of metals from the Printed Circuit Board is profitable, since these PCBs are used in almost every electronic gadget due to their high availability. The present study is focused on the bioleaching of metals from PCBs, which will readily help in recycling these metals in an environmentally friendly way.

MATERIALS AND METHODS

Sample Collection

Discarded Printed Circuit Boards (PCBs) from used mobile phones were collected from mobile phone repair shops in Richie Street, Chennai, Tamilnadu. Heavy metal contaminated soil samples were also collected from Ambattur industrial estate, Chennai which has a high cluster of small–scale industries. The samples were stored in a sterile container and transferred to the laboratory for further analysis.

Processing and Metal Content Analysis of E-Waste

The E-waste was processed according to the protocol of Khatri (2018) and particle size was reduced to have an approximate size of ≤ 200 to 250 µm, which was used for this study. 1g of finely powdered E-waste was taken in a 100 mL Erlenmeyer flask and digested using aqua regia, which can dissolve metals. Aqua regia is made up of Conc. HCl and HNO_3 in the ratio 3:1 (Sheng & Etsell 2007). The solution was allowed to stand for 48 hours and then was centrifuged at 5000 rpm for 5min. The supernatant was again filtered using Whatman filter paper and was stored at 4°C. (Pradhan & Kumar 2012). The total metal concentrations in the solution were analyzed using Atomic Adsorption Spectroscopy (AA-700 Shimadzu) at various wavelengths (nm) for analyzing the concentrations of different metals in the sample.

Isolation and Characterization of Microbes from Heavy Metal Contaminated Soil

Minimal salt media was used for the isolation of organisms. The composition of the media is given below (Tipre et al. 2004).

Composition of minimal media (1000 mL) pH-7

Ammonium chloride – 1.0 g
Magnesium sulfate – 0.2 g
Di Potassium hydrogen phosphate – 1.0 g
Calcium chloride – 0.1 g
Potassium chloride – 0.15 g
Yeast extract – 0.1
Ferrous sulfate – 1.0 mg
Zinc sulfate – 1.0 mg
Manganese sulfate – 1.0 mg

The printed circuit boards were finely powdered and sterilized by autoclaving at 121°C for 15 min before adding to the minimal media. After sterilization of the media, 1 g of autoclaved PCB was aseptically added to the media along with the soil sample and incubated for 7 days at 37°C. The culture was plated onto sterilized minimal agar plates and incubated at 37°C for 24 h and fungal isolation, minimal agar plates were supplemented with Ampicillin (10mg. mL^{-1}) and were incubated for 3-4 days. Individual bacterial colonies were sub-cultured further to obtain a pure culture of isolates and characterized based on biochemical properties identified based on classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg & Holt 1984). Also, identification of fungal isolates was based on colony size, shape, margin, elevation, consistency, opacity, and pigmentation and morphologically identified using Lacto phenol cotton blue staining.

Molecular Characterization of Isolates

Molecular identification of the isolates was performed using Forward primer (5′-TGGAGAGTTTGATCCTGGCTCAG-3′) and reverse primer (5′-TACCGCGGCTGCTGGCAC-3′) for amplification of 16S rRNA gene of bacteria (Hall et al. 2003) and internal transcribed spacer (ITS) region of nuclear ribosomal RNA genes for yeast using specific forward (ITS-1) and reverse (ITS-4) primers for PCR reactions (White 1990). Phylogenetic studies of the isolated samples were analyzed using the BLAST method to determine their approximate phylogenetic affiliation based on 16S rRNA gene sequence similarities for bacteria and ITS for fungal isolate (Altschul 1990, Engel 2005).

Bioleaching Studies-Two Step Process

The present study employs a two-step bioleaching process which was reported in previous studies to be appropriate to increase the metal leaching efficiency of micro-organisms from electronic waste (Mishra & Rhee 2014). For more efficient metal mobilization, direct growth of micro-organisms in the presence of electronic waste is not advisable due to its toxic effects (Brandl et al. 2001).

Bioleaching experiments were conducted in a 250 mL Erlenmeyer flask with 100 mL Minimal salt media at pH 7. The flasks were sterilized by autoclaving at 121 psi for 15 min. Post autoclave, the flasks were cooled at room temperature to inoculate the organisms, and once the organisms have reached the log phase, 1.0 g of sterilized electronic waste (1% w/v) was added to each flask under aseptic conditions. The flasks were then placed in a rotary shaker at room temperature for an incubation period of 30 days. In the post-incubation period, the samples were analyzed for any change in pH and total metal ion content. The same procedure was followed for all the organisms that were isolated.

Analysis of Bioleached Samples

15 mL of the bioleached sample were withdrawn from the flask and filtered through Whatman filter paper (0.45µm). The filtered sample was centrifuged at 10000 rpm for 10 min and the supernatant was used to study the total metal ions concentration of eight heavy metals (Cu, Ni, Mn, Pb, Fe, Cr, Zn & Co) in the sample using Atomic Adsorption Spectroscopy (AAS) and SEM analysis.

RESULTS AND DISCUSSION

Sample Collection

The waste discarded PCBs were collected from used mobile phones from mobile repair shops present in Richie Street, Chennai, Tamilnadu. Chennai is one of the important metropolitan cities in the south which is the largest producer of E-waste. No physical or mechanical separation processes were carried out before transportation to the laboratory (Fig. 1).

Screening and Identification of Heavy Metal Resistant Microorganisms

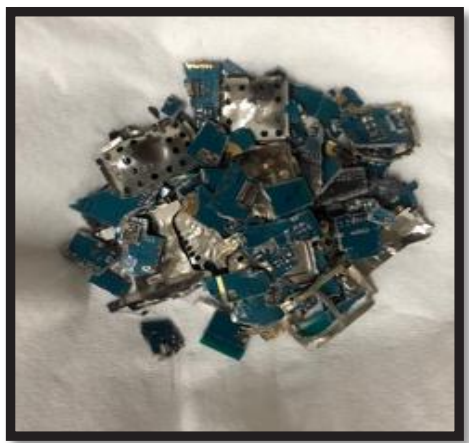

Fig. 1: Printed circuit boards from spent mobile phones.

Minimal media broth was inoculated with PCBs and the soil sample was incubated for 7 days at 37°C. On reaching an OD of 0.7 nm growth, the sample was streaked onto Minimal media agar and repeated sub-culturing was carried out to isolate pure colonies of microbes resistant to heavy metals (Figs. 2 & 3).

Cultural Characteristics of Heavy Metal Resistant Isolates

In our study, three pure cultures of microorganisms resistant to heavy metals were isolated using minimal media agar plates and labeled as PCB1, PCB2, and PCB3, out of which 2 were bacterial organisms and one was yeast (Table 1). Gram staining and biochemical tests were performed for the bacterial isolates to study their morphological and biochemical characteristics (Fig. 4 & Table 2).

The above results provided an idea of the morphology, colony characteristics, and biochemical nature of the isolated bacterial strains. Further, the isolated organisms were subjected to molecular amplification of partial 16s rRNA and the obtained sequence length for PCB2 was 1456 bp and PCB3 was 1420 bp.

The organisms were identified as *Stenotrophomonas maltophilia (*PCB2) and *Bacillus* sp. (PCB3). The constructed phylogenetic tree clearly shows that strain *Stenotrophomonas maltophilia* is closely related to *Pseudomonas* sp. (Fig. 5). Previously, several authors have reported the bioleaching potential of *Stenotrophomonas maltophilia* isolated from dif-

Fig. 2: Minimal broth inoculated with PCB and soil.

Table 1: Colony features of isolated heavy metal resistant organisms.

Strain No.	Colony Colour	Cell Feature
PCB 1	White	Yeast
PCB 2	White	Gram negative rods
PCB 3	White	Gram-positive rods

Fig. 3: Isolated pure colonies of heavy metal resistant organisms using minimal media agar.

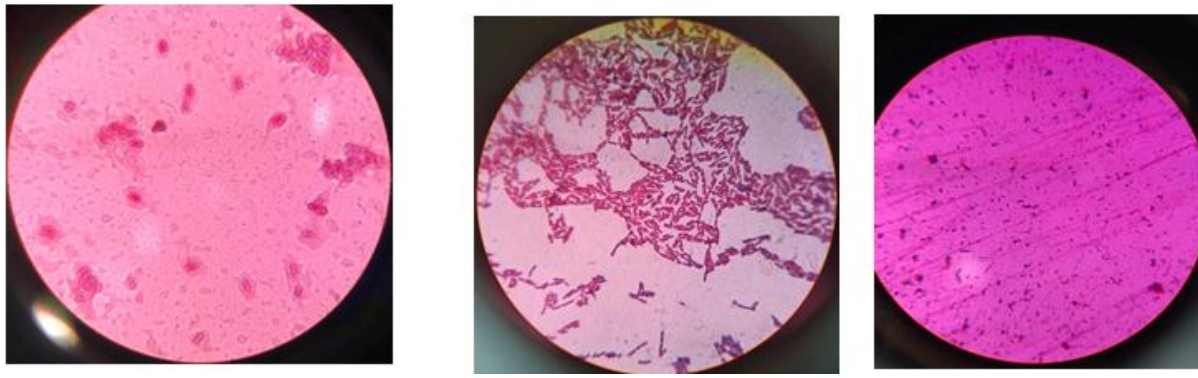

Fig. 4: Gram staining results of isolates PCB1, PCB2 and PCB3.

Table 2: Biochemical characteristics of isolated bacteria (+ = Positive; - = Negative).

	Biochemical tests							
Strain no	Catalase	Indole	MR	VP	Oxidase	TSI	Citrate	Gelatin utilization
PCB 2	+	-	+	+	+	A/A (no H_2S)	+	+
PCB 3	+	-	-	+	+	K/A (no H_2S)	+	+

ferent sources (Sajjad 2019, Venkidusamy & Megharaj 2016). However, *Stenotrophomonas maltophilia* was reported to be a potential Electrode respiring bacteria (ERB) widely used in microbial electrochemical remediation systems (MERS) because of their exoelectrogenic capabilities to degrade xenobiotic pollutants, especially heavy metals. Similarly, *Bacillus* sp. (PCB3) was closely associated with *Bacillus cereus* (Fig. 6). *Bacillus* sp. were isolated from sponges and had a high potential for bioleaching electronic waste and producing copper nanoparticles, according to a study (Rozas 2017). Several other studies have cited the potential effects of the E-waste bioremediation process using different *Bacillus* sp. (Arshadi & Mousavi 2015, Karwowska 2014).

PCB1 displayed normal yeast budding structures when stained with Gram. As demonstrated in Fig. 7, amplification of the ITS (Internal transcribe regions) gene verified the same, which is closely linked to Candida tropicalis. Though various articles have reported on the possible use of fungal species in bioremediation (Díaz-Martínez 2019, Ren et al. 2009), yeast as a bioleaching agent has received little attention, particularly in the context of printed circuit boards (PCBs).

Bioleaching of Heavy Metals from PCB

Heavy metals present in PCB were bioleached by adopting the shake flask method at room temperature using Minimal

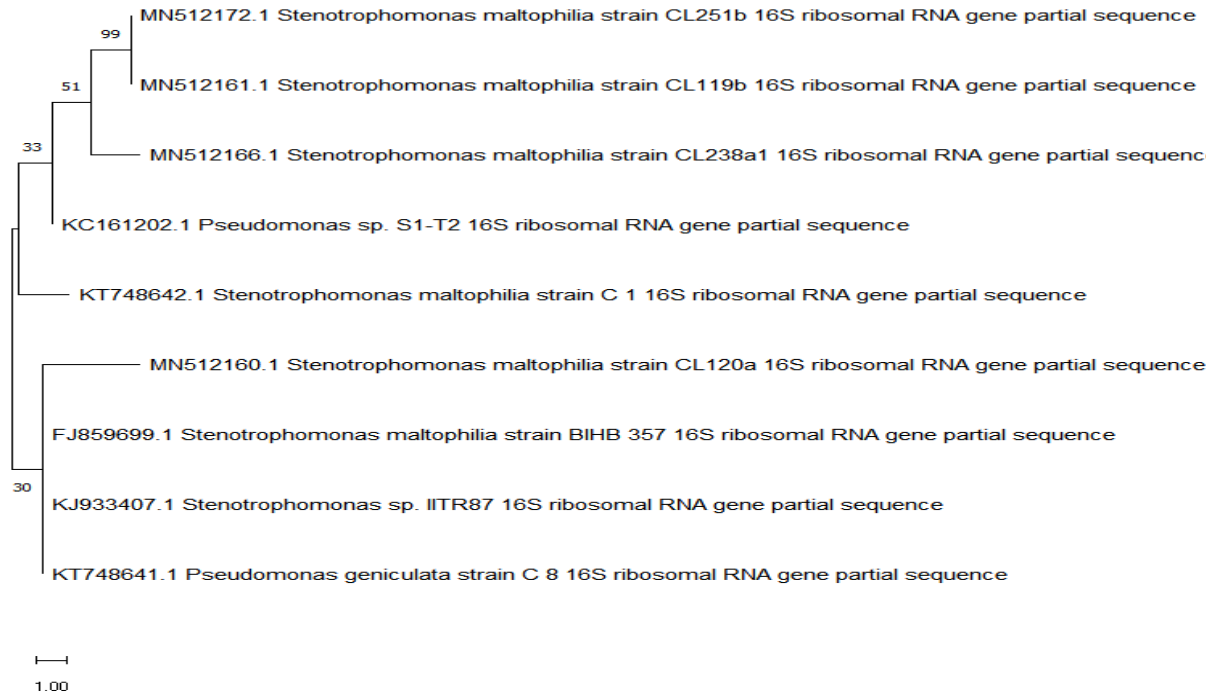

Fig. 5: Phylogenetic analysis of PCB 2.

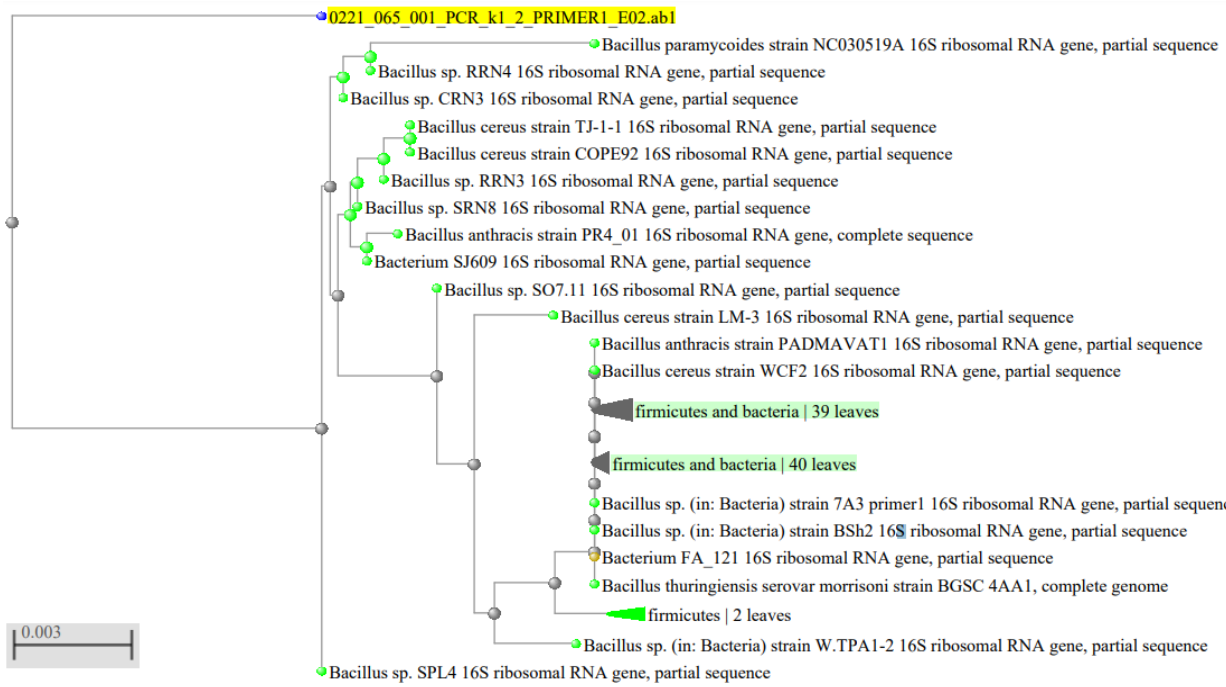

Fig. 6: Phylogenetic analysis of PCB 3.

salt media at pH 7. Organisms use PCBs as carbon sources for their growth. Pre- and Post-incubation, the samples were subjected to Atomic adsorption spectroscopy studies to analyze the bioleaching of metal content pre- and post-two-step leaching process using isolated organisms PCB1 PCB2 and PCB3. The pH of the samples after the bioleaching procedure varied from 5.5-7 for all metals, ranging from slightly acidic to neutral (Table 3).

Overall, the metal recovery content of different types of metals contained in the sample was considerable before and after bioleaching. Organism PCB3 had the highest metal recovery in copper, chromium, and cobalt, while organism PCB2 had the highest metal recovery in manganese, zinc, and lead. PCB1 had a greater zinc and nickel recovery rate. Using microorganisms in a consortium for improved results has only been documented in a few studies (Lima 2012, Sajjad 2019).

SEM Analysis

Figures 8a, 8b and 8c show scanning electron microscope (SEM) images of PCBs before bioleaching, which demonstrate crystalline structures and the presence of rough surfaces. The heavy metals in the PCBs were transformed to a granular shape with a smooth surface during the bioleaching process (Fig. 8d, 8e & 8f). This structural change confirms the bioleaching treatment by the isolated organisms. Previously a similar type of result was reported in another study (Zhao et al. 2008).

CONCLUSION

The findings of the current study revealed that microbes play an important role in metal recovery. The metal concentrations were drastically reduced from their initial concentration because of the detoxification process and metabolic activity of microbial isolates (PCB1, PCB2 & PCB3). The isolates were characterized and identified as *Candida tropicalis*, *Stenotrophomonas maltophilia* and *Bacillus* sp. The recovery of the metal from PCB was confirmed using Atomic adsorption studies and SEM analysis. Maximum bioleaching was obtained in the recovery of copper and nickel when compared to other metals. Three isolates exhibited their affinity for

Table 3: AAS results of the metal content analysis before and after bioleaching.

Metals	Concentration [ppm] before treatment	Concentration [ppm] after treatment		
		PCB1	PCB2	PCB3
Copper	78.51	3.401	11.18	1.904
Manganese	64.50	0.072	0.053	0.061
Chromium	63.45	0.876	2.055	0.391
Zinc	25.26	0.071	0.225	0.275
Nickel	21.60	1.413	11.377	2.494
Cobalt	4.30	0.844	0.045	0.031
Iron	26.32	0.003	0.056	0.314
Lead	52.51	3.66	0.04	0.165

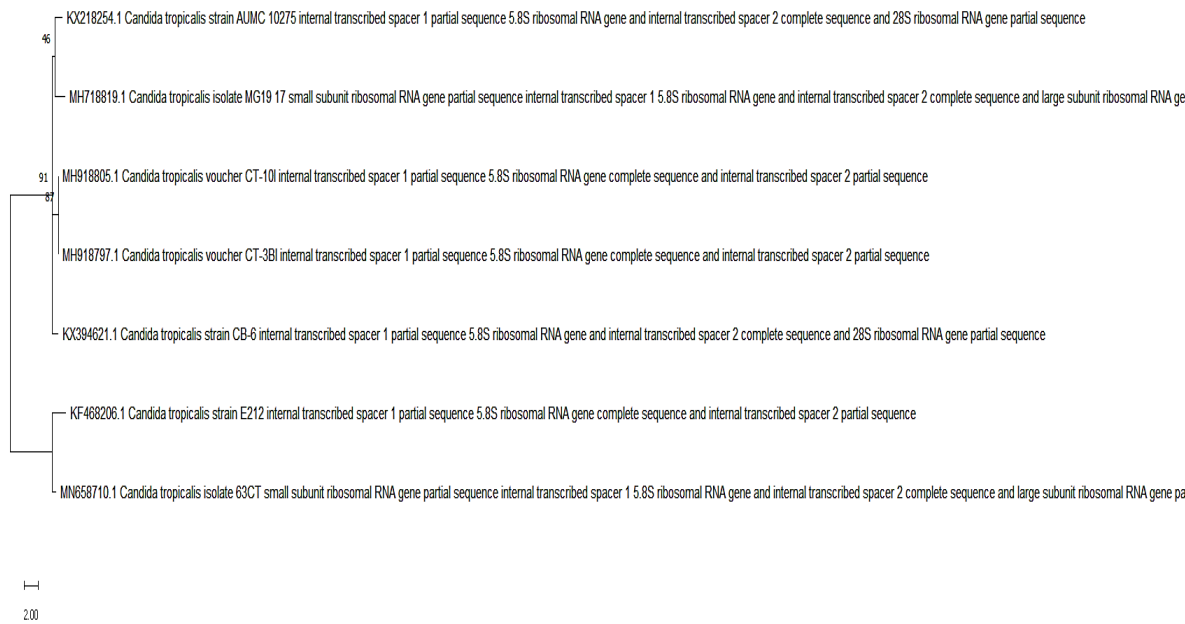

Fig. 7: Phylogenetic analysis of PCB 1.

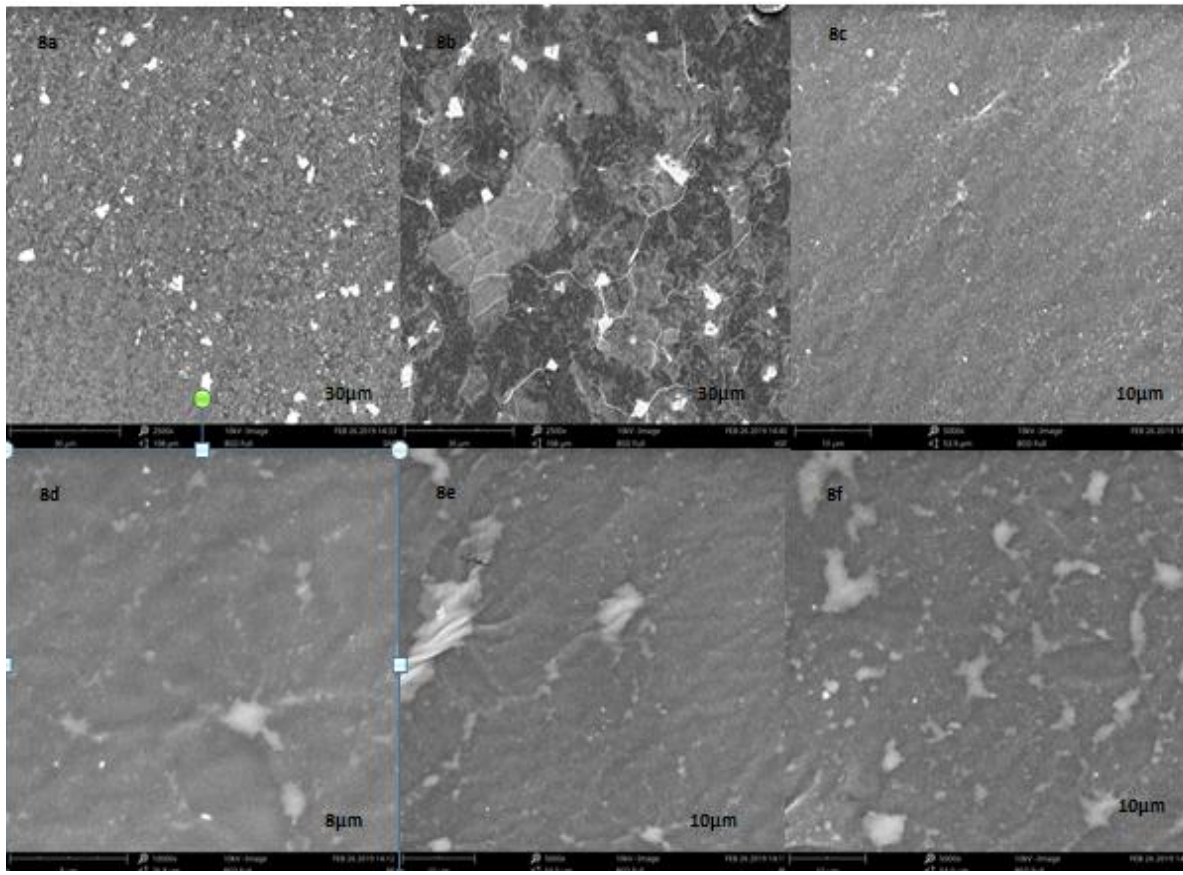

Fig 8a,8b,8c: SEM images of samples before bioleaching process using PCB1,PCB2 & PCB3.
Fig 8d,8e,8f: SEM images of samples after bioleaching process using PCB1,PCB2 & PCB3.

bioleaching different metals; the efficacy of leaching activity can be further improved by using a consortium of microbes which can be used as an efficient source in the removal of metals from discarded PCBs in an economic and eco-friendly perspective.

ACKNOWLEDGEMENTS

The authors would like to thank the Management and Principal Dr. Lilian Jasper of Women's Christian College, Chennai for providing the seed grant to carry out the research work.

REFERENCES

Altschul, S.F. 1990. Basic local alignment search tool. J. Mol. Biol., 215(3): 403-410.

Arshadi, M. and Mousavi, S.M. 2015. Enhancement of simultaneous gold and copper extraction from computer printed circuit boards using Bacillus megaterium. Bioresour. Technol., 175: 315–324.

Brandl, H. and Faramarzi, M.F. 2006. Microbe-metal interactions for the biotechnological treatment of metal-containing solid waste. China Particuol., 4(2): 93-97.

Brandl, H., Bosshard, R. and Wegmann, M. 2001. Computer-munching microbes: Metal leaching from electronic scrap by bacteria and fungi. Hydrometallurgy, 59: 319-322.

Cui, J. and Forssberg, E. 2003. Mechanical recycling of waste electric and electronic equipment: Rev. J. Hazard. Mater., 99(3): 243-263.

Díaz-Martínez, M.E. 2019. Microbial bioleaching of Ag, Au and Cu from printed circuit boards of mobile phones. Curr. Microbiol., 76(5): 536-544.

Engel, A. 2005. Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic epsilon proteobacteria. FEMS Microbiol. Ecol., 51: 31-53.

Greenfield, A. and Graedel, T.E. 2013.The omnivorous diet of modern technology. Resour. Conserv. Recycl., 74: 1-7.

Hagelüken, C. and Meskers, C. 2008. Mining Our Computers: Opportunities and Challenges to Recover Scarce and Valuable Materials. In: OECD-UNEP Conference on Resource Efficiency, 23-25 April 2008, Paris, France, OECD, Paris, pp. 1-22

Hall, L., Kelly A.D., Sherri, L., Wohlfiel, R. and Glenn D.R. 2003. Evaluation of the MicroSeq system for identification of mycobacteria by 16S Ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. J. Clinic. Microbiol., 41(4): 1447-1453.

Karwowska, E. 2014. Bioleaching of metals from printed circuit boards supported with surfactant-producing bacteria. J. Hazard. Mater., 264: 203-210.

Kaya, M. 2016. Recovery of metals and nonmetals from electronic waste by physical and chemical recycling processes. Waste Manag., 57: 64-90.

Khatri, B.R. 2018. Chemical and microbial leaching of base metals from obsolete cell-phone printed circuit boards. Sustain. Environ. Res., 28(6): 333-339.

Krieg, N.R. and Holt, J.G. 1984. Bergey's Manual of Systematic Bacteriology. Volume 1, Williams & Wilkins Co., Baltimore, pp. 161-172.

Lima, R. D. 2012. Bioleaching of copper sulfide flotation concentrates in batch reaction system using mesophile and thermophile microorganisms. Dyna, 72: 133-148.

Mishra, D. and Rhee, Y. 2014. Microbial leaching of metals from solid industrial wastes. J. Microbiol., 52: 1-7.

Needhidasan, S., Melvin, S. and Ramalingam, C. 2014. Electronic waste: An emerging threat to the environment of urban India. J. Environ. Health Sci. Eng., 12(1): 36.

Pradhan, J. and Kumar, S. 2012. Metals bioleaching from electronic waste by Chromobacterium Violaceum and Pseudomonads Sp. Waste Manag. Res. – J. Int Solid Wastes Public Clean. Assoc., 30(11): 1151-1159.

Ren, W., Li, P., Geng, Y. and Li, X. 2009. Biological leaching of heavy metals from contaminated soil by *Aspergillus niger*. J. Hazard. Mater., 167(1–3): 164-169.

Rozas, E.E. 2017. Bioleaching of electronic waste using bacteria isolated from the marine sponge *Hymeniacidon heliophila* (Porifera). J. Hazard. Mater., 329: 120-130.

Sajjad, W. 2019. Metals extraction from sulfide ores with microorganisms: The bioleaching technology and recent developments.Trans. Indian Inst. Metals, 72(3): 559-79.

Sheng, P.P. and Etsell, T.H. 2007.Recovery of gold from computer circuit board scrap using Aqua Regia. Waste Manag. Res. – J. Sustain. Circular Econ., 25(4): 380-383.

Tipre, D., Vora, S. and Dave. S. 2004. Medium optimization for bioleaching of metals from Indian bulk polymetallic concentrate. Indian J. Biotechnol., 3: 86-91.

Venkidusamy, K. and Megharaj, M. 2016. Identification of electrode respiring, hydrocarbonoclastic bacterial strain *Stenotrophomonas maltophilia* MK2 highlights the untapped potential for environmental bioremediation. Front. Microbiol.,7: 1-12.

White, T. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In Sninsky, J.J., Innis, M.A., Gelfand, D.H. and White, T. J. (eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, Cambridge, MA, pp. 315–322.

Widmer, R. 2005. Global perspectives on e-waste. Environ. Impact Assess. Rev., 25(5): 436-458.

Zhao, G., Wang, Z., Dong, M.H., Rao, K., Luo, J., Wang, D., Zha, J., Huang, S., Xu, Y. and Ma, M. 2008. PBBs, PBDEs, and PCBs levels in hair of residents around e-waste disassembly sites in Zhejiang Province, China, and their potential sources. Sci Total Environ 397: 46-57.

Web reference: https://unitar.org/about/news-stories/press/global-e-waste-surging-21-cent-5-years