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Bioleaching of Metals from Printed Circuit Boards by Mesophilic *Lysinibacillus sp.*

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ABSTRACT

In view of the rapidly increasing e-waste, bioleaching of metals from e-waste is an economical and ecologically conscientious option to address the issue of its disposal and/or recycling. Bioleaching of heavy metals by using bacteria of the Bacillus genus has been reported in many studies; however, the bioleaching potential of the Lysinibacillus genus is unexplored. In the present study, Lysinibacillus sp. SDG4 was isolated, identified, analysed and used for leaching toxic metals from e-waste printed circuit boards (PCBs). The bioleaching of metals was deciphered and analysed by using scanning electron microscopy (SEM) along with energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FTIR) and inductively coupled plasma-optical emission spectrometry (ICP-OES). The presence of organic acids in the Lysinibacillus primary metabolites was established by FTIR analysis. The presence of functional groups like C–O and C–N in the transmittance band range of 1037.70 to 1658.78 cm^{-1} wavelength, C–H at 2746.63 and 2964.59 cm^{-1}, O–H at 3412.08 cm^{-1} and RCO–OH at 582.50 cm-1 suggested the production of metal chelating functional groups by the bacterial strain. The heavy metal profile was determined using ICP-OES analysis. This revealed bioleaching of Al (99.74%), Zn (99.60 %), Cu (93.75%) and Fe (59.24%) in 30 days with a PCB concentration of 1 gm/ml by Lysinibacillus sp. SDG4. These findings confirmed the display of morphological changes by accumulation of metals by *Lysinibacillus sp*. SDG4 using SEM-EDX analysis. Thus, the study exhibited the potential of the *Lysinibacillus* genus in bioleaching metals from e-waste.

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INTRODUCTION

Exponential population growth coupled with technological advancement has substantially increased and increasing the use of electrical and electronic equipment (EEE). The disposal and/or recycling of obsolete and discarded EEE is a major environmental challenge having potential health hazards (Robinson, 2009; Sansotera *et al.*, 2013; Cesaro *et al.*, 2018; Islam *et al.*, 2020; Kang *et al.*, 2020; Abalansa *et al.*, 2021; Guin & Deswal 2023). The discarded EEE are commonly referred to as electronic waste or e-waste (EU, 2012; Grant *et al.*, 2013; Garlapati, 2016; Kudrat-E-Khuda, 2021; MoEFCC, 2022). The annual global e-waste generation in the year 2019 was 59.08 million tonnes (7.37 kg per capita) and is projected to be 82.34 million tonnes by 2030 (Leur & Walter 2019; Forti *et al.*, 2020; Abalansa *et al.*, 2021).

Printed circuit boards (PCBs), the basic and essential component of EEE, constitute about 1.7 to 3.1 per cent by weight of the total e-waste scrap (Karwowska *et al.*, 2014), and are the leading carriers of numerous valuable and toxic metals of e-waste (Narayanaswamy *et al.*, 2018). The major metallic elements of PCBs, from toxic and non-toxic groups, include

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aluminium (Al), arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), gold (Au), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), silver (Ag), tin (Sb), zinc (Zn), etc. having varying amount/content of metals ranging from about 0.2 – 20% (Narayanaswamy *et al.*, 2018 & 2021; Patel *et al.*, 2023). Most of these heavy metals are extremely poisonous, even in very low quantities, and cause severe harm to living things by getting into the food chain (Manna *et al.*, 2018). When present in excess they have a negative impact on the environment and the living things (BAN, 2002; Srinath *et al.*, 2002; Widmer *et al.*, 2005; Zaved *et al.*, 2008). E-waste cannot be degraded biologically, therefore it builds up in the environment and poses a major threat to ecosystems and living things. Due to budgetary restrictions and no substantial environmental rules in developing nations, the problem is worsened as growing internet infiltration has increased the generation of e-waste, which results in the dumping of garbage in landfills without proper treatment (Guin & Deswal, 2023).

Many physicochemical strategies have been used, including landfill disposal, chemical oxidation, reduction, adsorption, and precipitation. However, these strategies have some downsides and limitations as well (Parga *et al.*, 2007; Ariffin *et al.*, 2017; Uddin, 2017; Cheng *et al.*, 2019; Crini & Lichtfouse, 2019; Ang & Mohammad, 2020; Torres, 2020; Kalia *et al.*, 2022; Kumar *et al.*, 2023; Rehman *et al.*, 2023). Therefore, there has been increasing focus on finding alternative effective, economical, and environmentally friendly processes for removing metals from e-waste. In this context, biological processes offer economically viable and environment-friendly options for bioleaching heavy metals that do not require inputs of energy and/or chemicals (Fourest & Roux, 1992; Abatenh *et al.*, 2017; Deswal & Deswal, 2017; Igiri *et al.*, 2018; Kumar & Deswal, 2020). These biological methods use an organism's innate ability to absorb, change, or immobilize pollutants in the soil, water, and air. Pollutants can be broken down by some bacteria and fungus into less dangerous forms. For instance, some bacteria and fungi can break down or change contaminants into less toxic forms and then absorb the byproducts (Atuchin *et al.*, 2023; Alabssawy & Hashim, 2024). In fact, long-term waste disposal gives microorganisms a significant advantage in combating the combined polycyclic aromatic hydrocarbons (PAHs) and heavy metal pollution (Liu *et al.*, 2017). The biological methods involve natural metabolic bioprocesses of microorganisms to transform harmful compounds into non-toxic forms. Studies have reported that some microorganisms can develop particular resistance mechanisms, such as efflux and absorption mechanisms, extracellular precipitation, etc., to protect themselves against the toxicity of heavy metals even at high concentrations (Leedjarv *et al.*, 1996; Madoni *et al.*, 1996; Canovas *et al.*, 2003; Kumar & Deswal, 2021).

Bacterium *Strenopha monasmaltophilia* biologically removed Cu as much as 10 mg/L along with many PAHs as reported by Chen *et al.* (2014). Bacteria *Acromobacter, Pseudomonas and Enterobacter* are reported to successfully remove Pb, Cr and Cd up to a concentration of 0.1 – 10 mM (millimolar) from a given sample (Patel *et al.*, 2012). Bacteria *Mycobacterium* and *Pseudomonas* biologically removed Cd and Cu to the extent of 9.6 g/Kg and 246 g/Kg respectively (Brito *et al.*, 2015). Apart from bacteria species, some fungal species have also been reported for the biological removal of many heavy metals including Cd, Pb and, Ni, such as *Trichoderma tomentosum* (Hoseinzadeh *et al.*, 2023). *Acremomonium*, a fungal species, has reportedly consumed Mn, Fe, Zn, Cu and Al up to 5 mg/l (Ma *et al.*, 2014). Atagana (2009) reported *Fusarium Flocciferum*, *Trichoderma*, *Trametes versicolor*, and *Pleurotus* fungal species to consume Cd and Ni up to a concentration of 50 – 500 mg/kg.

Waghmode *et al.* (2021) reported the potential of *Paenibacillus* sp. to bioleach heavy metals from e-waste; Kadivar *et al.* (2021) reported the potential of *Acidithiobacillus thiooxidans* to bioleach metals from PCBs of old mobile phones; whereas, Wang *et al.* (2009) and Chandane *et al.* (2020) reported the potential of *Acidithiobacillus thiooxidans* and *Acidiphilum acidophilum* respectively to bioleach copper from computer PCBs. *Bacillus* sp. (like *Bacillus cerus* and *Bacillus subtilis*), *Aspergillus niger* and *Micrococcus luteus* are reported to be most efficient

microorganisms typically used for bioabsorption of *heavy* metals from PCBs (Sinha *et al.*, 2012; Borthakur & Sinha 2013; Aka & Babalola 2016; Narayansamy *et al.*, 2018; Narayansamy *et al.*, 2021; Wrobel *et al.*, 2023).

Although the bioleaching potential of *Bacillus genus* bacteria has been reported in many studies; however, the active participation of the *Lysinibacillus genus* as a dynamic metal-removing microorganism is unexplored. Though, *Lysinbacillus* sp. and *Bacillus* sp. are from the same family *Bacillacea*, but genus *Lysinibacillus* contains lysine and aspartate as essential amino acids in cell wall peptidoglycan instead of meso-diaminopimelic acid as in the case of genus *Bacillus*. In the present study, the bioleaching potential of *Lysinibacillus sp.* SDG4 (Genus: *Lysinibacillus*; Family: *Bacillaceae*) isolated from red mud having highly alkaline conditions has been explored for leaching heavy metals – iron, copper, aluminium and zinc from e-waste PCBs. Scanning electron microscopy (SEM) along with energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectroscopy (FT-IR) and inductively coupled plasma-optical emission spectrometry (ICP-OES) are the most widely used techniques to investigate the efficiency of microorganisms to bind with metal and bioleaching efficiency (Michalak *et al.*, 2011; Waghmode *et al.*, 2021); and therefore, being used in the present study.

MATERIALS AND METHODS

E-waste was obtained from depreciated e-waste of the National Institute of Technology (NIT) Kurukshetra, Haryana (India) stored in a room for subsequent transfer to an authorized disposal agency. This e-waste comprises of desktop units, laptops, UPS, keyboards, printers, scanners, batteries, etc. For the present experimental study, the plastic bodies were separated from the metallic parts of the e-waste units and printed circuit boards (PCBs) were collected. The collected PCBs were then crushed by using a Tilting Wolf-bench grinder for 20 minutes, and the crushed powder was sieved through a 120-µm pore size sieve. The sieved powder was autoclaved at 121°C and 15 psi of pressure for 30 minutes and then stored in the refrigerator for experiments to be carried out for the present study.

The red mud samples were randomly collected from a bauxite processing plant functioning in an active mining site of Koraput district (18°51'0" N 83°1'6" E) located in the southern-western part of Odisha state in India. The red mud samples were aseptically collected in sterile polythene bags at room temperature and transferred to the laboratory at NIT Kurukshetra for identification and isolation of mesophilic bacterial strains. The indigenous *Lysinibacillus sp.* was isolated from the red mud samples following the method of serial dilution in sterilized normal saline. For this, 1.0 g of bauxite soil sample was serially diluted nine times in distilled water, and an aliquot of 0.1 ml of the bauxite-soil sample of desired dilution was spread on Leuria- Bertani (LB) plates to achieve distinct colonies. Each dilution was plated on diluted LB medium which was enriched separately with different concentrations of salts of heavy metals, namely aluminium ($AlCl_3$; MW=133.34), ferrous ($FeSO_4 7H_2O$; MW=276.01) and copper ($CuSO_4 5H_2O$; MW=249.68) respectively for sensitivity or tolerance of bacterial growth. The agar plates were amended/enriched with Fe, Cu and Al salts at a concentration starting from 1 to 8 mM. The LB plates were then incubated at 37°C for 24 to 48 hours. The bacterial colonies which showed growth after this time frame at the highest concentration of Fe (7 mM), Cu (5 mM) and Al (4 mM) salts were considered to be tolerant to these heavy metals. After 24-48 hours, the bacterial colonies were then purified by grid streaking to separate the selected colonies based on their size, shape, and colour. Thereafter, the purified bacterial colonies were maintained at 4°C in a 20% glycerol stock (Jinal *et al.*, 2019) for experiments to be carried out for the present study.

Phenotypic characteristics of bacterial isolate exhibiting PCB tolerance, such as colony morphology, colour, bacterial shape and others were studied through visual observations.

Biochemical characterization was carried out with the help of several biochemical tests – including those for oxidase, catalase, methyl red, and H_2S generation using Hi-Biochemical test kits (Hi-Media, India) following the manufacturer's manual; whereas, gram staining was carried out with the help of freshly grown culture (Usharani *et al.*, 2017).

A molecular study was carried out for molecular characterization of the selected bacterial isolate exhibiting PCB tolerance. The selected bacterial isolate was identified by sequencing the 16S rRNA gene of the bacterial DNA. The bacterial DNA was isolated according to Green & Sambrook's (2012) methodology, and then the 16S rRNA gene was amplified using the primer pair 27F (5'-GAGAGTTTGATCCTGGCTCAG-30) and 1498R (5'CTACGGCTACCTTGTTACGA). For this, a 50 µL PCR reaction mixture was prepared that consisted of 5µL 10x Taq Buffer, 400 ng of each primer, 50 ng of template DNA, 2 µL of Taq Polymerase 93 units/µL (Genei, India) and the remaining amount of water. The Polymerase Chain Reaction (PCR) reaction mixture was performed in a thermocycler (Hi-Media Prima 96) programmed for an initial denaturation at 95°C for 3 minutes, 30 cycles of denaturation at 95°C for 45 seconds, annealing at 48°C for 1.30 minutes, elongation at 72°C for 1minute, and a final extension at 72°C for 7 minutes. The PCR product was separated through a 2% agarose gel and visualized using EtBr staining (Green & Sambrook, 2012). The desired bands were cut using a clean blade and eluted using a DNA gel extraction kit (Genei, India). The purified DNA was sent for sequencing to Genexplore-Diagnostics and Research Centre Pvt. Ltd., Ahmedabad, Gujarat (India).

The DNA sequence obtained in '*.ab1 format*' was then edited in BioEdit version 7.2.5 (Hall, 1999) so as to modify the sequence to eliminate low-quality nucleotide stretches from the ends. The obtained sequence was used as a template in the BLASTN tool (Altschul *et al.*, 1990) to find the most similar sequences. Further, MEGA X software was used to do the phylogenetic analysis of the 16S rDNA sequence (Kumar *et al.*, 2018); CLUSTAL W was used to perform the multiple sequence alignment (Thompson *et al.*, 1994); and the evolutionary distance matrix was computed using Kimura-2 parameter model. The maximum likelihood approach was used to build the evolutionary tree. Bootstrap analysis, using 1,000 re-samples, was utilised to assess the tree's statistical significance. The 16S rRNA gene sequence was also submitted to the National Center for Biotechnology Information (NCBI), and GenBank Database for accession numbers.

The 2-step bioremediation method has been reported to be an effective and efficient method, in comparison to 1-step bioremediation, for the bioremoval of metals (Verma *et al.*, 2012; Narayansami *et al.*, 2021). So, this bioleaching study was performed in a 2-step bioremediation process as per Narayansami *et al.* (2021). The isolated identified bacterial strain (*Lysinibacillus*) was grown in LB medium diluted to 10^{-1} times and grown in an incubator shaker at 32°C for 48 hours. Sterile powder of PCB was then added to the growing culture to obtain a final concentration of 0.1 gm/mL, 0.5 gm/ml and 1 gm/ml, and the flasks were again incubated for 30 days. A positive control with bacterial strain but without e-waste, and a negative control with e-waste but without bacterial strain were also included in the bioleaching experiments.

The morphological and elemental content of the PCB sample of e-waste were identified using scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDX) (JEOL, JSM-6390 LV) following the Bajestani *et al.* (2014) methodology. A fully dried PCB sample was loaded onto the copper stubs using carbon tape. Thereafter, the PCB sample was coated with 10 microns' gold particles using an auto-fine coater under vacuum at 40 mA (milliampere) for 60 seconds to examine the morphological and elemental content of the PCB sample. The morphology and elemental composition of the PCB sample residue were again analyzed after microbial/bioleaching treatment. The dried and microbe-treated PCB was fixed using 3% (v/v) glutaraldehyde and dehydrated over an ethanol gradient. The dried samples were then subjected to SEM-EDEX study, as mentioned in the preceding paragraph, to identify the morphology of bioleached bacteria and PCB.

Fourier Transform Infrared Spectroscopy (FTIR) study was used to detect the removal of organic molecules from the PCB samples. The bioleached experiments were carried out by following the method of Bullen *et al.* (2008), Sethurajan *et al.* (2012) and Narayanasamy *et al.* (2018 & 2021) with minor alterations. The treated PCB samples obtained after the bioremoval experiments were filtered using Whatman No.1 filter paper to eliminate solid particles. The residue was mixed with potassium bromide (KBr) and grounded to make thin tablets. The tablets were then subjected to the diamond plate of the FTIR spectrophotometer (Perkin Elmer, USA).

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was carried out to measure the heavy metal concentration before and after the bioremoval experiment by modifying the methodology used by Ehi-Eromosele *et al.* (2012). Aliquots of 0.1, 0.5 and 1.0 gm of the crushed e-waste sample were separately, mixed with 100 ml of Nutrient Broth (NB) medium and autoclaved. One per cent log phase batch bacterial cultures (A_{600} =0.8) were inoculated into the flasks. The batch cultures were kept in an orbital shaker operating at 130 rpm at 32^0 C. A positive control containing 1.0 gm PCB powder but without bacterial inoculation was taken. After 72 hrs., 10 ml of the suspension from each flask was filtered through Whatman filter paper No.1 and then centrifuged (Remi CM-8 plus) at 10,000 rpm for 10 minutes. The supernatant was taken and filtered through a 0.22-micron filter. The filtrate was digested with 10 ml of concentrated nitric acid and 2 ml of H_2O_2 on a hotplate at 120^{o}C till all the acid was evaporated. The residue was then cooled to ambient temperature and diluted to 100 ml with autoclaved Milli-Q water. The solution was then filtered through a 0.22-micron syringe filter. The filtrates were then subjected to ICP-OES measurement (Perkin Elmer) availing the facility present in Odisha University of Agriculture and Technology (OUAT), Bhubaneshwar, India.

RESULTS AND DISCUSSION

PCB samples collected from e-waste for this study contain many valuable metals and toxic heavy metals. The qualitative scanning of PCB samples using SEM with EDX revealed the presence of aluminium (Al), silicon (Si), manganese (Mn), iron (Fe), copper (Cu), magnesium (Mg), zinc (Zn), lead (Pb), potassium (K), carbon (C) and oxygen (O). The presence of these metals and elements in PCBs has been reported by earlier studies as well (Patel *et al.*, 2014; Zhang *et al.*, 2015; Liu *et al.*, 2017; Narayanasamy *et al.*, 2018; Yuan *et al.*, 2018).

The isolated bacteria showed proper growth in the enriched medium containing high concentrations of heavy metals, namely Fe (7 mM), Cu (5 mM) and Al (4 mM). The bacterial isolate (SDG4) was subjected to phenotypic, biochemical characterization and molecular identification through 16S rDNA sequencing (Table 1). The isolate was found to belong to the genera *Lysinibacillus*. The partial 16s rDNA sequence was submitted to GenBank under the accession number ON320410 (*Lysinibacillus* sp.; SDG4). The phylogenetic tree based on a partial 16S rDNA sequence is presented in Fig. 1. The tree shows *Lysinibacillus sp.* SDG4 is forming a monophyletic clade with other species of *Lysinibacillus* bacterial strains with a bootstrap value of 96.

Many members of the *Bacillus* family are reported to be chemolithotropic bacteria, and tolerant to extreme concentrations of heavy metals, for example, *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans*, *Thermoplasma acidophilum*, etc. (Paiment *et al.*, 2001; Wang *et al.*, 2009; Arshadi *et al.*, 2019; Chandane *et al.*, 2020; Kadivar *et al.*, 2021; Magoda *et al.*, 2022; Panigrahi & Panigrahi, 2023). Although, the potential of Bacillus species (such as *Bacillus cerus, Bacillus subtilis*, etc.) in the bioleaching of metal ions has been reported by many studies (Sinha *et al.*, 2012; Aka & Babalola, 2016); however, such studies are lacking for *Lysinibacillus* strains. The morphology of the e-waste PCB sample was studied through

Table 1. Phenotypic, Biochemical and Molecular Identification of Isolated Bacteria Strain

Isolate name	**SDG4**
GenBank accession number	**ON320410**
Identification of species	***Lysinibacillus* sp.**
Genus	*Lysinibacilus*
Family	*Bacillaceae*
Colony Characters	Round, rough, small colonies with irregular edges, off-white in color and opaque.
Gram reaction	Gram +ve
Cell shape	Rod-shaped
Motility	Motile

Biochemical Examination

Test		Principle	Response
Carbon source utilization	ONPG (ortho-Nitrophenyl-β-galactoside)	Detects β galactosidase activity	+
	Esculin	Esculin hydrolysis	+
	Malonate	Detects the capability of the organism to utilize Sodium malonate as a sole source of carbon	–
	Arabinose	Arabinose Utilization	–
	Xylose	Xylose Utilization	–
	Adonitol	Adonitol Utilization	–
	Rhamnose	Rhamnose Utilization	–
	Cellobiose	Cellobiose Utilization	–
	Melibiose	Mellibiose Utilization	–
	Saccharose	Saccharose Utilization	–
	Raffinose	Raffinose Utilization	–
	Lactose	Lactose Utilization	–
	Trehalose	Trehalose Utilization	–
	Glucose	Glucose Utilization	+
	Malonate Utilization	Detects the capability of the organism to utilize Sodium malonate as a sole source of carbon	–
Nitrogen source utilization	Lysine	Detects Lysine decarboxylation	–
	Nitrate utilization	Detects nitrate reduction	–
	Phenylalanine Deamination	Detects phenyl alanine deamination activity	–
	Ornithine Utilization	Detects Ornithine decarboxylation	–
	Urease	Detects Urease activity	+
IMViC Test Series	Voges Proskauer's	Detects section production	+
	Methyl Red	Detects acid production	–
	Citrate	Detects capability of the organism to utilize citrate as sole carbon source	+
	Indole	Detects deamination of Tryptophan	–
Sulphur source utilization	H_2S Production	Detects H_2S production	–
Oxidase		Cytochrome oxidase production	+

SEM before and after bacterial treatment. The study (magnification: 50 μm) revealed that the tiny components of e-waste PCBs are soft, rod-like particles with irregular crystals connected to their surfaces (Fig. 2a). In between the irregular crystals, multilaterally shaped particles have adhered to the surface. Bioremoval study after incubation with the *Lysinibacillus* sp. (SDG4) onto the PCBs is presented in Fig. 2b, c and d with 0.1, 0.5 and 1.0 gm aliquote of PCB respectively showing attachment of the bacteria in all these three SEM images. The adsorption/ attachment of bacterial strain onto the e-waste particles is correlated to an increased amount of bioleaching. SEM study thus confirmed the bioremediation ability of the *Lysinibacillus* sp.

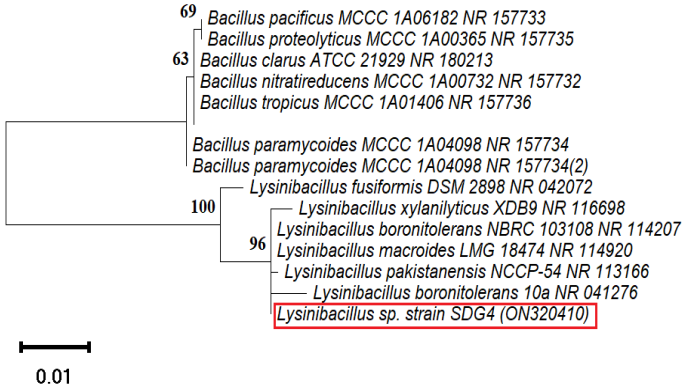


Fig. 1. Phylogenetic Tree based on Partial 16S rDNA Sequence. Numbers at Nodes Indicate Bootstrap Values (1,000 replicates). The Scale Bar Indicates Base Substitution per Site

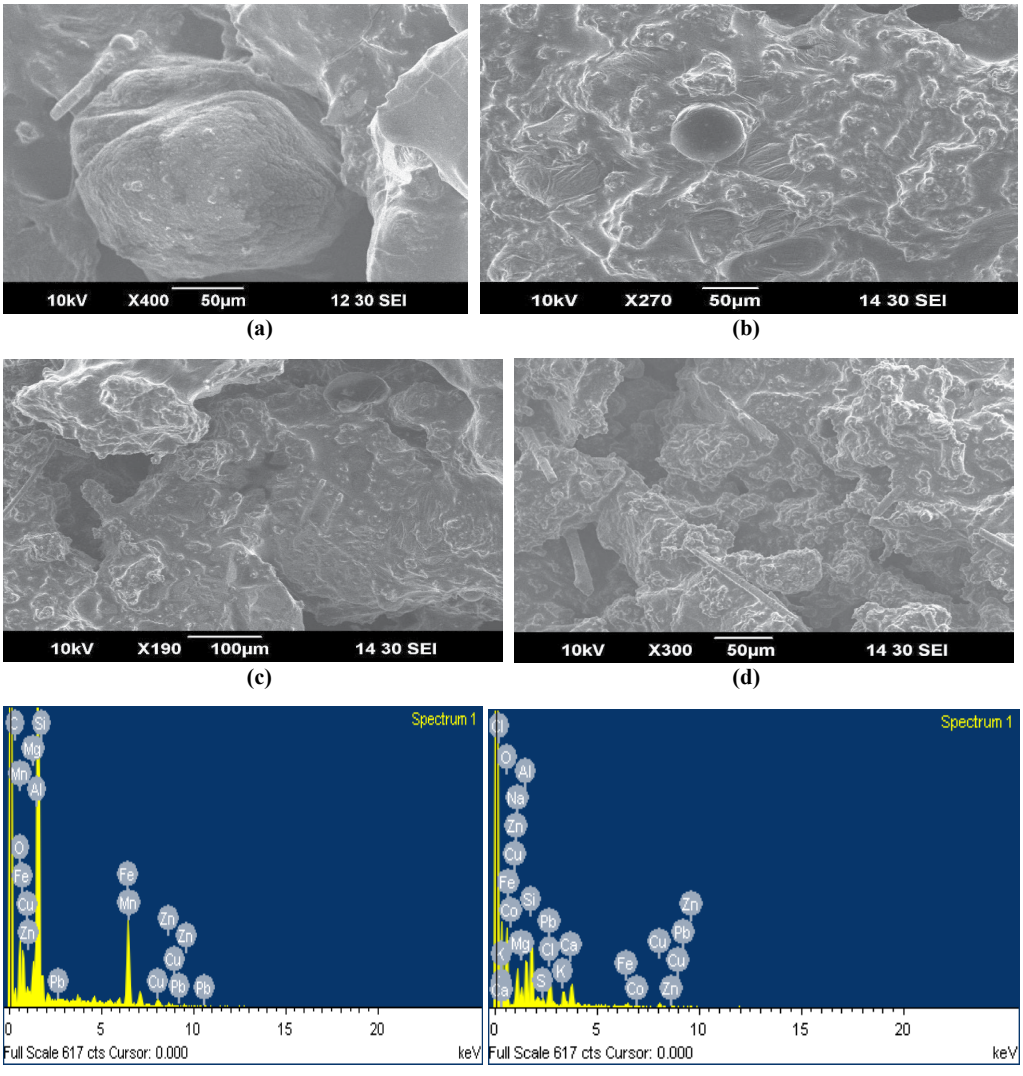


Fig. 2. SEM-EDX Spectrum of Biotreated Experiment with *Lysinibacillus* sp. Strain SDG4: (**a**). SEM Photograph of Negative Control; (**b**). SEM Photograph of Treated Residues in 0.1 gm Aliquote of E-waste PCB; (**c**). SEM Photograph of Treated Residues in 0.5 gm Aliquote of PCB; (**d**). SEM Photograph of Treated Residues in 1 gm Aliquote of PCB; (**e**). EDX Spectrum of the PCB Elements – negative control; (**f**). EDX Spectrum of the PCB Elements – treated residues

Table 2. Composition of Elements in Control and Treated E- waste PCB Samples

Element	Untreated (Control)		Treated	
	Weight %	Atomic %	Weight %	Atomic %
C K	12.49	24.51	43.03	54.91
O K	11.01	16.21	34.33	33.67
Na K			3.55	2.42
Mg K	0.97	0.94	1.37	0.89
Al K	**57.78**	**50.46**	**2.55**	**1.48**
Si K	1.53	1.29	4.56	2.55
S K			0.67	0.33
Cl K			2.48	1.10
K K			1.24	0.50
Ca K			2.46	0.96
Mn K	0.82	0.35		
Fe K	**12.51**	**5.28**	**0.62**	**0.18**
Co K			0.31	0.08
Cu K	**1.66**	**0.62**	**1.17**	**0.54**
Zn K	**0.86**	**0.31**	**1.69**	**0.40**
Pb M	0.35	0.04	-0.02	0.00

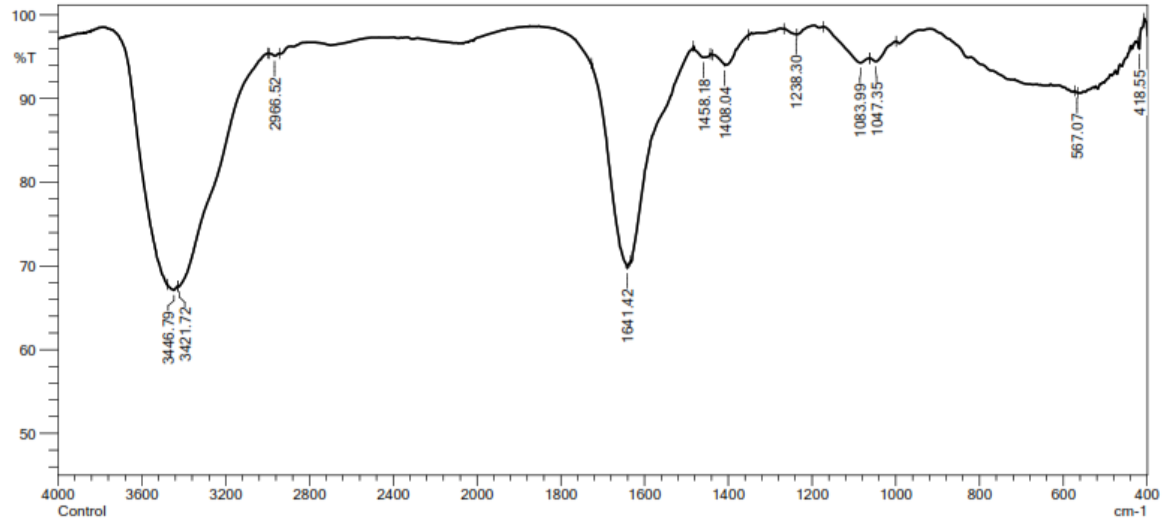
strain SDG4 to heavy metals.

The EDX analysis detected several elements including C, K, O, Mg and Si along with heavy metals like Al, Cu, Fe, Zn and Pb in the PCB sample (Fig. 2e). The EDX analysis also confirmed variations in metal quantities by the bioleaching process with *Lysinibacillus* sp. SDG4 (Fig. 2 f). The bioleaching of heavy metals from the e-waste PCB sample with *Lysinibacillus* sp. SDG4, in terms weight and atomic percentage, in comparison with control is presented in Table 2. After treatment with *Lysinibacillus* sp. SDG4, heavy metals Al, Fe and Cu have exhibited decreased weight and atomic percentage, and consequently, other elements exhibited the reverse trend (Table 2).

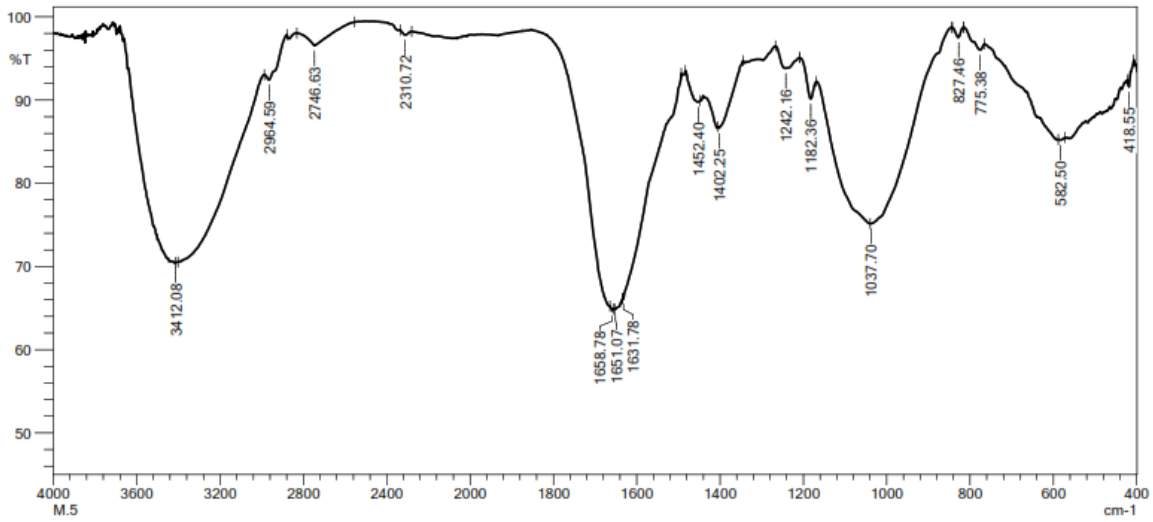
The findings of this work are consistent with reports that have demonstrated the removal of heavy metals using bacterial biomass and an ion exchange mechanism (Bueno *et al.*, 2007; Vishan *et al.*, 2017). Apart from this, the process by which the metals are freed from the PCBs depends upon the type of biomaterial, features of the metal fluid chemistry and environmental conditions. Das (2010) has also suggested that everything mentioned has an impact on the mechanism of metal biosorption irrespective of the biomass status (living or dead). Physico-chemical interactions between metal ions and the functional groups on the cell surface, such as electrostatic interactions, ions exchange, metal ion chelation, and complexation, serve as the foundation for most biosorption mechanisms (Ozer *et al.*, 2005) as it did in the case of the present study.

The production of acidic substances during bioleaching also contributes to the removal of metals from e-waste. FTIR spectrum reveals the range of transmittance bands that can be correlated towards active functional groups, including the ones associated with organic acids. Therefore, the presence of active functional groups serves as evidence that harmful heavy metals have been thoroughly eliminated during the evolution of biological systems. FTIR spectrum of control is presented in Fig. 3a, and that of the organic acid produced after the treatment of e-waste PCB sample with bacterial strains *Lysinibacillus* sp. SDG4 is presented in Fig. 3b for comparison and analysis.

The FT-IR spectrum of the e-waste PCB negative control sample (Fig. 3a) showed a range of transmittance bands which are correlated toward active functional groups C-H, O-H and PNH_2 (2966.52 to 3446.79 cm^{-1}); and C-O, C-C and C-N groups (1047.35 to 1641.42 cm^{-1}). In



(a)



(b)

Fig. 3. FT-IR spectrum of bioleached experiment: (**a**). Negative Control without Bacterial Treatment; (**b**). Bioleached Residues by Treatment with *Lysinibacillus* sp. Strain SDG4

the sample treated with *Lysinibacillus* sp. strain SDG4, the FTIR spectrum (Fig. 3b) showed stretching of C–N and C–O bonds and installation of many new functional groups. Bonds involving a lot of C–O and C–N groups can be observed in the range of 1037.70 to 1658.78 cm^{-1}. The treatment by strain *Lysinibacillus* sp SDG4 has shown numerous transmittance bands in the range of 1631.78 to 1658.76 cm^{-1}, indicating the formation of C = C bonds. There is also the formation of strong C–H bonds at transmittance bands at 2746.63 and 2964.59 cm^{-1}, O-H bond at 3412.08 cm^{-1} and RCO-OH bond at 582.50 cm^{-1} which confirms the production of organic acids. Therefore, according to the FTIR study, protonated carbonyl and carboxyl groups were crucial to biosorb metals. Such observation was also reported by Pethkar *et al.* (2001) where similar findings were noted during the adsorption of gold and silver by two fungal strains *Cladosporium cladosporoides* (strain 1 and strain 2). A similar report has been given by Santhiya & Ting (2005) in the bioleaching of spent refinery processing catalyst using

Table 3. ICP-OES analysis of heavy metals Fe, Cu, Al and Zn bioleached by *Lysinbacillus* sp SDG4

	Amount of aliquot of e-waste PCB powder sample (gm/ml)	Analytes' Residual Concentration (mg/L)			
		Fe	Cu	Al	Zn
Lysinbacillus sp SDG4	0.1	0.070	1.987	0.073	0.250
	0.5	0.091	1.829	0.057	0.190
	1.0	0.064	0.250	0.100	0.128
Control	1.0	0.157	4.000 (Saturated)	38.05	32.29

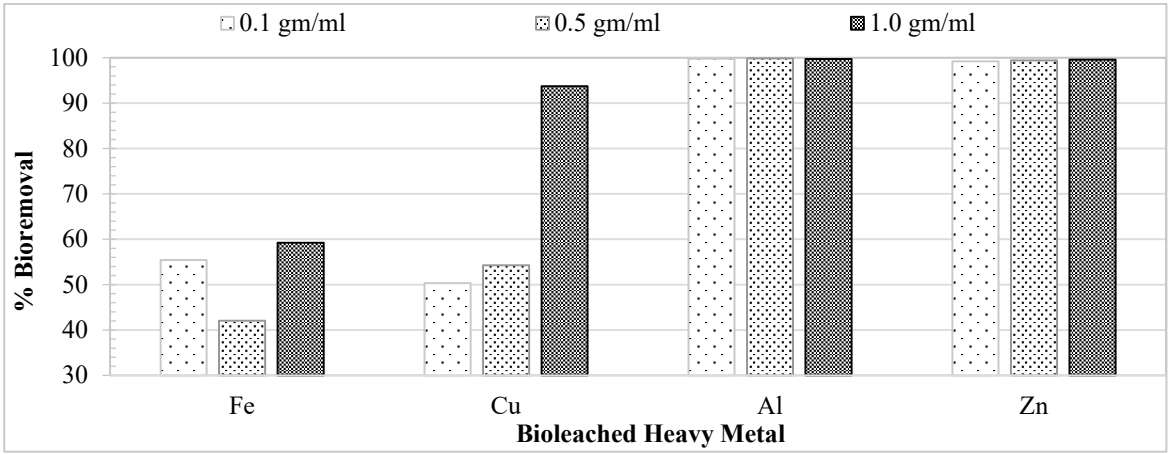


Fig. 4. Per Cent of Bioleached Heavy Metals with Varying Amount of Aliquot of E-waste PCB Sample

Aspergillus niger and Narayanaswamy *et al.* (2018) for the bioleaching of metals with the help of acidophilic *Aspergillus niger*.

The inductively coupled plasma-optical emission spectrometry (ICP-OES) study was performed to measure the bioremoval capacity of the *Lysinibacillus* sp. SDG4. The results are presented in Table 3. The result depicts the ability of *Lysinibacillus* sp. to remove heavy metals iron, copper, aluminium and zinc from e-waste PCBs. The bioleaching of metals increased with an increase in metal concentration in the medium (Fig. 4). The results are in accordance with the studies, such as Shabani *et al.* (2013), Narayanaswamy *et al.* (2018) and Chandane *et al.* (2020). The highest amount of bioremoval of metals could be observed when 1 gm/ml e-waste powder was present in the medium. With 1.0 gm/ml of the aliquot of PCB powder, *Lysinibacillus* sp SDG 4 bioleached Al, Zn, Cu and Fe to an extent of 99.74 % (37.950 mg/L), 99.60 % (32.162 mg/L), 93.75 % (3.750 mg/L) and 59.24 % (0.093 mg/L) respectively (Fig. 4) with the corresponding residual concentration of 0.100, 0.128, 0.250 and 0.064 mg/L (Table 3).

The results are encouraging and comparable with other reported bioleached studies. Kadivar *et al.* (2021) reported the potential of *Acidithiobacillus thiooxidans* to bioleach Cu (98%) and Ni (82%) at 30 °C from PCBs of old mobile phones. The bioleaching of computer PCBs by treatment with *Acidithiobacillus thiooxidans* and *Acidiphilum acidophilum* resulted in 75 and 100 % Cu removal respectively (Wang *et al.*, 2009; Chandane *et al.*, 2020). Kaliyaraj *et al.* (2019) reported the recovery of 68 % of Cu and 42 % of Fe from PCBs by treatment with *Streptomyces albidoflavus* TN10. Bacterium *Strenopha monasmaltophilia* biologically removed copper (Cu) as much as 10 mg/L along with many polycyclic aromatic hydrocarbons (PAHs) as reported by Chen *et al.* (2014). Bacteria *Mycobacterium* and *Pseudomonas* biologically removed Cd and Cu to the extent of 9.6 g/Kg and 246 g/Kg respectively, Brito *et al.* (2015). Apart from bacteria

species, some fungal species have also been reported for the biological removal of many heavy metals. *Acremomonium*, a fungal species, has reportedly consumed manganese (Mn), iron (Fe), zinc (Zn), copper (Cu) and aluminium (Al) up to 5 mg/L (Ma *et al.*, 2014).

CONCLUSION

In the current study, the effectiveness of isolated native bacterial species *Lysinibacillus* sp. SDG4 of *Lysinibacillus genus* of *Bacilaceae family* in the bioleaching of heavy metals from e-waste PCB has been reported. The results are in agreement with the reported studies on the bioleaching of heavy metals from e-waste by bacteria of the *Bacillus genus* and other microorganisms. The findings of the study exhibited the potential of the *Lysinibacillus genus* in bioleaching and/or degrading heavy metals from e-waste PCBs, and suggest carrying out more research on the *Lysinibacillus genus*. The findings recommend bioleaching and biodegradation for recycling and recovery of metals from e-waste as an economical and ecologically conscientious technique, particularly in developing countries that have potential scope of startups for the sustainable recycling of e-waste.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practised in this research.

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