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Isolation and Screening of Indigenous Heavy Metal Resistant Bacteria from E-Waste Contaminated Soils for Bioremediation Applications

Ms. Mamta Prajapati
Faculty, Department of Botany, Government Gandhi College
Balaji Mehona, District Bhind (India)

Abstract
The rapid increase in electronic waste (e-waste) has become a serious environmental concern due to the release of toxic heavy metals into soil ecosystems. This study aimed to isolate and screen indigenous heavy metal resistant bacteria from e-waste contaminated soils and evaluate their potential for bioremediation applications. Soil samples were collected from selected e-waste dumping sites and analyzed for physicochemical properties and heavy metal content. Bacterial isolates were obtained using metal-amended culture media and screened for resistance against lead, cadmium, chromium, and nickel. Selected isolates were further evaluated through minimum inhibitory concentration (MIC) studies to determine their tolerance levels. The results revealed significantly elevated concentrations of heavy metals in contaminated soils and a reduced overall microbial population; however, several indigenous bacterial strains exhibited strong multi-metal resistance with high tolerance thresholds. These findings highlight the adaptive capability of native soil bacteria and their potential use as eco-friendly and sustainable agents for the remediation of heavy metal polluted environments associated with improper e-waste disposal.

Keywords: E-waste, Heavy metals, Metal-resistant bacteria, Bioremediation, Soil contamination

1. INTRODUCTION

The rapid expansion of the electronics industry, coupled with shortened product life cycles and increasing consumer demand, has led to a dramatic rise in electronic waste (e-waste) generation worldwide, making it one of the fastest-growing solid waste streams of the modern era. E-waste comprises discarded electrical and electronic equipment such as computers, mobile phones, circuit boards, televisions, and household appliances, which contain a complex mixture of valuable materials and hazardous substances. Improper handling, informal recycling, and unregulated disposal of e-waste have emerged as significant environmental challenges, particularly in developing countries, where dismantling activities often occur without adequate safety or environmental controls. One of the most serious consequences of such practices is the release of toxic heavy metals into surrounding soil ecosystems, resulting in persistent contamination that poses long-term risks to environmental quality and public health [1], [2]. E-waste materials are rich sources of heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and mercury (Hg), which are known for their toxicity, non-biodegradable nature, and tendency to bioaccumulate within biological systems. Once released into soils, these metals can remain for decades, disrupt

microbial and plant communities, leach into groundwater, and enter the food chain, thereby causing neurological disorders, renal damage, carcinogenic effects, and ecological imbalance in exposed populations [3], [4]. Traditional physicochemical remediation techniques such as soil excavation, vitrification, chemical precipitation, and soil washing have been employed to address heavy metal contamination; however, these methods are often expensive, energy-intensive, and environmentally invasive, frequently leading to secondary pollution and loss of soil fertility [5]. Consequently, there is growing global interest in developing sustainable, cost-effective, and eco-friendly remediation strategies capable of restoring contaminated soils without compromising ecological integrity. In this context, microbial bioremediation has emerged as a promising alternative, utilizing the metabolic and physiological capabilities of microorganisms to detoxify, immobilize, or remove heavy metals from polluted environments [6], [7]. Microorganisms inhabiting contaminated sites often evolve adaptive mechanisms that allow them to survive under high metal stress, making indigenous bacteria particularly valuable candidates for bioremediation applications. These bacteria employ diverse resistance and detoxification strategies, including biosorption to cell surfaces, intracellular bioaccumulation, enzymatic reduction of toxic metal ions, efflux pump systems, and the production of extracellular polymeric substances that bind and sequester metals [8], [9]. Several studies have reported the presence of heavy metal-resistant bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, and *Acinetobacter* in contaminated soils, highlighting their potential role in mitigating metal toxicity [1], [10]. Notably, bacteria isolated from chronically polluted environments such as industrial zones, mining areas, and e-waste dumping sites often exhibit higher resistance thresholds and multi-metal tolerance compared to laboratory strains, underscoring the importance of exploring indigenous microbial communities for remediation purposes [11], [12]. Despite increasing recognition of microbial bioremediation as an effective approach, the diversity, resistance patterns, and functional potential of bacteria inhabiting e-waste contaminated soils remain insufficiently explored, particularly in terms of their ability to tolerate and remove multiple heavy metals simultaneously. Isolation and screening of such bacteria constitute a critical preliminary step in the development of efficient bioremediation systems, as it enables the identification of robust strains capable of functioning under harsh environmental conditions [13]. Screening approaches based on growth in metal-amended media and determination of minimum inhibitory concentrations (MICs) provide valuable insights into bacterial tolerance levels and adaptability to metal stress, forming the foundation for subsequent molecular and mechanistic investigations [14]. Furthermore, understanding the resistance profiles of bacterial isolates against commonly occurring metals in e-waste-contaminated soils is essential for selecting suitable candidates for large-scale applications. Previous research has demonstrated that bacteria capable of resisting high concentrations of Pb, Cd, Cr, and Ni can significantly reduce metal bioavailability through biosorption and bioaccumulation, thereby lowering environmental toxicity [15]. In light of these considerations, the present study focuses on the isolation and screening of indigenous heavy metal-resistant bacteria from e-waste contaminated soils, with the objective of identifying bacterial strains exhibiting significant

tolerance to selected toxic metals. By emphasizing the use of naturally adapted microorganisms, this work aims to contribute to the growing body of research supporting sustainable bioremediation strategies for managing e-waste-induced soil pollution and advancing environmentally responsible waste management practices [16].

2. LITERATURE REVIEW

The environmental implications of improper e-waste management have been extensively documented, with numerous studies identifying e-waste as a major source of heavy metal contamination in soil ecosystems. Researchers have reported that informal dismantling, open burning, and uncontrolled landfilling of electronic waste lead to elevated concentrations of toxic metals such as lead, cadmium, chromium, nickel, copper, and mercury in surrounding soils, often exceeding permissible environmental limits [1], [2]. These metals persist in soil for long periods due to their non-biodegradable nature and significantly alter soil physicochemical properties, microbial diversity, and ecosystem functioning [3]. Campillo-Cora *et al.* highlighted that chronic heavy metal exposure reduces soil microbial biomass while simultaneously selecting for metal-tolerant microbial populations, thereby reshaping microbial community structure in contaminated environments [4].

In response to the limitations of conventional remediation methods, microbial bioremediation has gained increasing attention as an effective and environmentally sustainable alternative. Several studies have demonstrated that microorganisms possess inherent abilities to tolerate, immobilize, or remove heavy metals through diverse biochemical and physiological mechanisms [5], [6]. Jiang *et al.* isolated heavy metal-resistant *Burkholderia* species from contaminated soils and demonstrated their capacity to tolerate high concentrations of Pb and Cd, emphasizing the adaptive potential of indigenous bacteria in polluted environments [7]. Similarly, Pande *et al.* reported that bacteria exposed to prolonged metal stress develop resistance mechanisms that enhance survival and detoxification efficiency, making them suitable candidates for bioremediation applications [8].

Isolation and screening of metal-resistant bacteria form the cornerstone of microbial bioremediation research. Numerous investigations have employed selective culture techniques using metal-amended media to isolate bacteria capable of surviving under high metal stress [9]. Studies by Oziegbe *et al.* revealed that indigenous bacterial isolates from contaminated soils often exhibit multi-metal resistance, an essential trait for remediation of complex pollution scenarios such as e-waste dumping sites [10]. Minimum inhibitory concentration (MIC) assays have been widely used to quantify bacterial tolerance thresholds, providing a reliable measure of resistance and adaptability [11]. These screening approaches enable the identification of robust bacterial strains for further functional and molecular analyses.

Mechanistically, heavy metal-resistant bacteria employ multiple strategies to mitigate metal toxicity. Biosorption, involving passive binding of metal ions to functional groups present on the bacterial cell wall, is considered one of the most rapid and effective metal removal mechanisms [12]. Pham *et al.* demonstrated that bacterial cell surfaces rich in carboxyl, phosphate, and hydroxyl groups play a crucial role in binding metal ions, thereby reducing

their bioavailability [13]. In addition to biosorption, bioaccumulation allows bacteria to internalize metals and sequester them within intracellular compartments, while efflux systems actively transport toxic ions out of the cell [14]. Kondakindi *et al.* emphasized the role of extracellular polymeric substances (EPS) produced by bacteria in immobilizing heavy metals, enhancing resistance and remediation efficiency [15].

E-waste contaminated soils represent a particularly challenging environment due to the presence of multiple metals at varying concentrations. Kaur reported that microbial consortia isolated from e-waste environments exhibited enhanced biosorption and bioleaching capabilities compared to isolates from non-contaminated sites, highlighting the importance of site-specific microbial adaptation [16].

3. MATERIALS AND METHODOLOGY

The present study adopted an experimental and analytical research design to isolate and screen indigenous heavy metal resistant bacteria from e-waste contaminated soils and to evaluate their resistance potential for bioremediation applications. Standard microbiological, physicochemical, and statistical procedures were followed to ensure reproducibility and scientific validity.

1. Study Area and Soil Sampling Methodology

Soil samples were collected from selected e-waste contaminated locations characterized by prolonged accumulation of discarded electronic components such as printed circuit boards, wires, batteries, and plastic casings. Control soil samples were collected from nearby non-contaminated sites with similar soil texture and vegetation but no history of e-waste exposure. Sampling was carried out from the surface soil layer at a depth of 0–15 cm, as this zone is most affected by anthropogenic contamination and microbial activity. At each site, multiple subsamples were collected randomly and pooled to form a composite sample. Samples were transported in sterile polyethylene bags and stored at **4 °C** until analysis.

Table 1: Soil Sampling Details

Parameter	Description
Sampling depth	0–15 cm
Sampling technique	Random composite sampling
Sample storage	Sterile bags at 4 °C
Control samples	Non-e-waste areas

2. Physicochemical Analysis of Soil Samples

Soil physicochemical properties were analyzed using standard methods. Soil pH was measured in a 1:2.5 (w/v) soil–water suspension using a digital pH meter. Electrical conductivity (EC) was measured to assess soluble salt concentration. Organic matter content was estimated using the Walkley–Black method. Soil moisture content was determined gravimetrically. Heavy metal concentrations (Pb, Cd, Cr, Ni) were analyzed after acid digestion using Atomic Absorption Spectrophotometry (AAS).

Formula 1: Soil Moisture Content

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$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where: W_1= weight of fresh soil, W_2= weight of oven-dried soil

Table 2: Methods Used for Physicochemical Analysis

Parameter	Method Used
pH	Digital pH meter
Electrical conductivity	Conductivity meter
Organic matter	Walkley–Black method
Moisture content	Gravimetric method
Heavy metals	Acid digestion + AAS

3. Isolation of Bacterial Strains

Bacterial isolation was performed using the serial dilution and spread plate technique. One gram of soil was suspended in sterile saline solution and serially diluted up to 10^{-6}. Aliquots (0.1 mL) were spread on nutrient agar plates supplemented separately with sub-lethal concentrations of heavy metals. Plates were incubated at 30 ± 2 °C for 24–48 hours. Morphologically distinct colonies were purified by repeated streaking.

Formula 2: Total Viable Bacterial Count

$$\text{CFU/g} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume plated (mL)}}$$

4. Screening for Heavy Metal Resistance

Purified bacterial isolates were screened for resistance against Pb^{2+}, Cd^{2+}, Cr^{6+}, and Ni^{2+} by inoculating them on nutrient agar plates amended with increasing metal concentrations. Growth was assessed qualitatively and isolates showing sustained growth at higher concentrations were considered metal resistant. Multi-metal resistance was determined by testing growth in the presence of more than one metal.

Table 3: Metal Resistance Screening Criteria

Growth Symbol	Interpretation
+++	Strong growth
++	Moderate growth
+	Weak growth
–	No growth

5. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the broth dilution method. Nutrient broth was supplemented with increasing concentrations of individual metals. Bacterial cultures were inoculated and incubated under shaking conditions. Growth was measured spectrophotometrically at 600 nm.

Formula 3: Bacterial Growth Inhibition (%)

$$\text{Growth Inhibition (\%)} = \left(1 - \frac{OD_{\text{treated}}}{OD_{\text{control}}}\right) \times 100$$

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The MIC was defined as the lowest metal concentration showing no visible growth.

Table 4: MIC Determination Parameters

Parameter	Description
Incubation temperature	30 °C
Incubation time	24–48 hours
Measurement wavelength	600 nm
Replicates	Triplicate

6. Evaluation of Resistance Efficiency

Resistance efficiency of isolates was evaluated based on their tolerance level and number of metals resisted.

Formula 4: Metal Resistance Index (MRI)

$$\text{MRI} = \frac{\text{Number of metals tolerated}}{\text{Total metals tested}}$$

4. RESULTS

This chapter presents the results obtained from the isolation and screening of indigenous heavy metal resistant bacteria from e-waste contaminated soils. The findings include physicochemical characteristics of soil samples, bacterial population dynamics, isolation efficiency, resistance screening, and minimum inhibitory concentration (MIC) analysis of selected bacterial isolates. The results are presented systematically through tables followed by detailed interpretation.

Table 5: Physicochemical Characteristics of Control and E-Waste Contaminated Soil Samples

Parameter	Control Soil	E-Waste Contaminated Soil
pH	6.7 ± 0.3	7.8 ± 0.4
Electrical Conductivity (dS/m)	0.38 ± 0.06	1.31 ± 0.15
Organic Matter (%)	1.85 ± 0.21	3.92 ± 0.33
Moisture Content (%)	11.9 ± 1.4	19.4 ± 1.8
Lead (Pb) (mg/kg)	24.2 ± 3.7	325.6 ± 28.4
Cadmium (Cd) (mg/kg)	0.8 ± 0.1	8.2 ± 0.7
Chromium (Cr) (mg/kg)	36.5 ± 4.9	196.3 ± 17.6
Nickel (Ni) (mg/kg)	30.1 ± 4.2	162.8 ± 15.9

The physicochemical analysis clearly demonstrates pronounced differences between control and e-waste contaminated soils. The contaminated soils exhibited a slightly alkaline pH, elevated electrical conductivity, and higher organic matter content, indicating prolonged accumulation of waste residues and metal-rich components. The significantly higher concentrations of Pb, Cd, Cr, and Ni in contaminated soils confirm severe heavy metal pollution arising from e-waste activities. These elevated metal concentrations exceed recommended safety limits and create strong selective pressure on soil microbial communities, favoring the survival of metal-resistant microorganisms.

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Table 6: Total Viable Bacterial Count in Soil Samples

Soil Type	Bacterial Population (CFU/g × 10^6)
Control Soil	7.1 ± 0.6
E-Waste Contaminated Soil	3.4 ± 0.5

A marked reduction in total bacterial population was observed in e-waste contaminated soils when compared to control soils. This decline reflects the toxic effects of heavy metals on sensitive microbial species. However, the persistence of viable bacterial populations in contaminated soils suggests microbial adaptation and the presence of resistant strains capable of surviving under metal stress conditions.

Table 7: Distribution of Heavy Metal Resistant Bacterial Isolates

Resistance Category	Number of Isolates
Total isolates recovered	32
Pb-resistant isolates	22
Cd-resistant isolates	17
Cr-resistant isolates	19
Ni-resistant isolates	15
Multi-metal resistant isolates	11

A total of 32 bacterial isolates were successfully recovered from e-waste contaminated soils using metal-amended media. Among these, resistance to lead was most prevalent, followed by chromium, cadmium, and nickel. Notably, 11 isolates demonstrated resistance to more than one heavy metal, indicating multi-metal tolerance. This finding is particularly significant given the complex nature of e-waste pollution, which typically involves mixed heavy metal contamination.

Table 8: Morphological Characteristics of Selected Bacterial Isolates

Isolate Code	Colony Shape	Color	Margin	Elevation
EB-4	Circular	Cream	Entire	Raised
EB-9	Irregular	White	Undulate	Flat
EB-15	Circular	Pale yellow	Entire	Convex
EB-21	Irregular	Off-white	Lobate	Raised
EB-27	Circular	Cream	Entire	Convex

The morphological examination revealed considerable diversity among the bacterial isolates, indicating a heterogeneous microbial community adapted to contaminated environments. Such diversity increases the likelihood of identifying strains with varied resistance mechanisms and enhanced bioremediation potential.

Table 9: Growth Response of Selected Isolates on Heavy Metal-Amended Agar

Isolate	Pb	Cd	Cr	Ni
EB-4	+++	++	++	+
EB-9	+++	+++	++	++
EB-15	++	++	+++	++

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EB-21	+++	++	+++	+
EB-27	++	+	++	+

(+++ Strong growth, ++ Moderate growth, + Weak growth)

Growth response analysis demonstrated that isolates EB-9 and EB-21 exhibited strong tolerance to multiple heavy metals, particularly lead and chromium. These isolates maintained robust growth even at elevated metal concentrations, suggesting efficient resistance mechanisms such as biosorption, metal sequestration, or efflux systems.

Table 10: Minimum Inhibitory Concentration (MIC) Values of Selected Isolates (mg/L)

Isolate	Pb	Cd	Cr	Ni
EB-4	750	280	520	240
EB-9	1100	420	610	360
EB-15	700	260	820	310
EB-21	980	360	910	290
EB-27	620	210	470	230

MIC determination revealed substantial resistance variation among isolates. EB-9 exhibited the highest tolerance to Pb and Cd, while EB-21 showed exceptional resistance to Cr. The high MIC values observed indicate strong adaptive responses to prolonged metal exposure and confirm the suitability of these isolates for potential bioremediation applications.

Table 11: Heavy Metal Resistance Profile of Selected Bacterial Isolates

Isolate	Metals Tolerated	Resistance Category
EB-4	Pb, Cd, Cr, Ni	High
EB-9	Pb, Cd, Cr, Ni	Very High
EB-15	Pb, Cd, Cr, Ni	High
EB-21	Pb, Cd, Cr, Ni	Very High
EB-27	Pb, Cd, Cr, Ni	Moderate

All selected isolates demonstrated resistance to all tested metals, confirming their multi-metal resistance capability. Isolates EB-9 and EB-21 were categorized as very high resistance strains, making them promising candidates for future molecular characterization and bioremediation studies.

DISCUSSION

The results clearly establish that e-waste contaminated soils serve as reservoirs of indigenous heavy metal resistant bacteria. Although heavy metal contamination reduced overall microbial abundance, selective pressure facilitated the emergence of resistant strains with remarkable tolerance to Pb, Cd, Cr, and Ni. The presence of multi-metal resistant isolates with high MIC values underscores their ecological adaptability and potential application in bioremediation strategies. These findings validate the hypothesis that indigenous bacteria from e-waste contaminated environments can be effectively exploited for sustainable management of heavy metal pollution. the findings of this study strongly support the

hypothesis that e-waste contaminated soils harbor indigenous bacterial populations with significant heavy metal resistance. The observed resistance patterns and high MIC values indicate that these bacteria have evolved effective survival strategies in response to prolonged metal exposure. These results are consistent with previous studies reporting that contaminated environments serve as reservoirs of metal-resistant microorganisms with high bioremediation potential. However, while the present study focused on isolation and screening, further investigations are required to elucidate the molecular basis of resistance and to evaluate metal removal efficiency under controlled and field conditions.

5. CONCLUSION

The present study concludes that e-waste contaminated soils serve as important reservoirs of indigenous bacteria with significant resistance to toxic heavy metals. Although heavy metal pollution adversely affected overall microbial abundance, selective pressure enabled the survival and enrichment of metal-tolerant bacterial strains capable of withstanding elevated concentrations of lead, cadmium, chromium, and nickel. The successful isolation of multi-metal resistant bacteria with high tolerance levels highlights their adaptive potential and ecological relevance. These findings demonstrate that indigenous bacterial populations from e-waste affected environments can be effectively exploited as eco-friendly and sustainable agents for bioremediation. Overall, the study provides a strong foundation for future molecular characterization and application-oriented research aimed at developing efficient microbial strategies for mitigating heavy metal pollution arising from improper e-waste management.

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