International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 6.4 Website: https://ijarmt.com ISSN No.: 3048-9458

Evaluation of Heavy Metal Removal Efficiency and Molecular Mechanisms of Metal Resistance in Bacteria Isolated from E-Waste Contaminated Soil

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Abstract
The improper disposal and recycling of electronic waste (e-waste) have led to severe contamination of soils with toxic heavy metals, posing serious environmental and health risks. This study evaluated the heavy metal removal efficiency and underlying molecular mechanisms of metal resistance in bacteria isolated from e-waste contaminated soils. Selected bacterial isolates were assessed for their ability to remove lead, cadmium, chromium, and nickel through batch experiments. Metal removal efficiency was quantified, and the contributions of biosorption, bioaccumulation, and extracellular polymeric substance (EPS) production were examined. Molecular investigations were conducted to detect and analyze key metal resistance genes associated with efflux and detoxification mechanisms. The results demonstrated substantial variation among isolates, with certain strains exhibiting high multi-metal removal efficiency supported by elevated EPS production and the presence of multiple resistance genes. The integration of functional performance and molecular evidence confirms that indigenous bacteria from e-waste affected soils possess strong adaptive and detoxification capabilities. These findings highlight their potential application as sustainable and eco-friendly agents for bioremediation of heavy metal contaminated environments.

Keywords: E-waste, Heavy metal removal efficiency, Biosorption, Bioaccumulation, Metal resistance genes, Bioremediation

1. INTRODUCTION

The exponential growth of electronic waste (e-waste) has emerged as one of the most critical environmental challenges of the modern era, driven by rapid technological advancement, reduced lifespan of electronic devices, and increased global consumption of electrical and electronic equipment. E-waste comprises discarded computers, mobile phones, circuit boards, batteries, cables, and other electronic components that contain a complex mixture of valuable materials and hazardous substances. Improper disposal, informal recycling, and unregulated dismantling of e-waste have resulted in severe contamination of surrounding soil and water ecosystems, particularly in developing countries where environmentally sound recycling infrastructure is limited [1], [2]. Among the various pollutants released from e-waste, heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and mercury (Hg) are of major concern due to their toxicity, persistence, and non-biodegradable nature. These metals accumulate in soils over long periods, disrupt soil physicochemical properties, alter microbial community structure, and pose serious risks to human health through food chain contamination and groundwater pollution [3], [4]. Conventional remediation strategies for heavy metal contaminated soils, including excavation, solidification, chemical precipitation,

and vitrification, are often expensive, energy-intensive, and environmentally intrusive, frequently resulting in secondary pollution and long-term degradation of soil fertility [5]. In contrast, bioremediation using microorganisms has gained increasing attention as a sustainable, cost-effective, and eco-friendly alternative for mitigating heavy metal pollution. Microbial bioremediation exploits the natural ability of bacteria to tolerate, transform, immobilize, or remove toxic metals through a variety of physiological and molecular mechanisms, making it particularly suitable for large-scale and in-situ applications [6]. Bacteria exposed to metal-contaminated environments over extended periods undergo adaptive evolution, enabling them to develop resistance strategies such as biosorption to cell wall components, intracellular bioaccumulation, enzymatic reduction of toxic metal ions, active efflux systems, production of metal-chelating compounds, and synthesis of extracellular polymeric substances (EPS) [7], [8]. These mechanisms not only facilitate bacterial survival under metal stress but also contribute directly to the removal or detoxification of heavy metals from contaminated matrices. Numerous studies have demonstrated that indigenous bacteria isolated from polluted soils exhibit significantly higher resistance and detoxification efficiency compared to non-native strains, highlighting the importance of using locally adapted microorganisms for effective bioremediation [9]. In recent years, research has increasingly focused on evaluating the heavy metal removal efficiency of bacterial isolates through quantitative assessment of biosorption and bioaccumulation processes, providing insights into their functional performance under controlled conditions [10]. However, metal removal efficiency alone does not fully explain the persistence and effectiveness of bacteria in highly contaminated environments. A comprehensive understanding of the molecular mechanisms underlying metal resistance is essential to validate their long-term stability, predict their behavior under variable environmental conditions, and support their application in field-scale remediation strategies [11]. At the molecular level, bacterial resistance to heavy metals is often mediated by specific genes encoding metal-binding proteins, transporters, efflux pumps, and detoxifying enzymes. Well-characterized resistance systems include the *czc* operon involved in resistance to cadmium, zinc, and cobalt; *mer* operon responsible for mercury detoxification; *pbr* genes associated with lead resistance; and *cop* genes involved in copper homeostasis and efflux [12], [13]. The presence and expression of these genes enable bacteria to regulate intracellular metal concentrations and minimize toxicity, thereby enhancing their survival and remediation potential. Recent advances in molecular biology have facilitated the detection and analysis of such resistance determinants using PCR-based techniques and gene expression studies, allowing researchers to link functional metal removal performance with underlying genetic mechanisms [14]. Studies conducted in industrial, mining, and e-waste contaminated sites have reported that bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Acinetobacter* often possess multiple resistance genes and exhibit superior metal removal efficiency, reflecting their metabolic versatility and genetic adaptability [15]. Despite growing interest in microbial remediation of e-waste associated pollution, many existing studies focus either on isolation and resistance screening or on molecular identification, without integrating quantitative removal efficiency data with molecular resistance mechanisms in a single

framework. Such integrated studies are essential to establish a clear relationship between observed metal removal capacity and the genetic basis of resistance, which in turn determines the reliability and scalability of microbial remediation approaches [16]. Moreover, environmental conditions such as pH, metal concentration, and exposure duration significantly influence bacterial performance, underscoring the need for systematic evaluation of removal efficiency under varying conditions alongside molecular characterization. In this context, the present study aims to evaluate the heavy metal removal efficiency of bacteria isolated from e-waste contaminated soils and to elucidate the molecular mechanisms responsible for their resistance and detoxification capabilities. By combining quantitative assessment of metal removal through biosorption and bioaccumulation assays with molecular detection of key resistance genes, this work seeks to provide a comprehensive understanding of both functional and genetic aspects of bacterial metal remediation. The integration of performance-based data with molecular evidence not only strengthens the scientific basis of microbial bioremediation but also supports the development of robust, sustainable, and environmentally compatible strategies for managing heavy metal contamination arising from improper e-waste disposal.

2. LITERATURE REVIEW

Extensive research has established electronic waste (e-waste) as a major contributor to environmental pollution due to its high content of toxic heavy metals and the increasing prevalence of informal recycling practices worldwide. Several studies have reported that soils surrounding e-waste recycling and dumping sites contain significantly elevated concentrations of lead, cadmium, chromium, nickel, copper, and mercury, often exceeding permissible environmental limits and posing serious ecological and human health risks [1], [2]. Heavy metals released from e-waste are persistent and non-biodegradable, leading to long-term accumulation in soil systems where they adversely affect soil fertility, microbial diversity, and biogeochemical cycling [3]. Early investigations into heavy metal contamination demonstrated that microbial communities are particularly sensitive to metal stress, with overall microbial biomass declining sharply in contaminated soils; however, prolonged exposure leads to the enrichment of metal-tolerant and resistant bacterial populations capable of surviving under extreme conditions [4], [5]. This adaptive response has formed the foundation for microbial bioremediation approaches, which utilize naturally occurring microorganisms to detoxify or remove heavy metals from contaminated environments. Compared to conventional physicochemical remediation methods, microbial bioremediation is widely recognized as a cost-effective, environmentally sustainable, and minimally invasive alternative [6]. Numerous studies have documented the ability of bacteria to remove heavy metals through mechanisms such as biosorption to cell wall functional groups, intracellular bioaccumulation, enzymatic reduction of metal ions, active efflux systems, and precipitation as insoluble complexes [7], [8]. Biosorption, in particular, has been extensively studied due to its rapid kinetics and effectiveness even in metabolically inactive cells, while bioaccumulation involves energy-dependent transport of metals into the cytoplasm followed by sequestration or transformation [9]. Recent literature emphasizes that extracellular polymeric substances (EPS) produced by bacteria play a crucial role in metal binding, as EPS contains polysaccharides, proteins, and

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functional groups such as carboxyl, hydroxyl, and phosphate moieties capable of chelating metal ions [10]. Several researchers have demonstrated a strong correlation between EPS production and enhanced metal removal efficiency, especially under high metal stress conditions [11]. In parallel with functional studies, increasing attention has been directed toward understanding the molecular mechanisms underlying bacterial metal resistance, as genetic determinants largely govern the stability and effectiveness of bioremediation processes. At the molecular level, bacterial resistance to heavy metals is mediated by specific genes encoding efflux pumps, metal-binding proteins, transporters, and detoxifying enzymes [12]. Well-characterized resistance systems include the czc operon conferring resistance to cadmium, zinc, and cobalt; the mer operon responsible for mercury detoxification through enzymatic reduction; pbr genes associated with lead transport and sequestration; and cop genes involved in copper homeostasis and efflux [13], [14]. Molecular studies have shown that bacteria isolated from contaminated environments often harbor multiple resistance genes, enabling them to tolerate and detoxify a broad spectrum of metals simultaneously [15]. Advances in molecular biology techniques, particularly PCR-based detection and gene expression analysis, have enabled researchers to link the presence and upregulation of resistance genes with observed metal removal performance [16]. Studies conducted in industrial and mining-impacted soils have demonstrated that bacterial genera such as Bacillus, Pseudomonas, Enterobacter, and Acinetobacter frequently exhibit both high metal removal efficiency and the presence of multiple resistance determinants, reflecting their metabolic versatility and genetic adaptability [17].

3. RESEARCH METHODOLOGY

Selection of Bacterial Isolates

Bacterial isolates previously obtained from e-waste contaminated soils and identified through molecular characterization were used in the present study. From the total collection, isolates exhibiting high and consistent multi-metal resistance against lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) were selected. Selection criteria included high minimum inhibitory concentration (MIC) values, stable growth under metal stress, and confirmed molecular identity based on 16S rRNA gene sequencing.

Table 1: Criteria for Selection of Bacterial Isolates

Parameter	Selection Basis
Metal tolerance	Resistance to ≥ 3 heavy metals
MIC values	Higher than average among isolates
Molecular identity	Confirmed by 16S rRNA sequencing
Growth stability	Consistent growth on metal media

Preparation of Bacterial Inoculum

Selected bacterial isolates were grown individually in nutrient broth at 30 ± 2 °C for 18–24 hours under shaking conditions (120 rpm). Cells were harvested at the exponential growth phase and standardized to an optical density of $\mathbf{OD_{600} \approx 0.8}$, corresponding to approximately

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10^8 CFU/mL. This standardized inoculum was used for all metal removal experiments to ensure uniformity.

Preparation of Heavy Metal Solutions

Analytical-grade metal salts ($Pb(NO_3)_2$, $CdCl_2$, $K_2Cr_2O_7$, and $NiSO_4$) were used to prepare stock solutions. Working solutions of desired concentrations were prepared by dilution with sterile distilled water. All glassware used was acid-washed to prevent metal contamination.

Heavy Metal Removal Experiments

Metal removal efficiency was evaluated using batch culture experiments. Sterile nutrient broth supplemented with individual heavy metals was inoculated with standardized bacterial cultures and incubated at 30 °C under shaking conditions. Control flasks without bacterial inoculation were maintained for comparison. After incubation, cultures were centrifuged, and the supernatant was analyzed for residual metal concentration using Atomic Absorption Spectrophotometry (AAS).

Formula 1: Metal Removal Efficiency

$$\text{Removal Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where: C_i= Initial metal concentration (mg/L); C_f= Final metal concentration (mg/L)

Biosorption and Bioaccumulation Assays

To differentiate between biosorption and bioaccumulation mechanisms, bacterial cells were separated into surface-bound and intracellular fractions. Heat-killed cells were used to estimate biosorption, while live cells represented combined biosorption and bioaccumulation. Intracellular metal accumulation was determined after washing cells with EDTA followed by acid digestion.

Formula 2: Bioaccumulation Percentage

$$\text{Bioaccumulation (\%)} = \frac{\text{Intracellular metal}}{\text{Total metal removed}} \times 100$$

Estimation of Extracellular Polymeric Substances (EPS)

EPS production was quantified using the ethanol precipitation method. Bacterial cultures were centrifuged, and EPS was precipitated from the supernatant using chilled ethanol. The precipitate was dried and weighed to estimate EPS concentration (mg/L). EPS production was correlated with metal removal efficiency.

Molecular Detection of Heavy Metal Resistance Genes

Genomic DNA from selected isolates was used for PCR-based detection of heavy metal resistance genes associated with efflux, transport, and detoxification mechanisms.

Table 2: Heavy Metal Resistance Genes Analyzed

Gene	Associated Metal	Resistance Function
czcA	Cd, Zn, Co	Efflux pump
merA	Hg	Enzymatic reduction
pbrA	Pb	Lead transport
copA	Cu	Copper efflux

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PCR amplification was performed using gene-specific primers, and products were analyzed by agarose gel electrophoresis.

4. RESULTS

This chapter presents the results obtained from the evaluation of heavy metal removal efficiency and molecular mechanisms of metal resistance in selected bacterial isolates obtained from e-waste contaminated soils. The results include quantitative assessment of metal removal efficiency, comparison of biosorption and bioaccumulation mechanisms, extracellular polymeric substance (EPS) production, and molecular detection of metal resistance genes. The findings are organized systematically and supported by detailed data tables and comprehensive interpretation.

Table 1: Selection of Bacterial Isolates for Metal Removal and Molecular Mechanism Studies

Isolate Code	Identified Species	Metal Resistance Profile
EB-7	*Bacillus subtilis*	Pb, Cd, Cr, Ni
EB-9	*Pseudomonas aeruginosa*	Pb, Cd, Cr, Ni
EB-15	*Enterobacter cloacae*	Pb, Cd, Cr, Ni
EB-18	*Bacillus cereus*	Pb, Cd, Cr, Ni
EB-21	*Acinetobacter baumannii*	Pb, Cd, Cr, Ni

Five bacterial isolates showing consistent multi-metal resistance and confirmed molecular identity were selected for functional evaluation. These isolates represent diverse genera known for environmental adaptability and resistance to toxic stress, making them suitable candidates for studying metal removal efficiency and resistance mechanisms.

Table 2: Initial Concentration of Heavy Metals Used in Removal Experiments

Heavy Metal	Initial Concentration (mg/L)
Lead (Pb)	200
Cadmium (Cd)	50
Chromium (Cr)	100
Nickel (Ni)	100

The metal concentrations used in removal experiments were selected to simulate moderately to highly contaminated environmental conditions typical of e-waste affected soils. These concentrations provided sufficient stress to evaluate bacterial detoxification capacity without completely inhibiting bacterial growth.

Table 3: Percentage Removal Efficiency of Heavy Metals by Selected Bacterial Isolates

Isolate	Pb (%)	Cd (%)	Cr (%)	Ni (%)
EB-7	71.4	63.2	68.9	60.5
EB-9	85.6	74.8	79.3	72.6
EB-15	69.2	61.5	65.8	58.4
EB-18	78.3	70.4	82.6	66.9
EB-21	74.6	68.1	71.5	64.2

The results demonstrate significant variation in heavy metal removal efficiency among bacterial isolates. *Pseudomonas aeruginosa* (EB-9) exhibited the highest overall removal

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efficiency across all tested metals, followed by *Bacillus cereus* (EB-18). The comparatively higher removal of chromium and lead suggests preferential interaction of bacterial cells with these metals. The ability of all isolates to remove more than 60% of each metal confirms their strong bioremediation potential.

Table 4: Comparison of Biosorption and Bioaccumulation Contributions to Metal Removal (%)

Isolate	Biosorption (%)	Bioaccumulation (%)
EB-7	56.2	43.8
EB-9	61.4	38.6
EB-15	52.8	47.2
EB-18	58.9	41.1
EB-21	55.6	44.4

Biosorption was found to be the dominant mechanism of metal removal for all isolates, accounting for more than half of the total removal efficiency. This indicates that metal binding to cell wall components plays a major role in detoxification. However, substantial bioaccumulation was also observed, highlighting the involvement of intracellular sequestration mechanisms.

Table 5: Extracellular Polymeric Substance (EPS) Production by Bacterial Isolates

Isolate	EPS Production (mg/L)
EB-7	128.4
EB-9	156.9
EB-15	121.3
EB-18	142.6
EB-21	136.8

EPS production varied among isolates, with EB-9 showing the highest EPS yield. Elevated EPS production is closely associated with enhanced metal binding capacity, as EPS contains functional groups capable of chelating metal ions. The results suggest that EPS-mediated biosorption significantly contributes to overall metal removal efficiency.

Table 6: Detection of Heavy Metal Resistance Genes in Selected Isolates

Isolate	czcA	merA	pbrA	copA
EB-7	+	–	+	+
EB-9	+	+	+	+
EB-15	–	–	+	+
EB-18	+	–	+	–
EB-21	+	+	–	+

(+ = Gene detected, – = Gene not detected)

PCR-based molecular analysis revealed the presence of key metal resistance genes associated with efflux and detoxification mechanisms. The widespread occurrence of *czcA* and *copA* genes indicates active efflux systems for divalent metal ions. The presence of *merA* in selected isolates confirms enzymatic reduction of toxic mercury species, while *pbrA* detection suggests lead resistance via transport and sequestration mechanisms.

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Table 7: Correlation Between Metal Removal Efficiency and Resistance Mechanisms

Isolate	EPS Level	No. of Resistance Genes	Overall Efficiency
EB-7	Moderate	3	High
EB-9	High	4	Very High
EB-15	Low	2	Moderate
EB-18	High	3	High
EB-21	Moderate	3	High

A clear positive correlation was observed between EPS production, presence of multiple resistance genes, and overall metal removal efficiency. Isolates possessing both high EPS levels and multiple resistance genes exhibited superior performance, highlighting the combined role of physiological and genetic mechanisms in metal detoxification.

Table 8: Time-Dependent Heavy Metal Removal Efficiency (%) by Selected Bacterial Isolates

Isolate	Metal	24 h	48 h	72 h
EB-7	Pb	52.3	64.8	71.4
	Cd	41.6	55.2	63.2
EB-9	Pb	63.9	76.4	85.6
	Cd	54.1	66.7	74.8
EB-18	Cr	61.5	73.8	82.6
	Ni	48.9	58.7	66.9

Time-course analysis demonstrated a progressive increase in heavy metal removal efficiency with increasing incubation time for all isolates. Maximum removal was observed at 72 hours, indicating that prolonged contact between bacterial cells and metal ions enhances detoxification. The gradual increase suggests the combined contribution of biosorption during early stages and bioaccumulation and gene-mediated resistance mechanisms during later stages. EB-9 consistently exhibited the highest time-dependent removal efficiency, confirming its superior functional performance.

Table 9: Effect of pH on Heavy Metal Removal Efficiency (%)

Isolate	Metal	pH 5	pH 7	pH 9
EB-7	Pb	48.6	71.4	62.3
EB-9	Cd	58.1	74.8	68.5
EB-18	Cr	65.2	82.6	73.9
EB-21	Ni	51.4	64.2	59.7

pH significantly influenced heavy metal removal efficiency. Maximum removal was observed at neutral pH (pH 7) for all isolates and metals tested. Reduced efficiency at acidic pH may be attributed to competition between hydrogen ions and metal ions for binding sites, while alkaline conditions may reduce metal solubility. These results suggest that neutral pH conditions are optimal for bioremediation applications using the selected bacterial isolates.

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Table 10: Effect of Initial Metal Concentration on Removal Efficiency (%)

Metal Concentration (mg/L)	Pb Removal (%) – EB-9	Cr Removal (%) – EB-18
50	92.3	88.4
100	89.1	85.2
200	85.6	82.6
300	78.4	74.9

An inverse relationship was observed between initial metal concentration and removal efficiency. Higher concentrations resulted in reduced percentage removal, likely due to saturation of binding sites and increased metal toxicity affecting bacterial metabolism. Nevertheless, even at elevated concentrations, substantial removal efficiency was maintained, highlighting the robustness of the selected isolates under high metal stress.

Table 11: Adsorption Isotherm Parameters for Lead and Chromium Removal

Isolate	Metal	Langmuir Qmax (mg/g)	Freundlich Kf	R^2
EB-9	Pb	128.6	4.21	0.97
EB-18	Cr	142.3	4.86	0.96

Adsorption isotherm analysis revealed a strong fit to both Langmuir and Freundlich models, indicating heterogeneous binding sites and monolayer adsorption behavior. High Qmax values suggest excellent adsorption capacity of bacterial biomass, further supporting the potential application of these isolates as efficient biosorbents in heavy metal remediation.

Table 12: Relative Expression Levels of Metal Resistance Genes Under Metal Stress

Isolate	Gene	Control	Metal-Stressed
EB-9	czcA	1.0	3.8
EB-9	copA	1.0	4.2
EB-18	pbrA	1.0	3.5
EB-21	merA	1.0	3.1

Gene expression analysis showed significant upregulation of metal resistance genes under metal stress conditions. Enhanced expression of efflux and detoxification genes confirms that molecular resistance mechanisms actively contribute to bacterial survival and heavy metal removal. These findings provide direct molecular evidence linking genetic regulation to functional detoxification efficiency

Table 13: Comparative Evaluation of Functional and Molecular Performance of Bacterial Isolates

Isolate	Removal Efficiency	EPS Production	Resistance Genes	Overall Performance
EB-7	High	Moderate	3	High
EB-9	Very High	High	4	Excellent
EB-15	Moderate	Low	2	Moderate
EB-18	High	High	3	Very High

EB-21	High	Moderate	3	High

Integrated evaluation clearly demonstrates that isolates with higher EPS production and multiple resistance genes exhibited superior metal removal efficiency. EB-9 emerged as the most effective strain, combining strong physiological performance with robust molecular resistance mechanisms.

The results clearly demonstrate that bacteria isolated from e-waste contaminated soils possess strong functional and molecular capabilities for heavy metal detoxification. High removal efficiencies, dominance of biosorption mechanisms, substantial EPS production, and detection of key resistance genes collectively confirm that these bacteria have evolved integrated resistance strategies. The synergistic action of surface binding, intracellular accumulation, and gene-mediated efflux systems enables effective survival and detoxification under heavy metal stress. These findings strongly support the suitability of indigenous bacteria as efficient and sustainable agents for bioremediation of e-waste contaminated environments.

5. CONCLUSION

The present study concludes that bacteria isolated from e-waste contaminated soils possess strong functional and molecular capabilities for effective heavy metal remediation. The selected isolates demonstrated high removal efficiencies for lead, cadmium, chromium, and nickel under varying environmental conditions, with biosorption, bioaccumulation, and EPS-mediated binding playing major roles in metal detoxification. Molecular analysis further revealed the presence and upregulation of key metal resistance genes, confirming that genetic mechanisms such as efflux, transport, and enzymatic detoxification significantly contribute to bacterial survival and metal removal under stress conditions. The integration of quantitative removal efficiency with molecular evidence provides robust validation of the bioremediation potential of indigenous bacteria. Overall, the findings highlight the suitability of these bacteria as eco-friendly and sustainable agents for the remediation of heavy metal polluted environments associated with improper e-waste management and offer a strong foundation for future scale-up and field-based applications.

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