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Biosorptive removal of selected metal ions from simulated wastewater using highly metal-resistant bacteria

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ABSTRACT

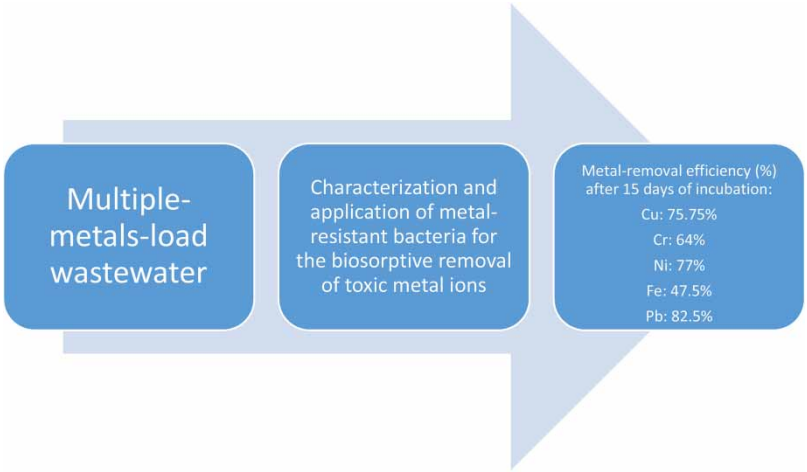
In the current scenario of the need for cost-effective remediation, our study aimed to assess the remedial potential of bacteria obtained from metal-rich wastewater. To simulate the conditions, we prepared wastewater containing five toxic metals (Cu, Cr, Ni, Fe, and Pb). Two types of metal-resistant bacteria were isolated from a prominent wastewater drain in Lahore, Pakistan. These isolated bacteria were thoroughly characterized, both phenotypically and genotypically. Subsequently, the isolated bacteria were exposed to the wastewater solution containing each of the aforementioned metals at a concentration of 250 ppm. The exposed isolates were then incubated for a duration of 15 days. After 5 days, we measured the uptake of metals by the bacterial isolates. Following the 15-day incubation period, we observed that the bacterial isolates demonstrated the maximum efficiency in removing metals, with approximately 47.5% of Fe, 77% of Ni, 75.75% of Cu, 64% of Cr, and 82.5% of Pb being removed. These findings have significant implications for the development of environmentally friendly and cost-effective strategies for metal ion remediation.

Key words: biosorption, economical remediation, metal-resistant microflora, toxic metals, wastewater

HIGHLIGHTS

- Assess the remedial potential of bacteria obtained from metal-rich wastewater.
- Isolated bacteria were thoroughly characterized, both phenotypically and genotypically.
- Bacterial isolates demonstrated maximum efficiency in removing metals, with approximately 47.5% of Fe, 77% of Ni, 75.75% of Cu, 64% of Cr, and 82.5% of Pb being removed.

GRAPHICAL ABSTRACT



INTRODUCTION

Environmentalists are dealing with the alarming accumulation of persistent and non-degradable contaminants like heavy metals in the environment. Heavy metals have widespread usage in modern-day industry, i.e. fertilizer, tanning, plastic/paper manufacturing, energy/fuel production, and other metallurgical processes (Kumar *et al.* 2023). Metals that have an atomic density of more than 5 g per cm^3 are considered heavy metals (Ali & Khan 2018). Albeit some of the heavy metals are essential for all life forms on earth, longer exposure to high concentrations of heavy metals can be toxic (Ghuge *et al.* 2023). Zn, Ni, Mn, Cu, and Co are required as trace minerals for the optimal growth of microbes; but at higher levels, they exert lethal effects on various organisms and human health (Isik *et al.* 2022; Kumar *et al.* 2023), whereas there are some other metals that do not have any physiological role and are deadly poisonous, even in very low quantities (Sundseth *et al.* 2017).

During the last three decades, efforts have been made to deal with environmental pollution caused by heavy metals. Decontamination of heavy metals from industrial and household sewage has been a major concern, and several methods, i.e. electroplating, precipitation, and ion-exchange methodology, have been devised to cope with pollutants. Although all of these methods have their own efficacy, yet at the same time, they have some cons like the production of toxic sludge/slurry. In modern-day science, biosorption provides an excellent remedial strategy having low operating costs with minimum environmental problems. In biosorption, microorganisms are utilized for the efficient uptake of metallic ions from agriculture and industrial wastewater (Ghaffar *et al.* 2023).

Some species of bacteria, algae, fungi, and yeast reportedly have the ability to tolerate varying levels of metals and decrease the amount of metals in aqueous solution. Microorganisms uptake heavy metal ions which are followed by the efflux of similar ions: these processes normally comprise of oxidation and reduction reactions, depending on the metal. It is also evident that several bacteria consume heavy metals for acquiring growth and energy through their extraordinary metabolic pathways, in which their enzymes specifically break down heavy metals (Ramírez Calderón *et al.* 2020). Different microbes having different tendencies toward heavy metals correspondingly perform significant roles in the removal of heavy metals via biogeochemical cycles (Ghaffar *et al.* 2023).

Microbes have become resistant to different toxic heavy metal ions by adopting various ways, such as bioaccumulation, biosorption, biomineralization, and biological transformation, to live successfully in highly contaminated environments (Priya *et al.* 2022). The use of microbes to treat heavy metal pollution is preferred over traditional techniques such as flocculation, chemical oxidation or reduction, ion replacement, coagulation, evaporation, reverse osmosis, etc., because of their insane operating costs (Priya *et al.* 2022). There are some factors that affect the sorption process, i.e. concentration of metal ions in solution, temperature of the solution, pH, etc. (Nilanjana *et al.* 2007). Bacteria are resistant toward heavy metals and are capable of continuing their life cycle even in high concentrations of heavy metals (Issazadeh *et al.* 2013; Yabalak *et al.* 2022). *Pseudomonas*, *Mycobacterium, Escherichia coli, Bacillus,* and *Streptomyces* have shown great efficiencies

toward heavy metals (Sharma 2021; Ramli *et al.* 2022). The current study is aimed at the isolation of a metal-resistant bacterial strain from a highly-polluted wastewater channel (Hadiara Drain) and employment of the isolated strain for the efficient removal of some toxic heavy metals from artificially prepared wastewater.

MATERIALS AND METHODS

Sampling and description of sampling site

The wastewater samples were collected from a pollution-rich channel of Hudiara Drain, Mohlanwal, Lahore, Pakistan (31° 24′ 35N 74° 8′ 27E). Wastewater from various industries, having a bulk variety of heavy metals, passes through the drain. This drainage channel ends in River Ravi, thus it plays a significant role in polluting the river's water. Therefore, Hudiara Drain is considered as a hotspot of pollution-resistant microbes. The wastewater samples were collected from six different localities of the drain in fresh sterile vials under sterilized conditions. Physical parameters such as pH and temperature were noted during sampling and were 7.3 and 32.4 °C, respectively. These samples were safely transported to the Applied and Environmental Microbiology Laboratory, Institute of Zoology, University of the Punjab, Lahore, Pakistan. The vials were placed in a refrigerator till further processing.

Physicochemical characterization of the collected samples

Physicochemical parameters of the samples were measured before isolating the microbes. Physical parameters include temperature, pH, electrical conductivity, total dissolved solids (TDS), total volatile solids (TVS), sulfates, phosphates, chlorides, carbonates, and bicarbonates. The parameters were measured according to the methods illustrated by Garg *et al.* (2001).

By using a digital pH meter (pH7110, Germany) and a digital thermometer, the pH and temperature of the samples were measured, respectively. The electrical conductivity was determined using a digital conductivity meter (UK) and readings were noted in S/m. For measuring TDS, the known volume (V) of samples was poured into Petri plates. The weight of the empty Petri plate (A) was measured before adding the desired volume of the samples. After adding known volume, the Petri plate was placed on a hot plate at 105 °C. After 2–3 h, the Petri plate was cooled down at room temperature and the final volume of the Petri plate was measured (B):

$$\text{TDS (mg/L)} = \frac{B - A \times 1,000 \times 1,000}{V}$$

where A is the weight of petri plate; B is the weight after 2–3 h of evaporation; V is the volume of the sample added in the Petri plate.

The residues of TDS were combusted at 550 °C in a muffle furnace for 1 h. After 1 h, the weight was noted according to Garg *et al.* (2001):

$$\text{TVS} = \frac{\text{Weight of TDS residues}}{\text{Weight after drying}} \times 100$$

For measuring the sulfate contents of the samples, the samples were filtered on a fine-quality Whatman's filter paper. Approximately 50 mL of the filtered wastewater sample was taken out and then mixed with a mixture consisting of 10 mL of NaCl–HCl solution and glycerol-ethanol solution. After that, 0.5 g of $BaCl_2$ (Barium Chloride) was added to the mixture with continuous stirring for half an hour. After that, the reading was noted as 420 nm under a digital spectrophotometer (Garg *et al.* 2001).

The phosphate content of the samples was measured by digesting the wastewater sample with perchloric acid, followed by oxidation using sodium hydroxide (Garg *et al.* 2001). The chloride content of the samples was measured by titrating chlorides against soluble silver nitrate ($AgNO_3$) in the presence of chromate. The titration resulted in the formation of silver nitrate (AgCl) precipitates. Following titration, free silver reacts with chromate to form sliver chromate, leaving chloride ions later on the precipitates. The end color of titration was reddish brown (Garg *et al.* 2001):

$$\text{Chlorides} \left(\frac{\text{Mg}}{\text{L}}\right) = \frac{\text{mL of titrant} \times N \times 35.5}{\text{mL of selected sample (50 mL)}} \times 1,000$$

where N is the titrant's normality.

Carbonates were estimated according to Garg *et al.* (2001) by titrating the selected sample with a strong acid solution (H_2SO_4) using phenolphthalein as an indicator:

$$\text{Carbonates (mg/L)} = \frac{\text{mL of phenolphthleine titrant}}{\text{mL of selected sample}} \times 1,000$$

Bicarbonate contents were estimated following Garg *et al.* (2001) using methyl orange solution as an indicator:

$$\text{Bicarbonates (mg/L)} = \frac{\text{mL of methyl orange titrant}}{\text{mL of selected sample}} \times 1,000$$

Isolation and pure culturing of multiple-metal-resistant bacteria

Five toxic metals (Pb, Ni, Fe, Cu, and Cr) in different concentrations were used to isolate multiple-metal-resistant bacteria (Figure 1). Stock solutions of the metals were prepared by using different required concentrations of salts as described in Table 1. Nutrient agar was used as a growth medium for maintaining bacterial growth. The medium was amended with different concentrations of toxic metals to isolate multiple-metal-resistant bacteria. The obtained metal-resistant bacterial isolates were pure cultured by streak-plate method and then characterized phenotypically as well as genotypically.

Phenotypic characterization of the bacterial isolates

Phenotypic characterization of the bacteria involved motility detection, Gram's, and endospore staining (Figure 2).

Molecular characterization of the bacterial isolates

To identify the bacteria, we extracted the total genomic DNA from the isolates (Hussain *et al.* 2014). We used specific primers, namely 27f (50-AGAGTTTGATCMTGGCTCAG-30) and 1492r (50-GGTTACCTTGTTACGACTT-30), to amplify the 16S rRNA genes, which are approximately 1.5 kb in length. Polymerase chain reaction (PCR) was carried out in 50 μL vials. The PCR reaction mixture consisted of 5 μL of DNA extract, 18 μL of DNA-free water, 5 μL of 1× Taq buffer, 2 U/mL of DNA Taq polymerase, 5 μL of each primer (5 pmol), 5 μL of dNTPs (1 mM), and 5 μL of $MgCl_2$ (25 mM). The PCR procedure involved the following steps: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 2 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 2 min.

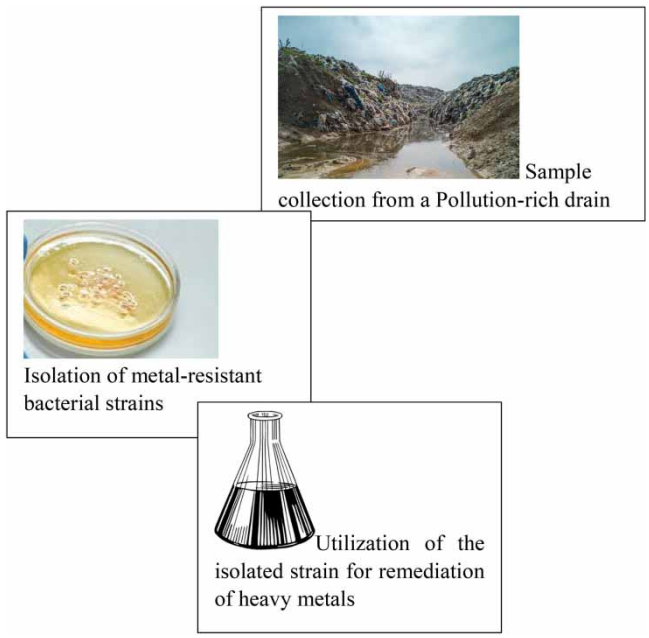


Figure 1 | Overview of bioremediation of heavy metals.

Table 1 | Preparation of different concentrations of five toxic metals from their respective salts

			Concentration of metal employed	
Sr. No.	**Metals under analysis**	**Salts used**	**Metal concentration (ppm)**	**Quantity of salt used (mg/L)**
1	Pb	$Pb(NO_3)_2$	5,000	8
2	Ni	$NiSO_4$	5,000	13.1
3	Fe	$FeSO_4$	5,000	13.6
4	Cu	$CuSO_4{\cdot}5H_2O$	5,000	19.53
5	Cr	CrO_3	5,000	9.61

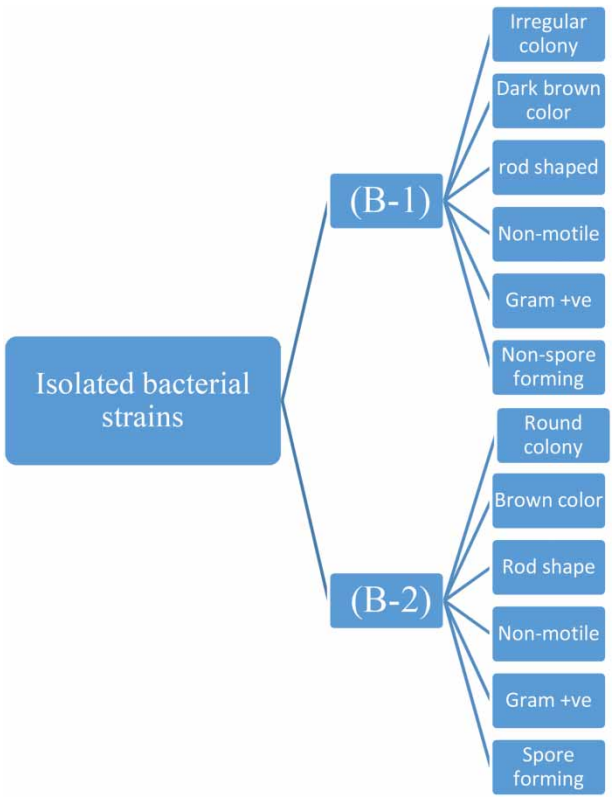


Figure 2 | Phenotypic characteristics of the isolated strains.

To visualize the PCR products, we loaded them onto a 1% (w/v) agarose gel containing ethidium bromide in TAE buffer and examined them using an electrophoresis chamber. Subsequently, we purified the PCR products using a Gene Purification Kit. The amplified sequences were then subjected to sequencing using the Big Dye Terminator v3.1 cycle (Macrogen, Korea) at the DNA sequencing facility in Korea. The obtained 16S rRNA sequences were assembled using Phrap (version 0.990319). To determine their similarity with other sequences, we performed a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/), and the resulting sequences were submitted to GenBank to obtain accession numbers.

Determination of multiple-metals-resistance of the isolate

The bacterial isolate *Bacillus* sp. was assessed for its resistance against different concentrations of randomly selected five toxic metals. For this purpose, a fresh inoculum of the isolated strain was prepared in nutrient broth. All wastewaters were prepared artificially using salts of the toxic metals. Five thousand ppm stock solutions of Cu, Cr, Ni, Pb, and Fe were

prepared by using $CuSO_4{\cdot}5H_2O$, CrO_3, $NiSO_4$, $Pb(NO_3)_2$, and $FeSO_4$, respectively. Then, stock solutions were further diluted into 250 ppm solutions and inoculated with 5% inoculum of freshly cultured bacterial strain and placed in an incubator at 30 °C. All experiments for the remediation purpose were conducted under batches, in triplicates. Metal-polluted wastewaters, without the addition of bacteria, served as the control. The remedial potential of the bacterial species was determined in a 15-day experimental trial. Samples were withdrawn and filtered for the analysis of metal reduction after an interval of every 5 days. The filtered samples were shifted to the M2 Lab Department of Environmental Sciences, Lahore, for atomic absorption spectroscopic analysis.

Statistical analysis

The obtained data were analyzed by General Linear Model (GLM) procedures using factorial arrangements according to a Completely Randomized Design (CRD). By using Duncan's Multiple Range (DMR), the means of obtained data were separated out by using SAS 9.1. The differences between the means of obtained data were considered significant at $P < 0.05$.

RESULTS

Physicochemical analysis of the sample

The collected samples were analyzed for different physicochemical parameters by following specific procedures. By using a digital pH meter (pH7110, Germany) and a digital thermometer, the pH and temperature of the samples were measured and found to be 7.3 and 32.4 °C, respectively. Organic contents were also present in quantity different from the spring water, but carbonates were absent. The value of TDS, electrical conductivity, and total volatile solids are mentioned in Table 2.

Isolation and phenotypic characterization of the bacterial isolates

Two metal-resistant bacterial strains were isolated from the collected wastewater sample. The bacterial isolates were designated as B-1 and B-2. The phenotypic characteristics of the bacterial isolates are shown in Figure 2.

Genotypic characterization of the bacterial isolates

Sample B-1

A target sequence of 1,224 bp of 16S ribosomal DNA has been obtained using the Sanger sequencing method. When it was BLAST (basic local sequence tool) matched with the already sequenced data available in GenBank of NCBI database, the sequence showed maximum base similarity with *Exiguobacterium sp*. XT-14 (KR063545), *Exiguobacterium profundum* (KX233849) and *Exiguobacterium profundum* (KF928335) that were isolated from China, Bangladesh and Pakistan. All possible 25 closest matches have been mentioned in Table 3.

Sample B-2

Another sequence of 1,224 bp of 16S ribosomal DNA has been obtained by using the Sanger sequencing method. When it was BLAST (basic local sequence tool) matched with the already deposited data in the GenBank of NCBI database, the

Table 2 | Physicochemical characterization of the selected samples

Parameters	Samples
pH	7.3
Temperature	32.4 °C
Electrical conductivity	4.37 S/m
Total dissolved solids (TDS)	2,980 mg/L
Total volatile solids (TVS)	0.07 mg/L
Sulfates	0.402 mg/L
Phosphates	0.078 mg/L
Chlorides	268.38 mg/L
Carbonates	Absent
Bicarbonates	800 mg/L

Table 3 | The closest matches to the bacteria isolated from sample B-1

Serial No.	Accession No.	Species name	Country	Query cover (%)	Identity %
1.	KR063545	*Exiguobacterium* sp. XT-14	China	99	99
2.	JF758868	*Exiguobacterium arabatum*	India	99	99
3.	KX233849	*Exiguobacterium profundum*	Bangladesh	99	99
4.	KF928335	*Exiguobacterium profundum*	Pakistan	99	99
5.	KJ456599	*Exiguobacterium* sp. NH-Q34	China	99	99
6.	JX987048	*Exiguobacterium profundum*	India	99	99
7.	FJ785505	*Exiguobacterium* sp. EB408	China	99	99
8.	JN642678	*Exiguobacterium* sp. YMD-1	China	99	99
9.	HM352336	*Exiguobacterium* sp. CmLB12	China	99	99
10.	GU815993	*Exiguobacterium* sp. BCH4	India	99	99
11.	EF108298	*Exiguobacterium* sp. R	China	100	99
12.	LN846823	*Exiguobacterium* sp. JC358	India	99	99
13.	KX185942	*Exiguobacterium profundum*	Pakistan	99	99
14.	KT074375	*Exiguobacterium* sp. RB 215	India	99	99
15.	KM873375	*Exiguobacterium profundum*	China	99	99
16.	KM215140	*Exiguobacterium profundum*	China	99	99
17.	KF070180	uncultured bacterium	USA	99	99
18.	KF070120	uncultured bacterium	USA	99	99
19.	KF269101	*Exiguobacterium profundum*	India	99	99
20.	KC668297	*Exiguobacterium* sp. E4	Pakistan	99	99
21.	JX112643	*Exiguobacterium profundum*	Pakistan	99	99
22.	FJ785504	*Exiguobacterium* sp. EB277	China	99	99
23.	AB681514	*Exiguobacterium* sp. NBRC 101652	Japan	99	99
24.	JN644510	*Exiguobacterium profundum*	India	99	99
25.	JF241394	uncultured bacterium	USA	99	99

present sequence showed close resemblance with *Bacillus* sp. (MG266290), *Bacillus* sp. (MG266289), and *Bacillus* sp. (MG266286), all from China. It has been supposed as a new species because its morphological, ecological, and molecular data did not match with the already reported species of the genus *Bacillus*. The closest matches have been mentioned in Table 4.

Biosorption of heavy metals

The isolated bacterial strain showed different biosorption against different metals. The following results have been observed after analysis:

The isolated bacteria absorbed 47.5% of Fe on the 5th day, followed by 38% on the 10th day, and minimum absorption (33%) of Fe was observed on the 15th day of incubation. The results of the present study showed maximum biosorption of Fe (47.5%) during the initial days and the biosorption rate decreased with the passage of time (Figure 3). The bacteria isolated in this study consumed 56.5% of Ni in 5 days, followed by 77% after 10 days, and 58.5% after 15 days of interval. Ni absorption showed a bell-shaped biosorption pattern where the maximum (77%) absorption of Ni was on the 10th day of incubation in Ni-polluted water. The biosorption of Ni by bacteria was significantly ($p < 0.05$) high at 10-day intervals in 250 ppm metal-polluted water.

The biosorption of Cu was found to be the maximum (75.75%) on the 5th day of incubation, followed by 43% after 15 days, and the lowest biosorption 30.5% was observed on the 10th day. The results suggest the maximum utilization of Cu during their earlier days of exposure to 250 ppm of artificially prepared Cu-polluted water. The bacterial isolate showed Cr reduction

Table 4 | The closest matches to the bacteria isolated from sample B-2

Serial No.	Accession No.	Species name	Country	Query cover (%)	Identity %
1.	MG266290	*Bacillus* sp.	China	100	99
2.	MG266289	*Bacillus* sp.	China	100	99
3.	MG266286	*Bacillus* sp.	China	100	99
4.	MG266282	*Bacillus* sp.	China	100	99
5.	KY082734	*Bacillus firmus*	China	100	99
6.	KY849422	*Bacillus firmus*	China	100	99
7.	KX783540	*Bacillus* sp.	Argentina	100	99
8.	KY928097	*Bacillus firmus*	India	100	99
9.	KX817957	*Bacillus* sp.	India	100	99
10.	KY672890	*Bacillus* sp.	India	100	99
11.	KY616401	*Bacillus firmus*	India	100	99
12.	KX108985	*Bacillus* sp.	Chile	100	99
13.	KX033474	*Bacillus* sp.	China	100	99
14.	KX033473	*Bacillus* sp.	China	100	99
15.	KX033472	*Bacillus* sp.	China	100	99
16.	KP992901	*Bacillus firmus*	China	100	99
17.	KX242398	*Bacillus firmus*	India	100	99
18.	KX181398	*Bacillus* sp. BAB-5835	India	100	99
19.	LC094998	*Bacillus* sp. B17Va	Japan	100	99
20.	LC094994	*Bacillus* sp. A46V	Japan	100	99
21.	KU693281	*Bacillus firmus*	Iran	100	99
22.	KU254653	*Bacillus firmus*	South Korea	100	99
23.	KM979152	*Bacillus* sp. H2-31	China	100	99
24.	KM979080	*Bacillus* sp. H1-87	China	100	99
25.	KJ544043	*bacterium* AM0334	USA	100	99

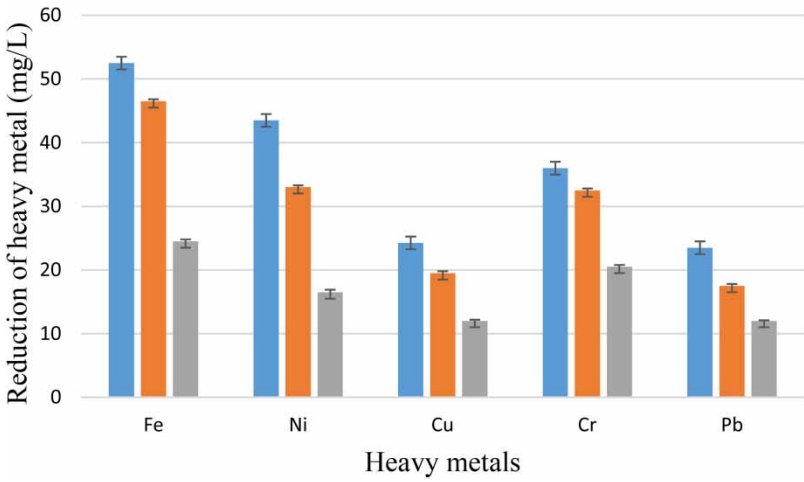


Figure 3 | Periodic removal of heavy metals at 250 ppm of the added metal after 5 (blue bar), 10 (red bar), and 15 (black bar) days. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wrd.2023.059.

in a linear fashion, i.e. it did not vary significantly with the passage of time. The maximum biosorption of Cr (64.5%) was observed on the 15th day of incubation followed by 64 and 62.5% on the 5th and 10th day, respectively.

In this study, the biosorption of Pb increased with the passage of time, but it did not vary statistically significantly. The results suggest that Pb utilization remained almost similar throughout, while the maximum Pb biosorption was 85%, which was observed after 15 days, followed by 82.5 and 76.5% after the 10th and 5th days of incubation, respectively. Statistical analysis revealed non-significant differences in Pb utilization by bacteria during intervals of 5, 10, and 15 days.

DISCUSSION

Industrialization has resulted in the contamination of the environment, by the emission of heavy metals (Figure 4). Lately, bacteria have been considered as a potent source of remediating heavy metal pollution. Biosorption is the most common mechanism used by bacteria to treat metal-polluted wastewaters. In the present study, two metal-resistant bacterial strains were isolated from a heavily polluted water channel that received heavy metals from industries, as observed from previous literature (Parvin *et al.* 2015; Gupta *et al.* 2017). Isolated bacterial strains were characterized phenotypically and the results showed consistency with those of the previous work (Abbas *et al.* 2014).

The physicochemical parameters observed in this study showed a resemblance to the previous work done by Ahmed *et al.* (2007). The incubation period played a role in the efficiency of biosorption bacterial strain that showed a maximum reduction on the 5th day of the incubation period for Fe and Cu, and on the 10th day for Ni. While for Cr and lead, maximum metal reduction was observed on the 15th or final days of the trial. Malik (2004) explained that during the exponential growth phase, metabolically active bacterial cells are involved in rapid biosorption of metallic ions to the negatively charged sites present on the cell wall. The rapid biosorption was further assisted by a slower energy-dependent entry into the cell (Malik 2004).

The bacterial strain under study leads to the reduction of heavy metals present in the aqueous samples by biosorption at different rates. During the present study, the maximum absorption of Pb was 23.5% and the lowest was recorded as 12%, while a Pb reduction of 35.77% was observed by *Paenalcaligenes hominis,* followed by *Proteus mirabilis* at a value of 32.75% (Sanuth & Adekanmbi 2016). However, 100% Pb removal was recorded by Ilhan *et al.* (2004) and Kumar *et al.* (2010) calculated 93% reduction by heavy metal acclimated *Staphylococcus* species, respectively. Recently, Zhou *et al.* (2023) employed phosphate solubilizing bacteria along with biochar that showed significant removal of lead. Shan *et al.*

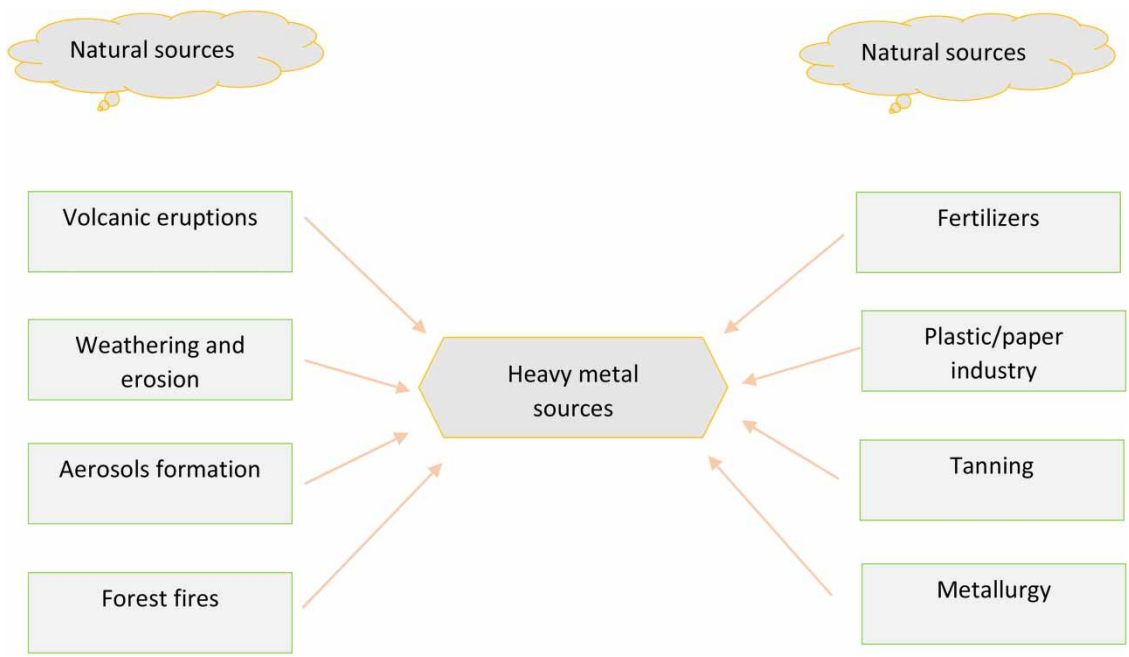


Figure 4 | Accumulation of heavy metals from natural and anthropogenic sources.

(2023) have reported that microbes can be efficiently used for the remediation of lead-polluted agricultural wastewaters. Peng *et al.* (2023) employed mixed bacteria passivation for the removal of metals such as cadmium, lead, and arsenic, and the results showed a maximum biosorption for arsenic.

Mathiyazhagan & Natarajan (2011) reported 7.01 and 6.68% Cr reduction by *Thiobacillus* and *Pseudomonas* species, respectively, which is much lower than that recorded during the present study, i.e. 36%, and results are in accordance with the findings of Sanuth & Adekanmbi (2016). Sun *et al.* (2023) reported that *Deinococcus wulumuqiensis* has tolerance against Cr up to 60 mg/L. Le *et al.* (2023) enhanced Cr reduction by combining *Paraclostridium bifermentans* G3 with cadmium sulfide nanoparticles. During the present study, the highest absorption of Ni was recorded as 43.5 and 16.5% was the minimum absorption. Öztürk *et al.* (2004) reported that the uptake of Ni^{2+} was a metabolism-independent passive binding process. An interesting result was found by Tsezos *et al.* (1996) that biosorption of Ni^{2+} was always lesser as compared to the other metals, and that it might be due to the steric hindrance by intrinsic chemical property (Tsezos *et al.* 1995). However, strains of *Pseudomonas* spp. have often been used for the biosorption of Ni^{2+} (Ramteke 2000; Malik 2004), where Ni^{2+} was biosorbed rapidly on cell surfaces. Li *et al.* (2022) reported that the immobilized microorganism group (that contained biochar and *Citrobacter* sp.) showed more reduction than the free bacteria in Ni-polluted soils. During the present study, the highest absorption rate of Cu was recorded as 24.25% and the lowest as 12%, respectively. Öztürk *et al.* (2004) observed that the initial metal ion absorption was directly proportional to the concentration of Cu^{2+}. Several organisms showed sensitivity to Cu toxicity (Gordon *et al.* 1994) because of hydroperoxide radicals (Rodriguez-Montelongo *et al.* 1993) whose characteristics made them highly toxic (Nies 1999). During the current study, the maximum percentage of Fe observed was 52.5 and the lowest recorded was 24.5%, respectively. The current study has revealed that *Bacillus* sp. showed a varied tolerance against all five metals with a maximum reduction of lead, i.e. 85%. Hence, *Bacillus* sp. can be a proficient microbe for the remediation of metal-polluted wastewaters containing multiple containments.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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