



RESEARCH

Isolation and Screening of Microorganisms from Waste Dumping Sites for Biosorption of Cadmium and Lead

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Abstract

Heavy metal poisoning of the environment poses a serious threat to ecosystems and human health. Landfills have been found to harbor diverse microbial communities capable of bioremediating such contaminants via biosorption. Unlike traditional methods of heavy metal remediation, which often involve costly and resource-intensive techniques, biosorption offers a promising alternative that harnesses the natural capabilities of microorganisms. The objective of this research was to identify and screen microorganisms from landfills for their ability to bio-sorb cadmium (Cd) and lead (Pb). The present study deals with the isolation and screening of the microorganisms capable of removing heavy metals from the area near Dehradun Municipal Solid Waste Management Pvt. Ltd. Among the 17 bacterial isolates and 2 fungal isolates, only 7 bacteria and one fungal strain were able to absorb the heavy metal concentration. Among the 7 bacterial strains, the percentage of microbes with the ability to absorb Pb ranged from 35% to 70%, while the percentage of microbes with the ability to absorb Cd ranged from 20% to 50%. On the other hand, the fungal strain had an absorption capacity of about 80% for both Pb and Cd. This study emphasizes the potential of microorganisms isolated from landfills as attractive candidates for Cd and Pb biosorption. These isolates show significant tolerance and efficient biosorption capacities, making them good candidates for further exploration and prospective application in the bioremediation of heavy metal-contaminated environments.



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Statement of Sustainability: The isolation and screening of microorganisms from trash disposal sites for cadmium and lead biosorption is a novel strategy with tremendous potential for long-term environmental management. We can lessen the detrimental impacts of heavy metal pollution and contribute to a cleaner and healthier ecosystem by exploiting the natural skills of microbes. We may turn these sites into important repositories for metal recovery and recycling by using microbes to extract and accumulate heavy metals. This method is consistent with the concepts of the circular economy, which consider waste as a valuable resource that can be reused, repurposed, or recycled.

1. Introduction

The presence of heavy metals in the environment is of great concern because it poses significant risks to both human health and the ecosystem (Järup, 2003). Industrial, agricultural, and domestic activities contribute to the accumulation of heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) in soil and water (ATSDR, 2020). Traditional methods of remediating heavy metal-contaminated sites involve the use of chemical treatments, which can be expensive and can have adverse effects on the environment (Das and Mondal, 2018). As a result, there is growing interest in developing cost-effective and environmentally sustainable approaches for heavy metal removal (Volesky, 2007). One such method is biosorption, which involves the use of natural or synthetic materials such as microorganisms, algae, fungi, and plants to bio absorb heavy metals from contaminated soil or water (Srivastava and Thakur, 2006).

Biosorption is a promising approach for heavy metal removal due to its low cost and environmental friendliness. In addition, the process is simple and does not require extensive infrastructure or specialized equipment (Das and Mondal, 2018). Furthermore, biosorption has the potential for metal recovery, which reduces the environmental impact of heavy

metal pollution (Volesky, 2007). Among the various biomaterials used for biosorption, microorganisms such as bacteria, fungi, and algae have received considerable attention (Das and Mondal, 2018). These microorganisms have a high surface area and a wide range of functional groups on their cell walls, making them ideal for heavy metal binding (Das and Mondal, 2018). Numerous studies have demonstrated the effectiveness of microorganisms in adsorbing heavy metals from contaminated sites. For example, *Bacillus subtilis*, a gram-positive bacterium, was found to be efficient in removing Pb ions from contaminated water (Alzahrani et al., 2021). Similarly, the green microalga *Chlamydomonas reinhardtii* has shown the potential in removing Cd from contaminated water (El-Sheekh et al., 2019). Several other microorganisms, including *Pseudomonas putida*, *Rhodococcus erythropolis*, and *Aspergillus niger*, have also been shown to have high biosorption capacities for heavy metals (Liu et al., 2018; Zhou et al., 2020; Chen et al., 2021). Microorganisms use various mechanisms to adsorb heavy metals, and one of the important mechanisms is the production of extracellular polymeric substances (EPS). EPS are complex macromolecules secreted by microorganisms that play an important role in biofilm formation and metal binding. These substances have a high binding capacity for heavy metals and effectively sequester metals from the surrounding environment (Gupta et al., 2019). In addition, some microorganisms can produce specific metal-binding proteins, such as metallothionein, that can selectively bind to heavy metals (Khan et al., 2022).

Several factors affect the effectiveness of biosorption by microorganisms, including pH, temperature, contact time, and initial metal concentration. The optimum pH for biosorption varies depending on the type of microorganism and the metal to be adsorbed. For example, the optimal pH for Pb adsorption by *Bacillus subtilis* was found to be 5.5 (Alzahrani et al., 2021), while the optimal pH for Cd adsorption by *C. reinhardtii* was found to be 7.5 (El-Sheekh et al., 2019). Temperature is also an important factor in biosorption, as it affects the metabolic activity of microorganisms and the rate of metal binding. Contact time is another critical factor, as it determines the duration required for maximum metal uptake.

The present study deals with the isolation of microorganisms from the landfill site capable of biosorption of heavy metals. Microorganisms can accumulate and immobilize heavy metals either on their cell surfaces or within their cell because of the specialized mechanisms as well as structures, such as cell walls or extracellular polymers, which enable them to bind heavy metals effectively. As per the concern of heavy metal deposition in the environment, microorganisms were isolated from the landfill site. The present study focused on the identification of specific strains that have a higher affinity and capacity for the biosorption of heavy metals.

2. Methodology

2.1. Collection of Soil Sample

The samples were collected in sterile, airtight plastic bags from the vicinity of Dehradun Municipal Solid Waste Management Pvt. Ltd. The soil samples were collected at the coordinates $30^{\circ}20'47''$ N and $77^{\circ}52'01''$ E at an altitude of 538 meters above sea level (Figure 1). The samples were collected randomly from different locations in the morning between 10:00 am and 12:00 pm.

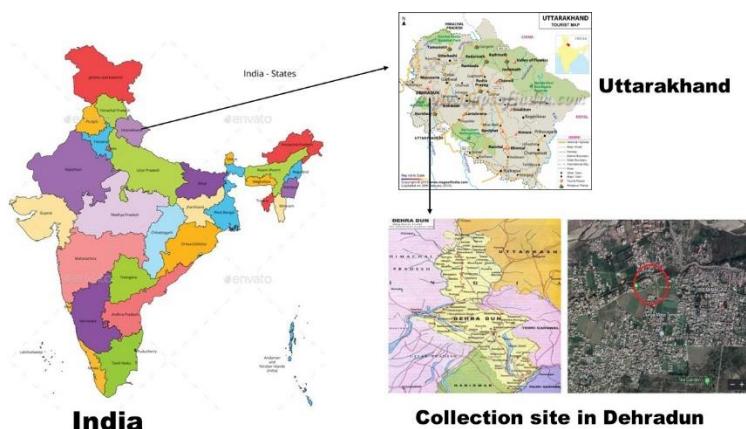


Figure 1. Collection site of the soil sample near Prem Nagar, Dehradun.

2.2. Isolation of Bacterial Strains

Bacteria were isolated from the soil sample using the serial dilution method by suspending 1 g of soil in 10 mL of sterile distilled water. The soil suspension was then serially diluted from 10^{-1} to 10^{-6} using sterile distilled water blanks. 0.1 mL of each dilution was transferred to sterile melted agar tubes and poured into labeled sterile Petri plates. The inoculated plates were then incubated at 37 °C for 24 h. After incubation, the plates were observed for bacterial growth and then purified by the streak plate method. The bacterial strains were maintained on agar slants containing nutrient broth and transferred weekly to a fresh medium to maintain metabolic activity. The purity of the strains was checked by microscopic examination (Aneja, 2007).

2.3. Isolation of Fungi Strains

Fungi were isolated from the soil sample using the serial dilution method with a suspension of 1 mg/10 mL sterile distilled water. The soil suspension was then serially diluted from 10^{-1} to 10^{-6} in sterile distilled water blanks. From each dilution, 0.1 mL of the sample was transferred to a sterile melted agar tube and poured into sterile, labeled Petri plates. The inoculated plates were incubated at 27 °C for 72 h. After incubation, the plates were observed for fungal growth followed by purification using the pour plate method. The fungal strains were maintained on agar slants containing nutrient broth and transferred weekly to a fresh medium to maintain metabolic activity and checked for purity by microscopy (Aneja, 2007).

2.4. Heavy Metal Sample Preparation for Fungi and Bacteria

Cd solution was prepared by dissolving Cd(NO₃)₂ salt in a 250 mL standard volumetric flask with deionized water at a concentration of 2000 ppm. Pb solution was prepared in the same way by using Pb(NO₃)₂. Forming the stock solution, experimental test solutions with concentrations of 600, 800, 1000, 1200, 1400, 1600, and 1800 ppm were prepared by diluting the primary stock solution with deionized water. From the stock solution, an experimental test solution was prepared at the specification of 50, 100, 150, 200, 250, 300, and 350 ppm by diluting the primary stock solution with deionized water.

2.5. Screening of the Microorganism for Biosorption

The heavy metal biosorption patterns of fungi and bacteria were evaluated using the agar well diffusion method (Hassen *et al.*, 1998). Metal salt solutions were prepared at a concentration of 0.05% (w/v) in distilled water and sterilized in a boiling water bath. Sterile plates were prepared, and wells (5 mm diameter) were drilled with a sterile drill. After inoculation with overnight grown indicator cultures, 45 µL of each metal salt solution was added to the wells. Plates were incubated at 25-27°C for 72 h for fungi and 37 °C for 48 h for bacteria. Inhibition zones were measured and isolates with a clear zone of 1 mm or less were considered resistant strains (Rani *et al.*, 2010).

2.6. Identification of Microorganisms

The resulting bacteria that were capable of biosorption were subjected to presumptive identification by their colony morphology and Gram staining. The identification of the fungal isolates involved a combination of methods, including the observation of their colonial morphology and microscopic characteristics. The colonial morphology was determined by evaluating their visual appearance, which included examining their color and mycelial formation. On the other hand, the microscopic characteristics were analyzed by placing a sample of the fungi on a microscope slide using an inoculation needle, staining it with 1-2 drops of lactophenol blue, and covering it with a coverslip. The resulting slide was then examined under a light microscope and identification was based on the type of mycelial structure and spore formation observed (Aneja, 2007).

3. Results

3.1. Isolation and Screening of Microorganisms

Microorganisms were successfully isolated and purified from a soil sample collected near the municipal waste disposal site in Dehradun. Enrichment techniques using nutrient agar medium (NAM) and Sabouraud dextrose agar (SDA) plates were used to culture these microorganisms. Initially, a total of 17 different bacterial strains and 1 fungal strain were isolated. However, at the beginning of the experiment, 17 bacterial strains and 2 fungal strains that showed slow growth were excluded from further analysis. The isolated microorganisms were then tested for their ability to tolerate high concentrations of Pb and Cd. Of the 15 bacterial strains tested, only 7 strains demonstrated the ability to

grow on NAM plates with a maximum concentration of Pb and Cd (350 ppm) using the well diffusion method. Similarly, the fungal strain showed growth on SDA plates with the highest concentration of Pb and Cd (1800 ppm) using the well diffusion method after the primary screening (Tables 1 and 2) (Figures 2 and 3).

Table 1. Morphological characteristics of heavy metal absorbing bacteria.

Isolates	Colony Color	Gram Stain	Shape	Margin / Elevation	Colony size	Texture	Colony Shape	Appearance / Pigmentation
Isolate 2	Pinkish	-Ve	Rod	Entire/ convex	Punctiform	Smooth, moist	Circular	Shiny/ pigmented
Isolate 4	Orange	-Ve	Rod	Entire /convex	Small	Smooth, moist	Circular	Shiny/non-pigmented
Isolate 11	Pale yellow	+Ve	Cocci	Entire/ umbonate	Large	Smooth, mucoid	Filamentous	Dull/non-pigmented
Isolate13	White	-Ve	Streptobacillus	Undulate/ flat	Moderate	Smooth moist	Irregular	Dull/ non-pigmented
Isolate 14	Yellow	-Ve	Rod	Entire/ convex	Small	Smooth, moist	Circular	Shiny/non-pigmented
Isolate15	White	-Ve	Rod	Entire /Undulate	Small	Smooth, mucoid	Irregular	Shiny / non-pigment
Isolate16	Yellow	+Ve	Diplococcus	Entire/ convex	Punctiform	Smooth, moist	Circular	Shiny/non-pigmented

Table 2. Morphological characteristics of heavy metal absorbing fungi.

Culture media	Fungal Isolate	Colony Characteristics	
		Color	Type of growth
Sabouraud Dextrose Agar	<i>Aspergillus</i> spp.	Black	Flat growth

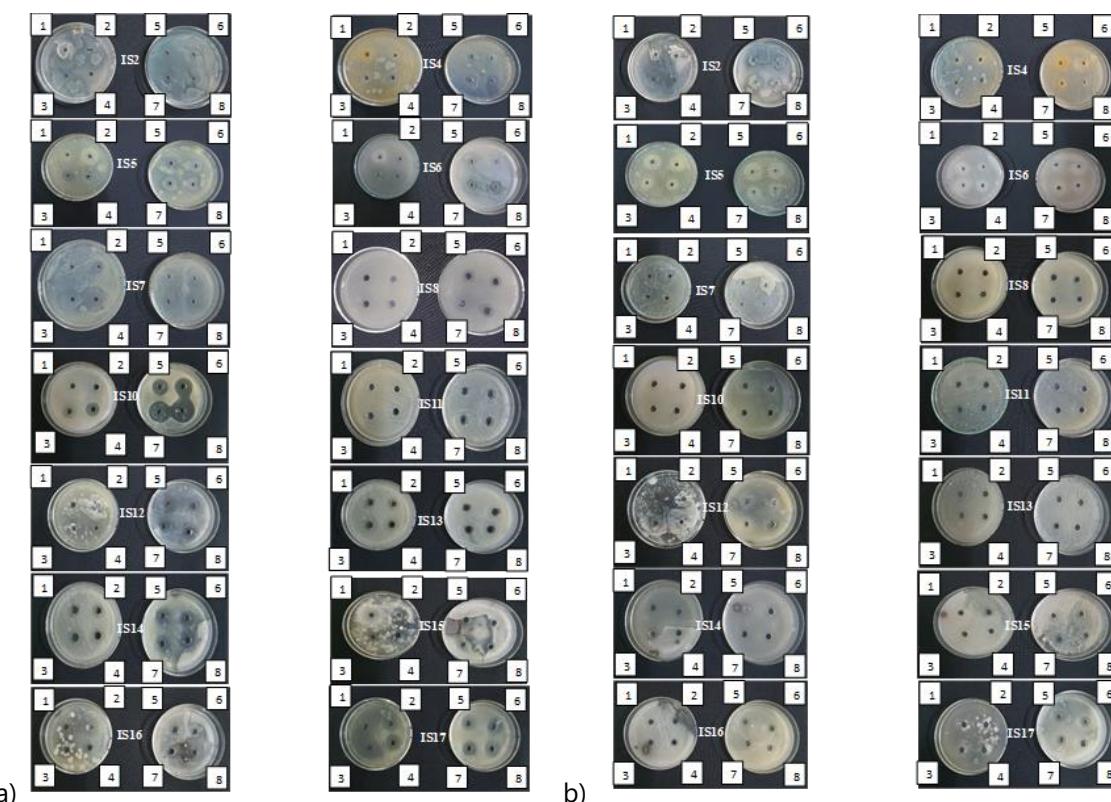


Figure 2. a) Screening of the bacterial isolates for the biosorption efficacy of Cd. (IS=Isolate; 1=Control; 2=50 ppm; 3=100 ppm; 4=150 ppm; 5= 200 ppm; 6= 250 ppm; 7= 300 ppm; 8= 350 ppm); b) Screening of the bacterial isolates for the biosorption efficacy of Pb. (IS=Isolate; 1=Control; 2=50 ppm; 3=100 ppm; 4=150 ppm; 5= 200 ppm; 6= 250 ppm; 7= 300 ppm; 8= 350 ppm).

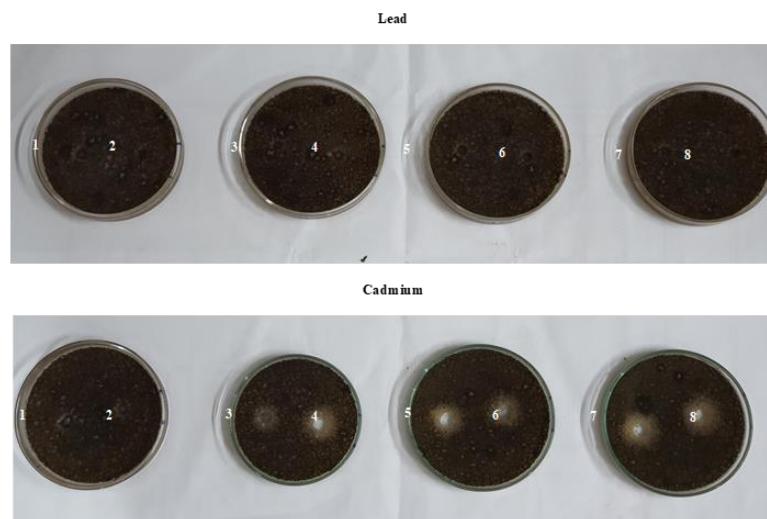


Figure 3. Screening of the fungal isolate for the biosorption efficacy of Pb and Cd (1=Control; 2=600 ppm; 3=800 ppm; 4=1000 ppm; 5= 1200 ppm; 6= 1400 ppm; 7= 1600 ppm; 8= 1800 ppm).

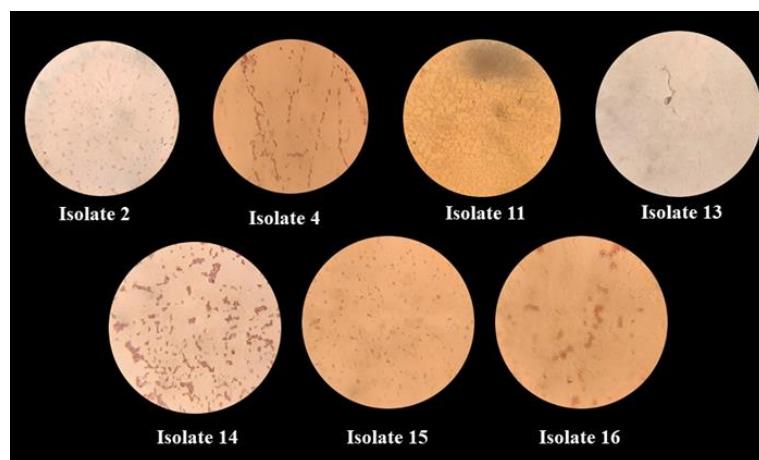


Figure 4. Gram staining results of absorbing isolates.

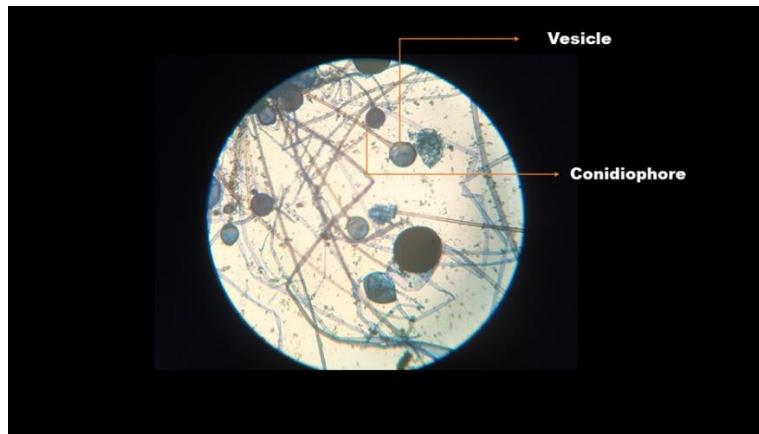


Figure 5. Lactophenol cotton blue staining of isolated fungi.

3.2. Preliminary Identification and Cd/Pb Biosorption Efficiency of Selected Isolates

The study revealed that amongst 17 isolated bacteria and 2 isolated fungus, 7 bacteria and 1 fungal strain is capable of absorbing Cd and Pb concentration. On preliminary identification among 7 isolated bacteria, 5 were Gram-negative and 2 were Gram-positive. The results revealed that Isolates 2 and 4 were found to be gram-negative rods having punctiform, circular, colonies with a smooth moist texture. The Isolate 11 was found to be Gram-positive cocci with large smooth mucoid dull filamentous colonies. Furthermore, isolate 13 appeared to be gram-negative bacilli with smooth

irregular moist colonies. Isolate 14 was found as a gram-negative rod with a smooth circular colony. Isolate 15 found as a gram-negative rod was like Isolate 14 but possesses an irregular colony shape. Moreover, isolate 16 was found to be gram-positive cocci with punctiform smoothy circular colonies (Figure 4). The absorbing fungus upon lactophenol cotton blue staining was found to be *Aspergillus* spp. (Figure 5). Upon evaluation, it was found that the selected microorganisms had different absorption capacities for Pb and Cd. Among the 7 bacterial strains, the percentage of microbes capable of absorbing Pb ranged from 35% to 70%, while the percentage of microbes capable of absorbing Cd ranged from 20% to 50%. On the other hand, the fungal strain had an absorption capacity of about 80% for both Pb and Cd (Figure 6).

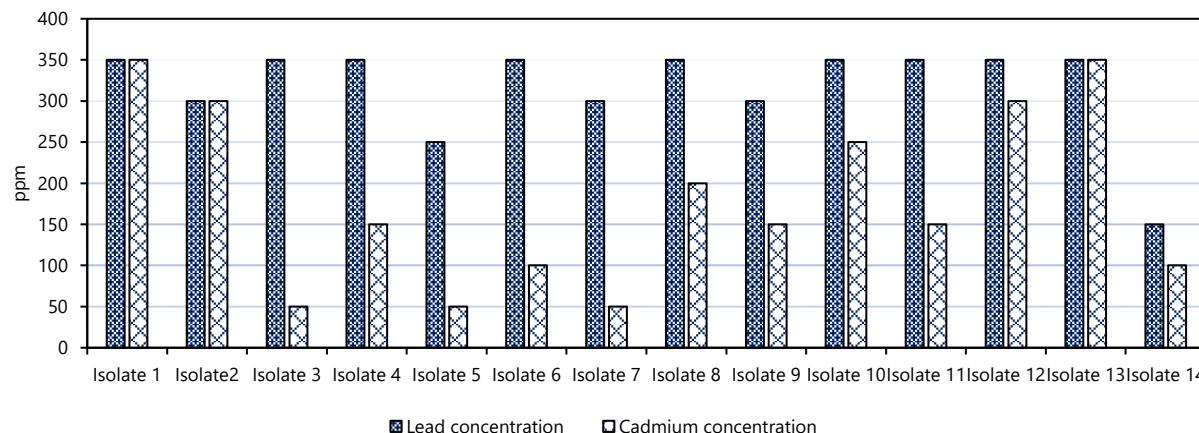


Figure 6. Comparison of biosorption of Pb and Cd by different isolates.

4. Discussion

Bacteria possess the remarkable ability to resist and biodegrade various substances, including organic compounds and heavy metals. This makes them highly valuable for applications in bioremediation. The focus of the present study was to develop cost-effective and efficient biosorbents using bacteria and fungi for the removal of Pb and Cd. To isolate suitable absorbing cultures, soil samples were collected near a municipal solid waste management site. Out of seventeen cultures tested, only seven bacterial strains (IS2, IS4, IS11, IS13, IS14, IS15, IS16) demonstrated the ability to bio-absorb both heavy metals. Additionally, one fungus (KM1) was isolated, which also exhibited biosorption capabilities for both metals. Metal tolerance assays revealed that these isolates were able to grow in the presence of metal ions at concentrations up to 350 ppm (bacteria) and 1800 ppm (fungus). However, their growth was inhibited at higher metal ion concentrations, indicating the toxicity of metals at elevated levels. Furthermore, the performance of these bacteria and fungi varied under different environmental conditions. They exhibited optimal performance when the pH and temperature conditions were closer to their respective optimum ranges. Therefore, their application would be most effective in environments where the pH and temperature align with their preferred conditions.

The potential efficacy of these bacterial and fungal isolates in removing metals is of great interest and requires further investigation. Utilizing these isolates in the metal removal process would not only be economically viable but also environmentally friendly, offering a versatile alternative to conventional methods of heavy metal removal. This suggests that different bacterial strains possess varying capacities for biosorption, depending on the type of heavy metal. The varying percentages of biosorption capacities reflect the affinity of different bacterial and fungal strains toward specific types of heavy metals. These findings highlight the significant potential of these bacterial isolates in remediating heavy metal-contaminated environments. By utilizing their unique biosorption capabilities, these isolates offer a promising approach for effectively removing toxic heavy metals. However, since this study represents a preliminary screening, the effectiveness of these isolates in real-world scenarios, such as complex environmental matrices or industrial wastewater, still needs to be determined. Furthermore, further research is required to uncover the underlying molecular mechanisms responsible for the biosorption process and to optimize the conditions to achieve maximum efficiency. A similar study was conducted by Ansari and Malik (2006), they conducted their research on the isolation of the microbes from the irrigation soil. They isolated 40 different strains among which 17 strains belong to Enterobacteriaceae and 10 were *Pseudomonas* spp. they performed experiments on their efficacy to biosorbent Cd and

Ni. Schott and Gardner (1997) demonstrated the recovery of light metals, such as aluminum, using *S. cerevisiae*. Brady et al. (1994) showed that *S. cerevisiae* cells treated with hot alkali were able to accumulate a broad range of heavy metal cations, including iron (Fe^{3+}), copper (Cu^{2+}), chromium (Cr^{3+}), Hg, Pb, Cd, cobalt (Co^{2+}), silver (Ag^{+}), nickel (Ni^{2+}), and ferrous iron (Fe^{2+}). The utilization of non-living biomass from *Aeromonas caviae*, which was isolated from raw water wells, has been reported for the biosorption of hexavalent chromium (Zouboulis et al., 2004).

4. Conclusion

These findings highlight the potential of these microorganisms, particularly the fungus strain, in bioremediation processes, as they possess the ability to tolerate and absorb significant concentrations of heavy metals commonly found in the vicinity of municipal waste management areas. As per the concern for the future perspective of the current investigation, this characterization indicates that different bacterial strains have varying capacities of biosorption depending upon the type of heavy metal. The different percentage of biosorption capacities indicates the affinities of different bacterial as well as fungal strains towards the heavy metals. These findings uncover the significant potential of these bacterial isolates for the remediation of heavy metal-contaminated environments. By harnessing their unique biosorption capabilities, these isolates offer a promising avenue for the efficient removal of toxic heavy metals. As it was the preliminary screening regarding the efficacy of these isolates, in real-world scenarios such as complex environmental matrices or industrial wastewater remains to be determined. Moreover, further investigation is needed to elucidate the underlying molecular mechanisms responsible for the biosorption process and to optimize the conditions for maximum efficiency. It would encompass a comprehensive analysis of surface properties, cell wall composition, and the identification of metal-binding proteins. Further research is required to address the limitations of this study and explore strategies to enhance the biosorption capabilities of these isolates.

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