

Removal Efficacy of Toxic Metals in Leachate through Micro-Organisms Isolated from the Natural Environment

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Landfill leachate is a serious contaminant for groundwater and surface water because of its potentially toxic metal content. In many countries, leachate is discharged into the natural environment without treatment because of the high disposal cost. However, this environmental problem can be solved by microorganisms, as they can adsorb the contaminants or convert them into end products, and this is cost-effective. This study focused on determining bacteria capable of efficiently removing toxic metals from leachates. Therefore, bacteria were isolated from nature that have a high adsorption and resistance capacity to a number of toxic metals. This potential was achieved by *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum*, among others. Their efficiency in removing toxic metals compared to raw leachate was Cd (78%, 67%, 78%), Ni (64%, 57%, 56%), Pb (99%, 75%, 76%), Cr (41%, 46%, 19%), Co (45%, 60%, 40%), and Cu (80%, 80%, 60%), respectively. According to the results, these bacterial strains proved to be very effective in the treatment of toxic metals from leachate. Therefore, they are good candidates for the treatment of wastewater by bioremediation methods.

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INTRODUCTION

Pollution of surface and groundwater by potentially toxic metals (PTMs) is a global problem. The PTMs pose chronic and even acute risks to the health of all living organisms, including humans (Imron *et al.* 2021). One of the sources of concern for human health and the environment is municipal solid waste landfills, which are a major source of soil and groundwater contamination in many regions of the world (Vodyanitskii 2016). Landfill leachate is also becoming increasingly problematic because of its potential for severe groundwater contamination (Vodyanitskii 2016). This is because many contaminants are highly toxic and dangerous to all forms of life. These include heavy metals, such as arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), and lead (Pb), which are toxic even at very low concentrations (Jayanthi *et al.* 2016; Rahman and Singh 2019). These PTMs have been identified as a major threat by international organizations such as the U.S. Environmental Protection Agency (EPA), the World Health Organization (WHO), the Agency for Toxic Substances and the Register of Diseases (ATSDR), and the United Nations Environment Program (UNEP) (ATSDR 2007; Rahman and Singh 2019;

Valdiviezo *et al.* 2021). Leaching of these PTMs from MSW landfills contaminates soil and groundwater, especially drinking water wells. The leachate volume of large landfills with a waste mass of several million tons can exceed 45,000 m³/year or more than 123 m³ per day (Masoner *et al.* 2016). It has been reported that PTMs leached from landfill leachate at a rate of 400 mm per year can persist in groundwater and surface water for about 150 years (Adelopo *et al.* 2018). Therefore, effective and cost-efficient leachate treatment technologies are needed for landfill management (Iravanian and Ravari 2020; Ozbay *et al.* 2021). Conventional treatment methods, although effective, are environmentally harmful to landfill leachate treatment and are also technologically expensive (Dursun 2006; Fan *et al.* 2008; Hermosilla *et al.* 2009). Although many chemical and physical methods have been used to treat leachate (Akgul *et al.* 2013; Gotvajn and Pavko 2015), many authors have confirmed, for example, the ability of carbon as an adsorbent to remove various inorganic and organic pollutants (Al-Saadi *et al.* 2013; Saleh and Gupta 2014). The use of adsorbents is a widely accepted approach for removing pollutants from wastewater. However, there is still much to be done to improve their efficiency, cost-effectiveness, and ecological performance (Gupta 1998; Saleh 2015a,b). To reduce the harmful effects of landfill leachate, many researchers are still conducting various experiments to treat the leachate effectively.

One of the most effective methods for treating leachate contamination is microbial treatment. One of these methods, bioremediation, is one of the most effective biotechnologies for removing PTMs. Recently, researchers have found that many microbes that break down waste into smaller forms are also among the most promising technologies due to their efficiency, relative cost-effectiveness, and environmental friendliness (Saetang and Babel 2012; Razarinah *et al.* 2014; Vijayaraghavan and Balasubramanian 2015), and these studies have identified the use of microbial metabolism as an essential part of biotechnology. The mechanism of PTM removal by microorganisms is a rather complex process. Some microorganisms have the potential to produce enzymes (Awasthi *et al.* 2017), surface binding (Sağ and Kutsal 1996; Tunali and Akar 2006), the mechanism of biosorption of metals, chelation, ion exchange, adsorption (Volesky 2007; Vijayaraghavan and Yun 2008; Wang and Chen 2009; Abdolali *et al.* 2014). In addition, microprecipitation of metals from outside the cell membrane to intracellular accumulation (Ahalya *et al.* 2003) suggests that there are related processes for the removal of heavy metals from contaminated areas. Bacteria, such as *Pseudomonas* sp., *Bacillus* sp., *Acinetobacter* sp., *Stenotrophomonas* sp., *Rhodococcus* sp., and *Burkholderia* sp., have been isolated from actively stored soil and leachate in the literature (Imron *et al.* 2019). It is also reported that *P. aeruginosa* isolated from leachate of solid waste landfills can remove about 29, 30, 35, and 28% of Pb, Cr, Ni, and Cd, respectively, and *P. aeruginosa* KHY2 and *Klebsiella pneumonia* KHY3 isolated from gold mine wastewater can reduce 1000 mg L⁻¹ Hg by up to 60% (Neneng and Gunawan 2018).

Little is known about the isolation of bacteria from the leachate of inactive landfills (Imron *et al.* 2021). Leaching metals in inactive landfills occurs not only by physicochemical leaching but also through biological leaching process that produces bacteria that are highly resistant to PTMs (Imron *et al.* 2021). In addition, bacteria isolated in contaminated areas can remove PTMs and are very resistant. Thus, indigenous microorganisms isolated in contaminated environments can resist and remove contaminants. One study reported that the combination of *E. hormaechei* and *Klebsiella* sp. is very effective in removing nitrate, even in the presence of heavy metals. These two

bacterial strains exhibit a remarkable ability to tolerate heavy metals, outperforming other denitrifiers. They have shown nitrate removal efficiencies above 99% and higher tolerance to Cu and Zn than many other denitrification strains. Thus, *E. hormaechei* and *Klebsiella* sp. are among the heaviest metal-tolerant denitrifying bacterial strains known to date (Amoako-Nimako *et al.* 2022). In addition, isolated strains of *Enterobacter hormaechei* have been reported to have significant potential for bioremediation in areas contaminated with nickel and various potentially toxic metals (Heidari *et al.* 2020), and the bioremediation potential of *E. hormaechei* and mutagen-exposed *E. hormaechei* (contaminated with Cd²⁺) was investigated in a one-week experiment. It is reported that mutant *E. hormaechei* exhibited excellent bioremediation potential (90.2%) compared to the wild strain (82.5%) on the sixth day of treatment. Moreover, the significant decrease of Cd²⁺ concentration in bioremediation started on the third day of treatment and decreased significantly until the sixth day of treatment (Lu *et al.* 2023). Recently, *P. aryabhatai* was reported to reduce the phytotoxicity of arsenic in plants and is the most promising PGPR showing great potential for new plant production strategies as it possesses several important PGP properties (Ghosh *et al.* 2018). *Priestia aryabhatai* has been shown to be able to degrade benzoate, methyl parathion, and polyethylene terephthalate (Dhaka *et al.* 2022; Esikova *et al.* 2021; Le *et al.* 2021). In addition, a specific strain of *Priestia aryabhatai*, namely KX-3, isolated from East Antarctica, was reported to be able to remove nitrogen under alkaline pH and low-temperature conditions (Kang *et al.* 2023). However, there are not many studies on the removal of potentially toxic metals from water by this bacterium. The Mycobacterium group includes pathogens that appear to be a source of infection to humans in the environment (Sanders and Wolinsky 1980), and their abundance is particularly noted in waters with heavy metal pollution (Falkinham *et al.* 1984; Davis-Hoover 1990), and they have been reported to have the ability to degrade methoxychloroethane, an organochlorine insecticide (Satsuma and Masuda 2012). In addition, effective results have been found in the removal of hydrocarbons, aromatics, and polycyclic hydrocarbons (Park *et al.* 1998). In conclusion, the diversity of mycobacteria, particularly prokaryotes, has been shown to offer the potential for adaptation to a variety of habitats, including environments heavily contaminated with hydrocarbons and heavy metals. Although mycobacteria are abundant in the environment, they have been overlooked for such an important use, a potential that is unexplored and unapplied in the bioremediation of hazardous chemicals (Azadi *et al.* 2017). This broad adaptability is also of great value for the bioremediation of degraded ecosystems.

This research aimed to determine which bacterial species have developed tolerance to heavy metals and adapted to natural conditions to obtain them for the National Center for Biotechnology gene bank. In addition, the removal of PTMs from leachate was investigated by producing low-cost and effective biological sand filters from these isolated bacteria. In addition, the removal efficiency of Cd, Pb, and Ni was to be determined under laboratory conditions.

EXPERIMENTAL

The Collection of Leachate and Soil Samples for the Isolation of Bacteria

Samples were collected from leachate and surrounding soils in the area where municipal waste and iron and steel industry waste were stored (41°10'40.20" N and 32°39

9.55" E) in Karabük province. The samples were transferred to sterile bags and brought to the laboratory on ice within 6 h. For isolation of bacterial species, 10 g of the soil samples were weighed and placed in test tubes containing 90 mL of isotonic water and then shaken for 15 min. After shaking, 10 mL of the soil solutions were taken, and serial dilutions were made between 10^{-1} and 10^{-5} . A total of 0.1 mL of the diluted solutions from 10^{-3} were placed on a culture medium (NA) and incubated at 37 °C for 48 h. At the end of the 48-h, samples were taken from different growing colonies to obtain pure cultures. All these procedures were also repeated for the leachate samples from the landfill. The pure cultures obtained were stored in a glycerol solution at -20 °C for research.

Determination of Potentially Toxic Metal Tolerance of Bacterial Isolates

The isolated 12 bacterial strains were inoculated in a culture medium containing solutions of 100, 200, 400, 550, and 600 ppm Pb (PbCl₂), Ni (NiCl₂·6H₂O), and 0.5, 1, 2, 3, 4, and 5 ppm Cd (CdCl₂·2.5H₂O) and then incubated at 37 °C for 48 h. This procedure was performed in three replicates in Petri dishes. At the end of the 48-h incubation period, the bacteria with the highest PTM concentrations were detected in a total of 576 Petri dishes. Three bacterial isolates that exhibited the highest heavy metal tolerance to Cd, Ni, and Pb were selected, and the species were identified and used for the experimental studies in this study.

16S rDNA Gene Region Polymerase Chain Reaction of Bacterial Isolates

To isolate DNA, bacterial isolates were incubated in the new medium at 30 °C for 24 h. After the incubation period, DNA isolation was carried out from each isolated strain, following the instructions given in the Invitrogen DNA isolation kit. 16S RDNA primers were used to amplify 16S RDNA regions from the obtained genomic DNA (27F5'-AGAGTTTGATCCTGGCTCAG and 1492R5'-GGTTACCTTGTTACGACTT) (Bal 2012). The total volume of the PCR response was set at 30 µL. Accordingly, 3 µL of 10x Buffer, 25 mM MgCl₂ (3 µL), 2 mM dNTP (3 µL), 10 pmol forward Primer (1.5 µL), 10 pmol reverse Primer (1.5 µL), 1.2 µL Tag DNA polymerase, and 2 µL DNA were used. The PCR conditions were performed with 35 cycles at 94 °C for 30 s, at 55 °C for 30 s, at 72 °C for 1.5 min, with an initial denaturation at 94 °C for 3 min, and a final elongation at 72 °C for 10 min. After imaging the gene region with a length of approximately 1400 bp obtained through PCR in the Agarose gel electrophoresis, sequences were performed in bidirectional sequence analysis. Sequential procedures were performed at Gaziantep University, Department of Molecular Biology.

Molecular Characterization of Bacterial Isolates Using 16S RDNA Gene Area

To determine the molecular species of the isolates obtained in the study areas, long bands of approximately 1000 to 1400 bps were obtained from the 16S RDNA gene region and analyzed. The sequence results obtained with the front and rear caps were evaluated in the FinchTV graphical viewer program (Geospiza Inc., Finch TV Version 1.4.0, Seattle WA, USA). The results from the sequence were recorded in the Seqtrace.exe program, resulting in a single sequence in FASTA format. The results in FASTA format were compared with the nucleotide sequences of bacterial strains recorded in the GenBank using the National Center for Biotechnology Information (NCBI) nucleotide BLAST program. Molecular identification of isolates was detected under MEGA7 (Kumar *et al.* 2016). A phylogenetic analysis was performed. The evolutionary history was established by

bootstrap consensus (1000 replicates) using the statistical method Joining (Felsenstein 1985; Saitou and Nei 1987). Evolutionary distances were determined using the p-distance method (Nei and Kumar 2000). *Clostridium butyricum* (EU621841.1) was autographed in the phylogenetic tree.

Biological Sand Filter Preparation

The biological sand filters were made of a 50-cm-long and 20-cm-wide polyvinyl chloride (PVC) pipe. The experiment was performed with 3 replicates for each bacterial strain. The soil and all other materials used for the filter were autoclaved at 150 °C for 2 h. Then, they were added to the tube as follows: I) It was filled with quartz sand filter (1.5 to 3 mm) at the height of 15 cm. II) Pebbles (5 to 12 mm) were placed 10 cm high. III) Larger pebbles (18 to 20 mm) were used at a height of 10 cm. IV) Soil was filled at a height of 15 cm. For the inoculation of soil bacteria, soil samples were mixed in using a sterile blender. On the first and second days, 50 mL of water was sprayed with a microbial load of 10^{-3} . The samples were incubated for a total of 48 h. After 48 h of incubation, the soil samples were placed on the filter systems. The leachate began to drain uniformly from the top, one drop in 10 s. After 24 h, water samples were collected from the bottom of the sand filters and analyzed for heavy metals.

F0: Original raw leachate.

F1: Only sterile soil and filter material was used; no bacterial strain was inoculated.

F2: Soil inoculated with *Enterobacter hormaechei* strains was used.

F3: Soil inoculated with *Priestia aryabhattai* strains was used.

F4: Soil inoculated with *Mycobacterium sacrum* strains was used.

Determination of the Absorption Capacity of Bacterial Strains in a Liquid Nutrient Medium Contaminated with Potentially Toxic Metals

Liquid media containing 10 ppm each of Ni, Pb, and Cd in 100 mL were prepared and their pH was adjusted to 6.5. Then they were poured into sterilized 50 mL incubation cups. Three isolated bacterial strains were inoculated into these sterilized incubation cups in three replicates (108 incubation cups in total) and incubated at 37 °C. After every 24 h (24, 48, 72, and 96 h), the inoculated three different bacterial broths were collected in three replicates and centrifuged at 3000 rpm for 10 min. Three types of leachates have been defined depending on the age of the landfill. Landfill leachate is specified between 6.5 and 7.5 in an age range of 5 to 10 years (Chian and DeWalle 1976). The original pH of the raw leachate from the sampling area was measured to be 6.8, which is consistent with what is reported in the literature. Therefore, the pH of the nutrient broth to which potentially toxic metals were added was adjusted to 6.5.

Toxic Metal Concentration Analysis of Leachate and Bacteria

Leachate passed through filters or raw leachate: Leachate samples were prepared for analysis by acid micro digestion in three steps. (Speed wave/MWS-2 Berghof products + Instruments Harreststr, Enien, Germany) (Mertens 2005). After sample preparation for analysis, the inductively coupled plasma optical emission spectroscope (ICP OES) (Perkinelmer, Optima 2100 DV, Shelton, CT, USA) was used to determine the PTM content. The same procedure was used for the centrifuged bacterial pellets.

Statistical Analysis

All data were analyzed using analysis of variance (ANOVA) SPSS 22 software (IBM, Armonk, NY, USA). Duncan's test was performed to detect significant differences in means ($p < 0.05$).

RESULTS

After molecular characterization of the bacterial isolates with the 16S rDNA gene region, they were compared and matched with the nucleotide sequences in the registered GenBank. The bacterial strains were assigned to the same group (*Priestia aryabhatai* (OP641851), *Mycobacterium sacrum* (OP641851), *Enterobacter hormaechei* (OP641850)) in the comparative phylogenetic tree with the reference strains found in NCBI (Fig. 1a, b). In addition, the strains were recorded using the Nucleotide BLAST program in NCBI (National Center for Biotechnology Information).

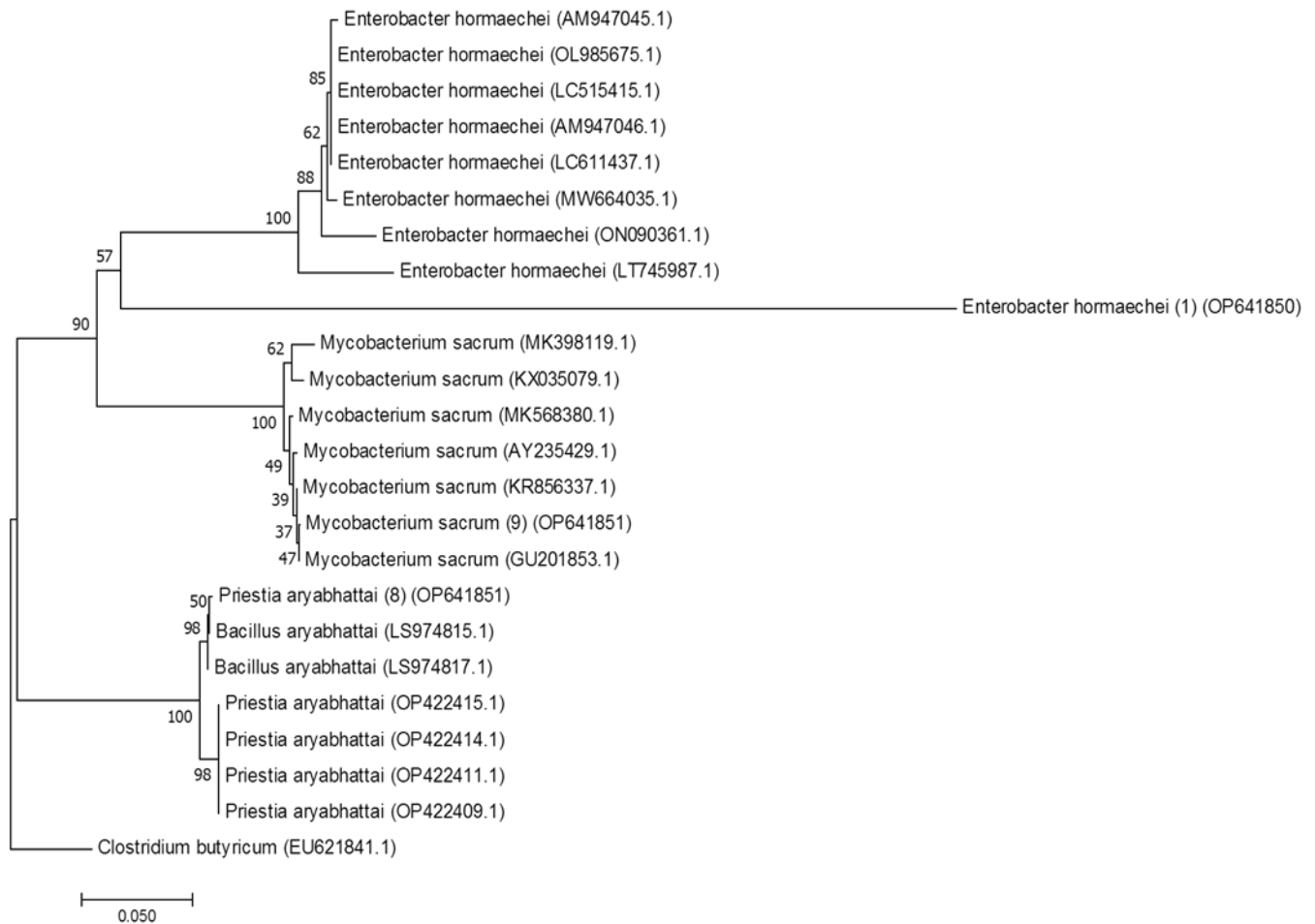


Fig. 1a. Phylogenetic tree based on 16S rDNA gene sequences

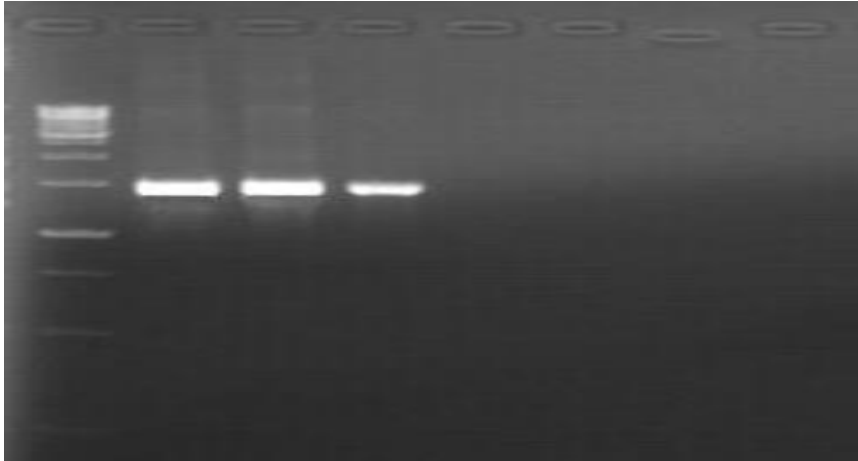


Fig. 1b. Agarose gel electrophoresis image

Statistically significant differences in heavy metal concentrations of leachate were found between F0, F1, and biological sand filters F2, F3, and F4 ($p < 0.05$) (Table 1).

Table 1. Changes in Heavy Metal Concentration in Leachate in Biological Sand Filters Inoculated with Different Bacteria and Without Bacteria

Bio-sand Filters	Cd	Ni	Pb	Cr	Co	Cu
F0	0.09 ± 0.01b	2.36 ± 0.01d	0.97 ± 0.01d	0.22 ± 0.01c	0.2 ± 0.01d	0.05 ± 0.01b
F1	0.08 ± 0.01b	1.28 ± 0.01c	0.17 ± 0.01b	0.19 ± 0.01b	0.16 ± 0.01c	0.02 ± 0.01a
F2	0.02 ± 0.01a	0.86 ± 0.01a	0.01 ± 0.01a	0.13 ± 0.01a	0.11 ± 0.01b	0.01 ± 0.01a
F3	0.03 ± 0.01a	1.01 ± 0.01b	0.25 ± 0.01c	0.12 ± 0.01a	0.08 ± 0.01a	0.01 ± 0.01a
F4	0.02 ± 0.01a	1.05 ± 0.01b	0.23 ± 0.01c	0.18 ± 0.01b	0.12 ± 0.01b	0.02 ± 0.01a

Duncan^{a,b} $p < 0.05$

Table 2. Potential for Absorption of Ni, Pb, and Cd of the Bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* into the Broth Medium

Bacteria	PTM	Time (h)			
		24	48	72	96
EH	Ni	0.10 ± 0.02d	1.59 ± 0.01c	4.24 ± 0.01b	4.58 ± 0.01a
PA		0.01 ± 0.001d	1.41 ± 0.01c	2.58 ± 0.01a	6.03 ± 0.01b
MS		0.13 ± 0.01c	1.24 ± 0.013b	1.98 ± 0.05a	2.00 ± 0.01a
EH	Pb	1.09 ± 0.01c	2.16 ± 0.01b	2.77 ± 0.01a	4.29 ± 0.01d
PA		0.37 ± 0.05c	0.38 ± 0.06b	0.75 ± 0.04c	1.09 ± 0.01a
MS		0.06 ± 0.01c	0.08 ± 0.01c	2.86 ± 0.04b	5.64 ± 0.04a
EH	Cd	0.45 ± 0.01c	0.76 ± 0.05c	1.76 ± 0.05b	1.83 ± 0.03a
PA		0.01 ± 0.002c	0.02 ± 0.01c	0.25 ± 0.01b	0.36 ± 0.06a
MS		0.01 ± 0.003b	0.02 ± 0.01b	0.04 ± 0.01a	0.05 ± 0.01a

The analyzed PTM concentrations of F0, F1, F2, F3, and F4 were determined: Cd (0.09 ± 0.01 , 0.08 ± 0.01 , 0.02 ± 0.01 , 0.03 ± 0.01 and 0.02 ± 0.01), Ni (2.36 ± 0.01 , 1.28 ± 0.01 , 0.86 ± 0.01 , 1.01 ± 0.00 and 1.05 ± 0.01), Pb (0.97 ± 0.01 , 0.17 ± 0.01 , 0.01 ± 0.00 , 0.25 ± 0.00 and 0.23 ± 0.01), Cr (0.22 ± 0.01 , 0.19 ± 0.01 , 0.13 ± 0.01 , 0.12 ± 0.00 and 0.18 ± 0.01), Co (0.20 ± 0.01 , 0.16 ± 0.01 , 0.11 ± 0.01 , 0.08 ± 0.00 and 0.12 ± 0.01), and Cu (0.05 ± 0.01 , 0.02 ± 0.01 , 0.01 ± 0.01 , 0.01 ± 0.00 and 0.02 ± 0.01) ppm, respectively.

Statistically significant differences were found between the absorption capacities of the isolated bacteria into the broth medium containing 10 ppm of Ni, Cd, and Pb and the removal efficiency over time ($p < 0.05$) (Table 2).

The concentrations of Ni absorbed by the bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* were determined by time series as follows: 24th h (0.10 ± 0.02 , 0.01 ± 0.00 , and 0.13 ± 0.01), 48th h (1.59 ± 0.01 , 1.41 ± 0.01 , and 1.24 ± 0.01), 72nd h (4.24 ± 0.01 , 6.03 ± 0.01 , and 2.00 ± 0.05), 96th h (4.58 ± 0.01 , 2.58 ± 0.01 , and 1.98 ± 0.01) as ppm (Fig. 2).

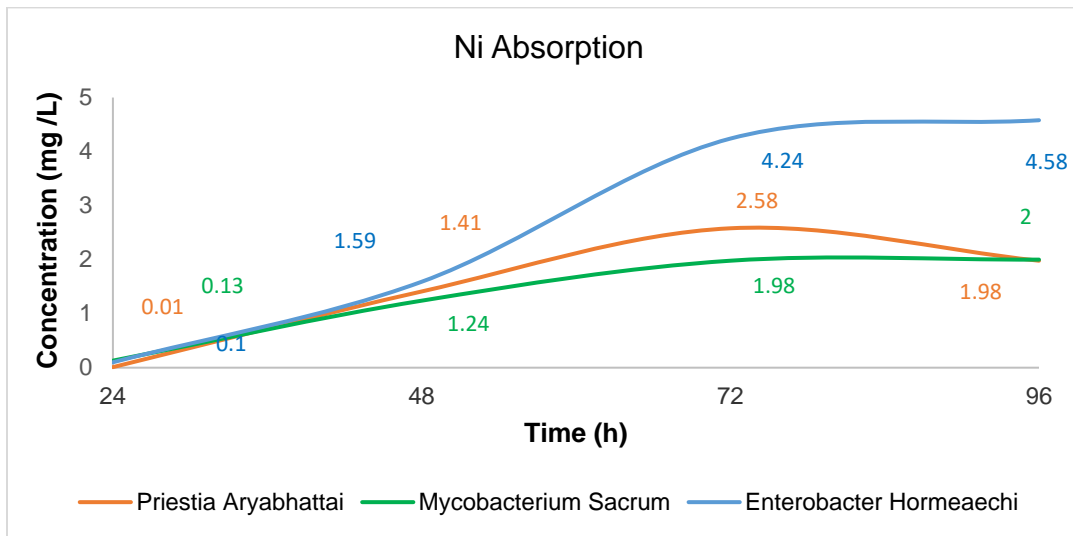


Fig. 2. Absorption capacities based on time series of bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* in media containing 10 ppm Ni

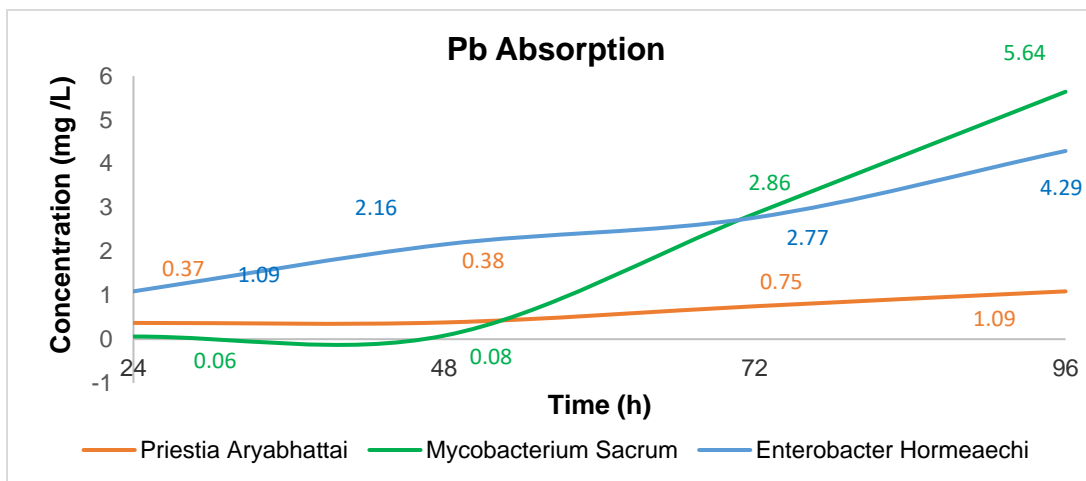


Fig. 3. Absorption capacities based on time series of bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* in media containing 10 ppm Pb

The concentrations of Pb absorbed by the bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* were determined by time series as follows: 24th h ($1.09 \pm .01$, $37 \pm .05$, and $06 \pm .01$), 48th h ($2.16 \pm .01$, 0.38 ± 0.06 , and 0.08 ± 0.01), 72nd h (2.77 ± 0.01 , 0.75 ± 0.04 , and 2.86 ± 0.04), 96th h (4.29 ± 0.01 , 1.09 ± 0.01 , and 5.64 ± 0.04) ppm (Fig. 3).

The concentrations of Cd absorbed by the bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* were determined by time series as follows: 24th h (0.45 ± 0.01 , 0.01 ± 0.00 , and 0.01 ± 0.00), 48th h (0.76 ± 0.05 , 0.02 ± 0.01 , and 0.02 ± 0.01), 72nd h (1.76 ± 0.05 , 0.25 ± 0.01 , and 0.04 ± 0.01), and 96th h (1.83 ± 0.03 , 0.36 ± 0.06 , and 0.05 ± 0.01) ppm (Fig. 4).

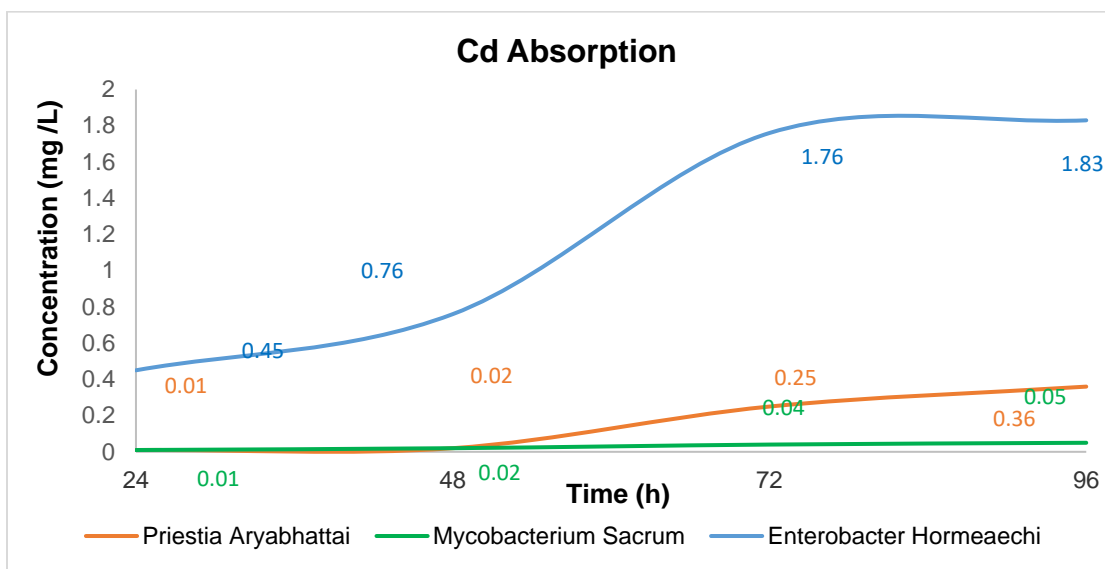


Fig. 4. Absorption capacities based on time series of bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* in media containing 10 ppm Cd

DISCUSSION

As an environmentally friendly technique, bioremediation has excellent potential for treating PTM in contaminated soils and waters. Moreover, microorganisms tolerant to toxic metals are essential for the bioremediation of contaminated areas with high specific levels of toxic metals (Yetunde Mutiat *et al.* 2018). Bacterial bioremediation can ameliorate contaminants in the environment through various mechanisms, such as adsorption, absorption, sequestration, detoxification, and conversion, to a bioavailable or less toxic and more soluble form (Zhang *et al.* 2020). This study observed a significant reduction in PTM concentration by F1, F2, F3, and F4 compared to the raw leachate (F0). The treatment rate of PTMs in biofilters was determined as follows: Cd (11%, 78%, 67%, and 78%), Ni (46%, 64%, 57%, and 56%), Pb (83%, 99%, 75%, and 76%), Cr (14%, 41%, 46%, and 19%), Co (20%, 45%, 60%, and 40%), Cu (60%, 80%, 80%, and 60%) F1, F2, F3, and F4, respectively. It was found that the bacterium *Enterobacter hormaechei* isolated from leachate was more effective than other bacteria in removing PTMs from leachate. The fact that this bacterium isolated from its natural environment was more effective than other bacteria in removing PTMs suggests that it can be used to treat PTMs in leachate, especially

wastewater. According to the analysis results of a study comparing the most commonly used systems for wastewater treatment in developing countries, the high-rate trickling filter systems are 0.3 to 0.45 m²/person, 0.5 to 1 W/person, and the construction cost is 40 to 70 US dollars/person. The cost of subsurface and rapid infiltration systems is reported to be 1 to 6 m²/person, 0 W/person, and construction cost is 5 to 15 US dollars /person. It has also been mentioned that conventional wastewater treatment systems have more disadvantages than subsurface and rapid infiltration systems, such as high energy consumption, sensitivity to toxic loads, needs for sophisticated operation, climate conditions, and high construction and operation costs (von Sperling 1996). An effective bacterial-biological sand filtration system at sites where leachate is generated will reduce these costs and provide an alternative solution to a significant environmental and health problem in developing countries. In addition, developing countries are generally located in tropical and subtropical regions. We assume that the microorganisms to be used in biological sand filters are hardly affected by temperature and climatic conditions since they usually operate below the soil surface. We can consider this as an advantage of this system. Furthermore, a constant supply of nutrients and substrate is not necessary due to the leachate content of the landfill. The disadvantage of this system is that it takes up more space, but if we take into account the surplus of unused space around the landfill, this will not prove to be a disadvantage. In addition, the results of the study showed that it is necessary to install a bacterial-biological sand water filter at a low cost in developing countries in areas where municipal waste is dumped. With the biological sand water filters to be installed, the pollutants in the leachate will prevent the pollution of surface water and groundwater and reduce the risk to public health. Some studies suggest that this bacterium should also be used to treat other pollutants, especially in wastewater. One study suggests that *E. hormaechei* JH can be used for organic deodorization and, along with other bacteria, has the potential to be effective in biological deodorization systems (Kim *et al.* 2008). In a study conducted in nitrate-polluted waters, *Enterobacter hormaechei* showed high removal potential with a denitrification rate of 3.0 C/N (98.7%) compared to methanol in the presence of sodium succinate. The results show that it can be accepted as a strong candidate for bio-denitrification of wastewater (Kebabi *et al.* 2018). Other studies show that the artificially induced mutation in *E. coli* has excellent tolerance to Cd metal and effectively absorbs Cd within the short duration of the bioremediation process (Kaur and Roy 2021), and *Priestia aryabhatai* is reported to be effective in the treatment of soil and water contaminated with organophosphates (Le *et al.* 2021). *Mycobacterium* is a genus of *Actinomycetes* that belongs to its own family, *Mycobacteriaceae*. There are more than 190 known species within the genus (King *et al.* 2017). The genus includes pathogens that can cause serious diseases in mammals, including tuberculosis in humans (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*) (Hussain 2007). For this reason, the use of this microorganism in soil and water purification is not recommended because it may pose serious risks to human health and life. However, it is expected that this species will be helpful for future studies if mutations are identified that do not pose a health risk.

After the 24th, 48th, 72nd, and 96th h, the concentrations of Ni, Cd, and Pb absorbed by the bacteria *Enterobacter hormaechei*, *Priestia aryabhatai*, and *Mycobacterium sacrum* were determined as follows: Ni (1%, 15.9%, 42.4%, and 45.8%), (0.1%, 14.1%, 25.3%, and 60.3%), (1.3%, 12.4%, 19.8%, and 20%); Cd (4.5%, 7.6%, 17.6%, and 18.3%), (0.1%, 0.2%, 2.5%, and 3.6%), (0.1%, 0.2%, 0.4%, and 0.5%), and Pb (10.9%, 21.6%, 27.7%, and 42.9%), (3.8%, 3.8%, 7.5%, and 10.9%), (0.6%, 0.8%, 28.6%, and 56.4%). In

the study conducted in liquid media, *Enterobacter hormaechei* and *Priestia aryabhatai* showed higher Ni, Cd, and Pb absorption potential than *Mycobacterium sacrum*. However, these three isolated bacterial strains did not show as high Cd absorption capacity as Ni and Pb. This might be due to the stronger toxic effect of Cd. In conclusion, *Enterobacter hormaechei* and *Priestia aryabhatai* show high potential for use in bioremediation. One study, the growth rate and bioremediation potential of isolated strains resembling *S. melonis* and *E. hormaechei* were tested in a medium containing Ni, Cu, Pb, and Cd under four metal mixtures and at different pH values. It is reported that they achieved the highest heavy metal removal after 48 h at pH 6 (Heidari *et al.* 2020). Previous study results have also shown that *Sphingomonas* strains can degrade hazardous compounds (Heidari *et al.* 2020) such as pentachlorophenol (Yang and Lee 2008) and Cd (Tangaromsuk *et al.* 2002). It has been reported that *E. hormaechei* strains are potential bioremediators of contaminated soils and can be used as PGPR and phosphate solubilizing bacteria in agriculture (Fahsi *et al.* 2021). Moreover, in one study, the isolated bacterial strain *Priestia aryabhatai*, which possesses toxic metal healing properties, was isolated from contaminated soil and water, and described as a potential agent for arsenic remediation (Kumar *et al.* 2021). In addition, the isolate of *Priestia megaterium*, one of the bacteria isolated from sediments transported to the Black Sea with some toxic metal salts, is reported to provide important evidence of potential isolates for bioremediation and biotechnological approaches (Kalkan 2022).

In this study, a process using microorganisms was carried out to treat the potentially toxic metals in landfill leachate without pretreatment or posttreatment. However, over time, the leachate-sewage sludge will accumulate due to the activity of these microorganisms. Therefore, the elimination of heavy metal accumulation in leachate sludge is important to maintain the efficiency and effectiveness of the bio-sand filter system. For this reason, there is a need for studies that address the applicability of microbiological leaching (Tang *et al.* 2020), hyperaccumulating endophytes (Wang *et al.* 2021), or other chemical leaching (Zhang *et al.* 2020) methods. Recently, it has also been reported that the combination of physicochemical processes followed by biological pretreatments is very efficient, and it has been shown that both COD and N-NH⁴⁺ were almost completely removed by a combination of reverse osmosis and activated sludge (Tałałaj *et al.* 2019). More recently, bioleaching has been developed to recycle various metals in leachate or solid waste residues treated by filtration (Zeng *et al.* 2016; Dunbar 2017).

CONCLUSIONS

1. *Enterobacter hormaechei* and mycobacteria are currently discussed in the literature for removing organic and inorganic pollutants. However, the efficiency of removal of toxic metals from wastewater, especially by *Priestia aryabhatai* strains, has yet to be discussed in the literature. Therefore, this study is unique and can be considered supportive of the literature concerning the other two isolated bacteria. Based on the research results, it is suggested that the effectiveness of these bacteria in removing heavy metals in the leachate at different pH, temperature, and contact time should be investigated in further field studies.
2. The current study found that the isolated bacteria *Enterobacter hormaechei* and *Priestia aryabhatai*, adapted to environmental conditions, were very highly efficient

in removing complicated, potentially toxic metals in the leachate at a rate of 68% and at 64%, respectively. Although, they have not been used for pre- or post-treatment of raw leachate. Therefore, it is supposed to be integrated or develop these bacterial sand filters with other conventional cost-effective systems to achieve safer wastewater treatment. Moreover, the researchers reported that this type of bacterial biofilter, besides its advantages, can be more than ten times cheaper than conventional wastewater treatment systems.

3. *Enterobacter hormaechei* and *Priestia aryabhatai* have already been used to remove organic pollutants. However, the efficiency of removal of toxic metals in wastewater, especially by *Priestia aryabhatai*, has yet to be discussed in the literature. Therefore, this study can be considered original in this regard. For this reason, according to the research results, it is important to include these bacteria, whose efficiency in removing of heavy metals in leachate is studied, in new studies on the efficiency of removal of other pollutant parameters in wastewater issues.

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