MedCrave
Step into the World of Research
Journal of Bacteriology & Mycology: Open access

Research Article

Open Access CrossMark

Removal of heavy metals from contaminated water by thermophilic bacteria isolated from hot springs in Saudi Arabia

Abstract

The xenobiotic and heavy metals are major source of pollutants cause severe illness to the human body and harm ecosystem. Therefore, a study was designed to investigate role of microbes in removal to these contaminants. Two thermophilic bacterial strains (*Brevibacillus borstelensis* AK1.) *and Anoxybacillus flavithermus* AK1) were isolated from the Hot Springs of Saudi Arabia and categorised as S11 and S40 respectively. The strains were tested against eight heavy metals via Minimal inhibitory concentrations (MICs) to determine heavy metal resistance. Study revealed that *B. borstelensis* AK1 efficiently biodegrade Chromium and Nickle with MICs of 3 mm while *A. flavithermus* actively involved in removal of Chromium with MIC of 3.5 mm. In distilled water, the maximum removal (90%) of Aluminum was achieved by both bacterial strains. The S40 strain was effectively remove 21.6% manganese and 50.9% cadmium. The strain S11 was capable to remove 6.1% manganese. The highest rate of removal of Lead (97.4%) and aluminum (98.5%) was observed by S11 strains. The whole study was conducted on 24-hour incubation utilizing antibiotics cinoxacin, ciprofloxacin (70 μgmL^{-1}) and ampicillin was (3 μgmL^{-1}) for both strains (S11 and S40) as positive control. The current study provides baseline data regarding role of thermophiles in heavy metals removal from water and in future more studies are required to identify the genetic aspects and related pathways associated with the heavy metals removal.

Keywords: trace metals, contamination, water, thermophilic bacteria

Volume 10 Issue 3 - 2022

Amjad Khalil,[1] Shahad Shaikh,[1] Bassam Tawabini[2]

[1]Department of Bioengineering, King Fahd University for Petroleum and Minerals (KFUPM), Saudi Arabia
[2]Geosciences Department, College of Petroleum Engineering and Geosciences, KFUPM, Saudi Arabia

Correspondence: Amjad Khalil, Professor, Department of Bioengineering, College of Chemicals and Materials, King Fahd University for Petroleum and Minerals (KFUPM), Dhahran, Saudi Arabia, Email amjadb@kfupm.edu.sa

Received: September 14, 2022 | **Published:** October 06, 2022

Abbreviations: Ar, arsenic; Al, aluminium; CAS, chemical abstracts service; Cd, cadmium; Cu, copper; Cr, chromium; MICs, minimal inhibitory concentrations; Mn, manganese; Ni, nickel; OD, optical density; Pb, lead; TT, tetrathionate; WHO, World Health Organization; Zn, zinc

Introduction

The increased urbanization and industrialization is actively related to the increased heavy metal pollution, which is a major concern for health and the environmental aspect. Hence, it is alarming for all living organisms in the ecosystem. The presence of heavy metals mainly associated with the nature, including soil, rocks, air, and water, and mainly originate from industrial waste, mining, sewage sludge, fossil fuel burning, pesticides, and fertilizers.[1] These heavy metals are chemically complexed nature and not easily biodegradable therefore it cause severe harmful impact on the human health if the level exceeded to the trace amount.[2] If the concentration of accumulated metal is exceeded to the certain limit which is set by the World Health Organization (WHO), these metals are responsible to disrupt functions of various systems within the human body including kidney, liver, or lung damage and nervous disorders.[3,4] Therefore, heavy metal removal and reduction are among the greatest challenges of this century. Hazardous heavy metals affecting ecosystems include arsenic (Ar), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), nickel (Ni), zinc (Zn), aluminium (Al), and manganese (Mn).[3] The hazardous nature of these metallic compounds has been associated with the physical and chemical properties of metal including, their atomic number, density, and chemical properties. In recent days the advancement in applied microbiology has revealed various microbial aspects which are capable to attain genetic resistance via various processes.[5] Microorganisms due to its less complex genome it is easier for researchers to study microbial genome, metabolic activities and respective pathways associated with the heavy metal accumulation.[6]

Bioremediation is the most effective low-cost and eco-friendly method of removing heavy metals, which uses biological mechanisms to reduce, degrade, detoxify, mineralize, or transform the concentration of pollutants to an innocuous level.[3,7] Microorganisms have adopted many diverse bioremediation mechanisms; each process is unique in its specific requirements, advantages, and disadvantages, and depends on the organism type and contaminants involved in the process. Bacteria are ubiquitous in the ecosystem with varying sizes and shapes. Different microbes have been proposed as efficient, economical alternatives for removing heavy metals from soil and water, and current study focuses on thermophiles.[7]

Thermophiles are organisms that grow at extreme temperatures, with an optimum temperature of 60-80 °C, and are typically found in hot springs. Since, they are capable of tolerating tolerate high temperatures due to presence of unique cell walls that provide special properties to bacteria that facilitate high metabolism, the production of physically and chemically stable enzymes, lower growth, and high end-product yields.[8,9] Microorganisms that inhabit in toxic metal environments may utilized further for developing various strategies for certain adaptations, such as active efflux systems, changes in ion permeability, adsorption, biotransformation.[10] The uptake of heavy metals by microorganisms typically involves two processes. The first is bioaccumulation, which requires the host cell to be alive and the microorganism actively taking up heavy metals into their intracellular component spaces. The second is biosorption, which is a passive, reversible uptake process that is independent of metabolism.[11,12] Therefore, current study was designed to determine the role of thermophilic bacteria (S11 and S40) in removal of heavy metal removal from the ground and distilled water.

Materials and methods

Chemicals

The antibiotics and metal salts used in this study were purchased from Sigma-Aldrich. The antibiotics were identified through the unique number assigned by the Chemical Abstracts Service (CAS) and included ampicillin (CAS number: 7177-48-2), cinoxacin (CAS number: 28657-80-9), and ciprofloxacin (CAS number: 0085721).

The following metal salts were used: chromium (III) chloride hexahydrate ($CrCl_3.6H_2O$), nickel (II) chloride ($NiCl_2.6H_2O$), copper sulfate pentahydrate ($CuSO_4.5H_2O$), lead chloride ($PbCl_2$), aluminum sulfate hexadecahydrate ($Al_2(SO_4)_3.16H_2O$), manganese (II) chloride tetrahydrate ($MnCl_2.4H_2O$), cadmium chloride ($CdCl.H_2O$), and zinc sulfate ($ZnSO_4$. H_2O). Tetrathionate (TT) Broth (1L) was prepared by adding 8g of tryptone, 4g of yeast extract, and 2g of NaCl to a flask.

Bacterial strains

Brevibacillus borstelensis AK1 (strain 11) and *Anoxybacillus flavithermus* AK1 (strain 40) were isolated from hot springs in Saudi Arabia.[13,14]

Antibiotic susceptibility

The minimal inhibitory concentration (MIC) of antibiotics was determined as the lowest concentration of antibiotics that prevented visible bacterial growth, and was determined following a modified version of a protocol described in the Manual of Antimicrobial Susceptibility Testing.[15]

Single colonies of *B. borstelensis* AK1 (11) and *A. flavithermus* AK1 (40) were inoculated in the liquid TT medium and incubated at 50 °C with shaking until the exponential phase was reached, corresponding to an optical density (OD) of 1.5 (OD600nm was measured using a Varian Cary 50 Scan UV-Visible Spectrophotometer) at 600 nm. The bacterial culture was diluted up to 0.1 OD600nm in TT medium supplemented with increasing concentrations (3-70 μgmL^{-1}) of antibiotics (ampicillin, cinoxacin, and ciprofloxacin) and incubated at 50 °C for 24 hrs. For each test, three independent experiments were conducted in triplicate. The MIC was determined from the OD600nm measurements after incubation for 24 hrs under optimal growth conditions.

Heavy metals resistance

To determine the MIC of heavy metal ions (Cd, Ni (II), Cr (III), Zn, Pb, Al, Mn (II), Cu), cell cultures were grown and diluted as described above. Heavy metals were added at increasing concentrations from 0.1 to 3.5 mm Two sets of experiments were prepared for this purpose; in the first set, distilled water was used to prepare the growth media (TT liquid media), while groundwater was used to prepare the growth media (TT liquid media) in the second set. The MIC values were determined as described above, and the reported values are the average of three independent experiments, each conducted in triplicate.

Heavy metal removal from distilled water

Heavy metal analysis

All samples were treated with 2% HNO_3 to ensure that all trace metals were available in the solution and were within the calibration range of ICP-MS, which was used for trace metal analysis, ICP-MS (iCAP-RQ, Thermo Scientific, UK). High-purity (99.999%) Argon (Ar) gas was used for plasma generation, while high-purity (99.999%) Helium (He) gas was included to limit interference in the KED mode. ICP-MS was calibrated using calibration standards containing metal mixtures with concentrations ranging from 1-1000 ppb. Table 1 below shows the starting concentration for each heavy metal.

Results

Antibiotic susceptibility

The antibiotic resistance results for the bacteria used in this study are listed in Table 1. The MIC for cinoxacin and ciprofloxacin for both strains was 70μgmL^{-1} while that for ampicillin was 3μgmL^{-1}.

Table 1 Starting concentration of each heavy metal

Heavy metal used by thermophiles	Concentration (mm)	Concentration (ppm)
Cadmium	0.1	11.2
Nickel	1.5	88
Chromium	2	104
Zinc	0.5	32.7
Lead	0.5	103.6
Aluminum	1.5	40.5
Manganese	1.5	82.4
Copper	0.15	9.5

MIC toward heavy metals

The efficacy of both strains S11 and S40 in removal of heavy metals. The MICs for the eight metals using both targeted strains revealed that Cd exhibited the lowest MIC of 0.2 mm for *B. borstelensis* AK1, while Cr and Ni exhibited the highest MICs of 3 mm. Cd also exhibited the lowest MIC for *A. flavithermus* AK1, which was 0.15 mm, while Cr exhibited the highest MIC of 3.5 mm (Figure 1).

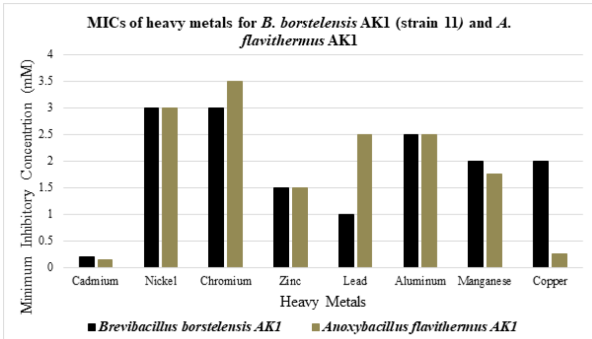

Figure 1 The representation of MICs of S11 and S40 against eight targeted heavy metals

Removal of heavy metals by both strains

Table 2 (distilled water) and 5 (groundwater) summarize the removal percentages of the eight tested heavy metals by strains S11 and S40.

Table 2 Antibiotic susceptibility for *Brevibacillus borstelensis* AK1 and *Anoxybacillus flavithermus* AK1 against Cinoxacin, Ciprofloxacin and Ampicillin

Antibiotic	MIC (µg) for *Brevibacillus borstelensis* AK1	MIC (µg) for *Anoxybacillus flavithermus* AK1
Cinoxacin	70	70
Ciprofloxacin	70	70
Ampicillin	3	3

Citation: Khalil A, Shaikh S, Tawabini B. Removal of heavy metals from contaminated water by thermophilic bacteria isolated from hot springs in Saudi Arabia. *J Bacteriol Mycol Open Access.* 2022;10(3):60–64. DOI: 10.15406/jbmoa2022.10.00328

Aluminum

Strain S11 (*B. borstelensis* AK1) degraded approximately 91.1% of Al within 24 h in distilled water, while 98.5% of Al was degraded in groundwater, indicating that S11 efficiently removed heavy metals from ground water in comparison to distilled water. However, strain S40 (*A. flavithermus* AK1) degraded 90.4% of the Al in distilled water within 24 h and 98.9% in groundwater which confirmed that S40 strain is also capably removed Al from the groundwater in comparison to distilled water. Generally, the Al removal performance of S40 was better than that of S11.

Chromium

In distilled water, strain S11 degraded approximately 74.9% of Cr within 24 h, while 79.9% of Cr was degraded in groundwater, indicating that S11 performed slightly better in groundwater than it did in distilled water. However, strain S40 degraded 77.6 % of Cr in distilled water within 24 h and 72.7% of Cr in groundwater, indicating that S40 performed better in distilled water than it did in groundwater. Generally, the Cr removal performance of S40 was better than that of S11.

Manganese

In distilled water, strain S11 degraded less than 6.1% of Mn within 24 h, while approximately 65% of Mn was degraded in groundwater within the same time period. Therefore, the performance of S11 in groundwater was much higher than that in distilled water. Additionally, strain S40 degraded 21.6% of Mn in distilled water within 24 h and 68.9% in groundwater, which is similar to strain S11, indicating that S40 performed better in distilled water than it did in groundwater. Moreover, the Mn removal performance of S40 was slightly higher than that of S11.

Nickel

Strain S11 degraded approximately 76.8% of Ni within 24 hrs in distilled water, while approximately 76.8% of Ni was degraded in groundwater within the same period. Therefore, the efficiency of S11 in groundwater was the same as that in distilled water. Additionally, strain S40 degraded 71.8 %of Mn in distilled water within 24 hrs and 75.3% in groundwater. The performance of the S40 strain in groundwater was slightly better than that in distilled water. Generally, S11 performed slightly better than S40 in the removal of Ni.

Copper

Neither strain S11 nor strain S40 could degrade Cu in distilled water. The Cu concentration slightly increased. However, in groundwater, almost 95.4% and 100% of Cu was removed by S11 and S40 in 24 hrs, respectively. Strains S11 and S40 remove Cu in distilled water but not in ground water, this is could be due to the presence of other ions (Ca, Mg, Na, K) in the ground water.

Zinc

In distilled water, strain S11 degraded approximately 68.5% of Zn within 24 hrs, while approximately 73.4% of Zn was degraded in groundwater within the same period. Therefore, the efficiency of S11 in groundwater was slightly higher than that in distilled water. Additionally, strain S40 degraded 68.2% of Zn in distilled water within 24 hrs, while 87.5% of Zn was degraded in groundwater. The performance of strain S40 in groundwater was significantly better than that in distilled water. Generally, S40 performed slightly better than S11 in the removal of Zn.

Cadmium

Strain S11 degraded approximately 50.9% of Cd within 24 hrs in distilled water, while approximately 45.5% of Cd was degraded in groundwater within the same period. Therefore, the efficiency of S11 in groundwater was slightly lower than that in distilled water. However, strain S40 degraded only 5.4% of Cd in distilled water within 24 hrs, while 40.9% of Cd in groundwater was degraded. Therefore, the Cd degradation performance of S40 in groundwater was much better than that in distilled water. Moreover, S11 generally performed better than S40 in the degradation of Cd.

Lead

Strain S11 degraded almost 96.7% of Pb within 24 hrs in distilled water, while 97.4% of Pb was removed in groundwater, indicating that the behavior of S11 in groundwater and distilled water was the same. Strain S40 degraded 70.4% of Pb in distilled water within 24 hrs, while that in groundwater was 97.7%, indicating that the performance of S40 in groundwater was superior to that in distilled water. Generally, the performances of S11 and S40 in groundwater were similar, while those in distilled water differed.

Discussion

In this study, the efficiency of heavy metal removal using two thermophilic bacteria isolated from hot springs in Saudi Arabia was investigated. The study revealed that the isolated strains exhibited relatively high rates of heavy metal removal in both distilled water and groundwater.

In distilled water, the highest removal rate was achieved by strains S11 and S40 was approximately 90% for Al within 24 h. S11 removed only 6.1% of Mn, while S40 removed 21.6% within 24 hrs. The lowest removal rate (using distilled water) achieved by S40 was observed for Cd, which was 5.4%, while S11 removed 50.9% of Cd. Neither strain removed Cu (Table 3).

Table 3 Results of the first experiment using distilled water

Removal of trace metals in distilled water								
Metals (μgmL^{-1})	Al	Cr	Mn	Ni	Cu	Zn	Cd	Pb
Initial concentration (ppm)	40.5	104	82.4	88	9.5	32.7	11.2	103.6
S11, 24 h	3.6	26.1	77.4	20.4	11.5	10.3	5.5	3.4
S11, 24 h, % removal	91.1	74.9	6.1	76.8	-21.1	68.5	50.9	96.7
S40, 24 h	3.9	23.3	64.6	24.8	12	10.4	10.6	30.7
S40, 24 h, % removal	90.4	77.6	21.6	71.8	-26.3	68.2	5.4	70.4

Al, aluminum; Cr, chromium; Mn, manganese, Ni, nickel; Cu, copper; Zn, zinc; Cd, cadmium; Pb, lead

In ground water, highest removal was for Al (98.5%) and Pb (97.4%) by S11 respectively, similar results were obtained from S40 for both metals Al and Pb. The lowest rate removal was for Cd (45.5%) by S11 and 40.9% by S40 (Table 4). Strains S11 and S40 remove Cu in distilled water but in ground water, this is could be due to the presence of other ions (Ca, Mg, Na, K) in the ground water.

The MICs for Cd by *B. borstelensis* AK1 and *A. flavithermus* AK1 were 0.2 and 0.15 mm, respectively. The MIC values for Cd reported here were lower than those in a study conducted by Hetzer et al,[16] using *Geobacills* (MIC ranging from 0.4 to 3.2 mm). Cd resistance was attributed to biosorption, that is, a phenomenon of metal binding to the microbial cell wall, which does not involve energy consumption.[10,17]

Citation: Khalil A, Shaikh S, Tawabini B. Removal of heavy metals from contaminated water by thermophilic bacteria isolated from hot springs in Saudi Arabia. *J Bacteriol Mycol Open Access.* 2022;10(3):60–64. DOI: 10.15406/jbmoa2022.10.00328

Table 4 Results of the experiment using ground water

Removal of trace metals in groundwater								
Metals (μgmL^{-1})	Al	Cr	Mn	Ni	Cu	Zn	Cd	Pb
Initial concentration (ppm)	40.5	104	82.4	88	9.5	32.7	11.2	104
S11, 24 h	0.61	20.87	28.9	20.4	0.44	8.7	6.1	2.68
S11, 24 h, % removal	98.5	79.9	64.9	76.8	95.4	73.4	45.5	97.4
S40, 24 h	0.43	28.42	25.64	21.77	0	4.09	6.62	2.2
S40, 24 h, % removal	98.9	72.7	68.9	75.3	100	87.5	40.9	97.9

Al, aluminum; Cr, chromium; Mn, manganese, Ni, nickel; Cu, copper; Zn, zinc; Cd, cadmium; Pb, lead

In addition to high temperature, the metal biosorption capacity of thermophilic bacteria is influenced by factors such as pH, initial metal concentrations, amount of biomass, and contact time, which should be investigated in future studies.[18]

Further studies on metal adsorption in thermophilic bacteria are required to evaluate their potential use in metal removal. The removal and recovery of heavy metals by biosorption mechanisms are important from environmental and economic perspectives.[19,20] Moreover, studies on thermophilic microorganisms can supplement our current knowledge regarding metal biosorption, which is based on mesophilic organisms.[10]

Antibiotic sensitivity

Cinoxacin, ciprofloxacin, and ampicillin were tested in this study, and the thermophilic strains exhibited sensitivity to both strains. This is consistent with the findings of Puopolo et al,[6] who reported that the genome of similar thermophilic bacteria, such as *G. stearothermophilus* 10, contains a sequence coding for a putative tetracycline major facilitator superfamily efflux (locus tag: GT50_ RS17520).

Heavy metal toxicity

A literature search indicated that heavy metal toxicity involves several mechanisms, such as destroying enzymatic functions, reacting as redox catalysts in the production of reactive oxygen species, destroying ion regulation, and directly affecting the formation of DNA as well as proteins. Therefore, the physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Different microbes have been proposed as efficient and economical alternatives for the removal of heavy metals from soil and water.[1]

Authors contribution

Shahad Shaikh: Study design, conceptualization, experimental design and data analyses

Bassam Tawabini: Experimental design, manuscript writing and data analyses

Amjad Khalil: Study design and final approval of manuscript

Data availability

The derived data supporting the findings of this study are available from the corresponding author on request

Ethical approval and consent to participate

Not applied to study

Acknowledgments

None

Conflicts of interest

The author declares no conflicts of interest.

References

1. Tarekegn MM, Salilih FZ, Ishetu AI. Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food & Agriculture*. 2020;6(1):1783174.
2. Jaishankar M, Tseten T, Anbalagan N, et al. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol.* 2014;7(2):60–72.
3. Jacob JM, Karthik C, Saratale RG, et al. Biological approaches to tackle heavy metal pollution: a survey of literature. *J Environ Manage.* 2018;217:56–70.
4. Kumar P, Kim KH, Bansal V, et al. Progress in the sensing techniques for heavy metal ions using nano materials. *J Indust Eng Chem.* 2017;54:30–43.
5. Pant G, Garlapati D, Agrawal U, et al. Biological approaches practised using genetically engineered microbes for a sustainable environment: a review. *Journal of Hazardous Materials*. 2021;405:124631.
6. Puopolo R, Gallo G, Mormone A, et al. Identification of a new heavy-metal-resistant strain of *Geobacillus stearothermophilus* isolated from a hydrothermally active volcanic area in southern Italy. *Int J Environ Res Public Health*. 2020;17(8):2678.
7. Kapahi M, Sachdeva S. Bioremediation options for heavy metal pollution. *J Health and Pollut.* 2019;9(24):191203.
8. Weralupitiya C, Wanigatunge R, Joseph S, et al. Anammox bacteria in treating ammonium rich wastewater: Recent perspective and appraisal. *Bioresource Technology*. 2021;334:125240.
9. Mehta R, Singhal P, Singh H, et al. Insight into thermophiles and their wide-spectrum applications. *3 Biotech*. 2016;6(1):81.
10. Özdemir S, Kılınç E, Poli A, et al. Biosorption of heavy metals (Cd2+, Cu2+, Co2+, and Mn2+) by thermophilic bacteria, *Geobacillus thermantarcticus* and *Anoxybacillus amylolyticus*: equilibrium and kinetic studies. *Bioremediation J*. 2013;17(2):86–96.
11. Diep P, Mahadevan R, Yakunin AF. Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Front Bioeng Biotechnol.* 2018;6:157.
12. Igiri BE, Okoduwa SI, Idoko GO, et al. Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. *J Toxicol*. 2018;2568038.
13. Khalil AB, Sivakumar N, Arslan M, et al. Insights into *Brevibacillus borstelensis AK1* through Whole Genome Sequencing: A Thermophilic Bacterium Isolated from a Hot Spring in Saudi Arabia. *BioMed Res Int*. 2018;5862437.
14. Khalil A, Sivakumar N, Arslan M, et al. Novel *Anoxybacillus flavithermus* AK1: A Thermophile Isolated from a Hot Spring in Saudi Arabia. *Arab J Sci Eng*. 2018;43:73–81.
15. Coyle MB. MIC Testing. In Manual of Antimicrobial Susceptibility Testing. *American Society for Microbiology*. 2005;53–62.
16. Hetzer A, Daughney CJ, Morgan HW. Cadmium ion biosorption by the thermophilic bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*. *Applied Environ Microbiol*. 2006;72(6):4020–4027.

17. Ranawat P, Rawat S. Metal-tolerant thermophiles: metals as electron donors and acceptors, toxicity, tolerance and industrial applications. *Environ Sci Pollut Res*. 2018;25(5):4105–4133.

18. Shamim S. Biosorption of heavy metals. *Biosorption*. 2018;2:21–49.

19. Barnard D, Casanueva A, Tuffin M, et al. Extremophiles in biofuel synthesis. *Environmental Technology*. 2010;31(8-9):871–888.

20. Bode HB, Müller R. The impact of bacterial genomics on natural product research. *J German Chem Society.* 2005;44(42):6828–6846.