Journal of Molecular Science

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Soil Remediation via Heavy Metal Immobilizing Bacteria: Effects on Arsenic and Chromium Co-Contamination

SN Praveena Yadavalli[1], Sreenivasulu Kamma[2] and Debasish Sahoo*[3] Tanushree Chatterjee[4]
[1]Ph.D Scholar, Department of Biotechnology, KL University Vaddeswaram, Andhra Pradesh, India.
[2]Department of Biotechnology, KL University Vaddeswaram, Andhra Pradesh, India.
[3*]Senior Scientist, BioInnovale Lifescience Pvt. Ltd. Bengaluru, India.
[4]Dean & HOD, Department of Biotechnology, Raipur Institute of Technology Raipur, Chhattisgarh, India.

Article Information
Received: 06-10-2025
Revised: 14-10-2025
Accepted: 24-10-2025
Published: 25-11-2025

Keywords
Bioaugmentation, Biosorption, Remediation, Heav metal, Soil enzyme

ABSTRACT
Heavy metal contamination, particularly with arsenic (As) and chromium (Cr), is a major environmental concern due to its persistence, toxicity, and potential for groundwater and food chain contamination. This study evaluates the potential of heavy metal-immobilizing bacteria for remediating As–Cr co-contaminated soils. Indigenous bacterial strains were isolated from contaminated environments and screened for their resistance and ability to immobilize As(V) and Cr(VI). The most effective strains exhibited high biosorption capacity, and bioaccumulation, significantly reducing the bioavailable forms of both metals. Soil microcosm experiments revealed that bacterial inoculation lowered metal mobility, suppressed plant uptake, and enhanced soil enzymatic activities and microbial biomass. These findings demonstrate that bioaugmentation with metal-tolerant bacteria not only mitigates the risks of As and Cr contamination but also improves soil health offering a sustainable and eco-friendly approach to rehabilitating polluted soils.

1. INTRODUCTION:

Soil contamination with heavy metals is a growing global concern, particularly in industrial and mining areas where toxic elements like arsenic (As) and chromium (Cr) are prevalent. These metals are hazardous to both human health and the environment, due to their persistence and toxicity. Arsenic, a naturally occurring element, and chromium, primarily released through industrial processes, can significantly degrade soil quality, affecting its fertility, biodiversity, and suitability for agriculture (Liu et al., 2020). Contaminated soils pose a severe risk to food safety, human health, and ecosystem stability, necessitating effective remediation strategies to restore soil functionality and prevent further environmental damage (Gao et al., 2021). Conventional methods of remediation, such as chemical treatments, excavation, and stabilization, are often expensive, environmentally disruptive, and not always effective in achieving long-term detoxification. In this context, bioremediation, particularly the use of heavy metal immobilizing bacteria, has emerged as a promising, cost-effective, and eco-friendly alternative.

Arsenic and chromium are both toxic heavy metals that can contaminate soil through industrial activities, mining operations, agricultural use of contaminated water, and improper disposal of waste. Arsenic contamination is widespread, with significant concentrations found in areas surrounding arsenic mining sites and regions utilizing groundwater containing high levels of arsenic (Saha et al., 2019). Arsenic exists in several oxidation states, with arsenite (As(III)) and arsenate (As(V)) being the most common in the environment. While As(V) is less toxic, As(III) is highly mobile and toxic to both plants and humans, easily infiltrating groundwater and agricultural systems. The carcinogenic properties of arsenic make its remediation critical to safeguard human health (Rahman et al., 2019). Chromium contamination mainly arises from industrial

effluents, such as those from electroplating, leather tanning, and pigment production. Chromium exists primarily in two valence states: hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)). While Cr(VI) is highly toxic, carcinogenic, and soluble in water, Cr(III) is less mobile and much less toxic (Zhou et al., 2020). However, the transformation between these two states in soil and groundwater complicates the remediation of chromium-contaminated sites. Cr(VI) is a potent oxidant, capable of damaging DNA and cell structures, leading to severe health risks including cancer, respiratory issues, and organ damage. Therefore, simultaneous contamination by both arsenic and chromium presents an even greater challenge for soil remediation, requiring a multifaceted approach to immobilize both metals and mitigate their toxic effects.

Traditional soil remediation strategies, such as soil washing, chemical stabilization, and excavation, involve significant financial costs and often lead to secondary environmental issues such as soil erosion, water pollution, and energy consumption. For instance, chemical stabilization techniques, which aim to reduce the mobility of contaminants, may involve the use of toxic stabilizers that pose their own environmental risks. Furthermore, physical removal or excavation of contaminated soil may not always be feasible, particularly in large-scale contamination cases or where the contaminated soil is deep underground (Sohail et al., 2021). In contrast, bioremediation offers an alternative that leverages natural processes to degrade or immobilize contaminants. The use of microorganisms, particularly bacteria, has gained attention due to their ability to transform or immobilize heavy metals through various mechanisms, such as biosorption, bioaccumulation, and precipitation (Vaishnav et al., 2024). Among these microorganisms, heavy metal immobilizing bacteria are particularly promising for remediating arsenic and chromium co-contamination in soil.

Heavy metal immobilizing bacteria utilize various biochemical mechanisms to reduce the mobility and bioavailability of toxic metals in contaminated soils (Gadd, 2010). These mechanisms include the production of extracellular polymeric substances (EPS) that can bind metal ions, the alteration of metal speciation through enzymatic reduction or oxidation, and the precipitation of insoluble metal compounds (Valls and De Lorenzo, 2002). By converting toxic forms of metals (such as Cr(VI) and As(III)) into less soluble and less toxic forms, these bacteria can effectively reduce the threat posed by heavy metals in contaminated environments. One of the key features of using bacteria for soil remediation is their adaptability and ability to survive in extreme environments, such as those with high levels of contaminants (Gupta & Joia, 2016). These bacteria can thrive in soil with low pH, high salinity, or high concentrations of heavy metals, making them suitable candidates for a wide range of contaminated sites. For example, certain species of *Pseudomonas*, *Bacillus*, *Rhodobacter*, and *Enterobacter* have been identified as capable of immobilizing both arsenic and chromium in soils through their metabolic processes (Singh et al., 2006). These bacteria can reduce Cr(VI) to the less toxic Cr(III), while simultaneously oxidizing As(III) to As(V), thereby decreasing the mobility and bioavailability of both metals. Bioremediation offers several advantages over conventional methods. First, it is cost-effective, as it typically requires fewer resources and less energy compared to chemical treatments or physical excavation. Second, bioremediation is environmentally friendly because it reduces the need for hazardous chemicals and minimizes the risk of secondary pollution. Third, the use of indigenous microorganisms for bioremediation ensures that the process is naturally integrated into the ecosystem, promoting the long-term sustainability of soil health. Additionally, bioremediation techniques can be applied in situ, meaning that contaminated soils do not need to be removed, thus reducing the environmental footprint of the remediation process (Saha et al., 2019).

2. MATERIALS AND METHODS:

Materials:

$CrCl_3 \cdot 6H_2O$ and $AsCl_3$ and other reagents were purchased from Chemicals and Reagent Guntur, Andhra Pradesh (India). All of the chemicals in our experiment are analytical grade.

Isolation of As and Cr resistant bacteria:

As and Cr resistant strains were obtained from As and Cr-contaminated soil at KL University in Guntur, Andhra Pradesh, India. The quick isolation method was as follows: 1 g of fresh soil was collected and agitated with 9 mL of sterilized water at 37 °C and 120 rpm for 1 hour. After shaking, the mixture was diluted with sterilized water and evenly distributed on Luria-Bertani (LB) culture medium (composition: yeast extract 5, peptone tryptone 10, and NaCl 10 g/L) containing 20 mg/L As and 100 mg/L Cr (concentrations comparable to soil contaminants) (Netherlands, 2008). The culture medium was then incubated at 37°C for 72 hours. Individual bacterial colonies were selected throughout this preliminary isolation phase to isolate As and Cr resistant bacteria. The As and Cr adsorption bacteria were isolated using a modified Jiang et al. (2013) technique. The isolated As and Cr resistant strains from the previous experiment

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

were inoculated into LB liquid culture medium with no heavy metal additions and cultured on a shaking table for 24 hours at 37 °C and 160 RPM. The mixture was then centrifuged at 3000 rpm for 5 minutes to remove the supernatant and collect the strains. To eliminate contaminants, strains were washed with 10 mM phosphate buffered saline (pH = 7.0). After that, strains were grown for 48 hours in LB liquid culture medium, which contained 20 mg/L As and 100 mg/L Cr. The bacterial cells were then extracted using centrifugation (3000 rpm for 10 minutes) and rinsed with sterilised deionised water. To determine heavy metal levels in strains, 0.2 g of strains were digested in a microwave with a mixture of HNO_3/HCl/$HClO_4$ (3:2:3, v/v/v) at medium high, medium, and low temperatures for 3 minutes each. Heavy metal levels in strains were measured by flame atomic absorption spectrometry (FAAS: Varian, SpectrAA 220FS) (Sungur et al., 2015). The heavy metal adsorption capacity of stresses on As and Cr was defined as follows:
The heavy metal adsorption ability of strains = Cs/Cp

The concentrations of heavy metals in strains and LB liquid culture medium were represented in the equation by Cs (mg/L) and Cp (mg/L), respectively. As a result, the higher value equation indicated that the strains had a higher adsorption efficiency. From this adsorption method, dominant strains with the ability to adsorb both As and Cr were identified. The minimum inhibitory concentration (MIC) of selected strains was then determined using Manasi et al.'s (2016) methods.

Characteristics of the isolated resistant bacteria:
The isolated dominant strains were identified using the previously described 16S rDNA sequence analysis approach (Ge et al., 2011). The obtained 16S rDNA sequences were then analysed using the NCBI BLAST program and sequence alignment in the GenBank database. The phylogenetic trees of strains were then generated using reference sequences retrieved from GenBank by the MEGA 7 tool.

Furthermore, a scanning electron microscope (SEM) and an Energy Dispersive Spectrometer (EDS) (JSM-5900LV, Japan) were used to monitor the surface features and heavy metal element distribution on isolated strains before and after adsorption (Ma et al., 2013a). Inductively Coupled Plasma Mass Spectrometry (ICP-MS, PerkinElmer NexION 350, USA) was also used to determine the absorption capacity of strains on heavy metals.

Soil and Pot experiment design:
To test the immobilisation capacity of the separated strains, they were put into the original contaminated soil (Table1). To remove extraneous materials from the soil, all samples were air-dried and sieved using a 2-mm sieve. The experiment was carried out in plastic pots (9 cm height, 10 cm base) with 0.5 kg of sieved soil. After comparing the heavy metal resistance and adsorption capabilities of isolated strains, two dominant strains (CS7 and CE2) were identified. Table 1 shows the specific experiment design in the pot experiment. 25 mL of the isolated strain suspensions (108 CFU/mL) and sterile deionised water were sprayed into the treatment and control groups, respectively. Afterwards, dirt was stirred to ensure that strains were evenly distributed. During the experiment, soil was sprayed with sterile deionised water to maintain a constant water retention capacity (13.06 ± 0.13%). Furthermore, after 30 days of inoculation, the same amounts of the isolated strains were re-inoculated into soil in T4 (inoculated with CS7), T5 (inoculated with CE2), and T6 (combined inoculated with CS7 and CE2) to assess the effects of re-inoculation on soil parameters (Vaishnav et al., 2025). Soil samples were collected every 15 days for four times during the pot experiment to track the dynamic changes in soil physical-chemical markers and bioavailable heavy metal levels.

Table 1 Design of immobilization pot experiment.

Treatment	Strain	Inoculation frequency
Control	None	None
T1	CS7	Once
T2	CE2	Once
T3	CS7 & CE2	Once
T4	CS7	Twice
T5	CE2	Twice
T6	CS7 & CE2	Twice

Bio-availabilities of As and Cr in contaminated soil:
The bio-available As and Cr contents in soil samples were measured using FAAS (VARIAN, SpectrAA 220FS). A 0.11 mol/L HOAc solution was expected to remove most water-soluble, exchangeable, and carbonate-bound metals from soil (Lu et al., 2007). Thus, the determination of HOAc-extractable As and Cr was used as an index to predict heavy metal bioavailability in soil (Pueyo et al., 2003). In particular, HOAc-extractable heavy metals were identified as follows: 1 g of air-dried soil sample and 40 mL of 0.11 mol/L HOAc were shaken at 25 °C and 250 rpm for 16 hours, then centrifuged at 3000 rpm for 20 minutes to collect the supernatant for HOAc-extractable heavy metal content analysis.

Soil enzyme activity determination:
Soil samples were taken at the end of the pot experiment in each treatment to assess soil enzyme activity. The activities of fluorescein diacetate (FDA) hydrolysis, acid phosphatase,

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

dehydrogenase, urease, and invertase were determined using Wang et al. (2020). The activity of FDA hydrolysis was spectrophotometrically evaluated at 490 nm and presented by the amount of fluorescein (μg) produced per hour and per gramme of soil (g-1 h-1). Acid phosphatase and invertase activities were spectrophotometrically evaluated at 410 nm and 508 nm, respectively, and expressed as the production of p-nitrophenol (pNP) and microgramme glucose per gramme soil per 24 hours. Dehydrogenase activity was determined spectrophotometrically at 492 nm and expressed as the synthesis of triphenylformazan (TPF) per gramme soil per hour. In addition, the activity of urease was spectrophotometrically measured at 578 nm and presented as microgram NH_4^+ per gram soil per hour.

Data analysis:
In this experiment, each treatment was replicated three times. The data were reported as value ± standard deviation. SPSS 21.0 with ANOVA was used to determine statistical significance. Means were compared using least significant differences (LSD) obtained at a significance threshold of $P < 0.05$ across treatments. All figures were created using the Origin V8.0 (USA) software and Photoshop CS (USA). MEGA 7 exports a phylogenetic tree of microorganisms.

3. RESULTS AND DISCUSSION

Isolation of As and Cr stress tolerant bacteria
In this experiment, five strains (CS2, CS7, CS9, CE2, and CE3) were isolated from contaminated soil. Re-isolation experiments yielded CS7 and CE2 strains with superior heavy metal removal properties (Table S2). Strains CS7 and CE2 showed adsorption rates of 25.10 ± 0.56% and 27.81 ± 0.21% in LB liquid culture medium solution, respectively. Strains CS7 and CE2 performed well on Cr removal, with adsorption rates of 61.41 ± 1.42% and 89.23 ± 2.01% in LB liquid culture medium, respectively. Compared to other heavy metal adsorption bacteria, the isolated strains had higher adsorption capacities for heavy metals in liquid. Tan et al. (2020) discovered that Bacillus sp. bacteria could reduce 68.5% of Cr in solution and collect approximately 0.5g/L of Cr in liquid. As a result, strains CS7 and CE2, which had relatively higher adsorption effects on both As and Cr, were chosen for the following experiments.

Identification of As and Cr adsorption bacteria:
The 16S rDNA sequences of CS7 and CE2 were presented in the supplementary information, and a phylogenetic tree of the isolated strains was shown in Figs. 1 and 2, indicating that strains CS7 belonged to Bacillus subtilis and CE2 to Bukholderia cenocepacia. Many strains of Bacillus sp. and Paenibacillus sp. have been shown to be capable of remediating heavy metal-contaminated soil via an absorbing and precipitating process (Jiang et al., 2009; Rau et al., 2009). Paenibacillus sp. might immobilise heavy metals such as lead (Pb), cobalt (Co), zinc (Zn), copper (Cu), as well as Cd and Ni by chemical reactions in soil (Prado et al., 2005) by spreading polysaccharides on the bacterial surface. Taking the heavy metal adsorption capacity and species of the isolated strains into account, strains CS7 and CE2 were chosen for the following experiment. Furthermore, the MICs of strains CS7 and CE2 for As were 100 mg/L and 650 mg/L, respectively. Meanwhile, the MIC of strains CS7 and CE2 towards Cr were 250 mg/L and 800 mg/L, respectively.

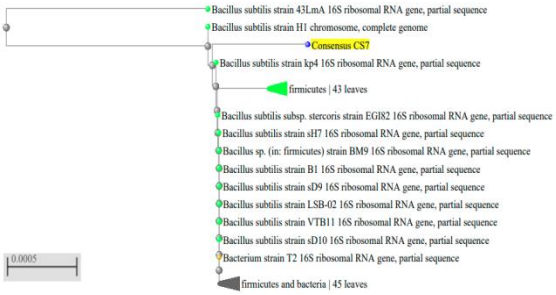

Figure 1: BLAST phylogeny tree of *Bacillus subtilis* (CS7).

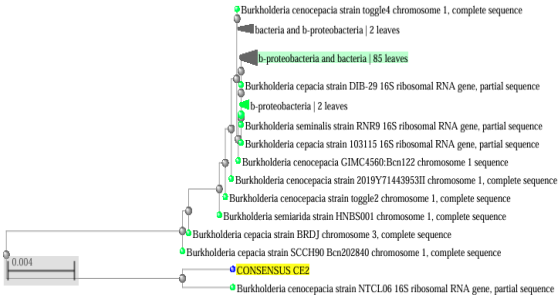

Figure 2: BLAST phylogeny tree of *Bukholderia cenocepacia* (CE2).

Surface characteristics of the isolated bacteria:
The surface properties of strains CS7 and CE2 were shown in Fig. 3 and 4. It was concluded that CS7 and CE2 were rods with a smooth surface prior to heavy metal addition (Fig. 3A & 4A). After heavy metal adsorption (Fig. 3B, 4B), strain CS7's appearance became deformed and rough. Heavy metals have been shown to elicit a variety of morphological changes in cells, which may be cell survival mechanisms or the expression of heavy metal poisoning (Wu et al., 2019b). Similar to the current experiment, Chakravarty et al. (2007) reported that strains survived from heavy metal threat were favourable from cell elongation and the reduction of cell surface volume ratio, This decreased the number of heavy metal binding sites on the cell surface. Furthermore, the morphology of strain CE2 became uneven, depressed, and wrinkled after As and Cr adsorption (Fig. 4b),

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

which may be attributed to heavy metal poisoning and was compatible with Tan et al.'s (2019) findings. Furthermore, in the presence of heavy metals, both strains CS7 and CE2 gathered and grew in clusters, which helped them resist adverse environmental impacts and bacteria synergies.

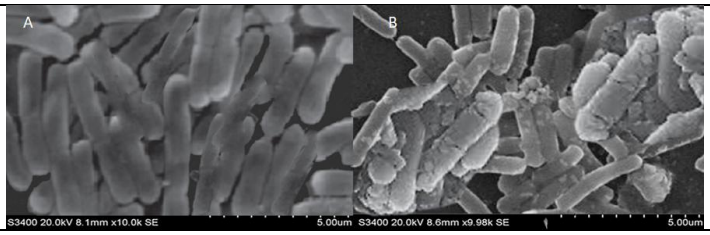

Fig. 3. SEM images of strains CS7 before and after heavy metal adsorption: A: strain in the absence of As and Cr; B: strain in the presence of As and Cr.

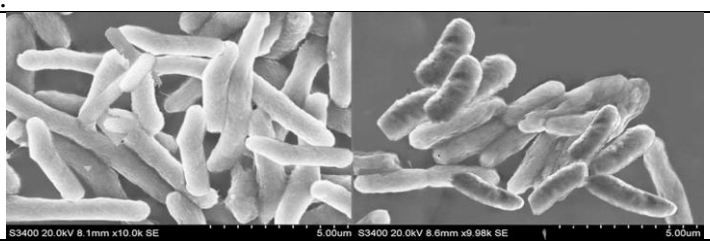

Fig. 4. SEM images of strains CE2 before and after heavy metal adsorption: A: strain in the absence of As and Cr; B: strain in the presence of As and Cr.

Functional groups analysis on immobilizing bacteria:

Figure 5 & 6 shows the energy spectrum results of strains CS7 and CE2 after As and Cr adsorption. As demonstrated in Fig. 3A & B and Fig. 4A & B, Cr and As were detected on the surfaces of both strains CS7 and CE2 after Cr and As treatment. However, Cr was not found on the surfaces of CS7 or CE2. As we know, Cr is involved in microbial metabolic processes and can influence enzyme activity, but its biological role is less defined than that of essential trace metals; some microorganisms have evolved mechanisms to convert toxic Cr(VI) to less toxic Cr(III), facilitating detoxification and survival in contaminated environments (Cervantes et al., 2001; Thatoi et al., 2014). Thus, Cr may have entered the internals of strains and been used. The Cr an As content of strains was evaluated by ICP-MS and shown in Fig. 7 & 8, which revealed that varied signal changes were achieved with the addition of different quantities of the strain digestion solutions and confirmed that Cr and As were absorbed into both strains CS7 and CE2.

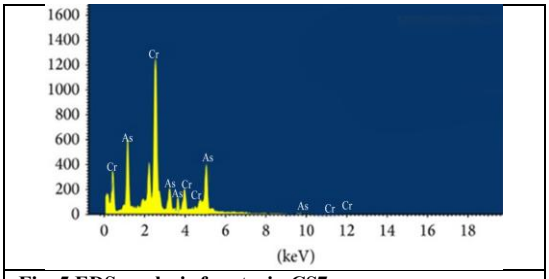

Fig. 5 EDS analysis for strain CS7

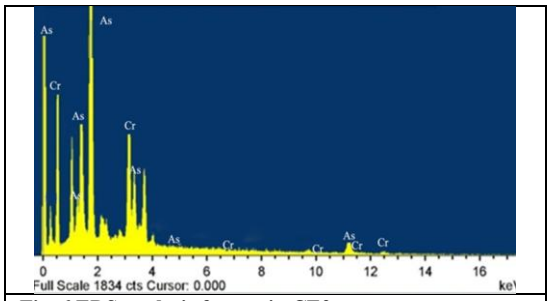

Fig. 6 EDS analysis for strain CE2

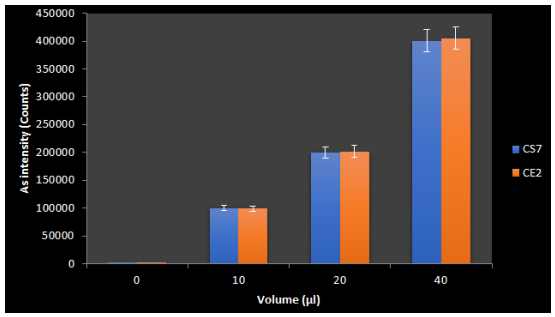

Fig. 7 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis of strains CS7 and CE2 after As adsorption.

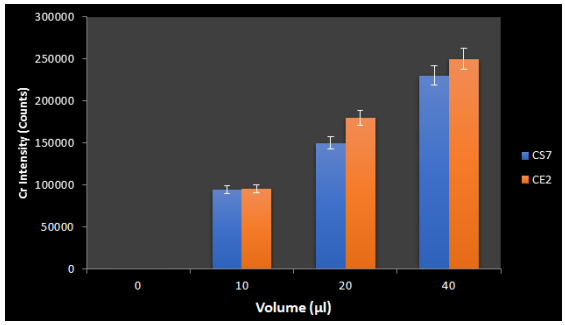

Fig. 8 Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis of strains CS7 and CE2 after Cr adsorption.

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

Alteration of HOAc-extractable heavy metal contents in soil:

Figure 9 depicts the dynamic content variations of soil HOAc-extractable As and Cr following immobilising bacterial inoculation for 60 days. Bacterial inoculation greatly reduced the concentration of HOAc-extractable heavy metals. After 30 days of bacterial inoculation, the contents of HOAc-extractable heavy metals remained stable in T1, T2, and T3. However, after reinoculation with bacteria (T4, T5, and T6), the levels of HOAc-extractable As and Cr continued to decline. At 60 days, the As and Cr contents in T1 - T6 dropped by 10.21-19.13% and 16.11-23.14%, respectively, compared to the control (no bacteria inoculation). This finding was consistent with the primary isolation experiment in LB liquid culture medium, which showed that the isolated isolates fared better on As immobilisation than Cr. Furthermore, it was observed that the contents of HOAc-extractable As and Cr fluctuated in the control treatment without bacterial inoculation, which could be attributed to the activity of indigenous microorganisms. Furthermore, re-inoculation of bacteria resulted in 52.17-88.14% and 13.17-42.36% increased immobilisation rates of As and Cr, indicating that re-inoculation was beneficial to the remediation process. Furthermore, compared to T1 and T2 treatments, the combined application of strains CS7 and CE2 individually resulted in 56.42% and 74.12% higher immobilising rates on As, respectively, which was consistent with previous research demonstrating that the combined application of microbes could promote microbes on contaminant tolerance and remediation (Gullotto et al., 2014). In particular, the simultaneous application of two fungus was found to produce superior remediation results on As and endosulfan polluted soil due to mutually beneficial effects on fungi development and toxin tolerance (Wang et al., 2017b).

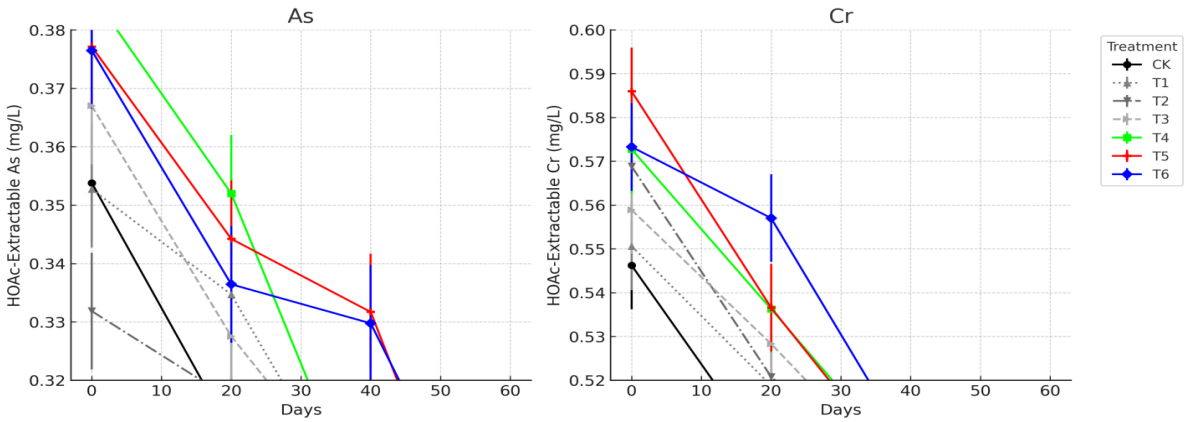

Fig. 9 The contents of heavy metals in soil with different bacteria inoculations.

Modification of enzyme activities in soil:

As demonstrated in Figure 6, the inoculation of bacteria resulted in a significant increase in soil enzymes after 30 days. After 60 days of strain inoculation, acid phosphatase activity increased by 15.09 – 30.11%, while FDA hydrolysis, urease, invertase, and dehydrogenase activities increased significantly ($p < 0.05$) by 7.65 – 31.08%, 55.32 – 131.53%, 68.11 – 212.52%, and 8.23 – 192.35%, respectively, indicating a decrease in heavy metal availability in the soil (Fig. 5). Wang et al. found that immobilising heavy metals could boost the activities of acid phosphatase and urease in soil. In the current study, the increase of soil enzyme activities could be explained by the following aspects: (1) the massive microorganisms in the added materials that had the capacity to stimulate the activity of soil enzymes; (2) the functional groups on the inoculated bacteria, such as carboxyl, phenolic, alcohol, and carbonyl, could react with heavy metal ions in soil by forming metal complexes to reduce the toxicity of heavy metals and improve the microbial activity (Wang et al., 2017b).

Soil enzyme activity was regulated by microbial activity, plant root system secretion, and the degradation of plant and soil fauna leftovers (Suneetha and Khan, 2010). In this experiment, no exogenous nutrients were supplied to the soil, therefore the amount of nutrients in the soil was limited, affecting the typical activities of heterotrophic bacteria. As indicated in Fig. 10, enzyme activities reduced after 60 days. According to Li et al. (2016), bacterial inoculation significantly increased the activities of FDA hydrolysis, acid phosphatase, and dehydrogenase in As and Cr co-contaminated soil. However, similar to our investigation, these enzyme activity showed a decrease in the final. Wang et al. (2016) found that the activity of ligninolytic enzymes in soil reduced with time, which was due to poor colonisation of injected bacteria, nutrient fatigue, and competition with indigenous microbes.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

Furthermore, many proteins released by bacteria could serve as substrates for soil enzymes (Rajkumar et al., 2012). As a result, it was remarkable that soil enzyme activities recovered significantly after 15 days of bacterial re-inoculation.

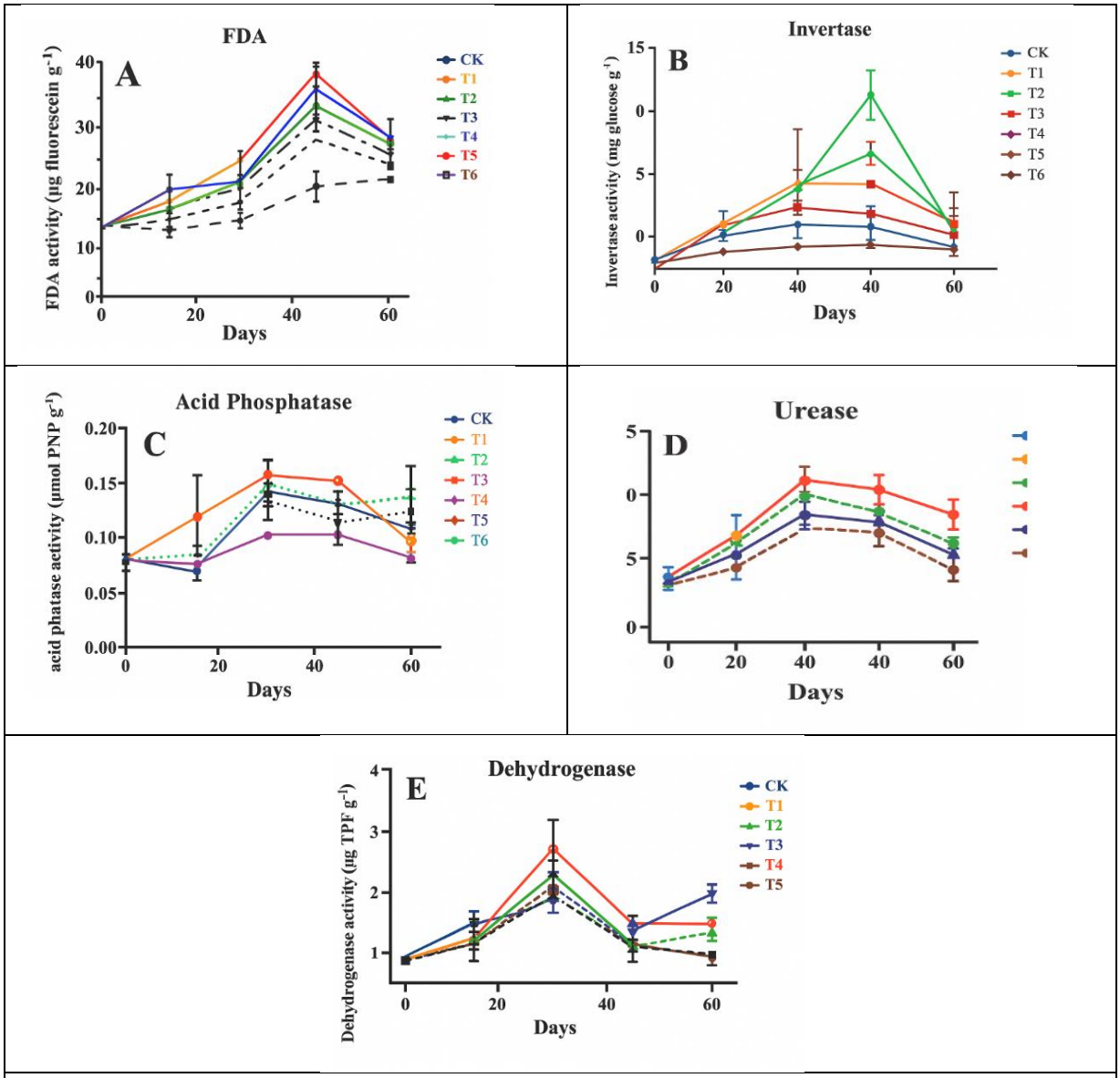

Fig. 10 The activities of soil enzymes with different bacteria inoculations (A: FDA; B: Invertase; C: Acid phosphatase; D: Urease; E: Dehydrogenase).

4. CONCLUSION

The remediation of arsenic and chromium co-contaminated soils presents a complex challenge due to the chemical and physical interactions between these two metals and the surrounding environment. Traditional remediation methods often fail to provide long-term, sustainable solutions. In contrast, the use of heavy metal immobilizing bacteria offers a promising alternative, leveraging natural processes to reduce the bioavailability and toxicity of these metals in contaminated soils. The effectiveness of this bioremediation approach, through mechanisms such as biosorption, bioprecipitation, bioaccumulation, and redox reactions, highlights the potential of using bacteria for the cleanup of toxic metal-contaminated environments. Future research focused on optimizing bacterial strains and understanding the interactions between bacteria and heavy metals will be crucial for enhancing the efficiency and applicability of this eco-friendly technology.

5. REFERENCE:

1. Alsamhary, K.E. (2025). Optimizing the process conditions for the biosorption of chromium (VI) by Bacillus subtilis in artificial wastewater, Electronic Journal of Biotechnology, Volume 76, 2025,Pages 22-38.
2. Chakravarty, R., Banerjee, D. K., & Lahiri, S. (2007). Morphological changes in an *Acidocella* strain in response

to heavy metal stress. *Research Journal of Microbiology*, *2*(9), 742–748.
3. Cervantes, C., Campos-García, J., Devars, S., Gutiérrez-Corona, F., Loza-Tavera, H., Torres-Guzmán, J.C., & Moreno-Sánchez, R. (2001). Interactions of chromium with microorganisms and plants. *FEMS Microbiology Reviews*, 25(3), 335–347. https://doi.org/10.1016/S0168-6445(01)00057-2
4. Gullotto, A., Fiorentino, A., Ambrosino, P., Faraco, V., & Rao, M. A. (2014). Biodegradation of naphthalenesulphonate polymers: The potential of a combined application of fungi and bacteria. *Environmental Technology*, *36*(5), 538–545. https://doi.org/10.1080/09593330.2014.954593
5. Gao, Z., Zhang, Z., & Hu, Y. (2021). Bioremediation of heavy metal contaminated soils: Mechanisms, methods, and applications. *Environmental Science and Pollution Research*, 28(15), 19062–19073.
6. Gupta, A., & Joia, J. (2016). Microbial extracellular polymeric substances: Functionality and their potential environmental applications. *Indian Journal of Microbiology*, 56(1), 1–10. https://doi.org/10.1007/s12088-015-0560-6
7. Ge, Z., Pei, H., Wang, L. Song, S., Fan, C. (2011) Electrochemical single nucleotide polymorphisms genotyping on surface immobilized three-dimensional branched DNA nanostructure. Sci. China Chem. 54, 1273–1276 (2011). https://doi.org/10.1007/s11426-011-4327-6
8. Gadd, G. M. (2010). Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology*, 156(3), 609–643. https://doi.org/10.1099/mic.0.037143-0
9. Jiang, J., Wang, Q., Wang, H., Wang, Y., Wang, Q., & Zhang, Z. (2015). Combined remediation of Cd–phenanthrene co-contaminated soil by *Pleurotus cornucopiae* and *Bacillus thuringiensis* FQ1 and the antioxidant responses in *Pleurotus cornucopiae*. *Ecotoxicology and Environmental Safety*, *120*, 386–393. https://doi.org/10.1016/j.ecoenv.2015.06.034
10. Jiang, X.T., Peng, X., Deng, G.H., Sheng, H.F., Wang, Y., Zhou, H.W., Tam, N.F. (2013) Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. Microb Ecol. 2013 Jul;66(1):96-104. doi: 10.1007/s00248-013-0238-8. Epub 2013 May 7. PMID: 23649297.
11. Jiang, C. X., Sheng, X. F., Qian, M., & Wang, Q. (2009). Immobilization of cadmium in soils by UV-mutated *Bacillus subtilis* 38 bioaugmentation and NovoGro amendment. *Journal of Hazardous Materials*, *167*(1–3), 1170–1177. https://doi.org/10.1016/j.jhazmat.2009.01.137
12. Kumar, S., Stecher, G., Tamura, K. (2016). Mega7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.
13. Liu, Y., Yang, L., & Li, Y. (2020). Bioremediation of arsenic and chromium contaminated soils: Current advances and future perspectives. *Frontiers in Microbiology*, 11, 500.
14. Lu, Y., Zhu, F., Chen, J. Gan, H., Guo, Y. (2007). Chemical fractionation of heavy metals in urban soils of Guangzhou, China. Environ Monit Assess 134, 429–439 (2007). https://doi.org/10.1007/s10661-007-9634-1
15. Manasi, R., Tripathi, A., & Srivastava, S. (2016). Evaluation of the genetic basis of heavy metal resistance in an isolate from electronic industry effluent. *Journal of Genetic Engineering and Biotechnology*, *14*(1), 177–180. https://doi.org/10.1016/j.jgeb.2016.07.002
16. Ma, L., Peng, Y., Wu, B., Lei, D., Xu, H. (2013). Pleurotus ostreatus nanoparticles as a new nano-biosorbent for removal of Mn(II) from aqueous solution, Chemical Engineering Journal, Volume 225,Pages 59-67.
17. Mori, H., Maruyama, F., Kato, H., Toyoda, A., Dozono, A., Ohtsubo, Y., Nagata, Y., Fujiyama, A., Tsuda, M., Kurokawa, K. (2014) Design and experimental application of a novel non-degenerate universal primer set that amplifies prokaryotic 16S rRNA genes with a low possibility to amplify eukaryotic rRNA genes. DNA Res. 2014;21(2):217-27. doi: 10.1093/dnares/dst052. Epub 2013 Nov 25. PMID: 24277737; PMCID: PMC3989492.
18. Netherlands, S., 2008. LB (Luria-Bertani) Bacterial Medium. Encyclopedia of Genetics Genomics Proteomics & Informatics 1087-1087.
19. Prado, A. M., Araujo, J. M., & Castro, P. M. L. (2005). Biosorption of copper by *Paenibacillus polymyxa* cells and their exopolysaccharide. *World Journal of Microbiology and Biotechnology*, *21*(7), 1157–1163.
20. Pueyo, M., Sastre, J., Hernández, E., Vidal, M., López-Sánchez, J.F., Rauret, G. (2003). Prediction of trace element mobility in contaminated soils by sequential extraction. J Environ Qual. 2003 Nov-Dec;32(6):2054-66. doi: 10.2134/jeq2003.2054. PMID: 14674527.
21. Rahman, M. M., & Akter, S. (2019). Arsenic contamination in groundwater and soil: Causes, effects, and remediation strategies. *Environmental Pollution*, 250, 705–718.
22. Rajkumar, M., Ae, N., & Freitas, H. (2012). Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnology Advances*, *30*(6), 1562–1574.
23. Rau, N., Sharma, A., & Singh, N. (2009). Evaluation of functional diversity in rhizobacterial taxa of a wild grass (*Saccharum ravennae*) colonizing abandoned fly ash dumps in Delhi urban ecosystem. *Soil Biology and Biochemistry*, *41*(4), 813–821. https://doi.org/10.1016/j.soilbio.2008.12.020
24. Sohail, M., Waqas, M., & Zhang, Y. (2021). Heavy metal contaminated soil: Environmental impacts and remediation strategies. *Environmental Toxicology and Pharmacology*, 77, 103400.
25. Singh, R., Paul, D., & Jain, R. K. (2006). Biofilms: Implications in bioremediation. *Trends in Microbiology*, 14(9), 389–397. https://doi.org/10.1016/j.tim.2006.07.001
26. Suneetha, V., Khan, Z.A., 2010. Actinomycetes: Sources for Soil Enzymes. Springer Nature
27. Saha, S., Roy, D., & Pal, A. (2019). Bioremediation of arsenic-contaminated soil: Role of microorganisms and their mechanisms. *Environmental Science and Pollution Research*, 26(11), 10979–10992.
28. Sungur, A., Soylak, M., Yilmaz, E., Yilmaz, S. and Ozcan, H. (2015) Characterization of Heavy Metal Fractions in Agricultural Soils by Sequential Extraction Procedure: The Relationship between Soil Properties and Heavy Metal Fractions. Soil and Sediment Contamination: An International Journal, 24, 1-15. https://doi.org/10.1080/15320383.2014.907238
29. Thatoi, H., Das, S., Mishra, J., Rath, B.P., & Das, N. (2014). **Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: A review.** *Annals of Microbiology*, 66(2), 1–19. https://doi.org/10.1007/s13213-014-1042-7
30. Tan, H., Wang, C., Zeng, G., Luo, Y., Li, H., Xu, H. (2020) Bioreduction and biosorption of Cr(VI) by a novel Bacillus sp. CRB-B1 strain, Journal of Hazardous Materials, Volume 386, 2020, 121628,
31. Vaishnav, V.K., Chatterjee, C., Agrawal, M. (2024). Ex-situ Bioremediation of Heavy Metal-Contaminated Soil Using Bacillus cereus and Micrococcus luteus Consortium: A Phytotoxicity Assessment. International Journal of Plant and Environment. 10(4), 122-130
32. Vardhan, S., Kumar, A., & Singh, R. (2018). Role of microbial communities in the bioremediation of chromium and arsenic contaminated soils. *Chemosphere*, 194, 474–485.
33. Valls, M., & De Lorenzo, V. (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiology Reviews*, 26(4), 327–338. https://doi.org/10.1111/j.1574-6976.2002.tb00618.x
34. Vaishnav, V. ., Chatterjee, T., & Agrawal, M. (2025). Evaluating the bioremediation potential of Bacillus cereus

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1301-1307
DoI-10.004687/1000-9035.2025.174

and micrococcus iuteus consortium in soil polluted by heavy metal. Asia-Pacific Journal of Science and Technology, 30(04), APST–30. https://doi.org/10.14456/apst.2025.50

35. Wu, M., Li, W., Zheng, R., Zhang, Y., Lin, M., & Zhang, C. (2019). Bioreduction of hexavalent chromium using a novel strain CRB-7 immobilized on multiple materials. *Journal of Hazardous Materials*, *368*, 412–420. https://doi.org/10.1016/j.jhazmat.2019.01.078
36. Wang, Y., Xu, Y., Wang, S., Liu, S., & Zhao, Y. (2017). Combined bioremediation of soil co-contaminated with cadmium and endosulfan by *Pleurotus eryngii* and *Coprinus comatus*. *Journal of Soils and Sediments*, *18*(6), 2136–2147. https://doi.org/10.1007/s11368-017-1692-7
37. Wang, C., Wang, Y., Li, J., Ren, N., Guo, C., Yu, Y., & Guo, W. (2016). Application of spent mushroom (Lentinula edodes) substrate and acclimated sewage sludge on the bioremediation of polycyclic aromatic hydrocarbon polluted soil. RSC Advances, 6(41), 37274–37285. https://doi.org/10.1039/C6RA01185E
38. Wang, Y., Luo, Y., Zeng, G., Wu, X., Wu, B., Li, X., Xu, H. (2020). Characteristics and in situ remediation effects of heavy metal immobilizing bacteria on cadmium and nickel co-contaminated soil, Ecotoxicology and Environmental Safety, Volume 192, 2020,110294,
39. Xu, N., Tan, G., Wang, H., Gai, X. (2016).Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure, European Journal of Soil Biology, Volume 74, 2016, Pages 1-8,
40. Zhou, X., Zhang, C., Wang, Y., Sun, L., & Liu, W. (2020). Bioremediation of heavy metal contaminated soil by using bacterial consortia: Mechanisms and practical applications. *Journal of Hazardous Materials*, 399, 123020. https://doi.org/10.1016/j.jhazmat.2020.123020