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Bioremediation of Lead and Cadmium from Liquid Media by Metal-Tolerant *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens NO*10

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Abstract

This study aimed to evaluate the bioremediation potential of two highly metal-tolerant bacterial isolates, SS1 and SS2, identified as Bacillus cereus MS54 and Bacillus amyloliquefaciens NO10, respectively, for the removal of lead (Pb^{2+}) and cadmium (Cd^{2+}) from contaminated environments. The isolates were identified using 16S rRNA gene sequencing and selected for their high resilience under heavy metal-contaminated conditions. Their ability to adsorb Pb^{2+} and Cd^{2+} ions from nutrient broth medium was assessed at varying concentrations (Pb^{2+}: 500, 2000, 6000 µg·ml^{-1}; Cd^{2+}: 25, 250, 500 µg·ml^{-1}) over different time intervals (24, 72, and 120 hours).

Bacillus cereus MS54 demonstrated high Pb^{2+} removal efficiency, achieving 35.43% removal at 24 hours and increasing to 98.65% after 120 hours at the lowest concentration (500 µg·ml^{-1}). At higher concentrations (2000 and 6000 µg·ml^{-1}), Pb^{2+} uptake continued but showed a slight reduction in efficiency, likely due to saturation effects. Conversely, *Bacillus amyloliquefaciens NO*10 exhibited strong Cd^{2+} removal, achieving 38.94% removal at 24 hours and reaching 96.90% at 120 hours at the lowest concentration (25 µg·ml^{-1}). Higher concentrations (250 and 500 µg·ml^{-1}) consistently increased metal uptake.

These findings highlight that both bacterial strains effectively removed their respective metals, with *B. cereus* MS54 excelling in Pb^{2+} and *B. amyloliquefaciens NO*10 in Cd^{2+} remediation. The mechanisms of metal uptake involve passive biosorption, active transport, biosurfactant production, and intracellular sequestration. This study demonstrates the potential of these strains as bioremediation agents for heavy metal contamination in both aqueous and soil environments.

Key words:

Bacillus cereus, *Bacillus amyloliquefaciens*, heavy metals, lead, cadmium, bioremediation.

Introduction

Pollution represents one of the most critical environmental challenges, posing a significant threat to human health and the stability of global ecosystems. Recently, heavy metal contamination has intensified markedly due to anthropogenic activities such as intensive agriculture, mining, and various industrial operations (1). Elevated concentrations of these toxic elements, which exceed permissible thresholds, can severely disrupt soil microbial communities and impair essential biochemical processes. Moreover, once introduced into the soil, heavy metals become persistent and non-biodegradable, resulting in long-term immobilization and detrimental ecological consequences (2).

Microbial removal (bioremediation) of heavy metals from the environment is the most cost-effective

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approach in the mitigation of elemental pollution. In connection with this study, Sharma and Shukla (2021) **(3)** stated that Bioremediation is an attractive and successful cleaning technique to remove toxic waste from a polluted environment. In support of this, Ali and Rawia (2024) **(4)** described bioremediation as a structured microbiological intervention capable of degrading or transforming hazardous contaminants into less toxic or non-toxic forms. Therefore, microorganisms serve as central agents in the restoration of polluted environments. Bacteria are particularly notable for their capacity to metabolize, detoxify, and accumulate heavy metals, frequently localizing them within the cell wall, like nutrient uptake.

From another point of view, microorganisms inhabiting metal-contaminated environments have evolved many adaptive mechanisms to tolerate and resist heavy metal stress. Among the most effective and economical strategies for mitigating heavy metal pollution is microbial bioremediation, particularly through processes such as bioaccumulation **(5)**. This involves the uptake and sequestration of metal ions both inside and on the surface of microbial cells, often through complexation and biosorption. The ability of microbial populations to develop resistance mechanisms makes them valuable tools for environmental detoxification.

Accordingly, the present study aims to identify two highly efficient heavy metal-tolerant bacterial strains, designated SS1 and SS2. The bioremediation efficiency of these isolates will be evaluated for the removal of Pb^{2+} and Cd^{2+} from the nutrient broth. This approach is designed to assess the bioremediation potential of the selected isolates under controlled laboratory conditions.

Materials and methods:

1. Heavy metals:

Two heavy metals, Pb^{2+} and Cd^{2+}, were used throughout the work as $Pb(CHCOOH)_2.3H_2O$ and $CdCl_2.H_2O$, respectively. Standard stock solutions of each metal salt were prepared and used to supplement the culture medium to attain the desired metal-ion concentrations.

2. Chemical analysis:

The total and available concentrations of Pb^{2+} and Cd^{2+} in soil and culture media were determined using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES), following the standard protocols of the Central Laboratory for Analysis of Pesticide Residues and Heavy Metals in Food (QCAP), affiliated with the Agricultural Research Center, Egyptian Ministry of Agriculture

3. Experiments:

3.1. Identification of the Two Highly Efficient Metal-Tolerant Bacterial Isolates SS1 and SS2:

This experiment aimed to identify two Gram-positive, spore-forming bacterial isolates, SS1 and SS2, previously obtained by Azhar and Mohamed (2023) **(6).** Molecular identification was conducted using 16S rRNA gene sequencing. About a 1.5 kb fragment of the 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') as described by Sambrook *et al*. (1989) **(7)**. Partial sequencing was performed at Gena Ti (King Abdulaziz University, Saudi Arabia), and the sequences were analyzed using the NCBI BLAST tool to determine similarity with other known sequences in the NCBI database **(8)**.

3.2. Efficiency of Pb^{2+} Remediation by *Bacillus cereus* and Cd^{2+} by *Bacillus amyloliquefaciens* Cultures from a Nutritive Broth Medium:

This experiment was designed to assess the efficiency of the two bacterial isolates: *Bacillus cereus* MS54 (Pb^{2+} -tolerant) and *Bacillus amyloliquefaciens NO*10 **(**Cd^{2+}-tolerant) to absorb lead and cadmium, respectively, from liquid medium. According to the study of Azhar and Mohamed (2023) **(6)**, three metal concentrations for both metals resulted in a reduction of the bacterial

count by >25%, *ca*.50%, and <90%, which were chosen for this experiment.

To evaluate bioaccumulation efficiency, six 100 ml flasks containing nutrient broth were prepared. Three flasks were amended with a known volume of lead acetate standard solution to attain final Pb^{2+} concentrations of 500, 2000, and 6000 µg.ml^{-1}, followed by inoculation with 1 mL of *Bacillus cereus* MS54 culture (23.80 × 10^7 CFU.ml^{-1}). The other three flasks were supplemented with cadmium monohydrate stock solution to achieve final Cd^{2+} concentrations of 25, 250, and 500 µg.ml^{-1} and inoculated with 1 mL of *Bacillus amyloliquefaciens* NO10 culture (26.93 × 10^7 CFU.ml^{-1}). All treatments were conducted in triplicate and incubated at 30°C for five days in a shaking incubator.

At intervals of 24, 72, and 120 hours, 20 ml samples from each treated flask were aseptically collected, centrifuged at 6000 rpm for 6 minutes, and then the residual metal concentrations in the supernatants were determined using ICP-OES. Metal uptake was calculated by subtracting the residual concentrations from the initial concentrations, and the percentage of metal uptake was subsequently calculated.

RESULTS:

1. Identification of the Two Highly Efficient Metal-Tolerant Bacterial Isolates SS1 and SS2

1.1. Amplification and partial sequencing of the 16S-rRNA gene

The 16S rRNA gene of the two selected bacterial isolates, SS1 and SS2, was amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), resulting in about 1.5 kb PCR product, as shown in Photo (1).

1.2. Sequencing of 16S-rRNA gene

Partial sequencing of the amplified fragments was performed, and the obtained sequences were analyzed using the NCBI BLAST tool to determine their closest taxonomic affiliations based on sequence similarity.

The BLAST results revealed that isolate SS1 shared 100% identity with several *Bacillus cereus* strains, including *B. cereus* strain MS54 (GenBank Accession No.: MT214299.1), as presented in Table 1 and Figure 2. Similarly, isolate SS2 exhibited 99.83% sequence identity with multiple strains of *Bacillus amyloliquefaciens*, including strain NO10 (GenBank Accession No.: MT377854.1), as detailed in Table 2 and Figure 3. These findings confirm the genotypic identification of the two isolates as *Bacillus cereus* (SS1) and *Bacillus amyloliquefaciens* (SS2). The two isolates are known for their resilience in heavy metal-contaminated environments.

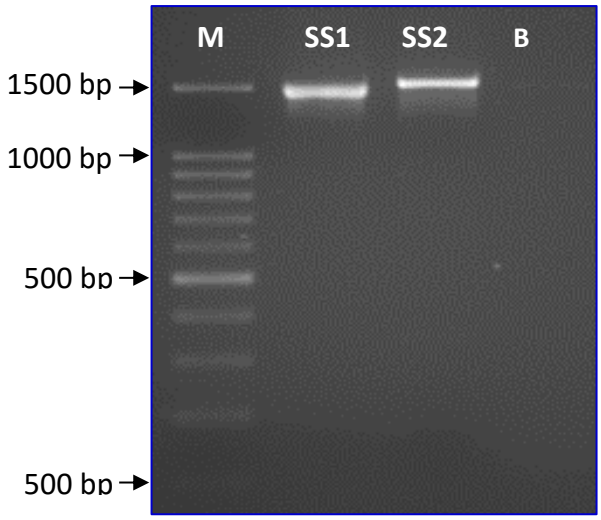

Photo (1): The agarose gel (2%) electrophoresis of 16S-rDNA PCR product of the two bacterial isolates (SS1 and SS2). Lane B: the negative control (Blank). Lane M: 100 bp DNA-Marker (Cleaver Scientific LTD, United Kingdom).

Table 1: The 16S-rRNA gene sequence alignment from the isolate SS1 (590 bp) in the NCBI GenBank database.

Description	Total score	Query Coverage	Identity %	GenBank Accession No.
Bacillus cereus strain MS54	1090	100%	100%	MT214299.1
Bacillus cereus strain NBRIVS16	1090	100%	100%	MN715826.1
Bacillus cereus strain DIF1	1090	100%	100%	MH351294.1
Bacillus cereus, isolate MB_M_20	1090	100%	100%	LT935744.1
Bacillus cereus strain FJAT-46079	1090	100%	100%	MG650865.1
Bacillus cereus strain X-7	1090	100%	100%	MF988730.1
Bacillus cereus strain BC 12A	1090	100%	100%	KX783552.1
Bacillus cereus strain PD1	1090	100%	100%	KY773584.1
Bacillus cereus strain a69	1090	100%	100%	KX057550.1
Bacillus cereus strain F5-1-35	1090	100%	100%	KX350016.1

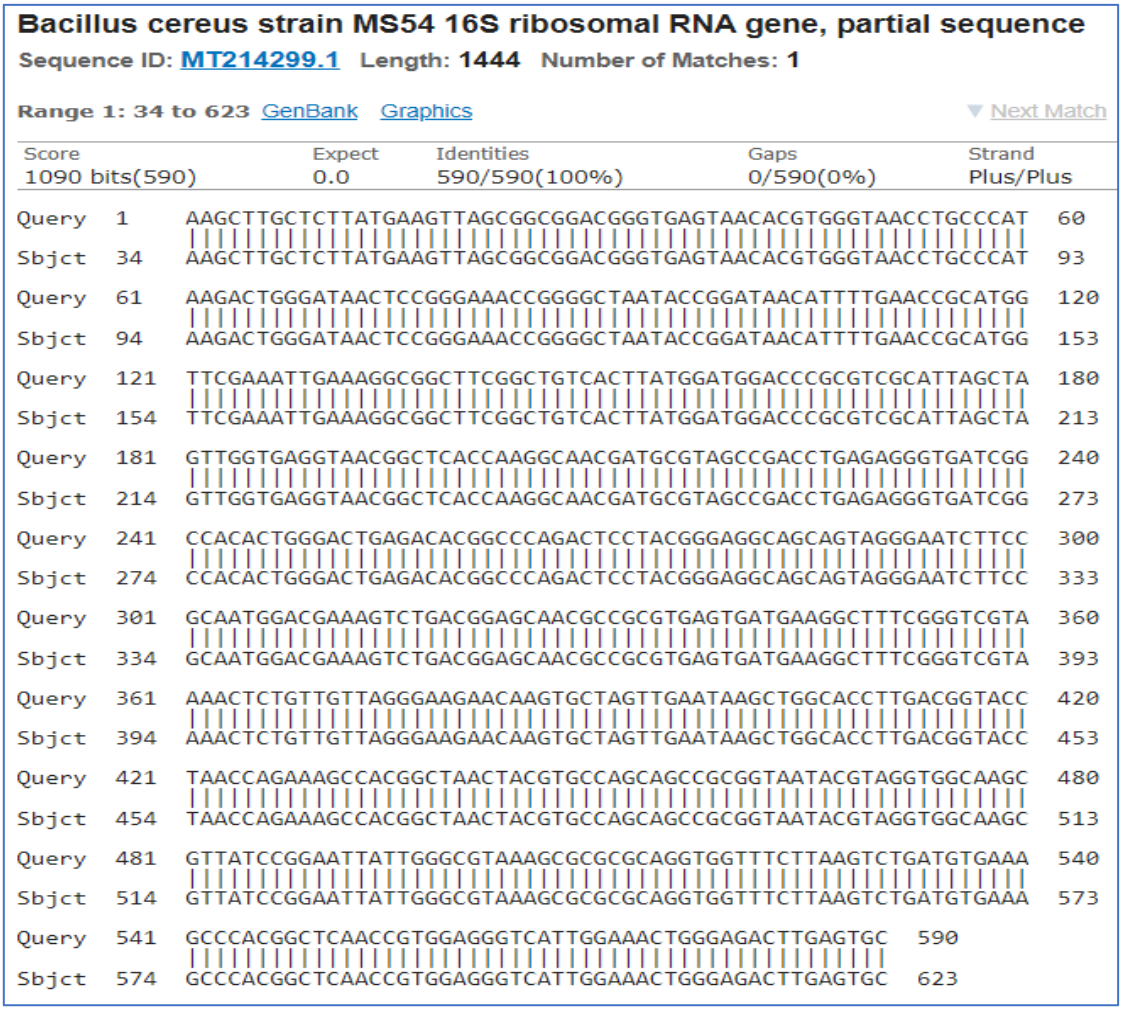

Figure (1): BLAST alignment output of the 16S rRNA sequence of isolate SS1 showing 100% identity with *Bacillus cereus* strain MS54 (GenBank Accession No: MT214299.1) and other closely related strains

Table 2: The 16S-rRNA gene sequence alignment from the isolate SS2 (600 bp) in the NCBI GenBank database.

Description	Total score	Query Coverage	Identity %	GenBank Accession No.
Bacillus amyloliquefaciens strain NO10	1103	100%	99.83%	MT377854.1
Bacillus amyloliquefaciens strain ER7	1103	100%	99.83%	MT124532.1
Bacillus amyloliquefaciens strain SRG15	1103	100%	99.83%	MK743994.1
Bacillus amyloliquefaciens strain LXZ	1103	100%	99.83%	MN759438.1
Bacillus amyloliquefaciens strain EH10	1103	100%	99.83%	MN750765.1
Bacillus amyloliquefaciens strain MPA 1034	1103	100%	99.83%	MN749804.1

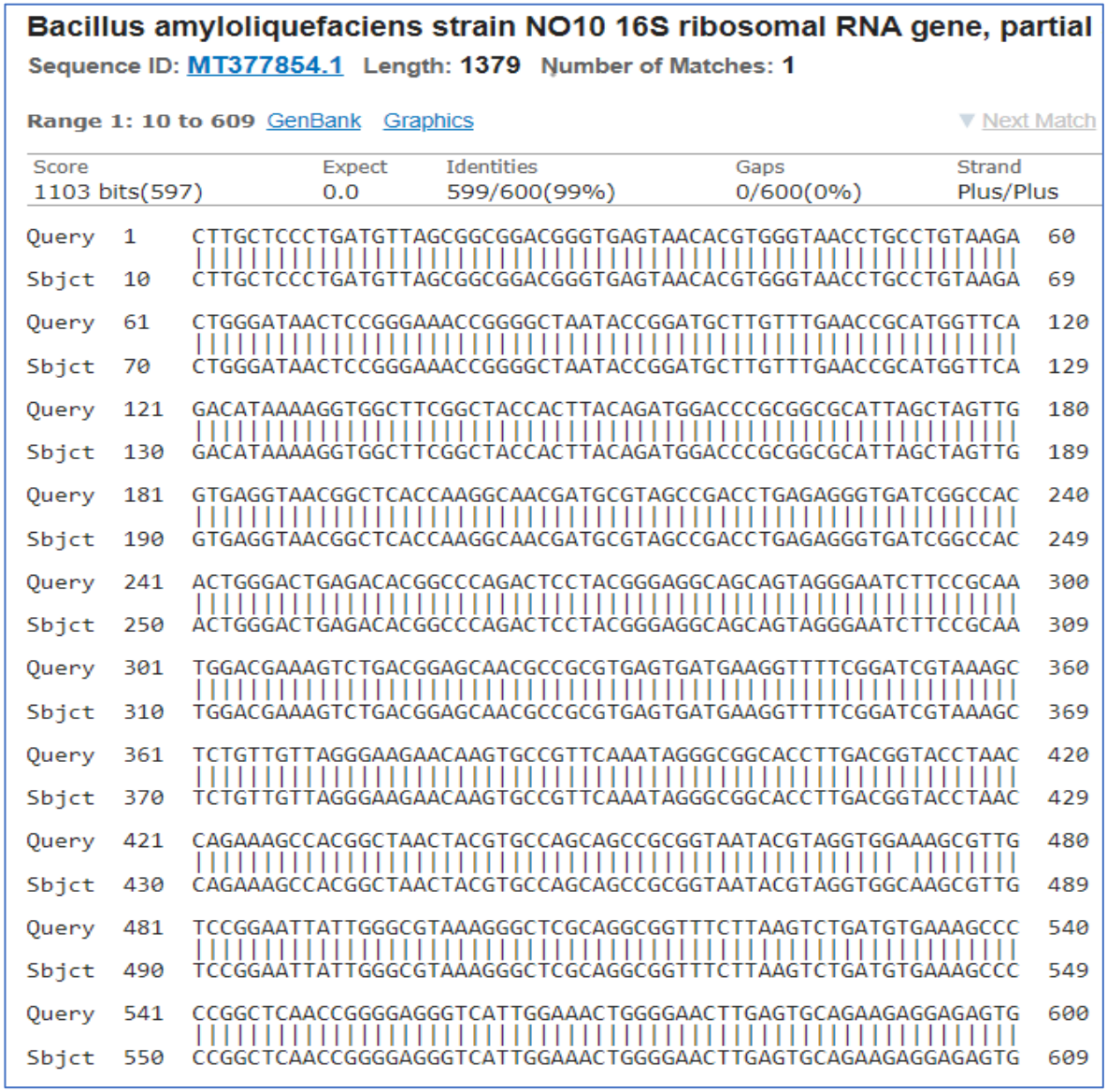

Figure (2): BLAST alignment output of the 16S rRNA sequence of isolate SS2 showing 99.83% identity with *Bacillus amyloliquefaciens* strain NO10 (GenBank Accession No: MT377854.1) and related strains.

Furthermore, given the growing importance of microbial-metal interactions in environmental remediation, the two genetically confirmed isolates, *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens NO*10, were selected for further evaluation of their ability to adsorb Pb^{2+} and Cd^{2+}, respectively. Their efficiency in metals remediation from a nutrient broth medium was assessed.

2. Efficiency of Pb^{2+} Remediation by *Bacillus cereus* and Cd^{2+} by *Bacillus amyloliquefaciens* Cultures from a Nutritive Broth Medium

The present experiment evaluated the bioremediation efficiency of *Bacillus cereus* MS54 (SS1) and *Bacillus amyloliquefaciens NO*10 (SS2) for lead (Pb^{2+}) and cadmium (Cd^{2+}), respectively, in both liquid (nutritive broth) and soil microcosm systems. The bacterial cultures were treated with three metal concentrations: 500, 2000, and 6000 $\mu g \cdot ml^{-1}$ for Pb^{2+} (T1, T2, and T3), and 25, 250, and 500 $\mu g \cdot ml^{-1}$ for Cd^{2+}. Metal uptake was measured at 24-, 72-, and 120-hours post-inoculation.

As shown in Table 3 and illustrated in Figures 3a and 3b, both bacterial strains demonstrated the ability to adsorb their respective target metals from the broth medium. The extent of metal removal depended on the metal concentration and exposure time.

For *Bacillus cereus* MS54, Pb^{2+} uptake increased over time and with higher metal concentrations. At the lowest concentration (T1), the strain removed 171.13 µg. ml^{-1} (35.43%) after 24 hours, which progressively increased to 433.00 $\mu g.ml^{-1}$ (98.65%) after 120 hours. At T2 and T3, the percentage uptake was slightly lower, with 87.94% adsorption at T3 after 120 hours, indicating saturation effects at higher concentrations.

Similarly, *Bacillus amyloliquefaciens NO*10 showed a strong capacity for Cd^{2+} remediation. In T1 (25 $\mu g.ml^{-1}$), the adsorbed Cd^{2+} increased from 38.94% at 24 hours to 96.90% at 120 hours. Even at higher concentrations (T2 and T3), a consistent upward trend in Cd^{2+} removal was observed throughout the experiment.

These results highlight significant differences in metal uptake efficiency between the two bacterial strains and suggest their selective suitability for different types of heavy metal contamination in aqueous systems. In connection with this point, and to assess the efficiency of the two isolates, *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens NO*10, in metal remediation in soil, a short-term microcosm experiment was carried out in parallel.

Table (3): Efficiency of *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens* *NO*10 in remediation of three Pb^{2+} and Cd^{2+} concentrations from the nutritive broth media.

Metal concentrations (µg.ml^{-1} medium)				Amount of Pb^{2+} and Cd^{2+} adsorbed (µg.ml^{-1}) by each bacterial culture at three time intervals					
0-time				24 hr.		72 hr.		120 hr.	
Added	Determined	Adsorbed(1)	%	Adsorbed(2	%(3)	Adsorbed(2	%(3)	Adsorbed(2	%(3)
Nutrient broth medium supplemented with three Pb^{2+} concentrations									
without bacterial inoculum				**Inoculated with 1 mL of *B. cereus* broth culture**					
500 (T1)	**483.00**	**17.00**	**3.40**	171.13	**35.43**	274.15	**56.76**	433.00	**89.65**
2000 (T2)	**1891.00**	**109.00**	**5.45**	800.10	**42.31**	1182.82	**62.55**	1711.73	**90.52**
6000 (T3)	**5730.00**	**270.00**	**4.50**	2508.02	**43.77**	4345.63	**75.84**	5038.96	**87.94**
Nutrient broth medium supplemented with three Cd^{2+} concentrations									
without bacterial inoculum				**Inoculated with 1 mL of *B. amyloliquefaciens* broth culture**					
25 (T1)	**23.88**	**1.12**	**4.48**	9.29	**38.94**	16.82	**70.45**	22.12	**92.63**
250 (T2)	**240.63**	**9.37**	**3.75**	102.79	**42.72**	164.68	**68.44**	203.95	**84.76**
500 (T3)	**476.70**	**23.30**	**4.66**	265.81	**55.76**	347.65	**72.93**	419.30	**87.96**

N.B. Metal adsorption at 0-time was recorded before inoculation by the bacterial culture.

(1): The amount of Pb^{2+} and Cd^{2+} adsorbed at 0-time (µg.ml^{-1}) was obtained by the difference (metal added at 0-time - determined at 0-time) before inoculation by the bacterial culture.

(2): Amount of Pb^{2+} and Cd^{2+} adsorbed at 24hr., 72hr. and 120-hour time intervals (µg.ml^{-1}) were obtained by the difference (determined at 0-time - determined at each time interval).

(3): Percentage of Pb^{2+} and Cd^{2+} adsorbed at 24hr., 72hr. and 120hr.-time intervals corresponding to amounts determined at 0-time

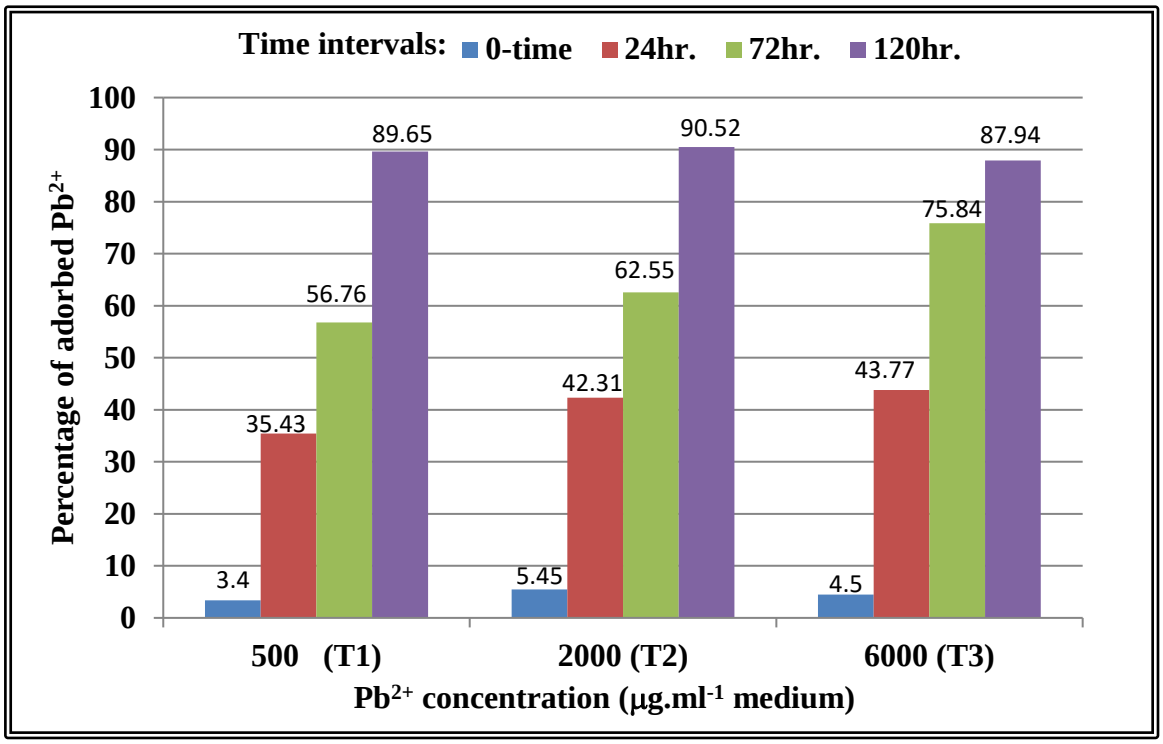

Figure (3.a): Percentage of Pb^{2+} adsorbed by *B. cereus* MS54 culture in a nutritive broth medium supplemented with three different Pb^{2+} concentrations

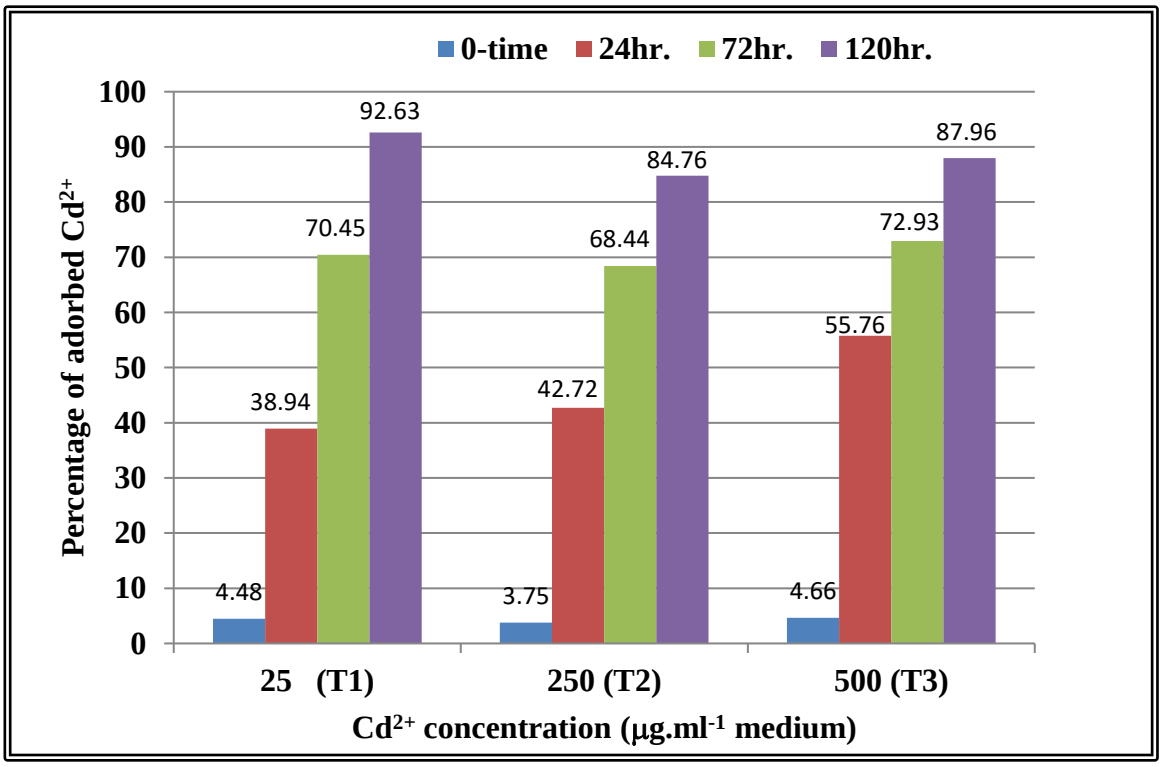

Figure (3.b): Percentage of Cd^{2+} adsorbed by *B. amyloliquefaciens NO*10 culture in a nutritive broth medium supplemented with three different Cd^{2+} concentrations.

DISCUSSION

1. Identification of the Two Highly Efficient Metal-Tolerant Bacterial Isolates SS1 and SS2

The genotypic identification of the bacterial isolates revealed that SS1 was identified as *Bacillus cereus* MS54 (100% identity), and SS2 was identified as *Bacillus amyloliquefaciens NO*10 (99.83% identity). This identification was in line with previous studies, which have demonstrated the efficacy of *Bacillus* species in metal uptake and bioremediation. For instance, Fakhar *et al*. (2020) **(9)** highlighted that *Bacillus* species possess several characteristics, including the ability to adsorb heavy metals through active sites in their cell walls, such as teichoic acids and carboxyl groups. These features play a significant role in the bioremediation process by facilitating the binding and sequestration of metal ions. Additionally, the spore-forming ability of these *Bacillus* species provides them with a unique advantage in surviving harsh environments, such as those contaminated with heavy metals. This characteristic allows the bacterial isolates to persist in polluted environments, making them promising candidates for the bioremediation of soil and water contaminated with metals **(10)**.

Given the high metal tolerance and bioremediation potential of both isolates, further investigation into their metal uptake mechanisms and their role in environmental cleanup is warranted **(11)**. The results suggest that these isolates may serve as effective tools in the remediation of contaminated sites, particularly due to their strong tolerance to Pb^{2+} and Cd^{2+} ions. Further research should focus on optimizing the conditions for their application in large-scale bioremediation projects.

2. Efficiency of Pb^{2+} Remediation by *Bacillus cereus* MS54 and Cd^{2+} by *Bacillus amyloliquefaciens NO*10 Cultures from a nutritive broth medium

Many bacterial species in environmental ecosystems exhibit the ability to accumulate significant quantities of metals within their cells. At elevated concentrations of heavy metals, bacteria can effectively adsorb metal ions due to their high surface area-to-volume ratio, facilitating both passive and active biosorption through a range of mechanisms.

This study confirmed the efficient bioremediation potential of *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens NO*10 in removing Pb^{2+} and Cd^{2+} from broth medium. Metal uptake by both strains was observed to increase with higher metal concentrations and longer exposure times, reaching its peak at 120 hours. These findings underscore the capacity of living bacterial cells to remove heavy metals through a variety of mechanisms.

For *Bacillus cereus* MS54, the removal of Pb^{2+} was particularly pronounced. Adsorption increased over time across all treatments, with the highest levels observed at lower concentrations. In the T1 (25 $\mu g.ml^{-1}$) treatment, the bacterium adsorbed 35.43% of Pb^{2+} after 24 hours and achieved 98.65% removal by 120 hours. In higher concentration treatments (T2 and T3), the initial adsorption rates were 42.31% and 43.77%, respectively, with final adsorption reaching 87.94% in T3. These results suggest that while the efficiency of adsorption was concentration-dependent, the removal percentage slightly decreased with increasing initial concentrations, likely due to site saturation or competitive binding effects. Similar observations were made by Qingrong *et al*. (2023) **(12)**, who reported a 90.8% removal of Pb^{2+} by *B. cereus* from a 100 µg/ml solution within 48 hours. The mechanism of Pb^{2+} removal by *B. cereus* likely involves multiple strategies. They also reported that the production of biosurfactants, amphiphilic compounds that can be complexed with metal ions, is a key contributor to lead solubilization and uptake. Additionally, intracellular sequestration of Pb^{2+} via metallothionein encoded by plasmid-borne genes (bmtA and smtAB) has been reported in lead-resistant strains **(3)**. This sequestration process serves as a protective mechanism against metal toxicity. Furthermore, bioaccumulation plays a critical role in Pb^{2+} removal. Fashola *et al*. (2016)

(13) proposed that Pb^{2+} can bind to the cell surface and subsequently be transported into cells, where it precipitates as crystalline Pb^{2+} phosphate, concentrating metals intracellularly and reducing their toxicity in the surrounding medium. They also reported 85% Pb^{2+} removal by a growing bacterial strain within 30 hours, emphasizing the importance of active growth in bioremediation.

In contrast, *Bacillus amyloliquefaciens NO*10 exhibited highly efficient biosorption of Cd^{2+} from the broth medium over 120 hours, with metal uptake increasing in a time- and concentration-dependent manner. At the lowest concentration (25 µg.ml^{-1}), removal reached 92.63% by 120 hours and peaked at 96.90%. Higher concentrations further enhanced metal uptake, demonstrating the strain's tolerance to and adaptive response against Cd^{2+} stress. These results are consistent with previous studies; for example, Qingrong *et al*. (2023) **(12)** showed that *Bacillus licheniformis NO*10 removed more than 98% of Cd^{2+} from solution, highlighting the potential of Gram-positive spore-forming bacteria as effective biosorbents. They also noted that bacterial spores play a significant role in metal biosorption due to their multilayered structure and surface chemistry, which enable strong and sometimes irreversible binding of metals. Several studies support the capacity of *Bacillus amyloliquefaciens* to remediate cadmium. Devanesan and Al-Salhi (2021) **(14)** reported that *B. amyloliquefaciens* HM28 achieved a maximum Cd^{2+} biosorption of 98.4% at a concentration of 100 µg/ml, consistent with the high removal efficiency observed in the present study. One possible explanation for the high biosorption capacity of this strain is the production of siderophores—low-molecular-weight compounds that can chelate metal ions like cadmium, enhancing their solubility and uptake. According to Roskova *et al*. (2022) **(15)**, siderophores contribute significantly to microbial metal remediation by improving metal availability for cellular absorption or immobilization. This mechanism likely contributed to the high uptake observed in this study. Moreover, the gradual increase in Cd^{2+} biosorption over time suggests the involvement of multiple mechanisms. Initially, passive adsorption to the cell wall likely dominates, involving functional groups such as carboxyl, hydroxyl, and phosphate. Over-extended exposure, however, active transport systems and intracellular sequestration are likely to enhance the total metal uptake, especially at higher concentrations.

Conclusion

Summing up, this study confirmed the high bioremediation efficiency of *Bacillus cereus* MS54 and *Bacillus amyloliquefaciens* NO10 in removing Pb^{2+} and Cd^{2+}, respectively, across varying concentrations and incubation times in the aqueous medium. Both strains demonstrated strong metal uptake over time, supporting their potential use as eco-friendly and cost-effective agents for *in situ* remediation of heavy metal-contaminated soils, especially in agricultural and industrial sites. Future research should assess their performance in real soil systems, including field trials, and explore synergistic effects with soil amendments or plant-microbe interactions. Environmentally, these microbial solutions offer a sustainable alternative to conventional chemical treatments, reducing ecological harm. Further studies into their genetic and metabolic traits could pave the way for enhanced microbial consortia or engineered strains, enabling broader application in environmental restoration and industrial bioremediation efforts.

Conflict of interest: NIL

Funding: NIL

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