Saudi Journal of Biological Sciences 30 (2023) 103740

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Saudi Journal of Biological Sciences

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Original article

Uptake of lead by bacteria isolated from industrial effluents and their potential use in bioremediation of wastewater

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ARTICLE INFO

Article history:
Received 31 May 2023
Revised 4 July 2023
Accepted 8 July 2023
Available online 13 July 2023

Keywords:
Heavy metals pollution
Pb-contamination
Bacillus cereus ID1
Bacillus sp. ID3
Bioremediation

ABSTRACT

Due to rising populations and human activities, heavy metals (HM) toxicity has become a serious problem for all life forms. The present study deals with isolating and identifying lead-resistant bacteria from contaminated wastewater of tanneries effluents. Two isolated strains were identified as *Bacillus cereus* (ID1), and *Bacillus* sp. (ID3), and both strains resisted a 25 mM concentration of Lead nitrate ($Pb(NO_3)_2$). After four days of treatment, *Bacillus cereus* (ID1) showed 80% lead uptake, and *Bacillus* sp. (ID3) showed 88%. Lead uptake was confirmed by Energy dispersive X-Ray (EDX) analysis. Fourier transform infrared spectroscopy (FTIR) showed that structural alterations had occurred in functional groups of the treated samples compared to the controls. Our research indicates that these *Bacillus* strains may be useful in bioremediating heavy metals from polluted environments. Further investigation into the processes involved in the uptake and homeostasis of heavy metals by these strains is required, as is the identification of the genes and enzymes responsible for Pb-bioremediation.

1. Introduction

Heavy metals are defined as elements with atomic numbers higher than 20 and atomic densities larger than 5 g/cm^3 (Zhong et al., 2022). They can be categorized as essential or non-essential for living things. For basic life activities like growth, metabolism, and organ development, several metals like cobalt, chromium, manganese, copper, iron, molybdenum, nickel, selenium, magnesium, and zinc are necessary (Tchounwou et al., 2012). Contrarily, non-essential heavy metals such as lead, cadmium, mercury, arsenic, and aluminum have no recognized biological purpose (Tchounwou et al., 2012).

One of the first metals to be found by mankind, lead (Pb), is a common substance (Ruckart et al., 2021). According to the United States, the safe blood lead levels for adults and children are 10 g/dL and 5 g/dL, respectively (Ruckart et al., 2021). Millions of fatalities worldwide have been attributed to excessive lead exposure, according to a 2022 WHO assessment (WHO, 2022). According to studies by Rees and Fuller (2020), lead exposure causes 4.6 percent of cardiovascular disease and 3 percent of chronic kidney disease, respectively, and accounts for 30 percent of the global burden of idiopathic intellectual disability.

Lead exposure negatively impacts human health, especially on the hematological, renal, and central neurological systems, which can cause serious issues (Kalia and Flora, 2005). According to Selvi et al. (2019), anthropogenic causes are to blame for lead's prevalence in aquatic and terrestrial ecosystems. The environment is harmed by the release of heavy metals, particularly lead, from refinery industries and vehicles (Yusuf et al., 2022). According to estimates, lead contributes around 10% of all environmental heavy metal contamination (Tchounwou et al., 2012). Upon encountering air, it starts to tarnish and, depending on the circumstances, forms a complicated variety of chemicals. To avoid such toxic chemicals, there is a need to reduce lead pollution, especially from water.

Compared to traditional techniques for removing metals from aqueous solutions, bio-sorption, a microbial treatment procedure,

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103740
1319-562X/

offers advantages. Conventional methods of treating low concentrations of metal-containing industrial effluents, such as chemical oxidation/reduction, precipitation, and ion exchange, have several drawbacks, including inefficiency, high prices, energy consumption, secondary waste creation, and impracticality (Aryal and Liakopoulou-Kyriakides, 2015). In contrast, bio-sorption has several benefits. It has great metal binding efficiency, and high selectivity for specific metals, especially lead, is simple to use, economical, and does not cause chemical sludges. The possibility of microbial therapy for metal removal has been noted in prior studies, highlighting its applicability to environmental remediation goals. Bio-sorption provides promise for tackling metal pollution issues in an effective and environmentally friendly way by utilizing the special skills of microorganisms (Javanbakht et al., 2014; Murthy et al., 2012).

Bacteria metabolize chemicals in a variety of ways, including bioaccumulation and reduction. Some naturally occurring microorganisms in metal-contaminated settings have adapted, grown, and function normally despite developing metal resistance. The current study aims to extract and characterize lead-reducing bacteria from tanneries to eliminate hazardous metal ions from the environment.

2. Material and methods

2.1. Sample collection

Wastewater samples were collected from the industrial effluents of Quaid-e-Azam Industrial Estate in Kot Lakhpat-Lahore, Pakistan, using sterilized screw bottles. The collection of water samples was carried out randomly, considering the type of industry. While collecting samples, we recorded several parameters, such as the pH of the wastewater, temperature, water color, and the site's location (Table S1).

2.2. Minimal salt medium

Minimal salt medium was prepared by adding 4.54 g KH_2PO_4, 0.50 g $MgSO_4$, 11.94 g of $Na_2HPO_4{\cdot}12H_2O$, 0.002 g $ZnSO_4$, 0.005 g $CaCl_2$, 0.001 g $MnSO_4$, 0.002 g $FeSO_4$, and 1.00 g NH_4Cl in 1 L of distilled water. 10 g glucose was also added to the minimal salt medium. A computerized measuring balance was used to weigh all the components correctly. The chemical was dissolved by vigorously shaking and heating in the oven (Ali et al., 2023).

2.3. Minimum inhibitory concentration

Bacteria were exposed to $Pb(NO_3)_2$ for metal stress in order to identify their minimal inhibitory concentration (MIC). At first, autoclaved LB agar medium was mixed with 5 mM lead nitrate. After that, the media was placed into sterilized Petri plates in a laminar flow and allowed to harden. Then, 25 µl of the bacterial culture was spread using a 0.9% saline solution on the LB agar plates that had been subjected to stress. The plates were incubated for 24 h at 37 °C. The bacterial culture was transferred to 10 mM stress-containing agar plates using a 0.9% saline solution after observing growth on the 5 mM stress agar plates. Repeated incubations and growth monitoring were done with the lead nitrate stress concentration steadily increasing to 25 mM. To avoid contamination, the entire procedure was carried out in sterile circumstances. Two of the six bacterial isolates, ID1 and ID3, were chosen for additional study (Table S1). For MIC of other heavy metals, different concentrations of $CdCl_2$, $NiCl_2.6H_20$, $ZnSO_4{\cdot}7H_2O$, and $CuSO_4{\cdot}5H_2O$, ranging from 1 to 10 mM, were added separately to the medium (Elahi et al., 2019).

2.4. Growth in minimal salt medium

A 250 ml flask containing 50 ml of M9 media was inoculated with bacterial culture from LB agar plates under 25 mM lead nitrate stress. Each flask was given a label and a bacterial culture inoculation. After that, the flasks were incubated at 37 °C in a shaking incubator. Control flasks were kept at -4°C. Compared to the control sample, turbidity readings from all six flasks showed growth after 72 h (Kalita and Joshi, 2017).

2.5. Lead uptake by bacteria

A lead processing experiment was conducted to evaluate the bacterial isolates' lead uptake. To screen for contamination, 15 ml glass test tubes with 6 ml LB broth were autoclaved and incubated at 37 °C for 24 h. Each test tube was inoculated with the bacterial culture, while the control was kept free of bacteria. The tubes were kept in an incubator at 37 °C for 14 h. After 14 h of incubation, 5 ml of the broth was transferred to 500 ml of M9 medium in a 1000 ml Erlenmeyer flask. Apart from the control flask, which was kept at −4°C, each flask was labeled and incubated at 37 °C for 24 h. The 2 mM $Pb(NO_3)_2$ stress was subsequently introduced by adding 1 ml of $Pb(NO_3)_2$ from a 1 M stock solution to 500 ml of M9 cultured media. The six flasks and the control M9 medium with 2 mM of $Pb(NO_3)_2$ were placed in a shaking incubator at 37 °C. During incubation, readings were taken at 2-day, 4-day, 6-day, and 8-day intervals. A 10 ml sample was collected at each interval in autoclaved glass test tubes using a micropipette. All samples, including the control, were centrifuged for 10 min at 4000 rpm. The supernatant was used to estimate the bacterial isolates' lead uptake by atomic absorption spectrophotometer (Varian, USA) at wavelength 217.0 and 357.9 nm, while the pellet was kept for further analysis.

The following equation was used to calculate the lead uptake on biomass (%).

$$q = \frac{(Ci - Ce)}{m} \times v$$

Ci = Initial concentration of the metal (mg/l)
Ce = Equilibrium concentration of the metal (mg/l)
V = Volume of the solution
m = Mass of the absorbent (Nur et al., 2016)

2.6. Bacterial identification and phylogenetic tree

Following the gram staining, the bacteria's genomic DNA was extracted using the technique outlined by Elahi and Rehman (2019), and the 16S rRNA gene was amplified through PCR using universal primers RS1 (5′AAACTCAATGAATTGACGG 3′) and RS3 (5′ACGGGCGGTGTGTA 3′). 25 µl of master mix, 1.5 µl of each primer, 10 µl of template, and 12 µl of nuclease-free water were combined to create a 50 µl reaction mixture. The PCR procedure involved an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 1 min at 94 °C; annealing was done at 50 °C for 45 sec and 72 °C for 1 min. Finally, PCR was set for a final extension at 72 °C. The PCR results were visualized on a 1% agarose gel (Bakht et al., 2020; Ali et al., 2023).

The 16S rRNA gene was purified using the Gene JETTM PCR purification kit (Ali et al., 2023). The binding buffer and the isolated DNA were added to a purification column. A quick washing procedure was used to get rid of the impurities. The isolated DNA was sent out for sequencing after being eluted from the column using the elution buffer (Bakht et al., 2020).

The NCBI BLAST database was used to compare the nucleotide sequences of the isolated bacteria to their neighbors. Multiple sequence alignments were carried out to find conserved areas

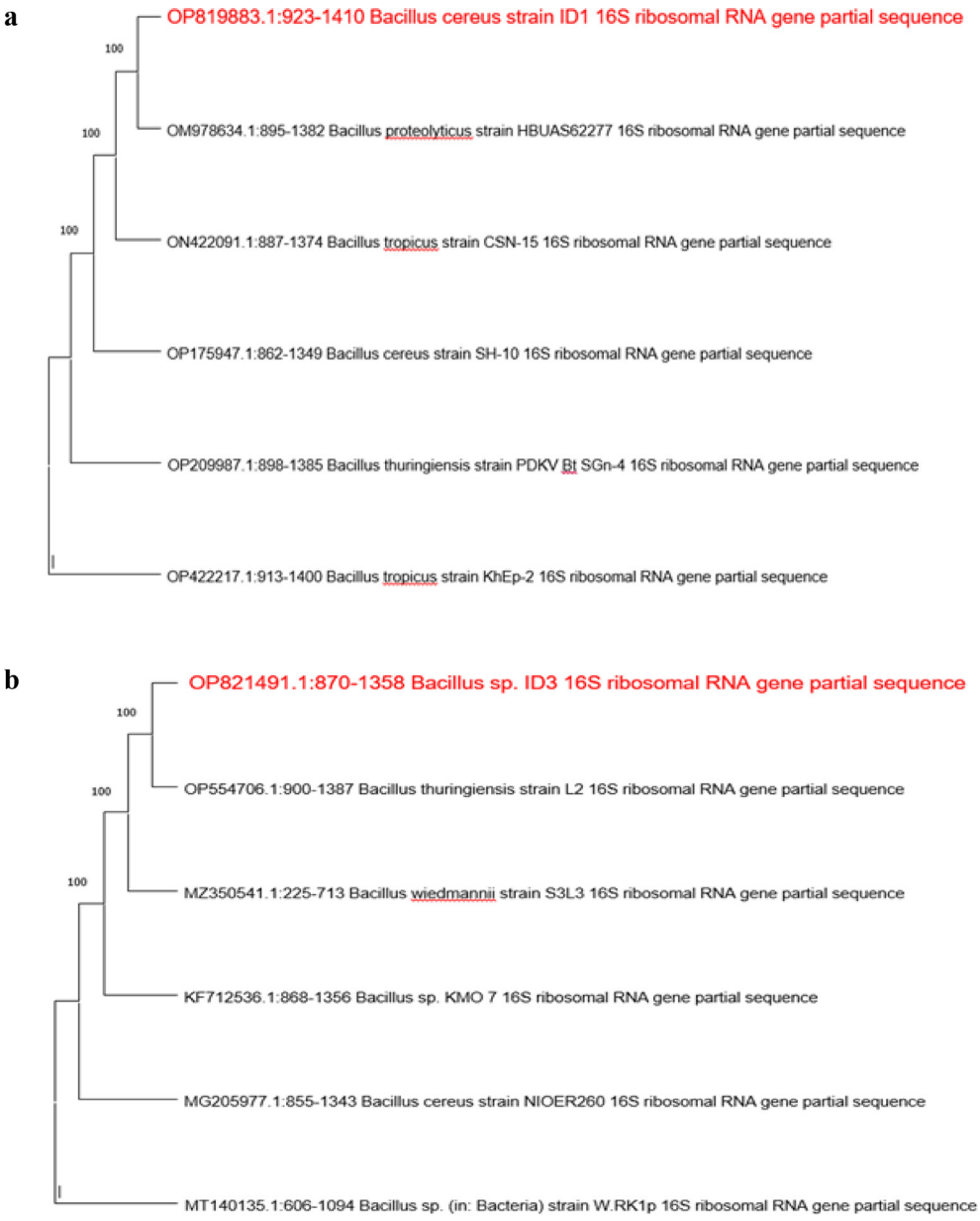


Fig. 1. Phylogenetic tree of (a) *Bacillus cereus* (ID1), and (b) *Bacillus* sp. (ID3) showing homology with other Bacillus strains.

using CLUSTAL W (Kwon et al., 2003). Following the sequencing of all the samples, it was discovered that strains ID1 and ID3 were members of the Bacillus genus.

A phylogenetic tree was created using the Neighbor-Joining approach to infer evolutionary history (Saitou and Nei, 1987). A bootstrap test with 1000 runs was performed to examine the clustering support, and the percentage of times the associated taxa grouped together was reported next to each branch (Felsenstein, 1985). The results of the Maximum Composite Likelihood approach (Tamura et al., 2004) were used to compute evolutionary distances, which were subsequently presented as the average number of base substitutions per site. MEGA11 software was used to carry out the evolutionary studies (Tamura et al., 2021).

2.7. FTIR and EDX (Energy dispersive X-ray) analysis

FTIR Fourier Transform Infrared (FTIR) analysis was performed to identify any functional groups in the bacterial structure. The lead processing experiment pellet was diluted at a 1:9 ratio (1 ml pellet and 9 ml distilled water). The diluted pellets were delivered to the Lahore University of Management Sciences (LUMS) for FTIR and EDX analysis. FTIR analysis is useful for identifying chemical bonds or functional groups in bacterial biomass, which can reveal information about the bacteria's potential metal-binding ability. The elemental composition of the biomass, including the presence of metals such as lead, is determined by EDX analysis. These investigations help to characterize and understand the bacterial isolates' interactions with lead ions and their uptake from the environment (Khan et al., 2015; Elahi et al., 2019).

2.8. Statistical analysis

Version 20.0 of the SPSS (Statistical Pack initial age for Social Sciences) software was performed for all statistical evaluations. The results were shown as the standard error of the mean, and the significance level was specified at ($p < 0.05$).

3. Results

3.1. DNA isolation and molecular characterization

The 16S rRNA gene was amplified successfully from DNA samples obtained using the bacterial DNA extraction procedure and PCR. Figure S1 displays the results of an agarose gel electrophoresis analysis of the extracted DNA samples and PCR product. The successful visualization of isolated DNA samples and the precise amplification of the 16S rRNA gene using PCR offer an established basis for subsequent investigation, such as sequencing and phylogenetic identification.

3.1.1. Phylogenetic relationship with reported strains

By comparing the nucleotide sequences of each species' 16S rRNA gene, available on NCBI, the Neighbor-Joining method in MEGA 11 software was used to deduce the evolutionary history of six different species. Fig. 2a and 2b illustrate the optimal tree structure, and next to each branch are the bootstrap support values (based on 1000 repeats), which demonstrate the frequency with which the linked taxa clustered. The average number of base substitutions per site was used to express the results of the Maximum Composite Likelihood method's estimation of evolutionary distances. After pairwise deletion deleted ambiguous positions, a final dataset of 550 distinct positions was produced. The phylogenetic tree revealed that *Bacillus* sp. (ID3) (accession no. OP821491) and *B. cereus* strain (ID1) (accession no. OP819883) had similarities with other *Bacillus* strains (Fig. 1a,b). MEGA11 software was used for the analysis (Tamura et al., 2021).

3.2. Minimum inhibitory concentrations (MICs)

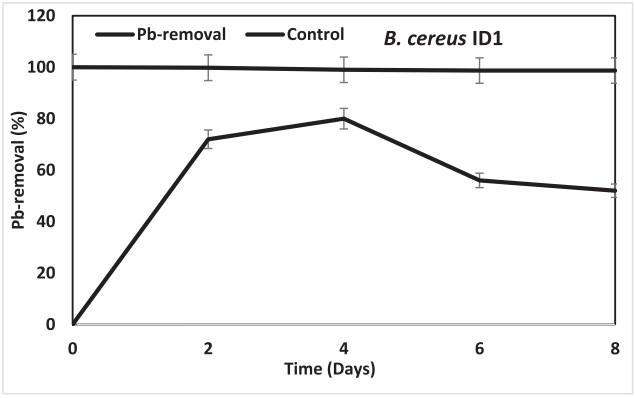
Both strains showed significant resistance up to 25 mM to Pb $(NO_3)_2$, and growth can be visualized on plates (Fig. S2b,c). No growth was observed on the control plate (Fig. S2a). *B. cereus* ID1 was capable to tolerate other heavy metals, viz., 4.0 mM (Zn^{2+}), 2.0 mM each (Cu^{2+}) and (Cd^{2+}), and 1.0 mM (Ni^{2+}). The order of resistance regarding heavy metals concentration was Pb^{2+}> Zn^{2+}> Cd^{2+}> Cu^{2+}> Ni^{2+}. Likewise, *Bacillus* sp. ID3 showed resistance to other heavy metal ions, i.e., 7.0 mM (Zn^{2+}), 4.0 mM each (Cu^{2+}) and 3.0 mM (Cd^{2+}), and 1.5 mM (Ni^{2+}). The order of resistance regarding heavy metals concentration was Pb^{2+}> Zn^{2+}> Cu^{2+}> Cd^{2+}> Ni^{2+}.

3.3. Lead uptake potential of bacterial strains

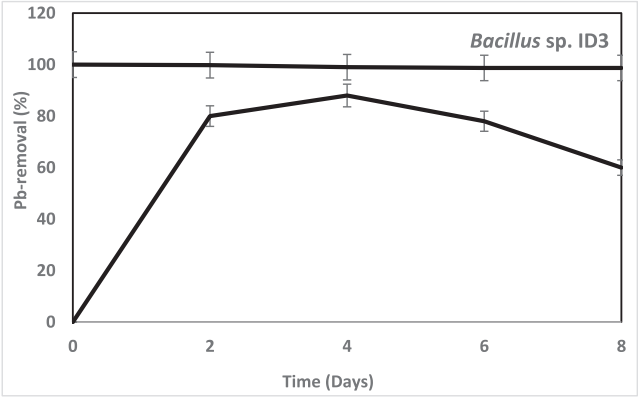
By lead processing essay, it was determined that on the 2nd day, both strains *B. cereus* (ID1) and *Bacillus* sp. (ID3) uptake 72% and 80% $Pb(NO_3)_2$, respectively through already prepared Pb-standard curve (Figure S3), from the medium. On the 4th day, strains *B. cereus* (ID1) and *Bacillus* sp. (ID3) showed 80% and 88% uptake, respectively. However, on the 6th day, $Pb(NO_3)_2$ increased in the medium to 44% (56% removal) for strains *B. cereus* (ID1) and 22% (78% removal) for *Bacillus* sp. (ID3), respectively. Similarly, on the 8th day, $Pb(NO_3)_2$ was raised in the medium to 48% (52% removal) for strains *B. cereus* (ID1) and 40% (60% removal) for *Bacillus* sp. (ID3), respectively, due to efflux, indicating metal homeostasis (Fig. S4a,b).

3.4. SDS-PAGE analysis

The bacterial strains treated with 1 mM $Pb(NO_3)_2$ and control cultures not treated with 1 mM $Pb(NO_3)_2$ were compared using SDS-PAGE. The analysis showed that the treated strains' protein profiles differed from the control strains, proving that lead exposure influenced the bacteria's protein structure. The various proteins in the samples are represented by the bands seen on the gel. Each band represents a distinct protein of a certain size. Pro-



(a)



(b)

Fig. 2. Pb-removal from the medium by (a) *B. cereus* ID1 and (b) *Bacillus* sp. ID3 after 2,4, 6, and 8 days of incubation at 37 °C. This metal removal was estimated by Atomic absorption Spectrophotometer at 217.0 wavelength.

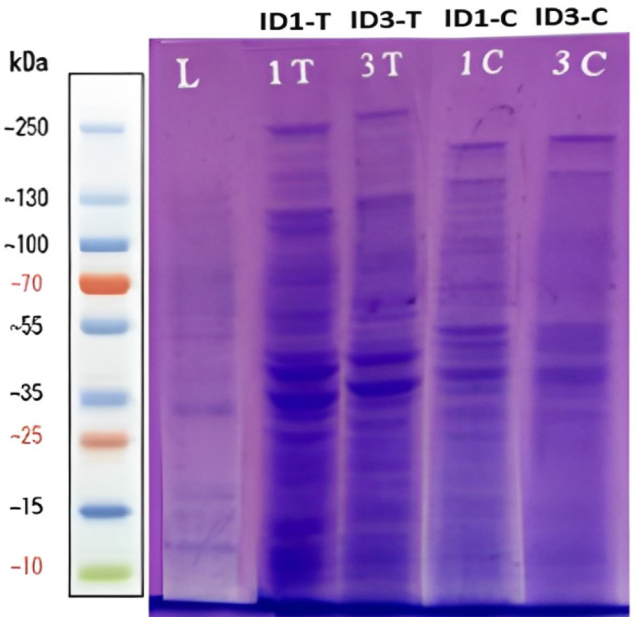


Fig. 3. SDS-PAGE analysis of *B. cereus* strain (ID1) and (b) *Bacillus* sp. strain ID3 with and without Pb-exposure.

teins with molecular weights of 15, 55, 70, 100, and 250 kDa were each detected as a separate band in the 1-T sample. There were bands at 55 kDa, 70 kDa, and 250 kDa in the 3-T sample. Two bands at 70 kDa and one at 130 kDa were visible in the 3-C sample, while three bands at 55 kDa were seen in the 1-C sample. Bands at 15, 25, 35, 55, 70, 100, 130, and 250 kDa were seen in the standard ladder. These varying-sized bands correspond to proteins of varying molecular weights (kDa) (Fig. 3).

3.5. FTIR and EDX analyses

The FTIR technique was employed to comprehend the qualitative and quantitative alterations in the constituents of every treated sample in relation to its corresponding control of the sample via overlapping. Both bacterial strains exhibited significant potential in terms of the structural alterations observed in their functional groups when subjected to Pb $(NO_3)_2$-induced stress. The experimental results indicate the presence of eight distinct peaks in the control sample, which can be attributed to the stretching of C = O, C–H, O–H, C = C, and C-O bonds, as well as the bending of C–H bonds, and the stretching of CO-O-CO and C-Cl bonds. The isolate ID1-T that was subjected to treatment exhibited five distinct peaks at the following wavenumbers: (1390–1310) for O–H bending, (980–960) for C = C bending, (2928) for N–H stretching, (1210–1163) for C-O stretching, and (1550–1500) for N-O stretching. The strain ID3-T, which was subjected to Pb treatment, exhibited ten peaks in its spectral analysis. These peaks were observed at specific wavelengths corresponding to various molecular vibrations, such as C–H stretching at 3016, C = C bending at 980–960, O–H bending at 1390–1310, C–H stretching at 1431, C-O stretching at 1210–1163, N-O stretching at 1550–1500, C = C stretching at 1642, C = O stretching at 1732, N–H stretching at 2928 and, C-I stretching at 500–600 as depicted in Fig. 4. The EDX analysis verified the uptake of Pb by the bacterial strains intracellularly,

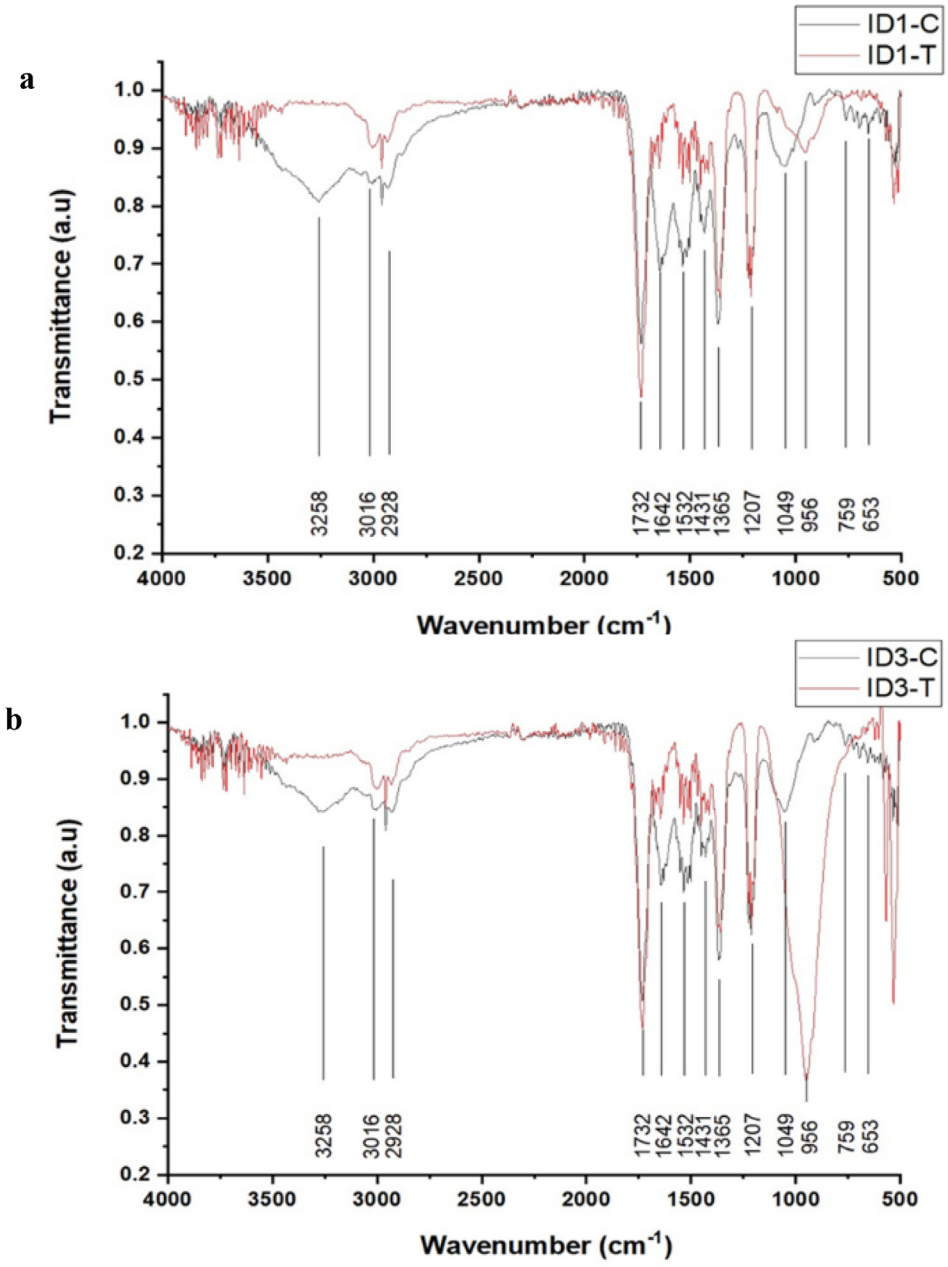


Fig. 4. The FTIR spectra of metal (Pb) treated with (a) *B. cereus* strain (ID1 and (b) *Bacillus* sp. strain (ID3).

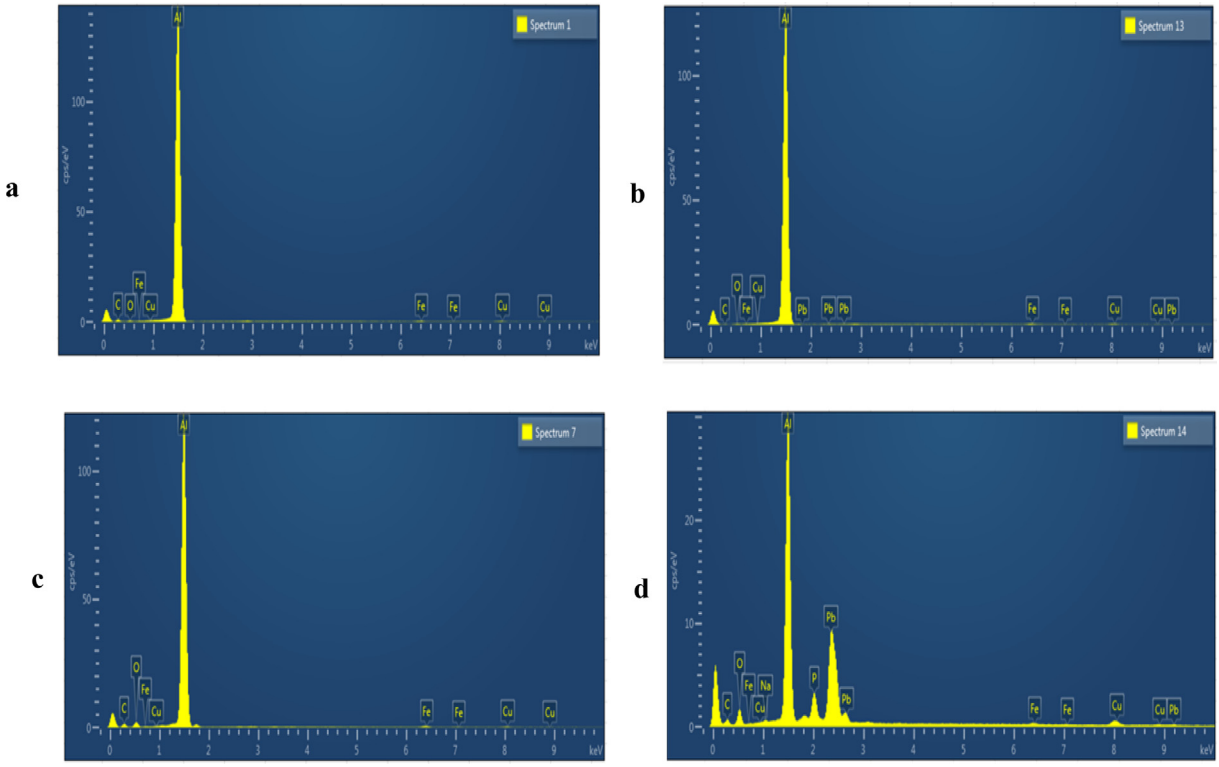


Fig. 5. EDX analysis with and without Pb-exposure; (a) and (c) represent control for ID1 and ID3 while (b) and (d) represent treated samples with Pb.

whereas no Pb concentration was detected in the control (Fig. 5).

4. Discussion

According to Briffa et al. (2020), metal significantly impacts the environment, posing a threat to both human health and the ecosystem. The process of remediating heavy metals is a complex mechanism that cannot be effectively addressed through physical or chemical means. At present, bioremediation represents a highly desirable approach for mitigating pollution. This methodology offers the possibility of heavy metal dissolution through regular biological processes. The process of utilizing biological agents, such as microorganisms like bacteria, fungi, or yeast, to clean contaminated soil and water is commonly referred to as bioremediation (Naik and Dubey, 2013). The current investigation was carried out on bacterial strains, which were obtained from industrial wastewater, with the aim of effectively remediating heavy metals.

In the current research investigation, bacterial samples were obtained from industrial wastewater, which was found to contain high concentrations of heavy metals such as Pb, Cd, and Cr. These specimens were subsequently exposed to Pb $(NO_3)_2$ stress. The bacterial strains that exhibited proliferation under lead-induced stress were subjected to characterization. Both strains were classified as gram-positive bacteria. Several studies have documented the isolation of gram-positive bacterial strains, including *Bacillus* sp. Pb15 (Pepi et al., 2016), as well as *Staphylococcus* sp., *Vibrio* sp., and *Enterobacter* sp. (Sowmya et al., 2014), from industrial wastewater.

In the current work, two bacterial strains, ID1 and ID3, were found to survive upto 25 mM of Pb $(NO_3)_2$. After sequencing, these two strains were identified as *B. cereus* strain (ID1) and *Bacillus* sp. (ID3). Utami et al. (2020) reported the isolation of *B. cereus* strains from contaminated soils, which could tolerate up to 10 mM Pb concentration. Compared to other investigations, the current findings are in consistent with earlier reports. As a result, the present work contributes to the knowledge of the potential of *Bacillus* strains in Pb-remediation. It proposes that these bacteria may be applied in bioremediation procedures for the eradication of toxic metal ions from the contaminated environment.

According to recent research, the capacity of bacteria to absorb heavy metals from contaminated environments has emerged as a promising bioremediation strategy. The lead processing essay's findings confirm this by being consistent with earlier research (Table 1) that mentioned *Bacillus* spp.'s potential for lead removal (Qiao et al., 2019). The results of the Pb-processing experiment provide important information about the uptake capability of two bacterial strains, *B. cereus* (ID1) and *Bacillus* sp. (ID3), in the presence of $Pb(NO_3)_2$. Over the course of 8 days, both strains demonstrated significant absorption efficiency, with *Bacillus* sp. (ID3) consistently showing higher uptake percentages than *B. cereus* (ID1). This shows that *Bacillus* sp. (ID3) may have more efficient mechanisms for absorbing metal from the medium.

Interestingly, the concentration of $Pb(NO_3)_2$ in the medium increased as the experiment progressed, showing the presence of efflux mechanisms in both strains. This phenomenon shows that bacterial strains actively maintain metal homeostasis by efficiently pumping lead out of their cells. The detected efflux rates differed

Table 1
Lead uptake potential of reported bacterial stains.

Sr. No	Organism	Pb-Uptake	Reference
1	*Bacillus xiamenensis*	2200 mg/l	Mohapatra et al. (2019)
2	*Acinetobacter* sp.	75.1%.	Ma et al. (2015)
3	*Stenotrophomonas* MB339	85.30%	Aslam et al. (2020)
4	*Staphylococcus* sp. MB371	88.33%	Aslam et al. (2020)
5	*Brevibacillus brevis* NBRC	1500 µg/ml	Bhagat & Thawait (2018)
6	*Bacillus cereus* ID1	80%	Present study
7	*Bacillus* sp. ID3	88%	Present study

between the two strains, with *B. cereus* (ID1) showing larger efflux percentages than *Bacillus* sp. (ID3). Furthermore, the observed outflow of Pb from the medium in the current work is consistent with prior studies demonstrating bacteria's ability to maintain metal homeostasis (Nies, 1999; Chandrangsu et al., 2017; Nies, 1999). These results imply that the bacteria I.e., *Bacillus cereus* (ID1) and *Bacillus* sp. (ID3) may be suitable for Pb-bioremediation.

The estimation of a protein's molecular weight is extremely important since it offers essential information about its size and structure. The molecular weights of the proteins present can be estimated by evaluating the detected bands in the samples and comparing them to the reference ladder. This information is critical in discovering and characterizing individual proteins, allowing for a better understanding of their functions and potential interactions within biological systems. The appearance of distinct bands with varying molecular weights indicates the presence of various proteins in the samples, each with its own size. The discrepancies in band patterns detected between the different samples (1-T, 3-T, 1-C, and 3-C) indicate probable variances in protein composition or abundance.

By using FTIR to characterize the bacterial isolates under Pb $(NO_3)_2$ stress for 48 h, the functional group analysis of the isolates distinguished the treated samples from the control samples. Similar outcomes are also provided by Gurbano et al. (2015). The findings of this study demonstrated that when exposed to the stress of $Pb(NO_3)_2$, both the *B. cereus* (ID1) and *Bacillus* sp. (ID3) strains demonstrated excellent results regarding the structural modifications in the functional groups. ID1-T, a treated isolate, displayed five peaks, whereas ID3-T displayed ten peaks. These findings agree with those of earlier studies examining the impact of heavy metal stress on bacterial strains. For instance, a study by Durve et al. (2014) discovered that FTIR analysis could distinguish between several bacterial strains subjected to heavy metal stress by detecting changes in the functional groups of their biomolecules. FTIR analysis might be used to assess changes in the metabolic composition of bacterial strains in response to heavy metal stress. The study discovered that the functional groups of bacterial strains under heavy metal stress differed greatly from their control counterparts.

5. Conclusion

In conclusion, *B. cereus* (ID1) and *Bacillus* sp. (ID3) strains showed significant resistance up to 25 mM against $Pb(NO_3)_2$. Moreover, both strains also significantly absorbed $Pb(NO_3)_2$ from the medium, *Bacillus cereus* (ID1) removed 80% while *Bacillus* sp. (ID3) removed 88% Pb after 4 days of incubation. On the other hand, an increase in $Pb(NO_3)_2$ concentration in the 6 and 8 days suggested the presence of metal homeostasis and efflux mechanisms. SDS-PAGE analysis visualized differences in protein profile between treated and control samples. The FTIR study shed light on the structural changes brought on by lead exposure in the bacterial strains' functional groups. This study's findings indicate that *B. cereus* (ID1) and *Bacillus* sp. (ID3) are promising bacterial strains for Pb-bioremediation from polluted environments. However, future research is needed to identify the mechanisms involved in these strains' uptake and homeostasis of heavy metals including lead.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103740.

References

Ali, S., Rehman, A., Hussain, S.Z., Bukhari, D.A., 2023. Characterization of plastic degrading bacteria isolated from sewage wastewater. Saudi J. Biol. Sci. 30, (5) 103628.

Aryal, M., Liakopoulou-Kyriakides, M., 2015. Bioremoval of heavy metals by bacterial biomass. Environ. Monit. Assess. 187, 1–26.

Aslam, F., Yasmin, A., Sohail, S., 2020. Bioaccumulation of lead, chromium, and nickel by bacteria from three different genera isolated from industrial effluent. Int. Microbiol. 23 (2), 253–261.

Bhagat, S., Thawait, L., 2018. Bioremediation of Lead by lead resistant bacterial isolates isolated from contaminated soils of Chhattisgarh. Res. J. Pharm. Technol. 11 (10), 4559–4562.

Briffa, J., Sinagra, E., Blundell, R., 2020. Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon 6 (9), e04691.

Chandrangsu, P., Rensing, C., Helmann, J.D., 2017. Metal homeostasis and resistance in bacteria. Nat. Rev. Microbiol. 15 (6), 338–350.

Durve, A., Chandra, N., 2014. FT-IR analysis of bacterial biomass in response to heavy metal stress. Int. J. Biotechnol. Photon. 112, 386–391.

Elahi, A., Rehman, A., 2019. Comparative behavior of two gram-positive Cr^{6+} resistant bacterial strains *Bacillus aerius* S1 and *Brevibacterium iodinum* S2 under hexavalent chromium stress. Biotechnol. Rep. 21, e00307.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gurbanov, R., Simsek Ozek, N., Gozen, A.G., Severcan, F., 2015. Quick discrimination of heavy metal resistant bacterial populations using infrared spectroscopy coupled with chemometrics. Analyt. Chem. 87 (19), 9653–9661.

Javanbakht, V., Alavi, S.A., Zilouei, H., 2014. Mechanisms of heavy metal removal using microorganisms as biosorbent. Water Sci. Technol. 69 (9), 1775–1787.

Kalia, K., Flora, S.J., 2005. Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. J. Occup. health 47 (1), 1–21.

Kalita, D., Joshi, S.R., 2017. Study on bioremediation of lead by exopolysaccharide producing metallophilic bacterium isolated from extreme habitat. Biotechnol. Rep. 16, 48–57.

Khan, Z., Nisar, M.A., Hussain, S.Z., Arshad, M.N., Rehman, A., 2015. Cadmium resistance mechanism in *Escherichia coli* P4 and its potential use to bioremediate environmental cadmium. Appl. Microbial. Biotechnol. 99, 10745–10757.

Ma, Y., Wang, P., Wang, C., Zhang, S.H., Cheng, S., 2015. Isolation and characterization of Pb-resistant strains and the removal of Pb (II). Fresenius Environ. Bull. 24 (3b), 1150–1157.

Mohapatra, R.K., Parhi, P.K., Pandey, S., Bindhani, B.K., Thatoi, H., Panda, C.R., 2019. Active and passive biosorption of Pb(II) using live and dead biomass of marine bacterium *Bacillus xiamenensis* PbRPSD202: Kinetics and isotherm studies. J. Environ. Manag. 247, 121–134.

Murthy, S., Bali, G., Sarangi, S.K., 2012. Biosorption of lead by *Bacillus cereus* isolated from industrial effluents. British Biotechnol. J. 2 (2), 73–84.

Naik, M.M., Dubey, S.K., 2013. Lead resistant bacteria: lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. Ecotoxicol. Environ. Saf. 98, 1–7.

Nies, D.H., 1999. Microbial heavy-metal resistance. Appl. Microbiol. Biotechnol. 51, 730–750.

Nur, T., Naidu, G., Loganathan, P., Kandasamy, J., Vigneswaran, S., 2016. Rubidium recovery using potassium cobalt hexacyanoferrate sorbent. Desalin. Water Treat. 57 (55), 26577–26585.

Pepi, M., Borra, M., Tamburrino, S., Saggiomo, M., Viola, A., Biffali, E., Balestra, C., Sprovieri, M., Casotti, R., 2016. A *Bacillus* sp. isolated from sediments of the Sarno River mouth, Gulf of Naples (Italy) produces a biofilm biosorbing Pb (II). Sci. Total Environ. 562, 588–595.

Qiao, W., Zhang, Y., Xia, H., Luo, Y., Liu, S., Wang, S., Wang, W., 2019. Bioimmobilization of lead by *Bacillus subtilis* X3 biomass isolated from lead mine soil under the promotion of multiple adsorption mechanisms. R. Soc. Open Sci. 6, (2) 181701.

Rees, N., Fuller, R., 2020. The toxic truth: children's exposure to lead pollution undermines a generation of future potential. UNICEF.

Ruckart, P.Z., Jones, R.L., Courtney, J.G., LeBlanc, T.T., Jackson, W., Karwowski, M.P., Cheng, P.-Y., Allwood, P.B., Svendsen, E., Breysse, P.N., 2021. Update of the blood lead reference value-United States, 2021. Morbid. Mortal. Weekly Rep. 70 (43), 1509–1512.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evolut. 4, 406–425.

Selvi, A., Rajasekar, A., Theerthagiri, J., Ananthaselvam, A., Sathishkumar, K., Madhavan, J., Rahman, P.K., 2019. Integrated remediation processes toward heavy metal removal/recovery from various environments-a review. Front. Environ. Sci. 7, 66.

Sowmya, M., Rejula, M.P., Rejith, P.G., Mohan, M., Karuppiah, M., Hatha, A.M., 2014. Heavy metal tolerant halophilic bacteria from Vembanad Lake as possible source for bioremediation of lead and cadmium. J. Environ. Biol. 35 (4), 655.

Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceed. Natl. Acad. Sci. (USA) 101, 11030–11035.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA 11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. https://doi.org/10.1093/molbev/msab120.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metal toxicity and the environment. Exp. Suppl. 101, 133–164.

Utami, U., Harianie, L., Dunyana, N.R., 2020. Lead-resistant bacteria isolated from oil wastewater sample for bioremediation of lead. Water Sci. Technol. 81 (10), 2244–2249.

World Health Organization WHO. (2022, August 31). *Lead poisoning*. https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health

Yusuf, A.A., Ampah, J.D., Soudagar, M.E.M., Veza, I., Kingsley, U., Afrane, S., Buyondo, K.A., 2022. Effects of hybrid nanoparticle additives in n-butanol/waste plastic oil/diesel blends on combustion, particulate and gaseous emissions from diesel engine evaluated with entropy-weighted PROMETHEE II and TOPSIS: Environmental and health risks of plastic waste. Energy Convers. Manag. 264, 115758.

Zhong, L., Zhang, H., Wu, L., Ru, H., Wei, N., Yao, F., Ni, Z., Duan, X., Li, Y., 2022. Copper and zinc treatments alter the thyroid endocrine system in Zebrafish embryos/larvae. Toxics 10 (12), 756.