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Quantitative Relationship Between Minimum Inhibitory Concentration (MIC) and Heavy Metal Biosorption Capacity of Native Resistant Bacteria Isolated from Industrially Contaminated Soils in Zanjan Province, Iran

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ABSTRACT

Background: Bacterial tolerance to heavy metals, commonly expressed as minimum inhibitory concentration (MIC), is often evaluated alongside biosorption capacity. However, the extent to which MIC can reliably predict metal absorption has rarely been quantified. This study investigated the quantitative relationship between MIC and biosorption capacity in native bacterial strains isolated from industrially contaminated soils.

Methods: Twenty morphologically and biochemically distinct strains were isolated. MIC values for lead, zinc, cadmium, and a mixture of the three metals were determined using the broth microdilution method. Biosorption capacity was assessed through triplicate batch experiments, and the amount of metal absorbed by biomass was quantified using ICP-OES. A cell-free abiotic control confirmed that metal recovery without biomass remained below 0.5 ppm. The most efficient strains were identified by 16S rRNA gene sequencing. We analyzed the relationship between MIC and biosorption using Pearson correlation, one-way ANOVA, and regression analysis of log-transformed MIC values.

Results: Biosorption capacity consistently increased with resistance level. All tested systems showed significant positive correlations: the multi-metal mixture ($r = 0.895$), lead ($r = 0.879$), cadmium ($r = 0.780$), and zinc ($r = 0.748$), all with $p < 0.001$. Strains with higher MIC values showed significantly greater biosorption. *Pseudomonas aeruginosa* and *Bacillus sp*. were the most efficient biosorbing strains.

Conclusion: MIC can serve as a simple and cost-effective criterion for the preliminary selection of promising bacterial biosorbents for heavy-metal bioremediation.

1. Introduction

Soil contamination by heavy metals is among the most persistent environmental challenges in mining and industrial regions. The lead-zinc belt of Zanjan in northwestern Iran is an important example. Surveys around the Zanjan Zinc Complex and the National Iranian Lead and Zinc Company have documented soils with high amounts of lead (Pb), zinc (Zn), and cadmium (Cd) loads that considerably exceed guideline limits, as a result of airborne emissions and tailings runoff (Abadi et al., 2023; Akhavan & Golchin, 2024; Jamal et al., 2019; Parizanganeh et al., 2010; Zamani et al., 2015). Beyond Zanjan, heavy-metal contamination of soils and surface waters has also been documented across northern Iran, including waste-disposal-affected soils and rivers in Guilan Province (Karimzadegan et al., 2008; Sadeghi Poor Sheijany et al., 2025). Because lead, cadmium, and zinc are non-degradable and accumulate through the food chain (Collin et al., 2022; Fu & Wang, 2011; Gadd, 2010), such pollution obviously imposes substantial

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health and economic costs in Iran (Sadeghi Poor Sheijany et al., 2020). Due to the efficiency and effectiveness of bioremediation methods, attention has shifted from costly physicochemical removal methods toward biological approaches that utilize the native microbial capacity of contaminated soils (Ayangbenro & Babalola, 2017; Gadd, 2009; Masoumi et al., 2016; Pande et al., 2022), although high heavy-metal concentrations can themselves depress soil enzyme activity and microbial biomass (Gul et al., 2024). In microbial treatment, two important elements should be considered: a strain's ability to survive under high heavy-metal concentrations and its ability to remove heavy metals from the environment by biosorption and related processes. Resistance is commonly expressed as the minimum inhibitory concentration (MIC), defined as the lowest amount of heavy metal concentration that prevents visible bacterial growth. Resistance is mediated by mechanisms such as efflux pumps, metal-binding regulatory genes, cell-wall modification, and the production of extracellular polymeric substances (Aslam et al., 2025; Bruins et al., 2000; Gadd, 1990; Nies, 2003). Metal removal mainly occurs through the binding of heavy metal ions to functional groups on the cell surface and, in some cases, through intracellular accumulation (Priyadarshanee & Das, 2021; Vijayaraghavan & Yun, 2008; Wang & Chen, 2009). If these two properties are positively associated, more resistant strains may also exhibit greater biosorption capacity. However, this assumption has rarely been tested quantitatively, especially in native bacteria obtained from extremely contaminated environments.

Determining the relationship between heavy-metal resistance and biosorption capacity is essential for practical applications. If a strong correlation exists, MIC could provide a rapid and cost-effective method for the preliminary screening of large collections of bacterial isolates. This would enable researchers to focus on the most promising candidates without labor-intensive sorption assays on strains unlikely to perform. Conversely, if the two properties are weakly or inconsistently associated, MIC-based pre-screening could lead to inaccurate predictions of biosorption performance. Therefore, the present study aimed to quantify the relationship between MIC and biosorption capacity in bacteria isolated from lead-zinc-contaminated soils in Zanjan, Iran, with respect to lead, zinc, cadmium, and a mixture of these metals, and to evaluate whether resistance level can serve as a screening proxy for biosorption potential.

2. Materials and Methods

To characterize the sampled soils and support ecological interpretation of the isolated community, physicochemical properties were determined for each of the ten stations following standard soil-analysis protocols. Soil pH and electrical conductivity (EC) were measured in a 1:5 (w/v) soil-to-water suspension; organic carbon was determined by the Walkley-Black wet-oxidation method; total nitrogen was determined by the Kjeldahl method; available phosphorus was extracted by the Olsen bicarbonate method; and cation exchange capacity (CEC) was determined by the ammonium acetate method (1 N NH4OAc, pH 7). Soil texture was assessed for each station during sampling. Total Pb, Zn, and Cd concentrations in the soils were determined after acid digestion by inductively coupled plasma optical emission spectrometry (ICP-OES). Across the ten stations, pH was near-neutral (mean 6.95 ± 0.40, range 6.25-7.41), EC was moderate to high (mean 597 ± 298 µS cm^{-1}, range 240-1085), CEC was moderate to low (mean 10.7 ± 1.5 cmol (+) kg^{-1}, range 9-14), organic matter content was low (0.8-1.2%), total nitrogen averaged 0.07 ± 0.04%, and available phosphorus averaged 16.8 ± 10.1 mg kg^{-1} (Table 1). Soil texture across the sampled stations was classified as loam, clay loam, and sandy loam. Total soil metal loads were markedly elevated and heterogeneous, ranging from 130 to 9100 mg kg^{-1} for Pb, 211 to 58,800 mg kg^{-1} for Zn, and 3.0 to 5800 mg kg^{-1} for Cd, consistent with previous surveys of this district (Abadi et al., 2023; Jamal et al., 2019; Parizanganeh et al., 2010; Zamani et al., 2015). The near-neutral pH generally favors the survival and activity of native soil bacteria, while the moderate-to-high EC indicates appreciable soluble salt content and osmotic stress that may constrain some strains, although native isolates are typically better adapted to such conditions than non-indigenous ones. The low organic-matter content limits the carbon and energy resources available to the microbial community and may keep bioremediation efficiency low without amendment, and the moderate-to-low CEC indicates limited cation-retention and buffering capacity, so that a larger fraction of the soil metal load is likely to remain mobile and exert pressure on the rhizosphere and on the isolated bacteria.

Table 1. Physicochemical characteristics of the sampled soils (n = 10 stations; mean ± SD)

Parameter	Unit	Mean ± SD	Range
pH	–	6.95 ± 0.40	6.25-7.41
Electrical conductivity	µS cm^{-1}	597 ± 298	240-1085
Cation exchange capacity	cmol (+) kg^{-1}	10.7 ± 1.5	9-14
Organic matter	%	-	0.8-1.2
Total nitrogen	%	0.07 ± 0.04	0.04-0.15
Available phosphorus	mg kg^{-1}	16.8 ± 10.1	6.7-30.0
Total Pb	mg kg^{-1}	2751 ± 3371	130-9100
Total Zn	mg kg^{-1}	18086 ± 21426	211-58800
Total Cd	mg kg^{-1}	789 ± 1802	3.0-5800

* Note: Pb, lead; Zn, zinc; Cd, cadmium.

The isolation and purification process involved suspending ten grams of each soil sample in 90 mL of sterile saline (0.85% NaCl). The samples were then serially diluted and plated onto nutrient agar at a pH of 7.0, and incubated at 30 °C for 48 to 72 h. In order to isolate resistant strains, filter-sterilized metal salts ($Pb(NO_3)_2$, $ZnSO_4$, and $CdCl_2$) were incorporated at graded concentrations. Colonies were purified by repeated streak-plating and characterized by Gram staining and standard biochemical tests, including catalase, oxidase, citrate, motility, and the Methyl Red-Voges-Proskauer (MR-VP) test. Ultimately, twenty strains with non-redundant profiles were selected, further purified, and then cryopreserved at -80 °C.

The MIC for each strain against lead (Pb), zinc (Zn), cadmium (Cd), and a combination of these metals was assessed using the broth microdilution method in 96-well microtiter plates. Relative to faster qualitative pre-screening methods such as disk diffusion or agar-based spot assays, broth microdilution MIC determination provides a quantitative, dose-resolved measure of resistance on a standardized two-fold dilution scale, is readily adapted to the 96-well format used here for parallel testing of many isolates, and yields a continuous variable that can be directly related to biosorption capacity; qualitative zone-of-inhibition methods do not readily support this type of quantitative pre-screening.

This involved conducting two-fold serial dilutions of the metal salts according to the Clinical and Laboratory Standards Institute (CLSI) protocol. Wells were inoculated to a standardized density of 0.5 McFarland and subsequently incubated. The MIC was determined as the lowest concentration of heavy metal at which no visible growth was observed. Each determination was conducted in triplicate, and the mean was reported.

Biosorption of heavy metals was evaluated in batch experiments in 250 mL flasks at 30 °C, with the medium pH adjusted to 6.5-7.0 and the inoculum standardized to 0.5 McFarland (approximately 1.5×10^8 CFU mL^{-1}, $OD_{600} \approx 0.1$). For each assay, the extracted biomass was resuspended to a fixed loading of 10 g L^{-1} (0.1 g biomass in 10 mL) to standardize the sorbent dose across strains. Three contact times (24, 48 and 72 h) were evaluated in preliminary trials, and sorption approached a plateau by 48 h. The values reported here therefore correspond to this near-equilibrium 48-h contact time. The suspensions were then gently centrifuged at 3,500 rpm for 10 min, a speed chosen to sediment the cells while preserving surface-adsorbed metal on the biomass, so that the total metal associated with the bacteria, both surface-bound and intracellular, was captured.

The harvested cell pellet was then washed twice with deionized water, with gentle resuspension between washes, to remove unbound and interstitial metal carried over from the medium and retain only the metal genuinely associated with the biomass. The washed biomass was digested in concentrated nitric acid (HNO_3, 65%), and the digest was diluted to a final volume of 25 mL with deionized water. The Pb, Zn, and Cd accumulated by the biomass were measured directly in the digestate by ICP-OES. Biosorption is therefore reported as the total metal accumulated by the bacterial biomass (mg L^{-1}). All assays were performed in triplicate. A cell-free abiotic control, containing the metal solution under identical conditions but no bacteria, was run alongside every assay so that any chemical precipitation at the working pH could be separated from biosorption.

Molecular confirmation by 16S rRNA sequencing was done on the five isolates that combined the highest resistance and sorption. As the main purpose of the study was to identify the best-performing isolates, for the remaining fifteen isolates, genus-level grouping relied on the morphological and biochemical characterization described above (Gram reaction, cell morphology, catalase, oxidase, citrate utilization, motility, and MR-VP), which is a standard, widely used scheme for preliminary bacterial grouping. Genomic DNA was extracted from pure cultures. The 16S rRNA gene was amplified using universal primers (27F/1492R). Purified amplicons were sequenced and compared with the NCBI database by BLASTn, with phylogenetic placement in relation to reference sequences (Weisburg et al., 1991). This molecular method was used to confirm strain identity at the species level rather than relying on phenotype alone.

The correlation between the minimum inhibitory concentration (MIC) and the potential biosorption ability of the strains was assessed using the Pearson correlation coefficient (per metal; n = 20). One-way ANOVA was used to analyze the sorption differences across MIC levels. Linear regression was also performed on log2-transformed MIC values to illustrate the dose-response relationship, acknowledging that MIC is on a two-fold dilution scale. We considered a threshold of significance of $p < 0.05$. All statistical analyses were conducted using SPSS version 16 and Python (SciPy).

3. Results and Discussion

The twenty strains exhibited significant and, in many cases, remarkable heavy metal tolerance. Minimum inhibitory concentration (MIC) values reached up to 4096 mg L^{-1} for lead, 2048 mg L^{-1} for zinc, and 1024 mg L^{-1} for cadmium, while the three-metal mixture had a maximum MIC of 512 mg L^{-1} (Table 2). The community was dominated by Gram-positive, bacillus-like, catalase-positive, and motile forms, which are well-adapted to handle oxidative and osmotic stress. The lower MIC ceiling in the mixed system relative to the single-metal systems is consistent with combined ionic stress and competitive toxicity, which is a pattern reported repeatedly for more real-world multi-metal exposure (Chellaiah, 2018; Harrison et al., 2007). The remarkable resistance observed indicates a microbiome that has been under intensive and sustained selective pressure, leading to the natural selection of only those strains that are equipped with effective mechanisms of tolerance and survival through evolutionary adaptation (Bruins et al., 2000).

In all four systems analyzed, resistance exhibits a strong predictive ability for biosorption capacity. The findings indicate a strong correlation between resistance and biosorption (Table 3 and Figure 1). The Pearson correlation between the minimum inhibitory concentration (MIC) and sorption was found to be strong and statistically significant. Specifically, for the multi-metal mixture ($r = 0.895$, $p = 1.0 \times 10^{-7}$). For lead, the relationship is also strong and clear ($r = 0.879$, $p = 3.4 \times 10^{-7}$). For cadmium, a strong relationship was observed ($r = 0.780$, $p = 4.9 \times 10^{-5}$), while the relationship for zinc was also remarkable ($r = 0.748$, $p = 1.5 \times 10^{-4}$). One-way ANOVA further confirmed these results, indicating that strains with higher MIC values consistently demonstrated a greater capacity for heavy metal removal in all tested systems. The results for lead ($F = 22.3$, $p = 3.6 \times 10^{-6}$), zinc ($F = 11.0$, $p = 8.5 \times 10^{-4}$), cadmium ($F = 20.3$, $p = 4.1 \times 10^{-5}$), and the mixture of these three heavy metals ($F = 28.3$, $p = 1.2 \times$

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10^{-6}) further support this finding. Moreover, a clear dose-response relationship was obtained through regression analysis of $\log_2$-transformed MIC values, where each incremental doubling of the tolerated concentration corresponded to a significant increase in sorption quantities. To quantify the strength and precision of these relationships beyond the correlation coefficients reported above, the corresponding coefficients of determination ($R^2 = r^2$) ranged from 0.56 (zinc) to 0.80 (multi-metal mixture), while 95% confidence intervals for each Pearson r, obtained by Fisher's z-transformation (n = 20), excluded zero in every case. Ordinary least-squares regression of sorption on $\log_2$-transformed MIC yielded significant positive slopes for all four systems, confirming the dose-response pattern described above; the regression equations, standard errors and 95% confidence intervals are reported in Table 4.

Table 2. MIC values for the twenty native strains in response to each heavy metal

Heavy Metal	MIC range (mg L^{-1})	Mean sorption (mg L^{-1})
Lead (Pb)	256-4096	770.0
Zinc (Zn)	512-2048	293.9
Cadmium (Cd)	32-1024	264.2
Multi-metal	32-512	230.1

Table 3. MIC and biosorption capacity Relationship

Heavy metal	Pearson r	*p**	ANOVA F	*P***
Multi-metal	0.895	< 0.001	28.3	< 0.001
Lead (Pb)	0.879	< 0.001	22.3	< 0.001
Cadmium (Cd)	0.780	< 0.001	20.3	< 0.001
Zinc (Zn)	0.748	< 0.001	11.0	< 0.001

*Pearson correlation test, **One-way ANOVA test.

The consistency of the positive correlation in four chemically distinct systems suggests that resistance and biosorption are not merely coincidentally correlated features; rather, it can be inferred that these represent different aspects of a unified cellular response to metal stress. Different kinds of cellular mechanisms can lead to this relationship. Carboxyl, phosphate, hydroxyl, and amine groups which are on the cell membrane control surface biosorption. The negatively charged metal-binding surface that captures ions also plays a crucial role in shielding the cell from those same ions. This means that a wall designed for durability also tends to function as a more efficient adsorbent (Flemming & Wingender, 2010; Gupta & Diwan, 2017; Priyadarshanee & Das, 2021). Additionally, the regulated systems that contribute to the cell's resistance, including metal-binding proteins, efflux pumps and extracellular polymeric substances, are capable of immobilizing or compartmentalizing heavy metals. Such a large overlap makes it difficult to distinguish between simply "surviving" and actually "removing" these metals (Ansari et al., 2011; Joo et al., 2010; Mathivanan et al., 2023; Nies, 2003). Since efflux pumps export metal ions from the cytoplasm, in isolation they would tend to lower intracellular accumulation; the net rise in total sorption with resistance therefore most plausibly reflects the dominance of cell-surface binding and extracellular sequestration over intracellular expulsion (Nies, 2003; Priyadarshanee & Das, 2021). Because the total metal content of the washed biomass was determined after acid digestion, the values reported here represent total cell-associated metal and do not, by themselves, distinguish surface-bound biosorption from intracellular bioaccumulation. Selective extraction or desorption protocols (for example, washing with a chelating agent such as ethylenediaminetetraacetic acid (EDTA) before digestion) would be required to partition these two fractions experimentally, and such protocols were not applied here. The dominance of surface binding and extracellular sequestration over intracellular accumulation is therefore inferred from the known efflux-driven reduction in intracellular metal reported elsewhere (Nies, 2003; Priyadarshanee & Das, 2021).

This relationship between MIC and biosorption was found to be most pronounced for lead. This observation is consistent with the inherent ability of lead to bind to cell-surface ligands and remain immobilized at the cell membrane, and it is plausible that the mechanisms underlying lead tolerance and lead sorption are closely linked (Gul et al., 2024). However, the correlational design of this study cannot establish causation, and alternative explanations, such as co-selection of independently arising resistance and sorption traits under the same contamination pressure, cannot be excluded.

Zinc is a trace element, an essential micronutrient that cells tightly control, especially at low concentrations. While the relationship with zinc remains significant, it is not as strong. This could be due to the way cells manage zinc, allowing for a distinction between tolerance to this heavy metal and its mere absorption. This approach is most reliable for metals such as lead, where the strong connection is observed, but it should be applied with more caution to actively regulated nutrients like zinc (Choi et al., 2024). The strong relationship between MIC and absorption observed in the multi-metal mixture is particularly significant and indicates that resistant strains retain their sorption advantage even under the severe conditions typical of real industrial contamination, rather than only in simplified assays focused on single heavy metals.

Although the mixed-metal system showed a lower absolute ceiling than the single-metal system and higher combined ionic toxicity relative to the single-metal systems, MIC-biosorption correlation was strongest for the multi-metal mixture (r = 0.895). This can be interpreted as a consequence of the more stringent and uniform selective pressure imposed by simultaneous multi-metal exposure. Under combined stress, only isolates with broad, overlapping resistance systems, such as efflux pumps with a broad substrate range, extracellular polymeric substances, and general stress-response regulons, can survive at all, whereas strains with narrower, metal-specific resistance adaptation are more likely to be eliminated regardless of their nominal single-metal MIC. As a result, the surviving population becomes more phenotypically coherent, which can sharpen the observed relationship between resistance and biosorption even when the tolerated concentrations are lower. A comparable sharpening of resistance-associated

phenotypes under combined multi-stress selection has been reported for biofilm-forming, multimetal-resistant bacteria (Harrison et al., 2007). In other words, the lower MIC ceiling reflects greater combined toxicity, while the stronger correlation suggests that multi-metal exposure more effectively filters for strains in which resistance and biosorption are tightly coupled.

The top strains identified in both resistance and sorption distributions were found to be Pseudomonas aeruginosa and Bacillus sp. (four *pseudomonas sp.,* two of which were identified as *pseudomonas aeruginosa*, *two* as other *pseudomonas spp.,* and one as *Bacillus subtilis*) based on 16S rRNA sequencing. These two species are frequently recognized as high-capacity metal biosorbents, with well-established resistance characteristics (Marzan et al., 2017; Nies, 2003). Recent studies have identified native strains from soils with long-term heavy metal contamination, indicating that these adapted isolates are not only tolerant to harsh environmental conditions but are also effective in removing heavy metals (Firincă et al., 2024; Tabak et al., 2005). The association between a high MIC and greater sorption observed in this study indicates a useful screening method and potential candidates for future applications.

3.1 Comparison with Previous Studies and Novel Contribution

Several previous studies have examined either the metal resistance or the biosorption capacity of native bacteria from contaminated sites, but few have explicitly quantified the statistical relationship between the two properties across multiple metals within the same isolate collection. Prior work on Curtobacterium sp. FM01 (Masoumi et al., 2016) and on tannery-effluent isolates (Marzan et al., 2017) reported high resistance or high sorption separately, without testing whether resistance level predicts sorption capacity; likewise, reviews of microbial biosorbents (Ayangbenro & Babalola, 2017; Priyadarshanee & Das, 2021) have generally treated tolerance and sequestration as related but distinct properties rather than statistically linked ones. By contrast, the present study explicitly tests and quantifies this relationship using twenty native isolates from an industrially contaminated lead-zinc district and four metal systems (lead, zinc, cadmium and their mixture), and reports regression equations, coefficients of determination and confidence intervals (Table 4) that allow the strength of the MIC-biosorption relationship to be judged quantitatively rather than only qualitatively. This quantitative, multi-metal screening framework, together with the demonstration that the relationship holds and is even strongest under the more industrially realistic multi-metal mixture, represents the principal novel contribution of this work relative to previous single-metal or descriptive studies.

3.2 Limitations of the Study

This study has some constraints that define its scope rather than undermine its findings. The isolate set was limited to twenty culturable strains, which was appropriate for a focused screening design and allowed direct comparison across metal systems. Although this sample size is sufficient for detecting strong trends, a larger isolate collection would improve the precision of regression estimates and broaden the ecological generalizability of the results. The batch-based laboratory design provided standardized conditions that minimized environmental variability and strengthened internal comparability among isolates. This approach was well suited to the study's primary objective, which was to test whether MIC can serve as a practical screening criterion for identifying promising biosorbents. However, because the experiments were conducted under controlled laboratory conditions, the findings may not fully capture the complexity of soil microcosms or field settings, where microbial interactions, nutrient gradients, and matrix heterogeneity can influence both resistance and sorption behavior. Although the digestion-based assay measured total biomass-associated metal rather than separating surface adsorption from intracellular accumulation, it offered a robust and reproducible measure of total metal association with the biomass. This was appropriate for the screening purpose of the study, since the goal was to identify strains with high overall metal-binding capacity.

Future work should incorporate selective extraction or desorption methods, as well as mechanistic assays such as efflux activity assays and extracellular polymeric substance analysis, to resolve the relative contributions of adsorption and accumulation. Taxonomic identification was performed using 16S rRNA sequencing for only the five best-performing isolates. This decision was consistent with the study's focus on screening high-performing candidates, but it means that the remaining isolates are identified only at the genus level based on phenotypic tests. Overall, these constraints do not invalidate the observed MIC-biosorption relationship. Rather, they define the conditions under which the findings are most appropriately interpreted and applied. In this sense, the study provides a controlled and transparent screening framework that can be extended in future work to broader collections and more detailed mechanistic analyses.

Table 4. Regression of biosorption on $\log_2$-transformed MIC, with coefficients of determination and 95% confidence intervals for Pearson r (n = 20 per metal)

Metal	Regression equation (Sorption vs $\log_2$ MIC)	R^2 ($\log_2$ model)	r^2 (Pearson)	95% CI for r	ANOVA F (p)
Lead (Pb)	Sorption = -4636.8 + 527.5 × $\log_2$(MIC)	0.54	0.77	0.714-0.951	22.3 (3.6×10^{-6})
Zinc (Zn)	Sorption = -1509.8 + 175.1 × $\log_2$(MIC)	0.52	0.56	0.457-0.894	11.0 (8.5×10^{-4})
Cadmium (Cd)	Sorption = -823.8 + 124.3 × $\log_2$(MIC)	0.32	0.61	0.516-0.909	20.3 (4.1×10^{-5})
Multi-metal	Sorption = -254.5 + 60.2 × $\log_2$(MIC)	0.56	0.80	0.748-0.958	28.3 (1.2×10^{-6})

* Note: R^2 ($\log_2$ model) is the coefficient of determination of the ordinary least-squares regression of sorption on $\log_2$-transformed MIC; r^2 (Pearson) is the square of the Pearson correlation coefficient computed on untransformed MIC and is reported in Table 3. The two values differ because they describe different models ($\log_2$-linear regression vs. linear correlation on raw MIC) fitted to the same underlying data. 95% confidence intervals for r were obtained by Fisher's z-transformation.

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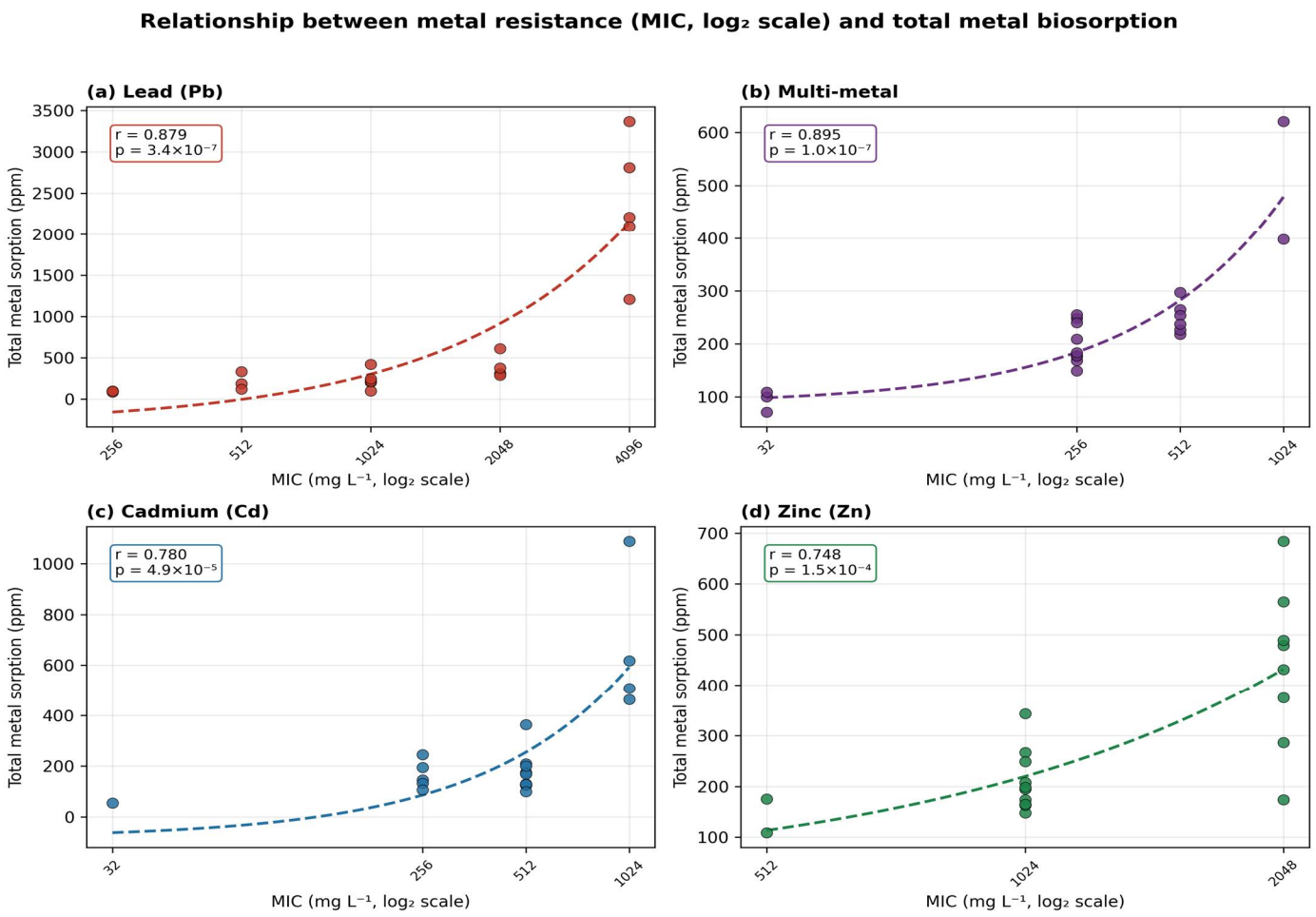

Figure 1. The Relationship between metal resistance (MIC) and biosorption illustrates a positive trend for lead and multi-metal mixtures

4. Conclusion

In native bacteria isolated from the heavily contaminated lead-zinc soils of Zanjan, metal resistance and biosorption capacity for lead, zinc, cadmium, and their mixture, were strongly and positively related. The correlation coefficients ranged from 0.75 to 0.90, indicating a consistently strong positive association across metals. According to the results, the coupling was tightest for lead and for the competitive multi-metal system, and the most resistant strains, *Pseudomonas aeruginosa* and *Bacillus sp.* were also the most efficient biosorbents. These results indicate that resistance and sequestration are strongly correlated and are likely co-selected or co-expressed rather than necessarily sharing a single mechanism. In addition, this suggests MIC as a simple screening criterion to pre-select promising biosorbents from contaminated environments. Future research should be focused on validating this relationship in larger isolate collections and on analyzing the equilibrium isotherm and biosorption kinetics with established models (Freundlich, 1906; Ho & McKay, 1999; Langmuir, 1918). Moreover, it is important to link this interaction with the specific molecular factors that regulate heavy metal homeostasis. This will improve the application of native strains in practical bioremediation studies and projects (Gholami Deljomanesh et al., 2025; Igiri et al., 2018).

Authors' Contributions

Seyed Alireza Fathi: Carried out the soil sampling; The laboratory work; The data collection; and prepared the first draft of the manuscript. **Hasan Karimzadegan:** Supervised the project; Shaped its overall design; and revised the manuscript critically. **Fatemeh Shariati Feizabadi:** Contributed to the experimental design and to the statistical analysis of the results. **Leila Ooshaksaraie:** Advised on the environmental interpretation of the findings and reviewed the manuscript. All authors read the final version and approved it for submission.

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Conflicts of Interest

The authors declare that they have no competing interests.

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Ethical Considerations

All ethical considerations were observed throughout the conduct of this research. This study is derived from a Ph.D. dissertation approved by the Department of Environment, La.C. Islamic Azad University, Lahijan, Iran. Research code: 20274810320087980000301630753 81.

Using Artificial Intelligence

Artificial intelligence–based tools were used only to improve the English language and translation of the manuscript.

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