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Research Article

Evaluating the efficiency of assisted treatments in the phytoremediation of multi metal-contaminated soil using Response Surface Methodology optimization approach

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ABSTRACT- Phytoremediation is a green, economic, and efficient tool for remediating soils contaminated with heavy metals. The present experiment was conducted to investigate the effect of growth regulator (GR) treatments (gibberellic acid (GA_3) and indole acetic acid (IAA)), biological treatments (*Thiobacillus* and *Bacillus subtilis*), and chemical treatments (nitrilotriacetic acid (NTA) and ethylene glycol tetraacetic acid (EGTA)) on the phytoremediation efficiency of zinc (Zn), cadmium (Cd), and nickel (Ni) using response surface methodology (RSM). The optimal chelate, bacterial strain, and GR for maximizing the translocation factor (TF) of Cd were NTA, *Thiobacillus*, and GA3, respectively. For the phytoremediation of Zn, the optimal combination was NTA, *Thiobacillus*, and IAA. For Ni, the highest TF was achieved without chelate, while using *Thiobacillus* and GA_3. Based on the TF values, the mechanisms governing metal uptake and translocation in maize appear to differ among metals and treatments. For example, the application of NTA increased the TF of Zn to above 1, indicating enhanced phytoextraction, whereas EGTA promoted Zn retention in the roots, reflecting phytostabilization. In the case of Cd, GA_3 enhanced metal accumulation in the shoots. Regarding Zn, IAA was more effective in promoting shoot accumulation. These findings demonstrate that individual metals exhibit distinct responses even within the same plant and under identical experimental conditions, highlighting the need for metal-specific assisted treatments to optimize phytoremediation.

INTRODUCTION

Heavy metals (HMs), including zinc (Zn), cadmium (Cd), and nickel (Ni), are classified as potentially toxic elements (PTEs) because of their persistence and non-biodegradable nature in the environment (Panda et al., 2025). Consequently, the accumulation of HMs in soils poses a serious threat to human health and reduces agricultural productivity (Bayuo et al., 2024). Therefore, effective remediation strategies are needed for HM-contaminated agricultural soils. Phytoremediation is an environmentally friendly, cost-effective, sustainable, and efficient technology that uses plants to remove HMs from contaminated soils. However, its practical application is constrained by two major limitations: the phytotoxic effects of high metal concentrations and the inherently slow rate of remediation (Bayuo et al., 2024). To overcome these limitations, various assisted phytoremediation approaches have been developed to improve remediation efficiency. These approaches include chemical, biological, physical, and integrated treatments. Among these strategies, chelator-assisted phytoremediation has received considerable attention for the remediation of HM-contaminated soils. Chelators are compounds that increase metal solubility by forming stable complexes with metal ions, thereby facilitating their uptake and translocation within plants during phytoremediation (Yin et al., 2024). Compared with non-biodegradable chelators, biodegradable alternatives offer significant environmental advantages since they are readily decomposed by microorganisms into environmentally benign products, thereby minimizing their ecological risks. The effectiveness of a chelator depends primarily on its optimal application rate and biodegradability. For example, Mohrazi et al. (2023) investigated ethylene glycol tetraacetic acid (EGTA)-assisted phytoremediation of Ni, Cd, and Pb using maize and sunflower and reported that the optimal chelator dosage depended on the plant species, target metal, and accompanying assisted treatments. Similarly, Keshavarz et al. (2021) demonstrated that the application of nitrilotriacetic acid (NTA) increased both ryegrass biomass and HM uptake. In contrast, Guo et al. (2020) reported that ethylene diamine tetraacetic acid (EDTA) reduced shoot and root biomass by 50% and 43%, respectively. Furthermore, EDTA inhibited soil respiration and metal uptake while enhancing the photosynthetic rate and increasing glutathione and phytochelatin (PC) contents. Because different assisted treatments target distinct chemical and physiological processes, combining chelators

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with beneficial microorganisms and plant growth regulators has the potential to produce synergistic improvements in phytoremediation efficiency. Plant growth-promoting bacteria (PGPB) have received considerable attention as biological agents for enhancing phytoremediation efficiency (Houida et al., 2024). These naturally occurring bacteria, which inhabit a wide range of environments including the rhizosphere, promote plant growth and reduce pollutant levels through mechanisms such as nitrogen fixation, phosphate solubilization, and phytohormone production (Fernández et al., 2023). In addition, certain bacterial strains can transform pollutants into less toxic or environmentally benign forms. Collectively, these mechanisms substantially enhance the efficiency and effectiveness of phytoremediation (Zhu et al., 2023).

Studies have demonstrated that the application of *Bacillus subtilis (B.S)* enhances plant growth, heavy metal uptake, and biosorption. Liu et al. (2018) reported that applying *B. subtilis* at a rate of 1 g pot^{-1} significantly improved plant growth and stress tolerance under HM-contaminated conditions. Similarly, Mohrazi et al. (2023) found that inoculation with *B. subtilis* increased Zn and Cd accumulation in maize and sunflower while simultaneously promoting plant growth. *Thiobacillus* spp. are chemotrophic sulfur-oxidizing bacteria that play an important role in enhancing phytoremediation (Ravichandra et al., 2007). By oxidizing elemental sulfur to sulfate, these bacteria not only increase sulfate availability for plant uptake and promote plant growth but also decrease soil pH, thereby enhancing the solubility and mobility of HMs. The increased mobility of HMs facilitates their uptake by plants, ultimately improving phytoremediation efficiency. Furthermore, the combined use of plants and microorganisms has been shown to be more effective for remediating HM-contaminated soils than the use of either alone (Agnello et al., 2016). For example, Keshavarz et al. (2021) reported that inoculation with *Thiobacillus* improved ryegrass growth and increased the uptake of Zn, Ni, and Cd, thereby enhancing phytoremediation efficiency. In addition to chelators and PGPB, plant growth regulators (PGRs) have recently attracted attention as promising agents for enhancing phytoremediation. Among them, Indole-3-acetic acid (IAA), Gibberellic acids (GAs), cytokinins, and salicylic acid have been identified as effective regulators for improving plant performance under HM stress. Their effectiveness depends on application rate, environmental conditions, and the physiological status of the plant. Further to promoting plant growth and biomass production, PGRs alleviate the toxic effects of HMs by improving plant tolerance to the environmental stress (Rostami and Azhdarpoor, 2019; Rostami et al., 2016; Shafigh et al., 2017). However, despite the growing body of research, comprehensive studies evaluating the combined effects of different assisted treatments and identifying their optimal application rates remain limited. Response Surface Methodology (RSM) has recently emerged as a powerful statistical tool for optimizing phytoremediation. By evaluating multiple factors and their interactions, RSM identifies optimal treatment combinations while reducing the number of experimental runs, thereby saving both time and resources.

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2 \quad \text{Eq. (1)}$$

Novelty statement

The present experiment was conducted to investigate the effects of growth stimulant treatments (GA and IAA), biological treatments (*Thiobacillus* and *B.S*), and chemical treatments (NTA and EGTA) on the phytoremediation efficiency of Zn, Cd, and Ni by maize plants grown on multi-metal-polluted soil.

MATERIALS AND METHODS

Experimental design

To achieve the objectives of this study, both individual and combined applications of three groups of assisted treatments were evaluated: the chelating agents EGTA and NTA, the bacterial inoculants *B.S* and *Thiobacillus*, and the plant growth regulators IAA and GA_3 for that purpose. These treatments were assessed for their effects on the phytoremediation performance of maize grown in soil contaminated with Zn, Ni, and Cd. The experiment was designed using RSM implemented in Design-Expert software. The treatment ranges, factor levels, and experimental design are presented in Table 1. The relationship between the independent variables and the response was described using the second-order polynomial model shown in Eq. (1): In this equation, y represents the response (dependent variable), whereas A (chelate), B (bacteria), and C (plant growth regulator) denote the independent variables. The terms AB, AC, and BC represent the two-factor interaction effects, while A^2, B^2, and C^2 are the quadratic terms. The coefficient b_0 is the intercept, b_1, b_2, and b_3 are the linear regression coefficients, b_{12}, b_{13}, and b_{23} are the interaction coefficients, and b_{11}, b_{22}, and b_{33} are the quadratic coefficients of the model.

Planting, maintenance, and harvesting

After the experimental design, soil samples were collected from the School of Agriculture, Shiraz university, Shiraz, Iran. The soil was air-dried, sieved, and thoroughly homogenized before equal amounts were placed into each experimental pot. The soil was then artificially contaminated with zinc, nickel, and cadmium using their sulfate salts at a concentration of 100 mg kg^{-1} (100 ppm). This contamination level was selected based on previous studies (Razmi et al., 2021; Keshavarz et al., 2021; Mohrazi et al., 2023; Paridar et al., 2023; Gol Soltani et al., 2024; Zarrabi et al., 2024; Mansourpour et al., 2024). After contamination, the soils were incubated for one month to allow equilibration of the added metals. Following the incubation period, fertilizer was applied according to the results of the soil analysis, and bacterial inoculation was performed at a rate of 1 g kg^{-1} soil in accordance with the experimental design. Four maize seeds were sown in each pot, and the seedlings were thinned to two plants per pot two weeks after emergence. The PGRs of GA_3 and IAA were applied as foliar sprays at a concentration of 1 mM during the fourth week after sowing. Foliar applications were repeated at two-week intervals over a four-week period to maintain treatment effectiveness. This application protocol was adapted from previous studies (Shafigh et al., 2016; Cabello-Conejo et al., 2013; Cassina et al., 2013; Hadi et al., 2013; Hadi et al., 2010). Chelating agents were applied to the soil at a rate of 1 mmol kg^{-1} during the final two weeks of the experiment. Plants were harvested two weeks after the final chelate application. Root and shoot samples were oven-dried to determine dry matter yield, after which the concentrations of Zn, Ni, and Cd were determined using atomic absorption spectrophotometry (AAS; Shimadzu AA-670G, Japan).

Calculations and statistical analysis

RSM was implemented using Design-Expert software to optimize the experimental conditions and evaluate the effects of the studied factors and their interactions. Compared with a full factorial design, which would require 81 experimental runs, the Box–Behnken design (BBD) reduced the number of runs to only 15, thereby considerably decreasing the time, labor, and resources required for experimentation. In this study, RSM was employed to model the relationships between the independent variables and the measured responses, design the experimental matrix, and identify the optimal treatment combinations that maximized phytoremediation efficiency. The BBD is a second-order response surface design based on three levels for each experimental factor and is widely used for optimization studies involving multiple variables. The bioconcentration factor (BCF) and translocation factor (TF), two important indicators of a plant's capacity to accumulate and translocate heavy metals, were calculated using Eq. (2) and Eq. (3), respectively.

$$TF = \frac{Concentration\ HMs\ in\ shoot(mg\ kg-1)}{Concentration\ HMs\ in\ root(mg\ kg-1)} \quad \text{Eq. (2)}$$

$$BCF = \frac{Concentration\ HMs\ in\ shoot(mg\ kg-1)}{Concentration\ HMs\ in\ soil(mg\ kg-1)} \quad \text{Eq. (3)}$$

RESULTS AND DISCUSSION

Prediction of growth parameters and phytoremediation criteria

The effects of the three independent variables, chelate, bacteria, and PGR, on the growth parameters of maize are presented in Table 2. According to the RSM based on the BBD, the experimental data were fitted to second-order polynomial models using multiple regression analysis. The final regression equations, obtained after eliminating statistically non-significant terms ($P \geq 0.05$), are presented in Eq. (4) and Eq. (5).

Shoot dry weight = + 2.78 + 1.96chelate + 0.77 bacteria + 0.71 * chelate * bacteria + 0.77 * chelate * GR + 1.67 $bacteria^2$ Eq. (4)

Root dry weight = +0.37 + 0.25chelate + 0.43bacteria + 0.98 chelate * bacteria + 0.41 $bacteria^2$ Eq. (5)

Eq. (4) and Eq. (5) indicate that the shoot and root dry weights of maize were significantly influenced by chelate and bacterial treatments. In contrast, the PGR alone had no significant effect on either parameter; however, its interaction with the chelate treatment significantly increased both shoot and root dry weights. The highest shoot and root dry weights were observed in Run 4 (EGTA, *Thiobacillus*, and GA_3), whereas the lowest values occurred in Run 7 (no chelate, *B.S*, and IAA) (Table 3). Notably, the RSM models also predicted the highest and lowest values for these parameters in Runs 4 and 7, respectively. These findings suggest that chelate application promoted plant growth and enhanced the effectiveness of bacterial inoculation. Among the three factors evaluated, chelate exerted the greatest influence on maize growth, while the interaction between chelate and PGR had a stronger effect than the other interaction terms. This indicates that the response of maize to each treatment depended on its interaction with the other treatments. ANOVA further demonstrated that the fitted models were significant ($P \leq 0.05$), whereas the lack of fit was not significant ($P \geq 0.05$). In addition, all model terms retained in the final equations were statistically significant ($P \leq 0.05$). These results confirm that the developed models adequately describe the effects of the treatments and can reliably predict the growth parameters of maize under the experimental conditions.

Table 1. Range and level of treatments in present study

Level			Factor
0	**1**	**2**	
Control	ethylene glycol tetraacetic acid	nitrilotriacetic acid	Type of chelate (A)
Control	*Bacillus subtilis*	*Thiobacillus*	Type of bacteria (B)
Control	Gibberellic acids	Indole-3-acetic acid	Type of growth regulators (GR) (C)

RUN	A	B	C
1	1	0	0
2	1	0	2
3	1	2	0
4	1	2	2
5	0	1	0
6	0	1	2
7	2	1	0
8	2	1	2
9	0	0	1
10	0	2	1
11	2	0	1
12	2	2	1
13	1	1	1
14	1	1	1
15	1	1	1

TF and BCF are two metrics that indicate the n of heavy metals (HMs) between the roots and shoots of plants (Yang et al., 2020). The nonlinear equations derived from the response surface methodology (RSM) model for the TF and BCF of Zn, Cd, and Ni are presented below (Eq. (6-11)).

TF of Cd = 0.11 + 0.7 chelate + 0.16 bacteria - 0.10 C - 0.017 chelate * bacteria - 0.22 chelate * GR 0.18 bacteria * GR + 0.39 $chelate^2$ + 0.43 $bacteria^2$ - 0.036 GR^2 Eq. (6)

TF of Zn = 0.66 + 0.080 chelate + 0.32 bacteria + 0.050 GR + 0.084 chelate * bacteria - 0.11 bacteria * GR + 0.5 $chelate^2$ + 0.46 $bacteria^2$ + $0.081GR^2$ Eq. (7)

TF of Ni = 0.63 - 0.14 chelate - 0.080 GR - 0.11chelate * bacteria + 0.63 $chelate^2$ + 0.2 $bacteria^2$ - 0.27 GR^2 Eq. (8)

BCF of Cd = 0.38 + 0.013 bacteria + 0.038 chelate * bacteria + 0.01 chelate * GR + 0.019bacteria *GR - 0.054 $chelate^2$ - 0.14 $bacteria^2$ -0.089 GR^2 Eq. (9)

BCF of Zn = 0.42- 0.21 chelate + 0.12 bacteria + 0.21 GR + 0.32 chelate * bacteria -0.63 GR*bacteria + 0.05 $chelate^2$ - 0.176 $bacteria^2$ -0.030 GR^2 Eq. (10)

BCF of Ni = 0.18 + 0.012 chelate - 0.016 GR -0.017 chelate * GR - 0.029 $bacteria^2$ Eq. (11)

Chelates and bacterial inoculation significantly increased the TF of cadmium and zinc. In contrast, the effects of the PGR varied among metals, reducing the TF and BCF values of Cd and Ni while increasing those of Zn. These findings indicate that the effectiveness of PGRs is strongly dependent on the type of metal. Among the evaluated factors, bacterial treatment had the greatest influence on the TF and BCF values of Zn and Cd, whereas chelate application exerted the strongest effect on those of Ni. Chelating agents enhance the solubility and bioavailability of heavy metals, thereby facilitating their uptake by plants and improving phytoremediation efficiency (Razmi et al., 2021). In the present study, Zn and Ni exhibited greater translocation from the roots to the shoots, whereas Cd was primarily retained in the roots. These results suggest that maize has potential for the phytoextraction of Zn and Ni and the phytostabilization of Cd. The BBD matrix for the three independent variables, together with the observed and predicted TF values, is presented in the table 3 and Fig. 1. The TF values further demonstrated that the translocation behavior of maize varied according to the applied treatments. For Zn, application of the NTA chelate increased the TF to values greater than 1, indicating enhanced phytoextraction, but EGTA helped with Zn retention in the roots, indicating phytostabilization. In the case of Cd, GA_3 enhanced root-to-shoot translocation, resulting in greater accumulation in the shoots. Similarly, IAA promoted Zn translocation and its accumulation in shoots.

Interaction effects of variables using 3D response surface plots, growth parameters, and phytoremediation criteria

The effects of the individual factors (chelate, bacteria, and PGR) and their interactions on the measured responses (growth parameters and phytoremediation indices) are illustrated in the three-dimensional response surface plots (Fig. 2, Fig. 3, and Fig. 4). As shown in Fig. 2, the response surface plots describing the interaction between bacteria and chelate at a fixed PGR level (IAA) indicate that both *Thiobacillus* and *B.S* improved maize growth compared with the control treatment. Moreover, the interaction between bacteria and chelate significantly increased both shoot and root dry weights. Although this interaction produced greater growth than bacterial inoculation alone, chelate application had an even stronger positive effect when applied individually. In contrast, the interaction between chelate and either bacteria or PGR reduced the TF of Cd, whereas the individual application of these treatments increased Cd translocation. Conversely, the interactions between bacteria and chelate and between bacteria and PGR enhanced the root-to-shoot translocation of Zn. However, the interaction between bacteria and chelate reduced the TF of Ni. These findings demonstrate that the effects of the treatments on heavy metal translocation depend not only on the type of treatment but also on the specific metal being remediated.

Response surface optimization

Growth parameters and phytoremediation criteria

The numerically optimized values for the growth parameters and phytoremediation indices, obtained using RSM, are presented in Table 4. Analysis of the experimental data using Design-Expert 10.0 identified the optimal conditions for maximizing shoot dry weight (11.51 g pot^{-1}) and root dry weight (1.37 g pot^{-1}) as NTA, *Thiobacillus*, and GA_3 for the chelate, bacterial inoculant, and PGR, respectively. These conditions differed from those that maximized the phytoremediation indices. Under the optimized conditions, the predicted shoot and root dry weights closely agreed with the observed values, indicating good model accuracy. The close agreement between the predicted and experimental values, together with the low prediction error, demonstrates that the RSM model adequately predicted both the growth parameters and the phytoremediation indices evaluated in this study. The optimal conditions for maximizing the TF of Cd were NTA, *Thiobacillus*, and GA_3 in order of appearance. For Zn, the optimal combination was NTA, *Thiobacillus*, and IAA, whereas for Ni, the highest TF was achieved without a chelate, using GA_3 and *Thiobacillus*. These findings indicate that the optimal assisted treatment depends on the target metal, highlighting the need for metal-specific strategies to maximize phytoremediation efficiency.

Table 2. Box–Behnken design matrix for the three independent variables with the actual and predicted responses for dry matter of maize

Run	Shoot		Root	
	Actual	Predicted	Actual	Predicted
1	4.04	3.77	0.44	0.43
2	4.55	4.49	0.57	0.53
3	3.47	3.52	0.45	0.48
4	6.77	7.03	0.97	0.97
5	2.67	2.63	0.21	0.17
6	3.66	3.41	0.42	0.41
7	0.78	1.02	0.18	0.18
8	4.45	4.48	0.5	0.53
9	3.91	4.21	0.52	0.56
10	4.7	4.68	0.77	0.77
11	3.25	3.26	0.59	0.59
12	5.4	5.09	0.94	0.89
13	2.03	2.78	0.33	0.37
14	3.02	2.78	0.37	0.37
15	3.3	2.78	0.41	0.37

Table 3. Box–Behnken design matrix for the three independent variables with the actual and predicted responses for Translocation Factor values of maize

Run	TF values of maize					
	Zn		Cd		Ni	
	Actual	Predict	Actual	Predict	Actual	Predict
1	1.024	0.9048	0.4936	0.4984	1.2184	1.244
2	1.014	1.0432	0.752	0.7568	1.2184	1.1936
3	1.462	1.3832	0.9472	0.9424	1.3704	1.3952
4	1.719	1.6976	1.1496	1.1448	1.0192	0.9936
5	0.910	0.9232	0.3336	0.34	1.0656	0.9992
6	0.995	0.996	0.7496	0.7552	0.7208	0.7032
7	0.959	0.9528	0.3584	0.3528	0.7832	0.8
8	1.146	1.1328	0.4056	0.3992	0.5776	0.644
9	0.606	0.572	0.2432	0.2328	0.5144	0.556
10	1.252	1.2672	0.9376	0.936	0.5064	0.5488
11	0.844	0.828	0.3472	0.3488	0.4864	0.444
12	1.138	1.1728	0.4664	0.4768	0.4448	0.4032
13	0.496	0.5272	0.1888	0.1824	0.5464	0.5016
14	0.511	0.5272	0.1776	0.1824	0.492	0.5016
15	0.574	0.5272	0.18	0.1824	0.4672	0.5016

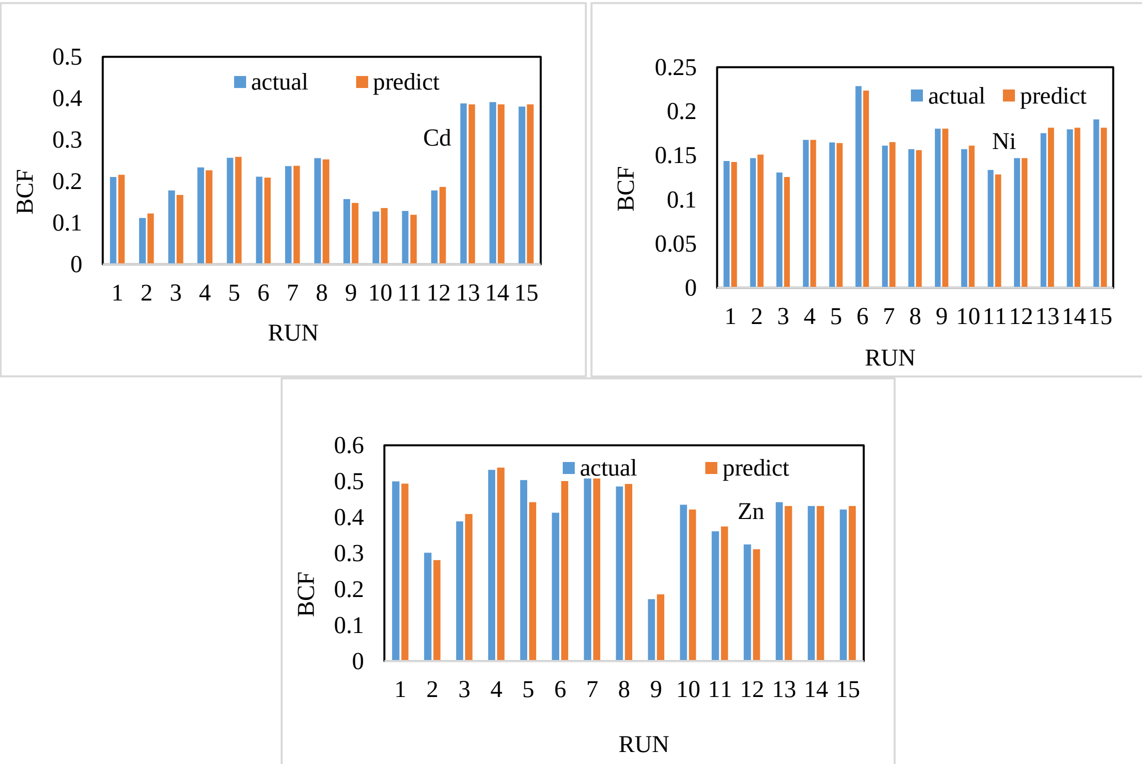

Fig. 1. Box–Behnken design matrix for the three independent variables with the actual and predicted responses for bio concentration factor.

Table 4. Response surface methodology result an optimum condition for growth parameters and phytoremediation criteria at desirability level of 1

Factors	A: Type of chelate	B: Type of bacteria	C: Type of growth regulator	Optimum value
Maize	**In range**	**In range**	**In range**	**Maximum**
Shoot dry weight	nitrilotriacetic acid	*Thiobacillus*	Gibberellic acid	7.34
Root dry weight	nitrilotriacetic acid	*Thiobacillus*	Gibberellic acid	1.37
Translocation Factor $_{Cd}$	nitrilotriacetic acid	*Thiobacillus*	Gibberellic acid	1.2
Translocation Factor $_{Zn}$	nitrilotriacetic acid	*Thiobacillus*	Indole-3-acetic acid	1.72
Translocation Factor $_{Ni}$	0	*Thiobacillus*	Gibberellic acid	1.39
bioconcentration factor $_{Cd}$	ethylene glycol tetraacetic acid	*Bacillus subtilis*	Gibberellic acid	0.29
bioconcentration factor $_{Zn}$	0	*Bacillus subtilis*	Indole-3-acetic acid	0.42
bioconcentration factor $_{Ni}$	nitrilotriacetic acid	*Bacillus subtilis*	0	0.17

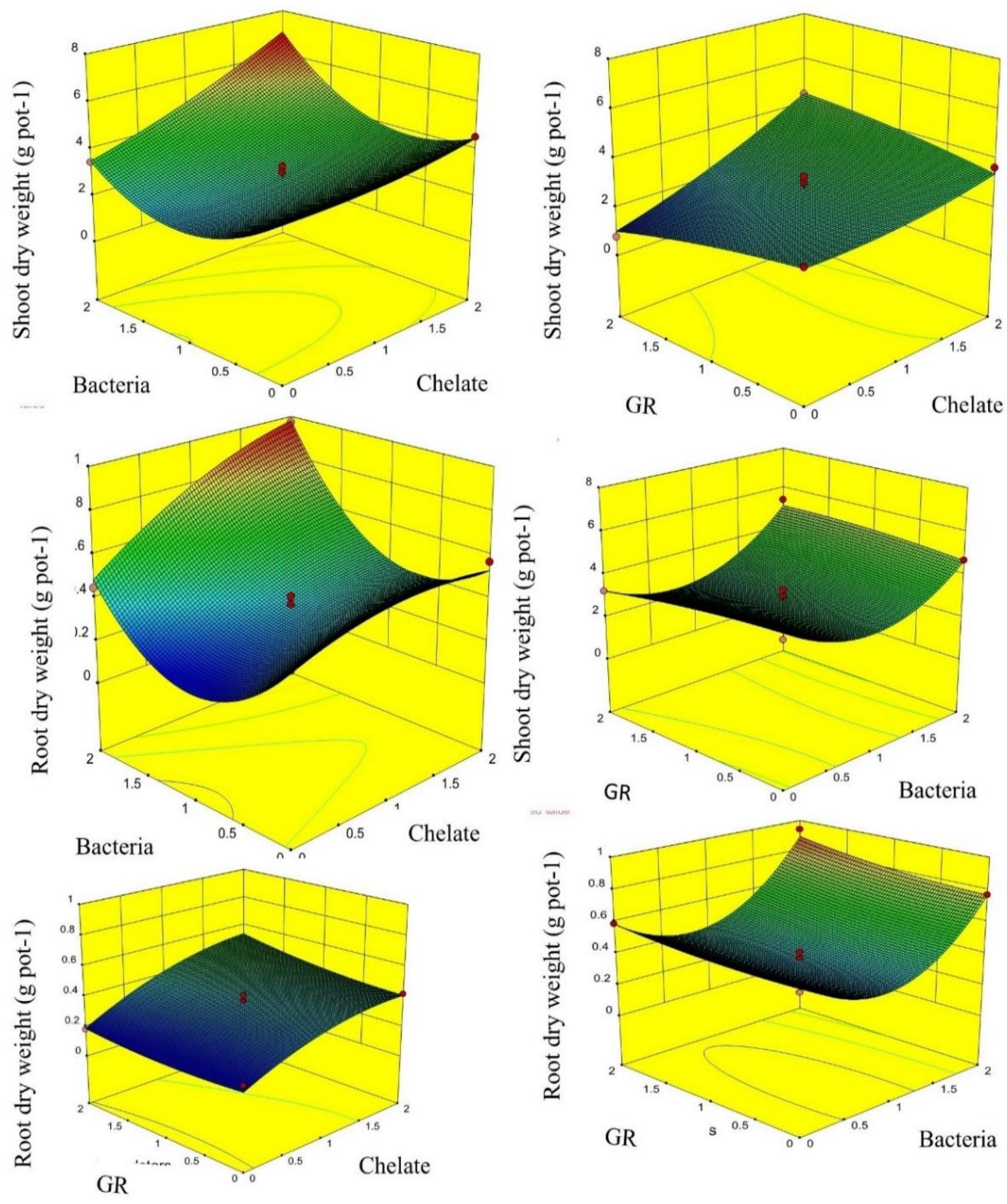

Fig. 2. 3D response surface plots of shoot and root dry weights.

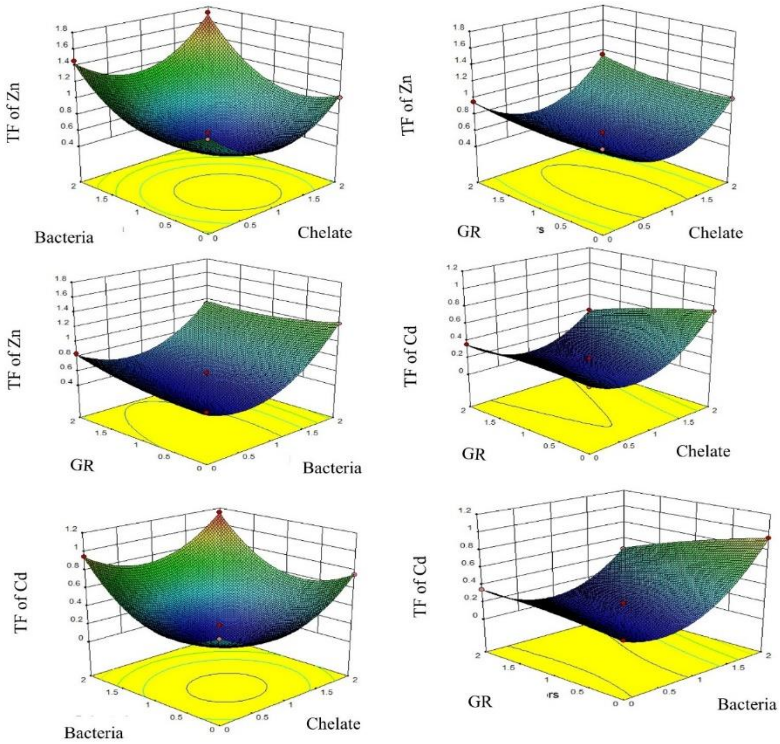

Fig. 3. 3D response surface plots of Translocation Factor of Zn and Cd.

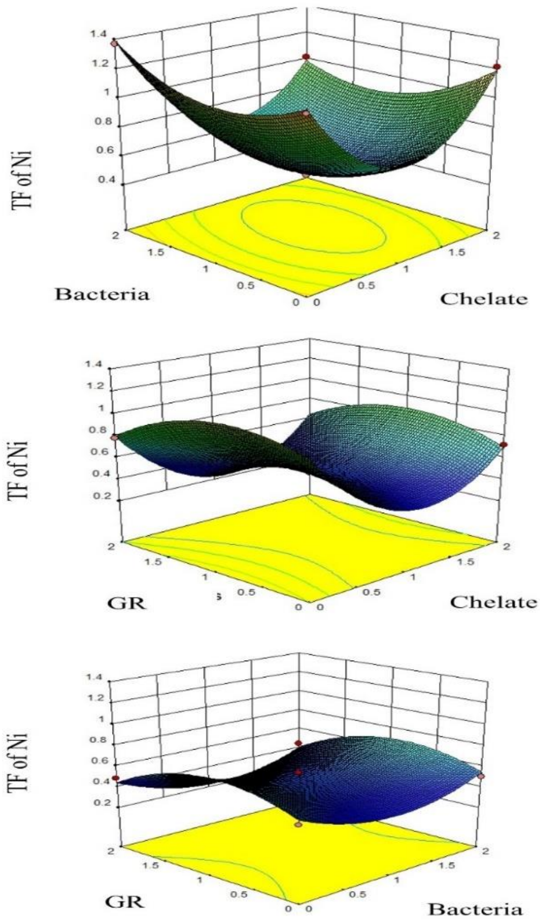

Fig. 4. 3D response plots for Translocation Factor of Ni.

Table 5. ANOVA for response surface quadratic model

Source model	Shoot dry weight	Root dry weight	Translocation Factor of Zn	Translocation Factor of Ni	Translocation Factor of Cd
	***P*-value**	***P*-value**	***P*-value**	***P*-value**	***P*-value**
	Prob > F	**Prob ≥ F**	**Prob ≥ F**	**Prob ≥ F**	**Prob ≥ F**
	0.0084	0.0008	< 0.0001	0.0005	< 0.0001
A: Chelate	0.0022	0.0005	0.0082	0.0050	< 0.0001
B: Bacteria	0.0262	0.0011	< 0.0001	0.6306	< 0.0001
C: growth regulator	0.5033	0.1106	0.0457	0.0416	< 0.0001
AB	0.0436	0.0143	0.0274	0.0467	0.0424
AC	0.0496	0.3466	0.2919	0.3456	< 0.0001
BC	0.2478	0.3885	0.0097	0.8116	< 0.0001
A^2	0.3986	0.0519	< 0.0001	< 0.0001	< 0.0001
B^2	0.0016	0.0001	< 0.0001	0.0021	< 0.0001
C^2	0.6199	0.3642	0.0338	0.0016	0.0031
Lack of fit	0.8009	0.3219	0.4944	0.2119	0.1885

CONCLUSION

According to the results of the present study, the highest dry weight of both shoots and roots of maize was recorded in run 4, which included EGTA as the chelate, *Thiobacillus* as the bacterial agent, and gibberellic acid as the plant growth regulator. Conversely, the lowest growth parameters were observed in run 7, which had no chelate, *B.S* as the bacterial agent, and IAA as the plant growth regulator. Notably, the maximum and minimum values for shoot and root dry weights were also predicted by the RSM models in runs 4 and 7, respectively. The results indicated that the application of chelates significantly enhanced plant growth and the effectiveness of bacterial treatments. Specifically, the assisted treatments increased the dry weight of roots and shoots by 50% and 70%, respectively, compared to the control treatment. The optimal conditions for maximizing the TF of cadmium involved using NTA as the chelate, *Thiobacillus* as the bacterial agent, and gibberellic acid as the plant growth regulator.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Ava Mohrazi and Reza Ghasemi-Fasaei; Methodology: Ava Mohrazi and Reza Ghasemi-Fasaei; Software: Ava Mohrazi; Validation: Reza Ghasemi-Fasaei; Formal analysis: Reza Ghasemi-Fasaei; Investigation: Ava Mohrazi and Reza Ghasemi-Fasaei; Resources: Ava Mohrazi and Reza Ghasemi-Fasaei; Writing—review and editing: Ava Mohrazi; Visualization: Reza Ghasemi-Fasaei; Supervision: Reza Ghasemi-Fasaei; Project administration: Reza Ghasemi-Fasaei.

DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

ETHICAL STATEMENT

Not applicable.

DATA AVAILIBILITY

The data that support the findings of this study are available on request from the corresponding author

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