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Original Research Article

Growth and cadmium and nickel uptake of maize (*Zea mays* L.) in a cadmium and nickel co-contaminated soil and phytoremediation efficiency using ethylenediamine tetraacetic acid

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ABSTRACT

Background: Anthropogenic activities release cadmium (Cd), nickel (Ni), and other heavy metals into soil. *Zea mays* can clean up contaminated soils, but little is known about how Cd and Ni co-contamination stress affects ethylenediamine tetraacetic acid (EDTA)-based phytoextraction and phytoremediation, hence this study was conducted.
Methods: The experiment involved nine treatment levels (0, 5, 10, 15, and 20 mg kg^{-1} Cd and 0, 15, 30, 45, and 60 mg kg^{-1} Ni), grouped into three categories: CT as the control group, P as Cd + Ni only, and CAP as Cd + Ni + EDTA (n=3). The treatments used $(CH_3COO)_2Cd{\bullet}2H_2O$ and $NiSO_4$ as the source of Cd and Ni, respectively, and EDTA was applied at two rates (0 and 0.5 g/kg). After the experimental period, measurements were taken for shoot length, biomass, and metal concentrations in both the roots and shoots using established procedures.
Results: The concentrations of metals in plants' roots and shoots increased as the concentrations in soil increased, but shoot length, biomass, bioconcentration factor (BCF), and translocation factor (TF) values decreased with increasing soil metal content. The application of EDTA increased metal uptake but led to greater root and shoot biomass loss. Generally, TF values for Cd and Ni were less than 1 but most of the BCF values were greater than 1.
Conclusions: The study found that phytostabilization is the main mechanism for phytoremediation of Cd-Ni-co-contaminated soils with *Zea mays*, with EDTA addition enhancing metal accumulation and reducing biomass yield.

Keywords: Phytotoxicity, Heavy metals, Translocation factor, Bioconcentration factor and biomass

INTRODUCTION

Heavy metals (HMs) are major causes of contamination on Earth because of anthropogenic activities such as mining, smelting, fertilizers and pesticide application, and sewage sludge.[1] HMs (such as Cd, Pb, Cr, As, Hg, Ni, Cu, and Zn) are a major threat to agriculture because they build up in soils and are absorbed by plants.[2] If not absorbed by plants or leached, these metals are resistant to deterioration and can linger in the soil for years.[3,4] While plants do not need certain metals, like cadmium (Cd), even at low concentrations, others, like nickel (Ni), are required in small amounts but can be toxic to plants in higher concentrations.

High concentrations of heavy metals impede plant growth and biomass production by inducing chlorosis, water and nutritional imbalance, reduced activity of Calvin cycle enzymes, CO_2 deficiency, protein denaturation, and possible plant death.[1,4] Tipu et al linked Nickel (Ni) to reduced growth, decreased photosynthetic pigments, and lower levels of phosphorus (P) and sodium (Na) in maize (*Zea mays* L.).[5] Maize plants treated with Cd exhibited reduced growth, substantial ultrastructural damage, lower biomass production, and decreased photosynthesis.[6] HMs can compete with nutrients for transport within the plant. Cd can hinder the absorption and use of important minerals such as iron, zinc, calcium, and magnesium by competing for the same transporters or binding sites. This might result in a notable decrease in growth and biomass production.[1]

This emphasizes the seriousness of heavy metal contamination and the pressing requirement for an effective solution to minimize the related dangers to crops and soils.[7]

Phytoremediation is an environmentally friendly and cost-effective way to break down, remove, or confine soil and water pollutants. This biological method uses green plants and related microorganisms to reduce metal contaminants in the environment through rhizo-degradation, phytodegradation, phytoextraction, rhizofiltration, phytovolatilization, and phytostabilization.[8] Metal bioavailability for plant absorption depends on soil pH, organic matter, competitive cations, and calcareousness, which limits traditional phytoremediation techniques.[9,10] Low soil metal bioavailability inhibits phytoremediation, although soil additives like metal chelates can improve heavy metal uptake by plants.[11] Tipu et al studied how EDTA affected phytoremediation efficacy and maize plant biochemical and physiological reactions in nickel-polluted soil.[5] They discovered that mixing EDTA with Ni boosted maize Ni accumulation to 50.23 mg/plant, compared to 40.62 mg/plant for Ni, 27.75 mg/plant for EDTA, and 15.51 mg/plant for the control group.

Oil drilling, industrial emissions, and other human activities have raised heavy metal levels in Nigeria's Niger Delta soil and water.[12,13] This has caused the loss of arable farmlands in several locations, hurting the local economy. Only a few remediation studies in the region have used plants and chelating chemicals to solve the problem.[14,15] Maize phytoremediation research in Cd and Ni-co-contaminated soils is rare, especially with chelators, and little is known about whether these additional methods can help plant species remediate these soils. This study investigated the remediation of Cd and Ni-co-contaminated soil using maize plants (*Z. mays*) and the influence of EDTA on phytoremediation performance. We also studied *Z. mays* shoot length, biomass, and Cd and Ni uptake and translocation in Cd-Ni co-contaminated soil.

METHODS

Study site

The study was done from March to May 2023 at Delta State University (DELSU) Campus 3 in Abraka, Nigeria. The coordinates for Campus 3 of DELSU are Latitude: 6° 30’ 59.99" N and Longitude: 3° 23’ 5.99" E. Abraka has an altitude of approximately 29 m and is about 49 km northeast of Warri. Warm, muggy summers and mild winters characterize Abraka's tropical wet (March–October) and dry (November–February) climate.

Preparation and characterization of soil

The soil sample was obtained from a depth of 0–20 cm near a coal-tarred road next to an agricultural field at Delta State University, Abraka, Nigeria. The sample was dried by exposure to air. A 2 mm diameter stainless steel screen was utilized as a sieve for the dried soil. The test soil underwent analysis for certain chemical qualities using standard methods. For instance, phosphorus and total nitrogen were analyzed using the Bray 1 method and the Kjeldahl measuring method, respectively.[16,17] Soil organic carbon (OC) was assessed using the Walkley-Black method.[18] The hydrometer technique was employed to ascertain the soil texture.[19] pH was determined using a pH meter in soil-to-water extracts of 1:1 w/v (10 g dry weight of soil in 10 mL distilled water).

Analysis of cadmium and nickel in soil

Determination of metal in soil was done following standard method described by Paunović et al: 0.5 g of air-dried and sieved soil sample was digested in the aqua regia with 15 ml of HCl (36%) and 5 ml of HNO_3 (65%) for 5 hours, at 80 °C, by microwave digestion (Speedwave XPERT, Microwave digestion system, BERGHOF, Germany).[20] After digestion, the sample was filtered through Whatman no. 42 filter paper and diluted with ultra-pure water to mark and the extracts were finally analyzed for Cd and Ni concentration using an atomic absorption spectrophotometry (Perkin Elmer 700, Boston, MA, USA) based on the standard methods.

Pot experiments

The pot experiment with *Zea mays* was carried out in a rain-protected net house for 60 days, under natural sunlight. Cadmium acetate $(CH_3COO)_2Cd{\bullet}2H_2O$ and Nickel sulphate ($NiSO_4$) were used as the source of Cd and Ni respectively. Different levels of $(CH_3COO)_2Cd{\bullet}2H_2O$ and $NiSO_4$ were added to 150 ml distilled water (DW), and then mixed with 2 kg soil to be placed in plastic pots (polythene bags). The study involved nine (9) treatment levels of Cd and Ni in pots, grouped into three treatment categories: CT, P, and CAP treatments. P was Cd + Ni only, while CAP was Cd + Ni + EDTA. The control (CT) was not spike-treated. In other words: CT=Cd_0 + Ni_0, P1=Cd_1 + Ni_1, P2=Cd_2 + Ni_2, P3=Cd_3 + Ni_3, P4=Cd_4 + Ni_4, CAP1=Cd_1 + Ni_1 + EDTA, CAP2=Cd_2 + Ni_2 + EDTA, CAP3=Cd_3 + Ni_3 + EDTA, and CAP4=Cd_4 + Ni_4 + EDTA (n=3). The levels of Cd were Cd_0=0, Cd_1=5, Cd_2=10, Cd_3=15 and Cd_4=20 mg kg^{-1}, respectively. For Nickel it was Ni_0=control, Ni_1=15, Ni_2=30, Ni_3=45 and Ni_4=60 mg kg^{-1}, respectively. The pots were incubated in a dark room with tap water for 14 days to retain 75% field capacity. After 10 days of growth in uncontaminated soil without fertilizer input, three *Zea mays* seedlings per pot were transferred to the test pots. Pots were irrigated twice or thrice a week and monitored regularly. The experimental design included the treatment of pots with EDTA twice (19d and 41d after transplant) during the experimental period. EDTA solution, 10% (w/v): 10 g sodium salt of EDTA (as Na_2-EDTA salt) was dissolved in 100 ml of distilled water. 20 ml of this solution was diluted to 100 ml with distilled water and added into each pot to get an amendment concentration of 0.5 g EDTA/kg of soil.[21] The plants were given the solution at the end of the lighting period so they had time to get used to it. At the end of the experimental period, the plants were meticulously harvested, their roots and shoots separated, their height measured, and then dried in an oven at 70 °C for 24 hours to obtain biomass. The concentrations of metals were then analyzed.

Analysis of cadmium and nickel in plant roots and shoots

Following the grinding of plant materials, 0.5 g portions of sieved plant matter were digested with a mixture of hydrochloric acid and hydrogen nitrate in a ratio of 3:1 by volume (HCl/HNO_3, 3:1, v/v). The concentrations of cadmium and nickel in the digests were determined through the use of atomic adsorption spectroscopy (AAS).[22]

Translocation and bioconcentration factors

The study evaluated *Z. mays*' capacity to absorb and transport Cd and Ni from soil using bioconcentration and translocation factors, as defined by Takarina and Pin and Usman et al.[23,24]

$$BCF_{shoot} = \frac{C_{shoot}}{C_{soil}} \quad \ldots. \quad \text{Equation 1}$$

$$BCF_{roots} = \frac{C_{root}}{C_{soil}} \quad \ldots. \quad \text{Equation 2}$$

$$TF = \frac{C_{shoot}}{C_{root}} \; or \; = \frac{BCF_{shoot}}{BCF_{root}} \ldots. \quad \text{Equation 3}$$

Where C_{shoot}, C_{root} and C_{soil} are the metal concentrations (mg/kg) in the shoot, root, and soil, respectively.

BCF values below one (BCF <1) suggest higher soil heavy metal concentrations than plant uptake, whereas BCF >1 implies higher crop or plant uptake. Plants with TF >1 have trace metals in their shoot biomass, while those with TF <1 have them in their root biomass.

Statistical analysis

All statistical analyses were performed using IBM statistical package for the social sciences (SPSS) statistics 27 and Microsoft excel 2016. All data reported are averaged values of three independent replicates (mean±SD; n=3). Data were statistically evaluated by one-way analysis of variance (ANOVA) and significantly different means were assessed by Tukey's HSD post-hoc test. Significance level was considered at $p<0.05$.

RESULTS

Selected physicochemical properties of the test soil

Table 1 shows the properties of the soil used for this experiment. The soil pH was 6.75 indicating a slightly acidic soil condition. Soil organic carbon was 0.19 %, total nitrogen and available phosphorous were 0.42, and 24.88 mg/kg, respectively. The soil texture was sandy and the background concentration of cadmium and nickel in the unspiked test soil was 6.95 and 20.80 mg/kg, respectively.

Table 1: Selected characteristics of the test soil used for the experiment.

Parameter (unit)	Value
pH (1.0:2.5 soil-water)	6.75
TOC (%)	0.19
Total nitrogen (mg/kg)	0.42
Available phosphorus (mg/kg)	24.88
Heavy metals (mg/kg)	
Total Cd	6.95
Total Ni	20.80
Particle size distribution (%)	
Sand	93.73
Silt	4.33
Clay	1.94

Effects of HMs and EDTA exposure on shoot length of Z. mays

Figure 1 displays the average shoot length of Zea mays in the treatment soils at the end of the experiment. The control plants had longer shoots than the Cd + Ni or Cd + Ni + EDTA plants. The plant height decreased as the metal content in the soil increased steadily. Even though the adverse impacts of Cd + Ni increased with dosage, the addition of EDTA resulted in amplified absorption of the metals.

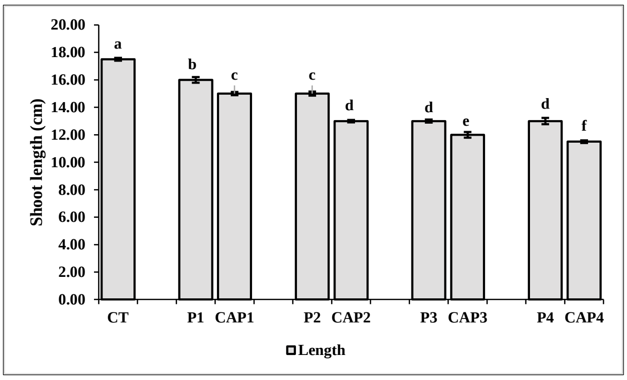

Figure 1: The effects of Cd + Ni and Cd + Ni + EDTA on *Zea mays* shoot length in soil. Mean values without a common letter are significantly different at p<0.05 (mean±SD; n=3).

Effects of HMs and EDTA exposure on biomass of Z. mays

CT plants had larger dry weights than all the other treatment plants: CT >Cd + Ni alone >Cd + Ni + EDTA alone (Figure 2). The lowest dry weights were found in Cd, Ni, and EDTA-contaminated roots and shoots. Generally, plant biomass decreased with increasing soil metal content. EDTA boosted HM absorption but also significantly diminished root and shoot biomass.

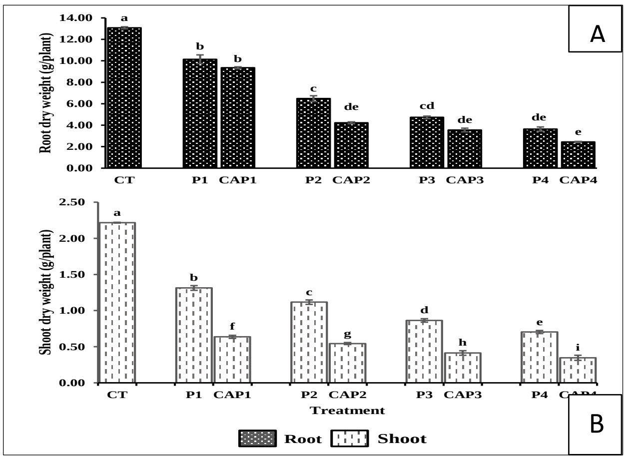

Figure 2 (A and B): Effect of Cd + Ni and Cd + Ni + EDTA on the dry biomass of *Zea mays* grown in soil. Mean values without a common letter are significantly different at p<0.05 (mean±SD; n=3).

Cadmium and nickel uptake and accumulation in Z. mays tissues

Cd and Ni distribution in *Z. mays* roots and shoots at the end of the experimental period are shown in Figures 3 and 4. All contaminated growth media plants had higher root-Cd and root-Ni levels than control plants. Cd and Ni were highest in Cd + Ni + EDTA-contaminated plants, followed by Cd + Ni alone and control plants. In like manner, contamination with Cd + Ni alone and Cd + Ni + EDTA considerably affected shoot-Cd and shoot-Ni contents (Figures 3 and 4). Cd + Ni + EDTA-contaminated soil exhibited the highest shoot-Cd and Ni concentration, followed by Cd + Ni alone. Plants with the lowest shoot Cd and Ni were controls. As soil cadmium and nickel levels increased, so did *Z. mays* root and shoot-Cd and Ni concentrations. In addition, the use of EDTA further increased HM absorption.

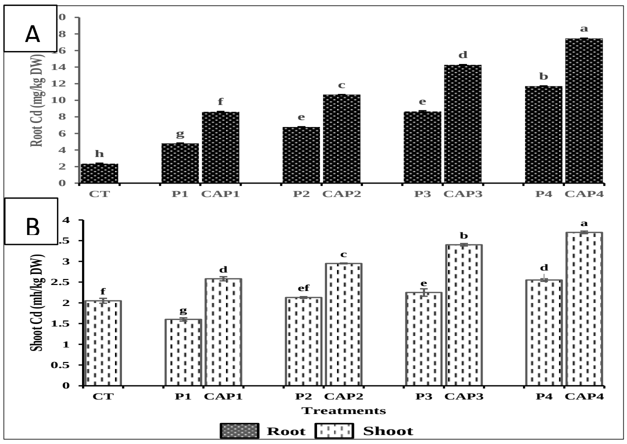

Figure 3 (A and B): Cadmium concentration in *Zea mays* roots and shoots grown in soil treated with Cd + Ni or Cd + Ni + EDTA. Mean values without a common letter are significantly different at p<0.05 (mean±SD; n=3).

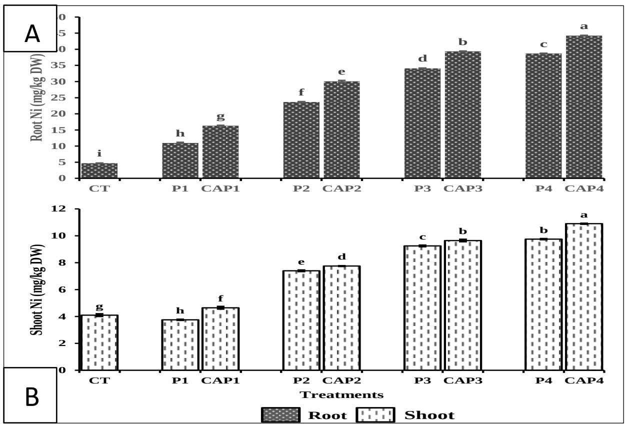

Figure 4 (A and B): Nickel concentration in roots and shoots of *Zea mays* grown in soil treated with Cd + Ni and Cd + Ni + EDTA. Mean values without a common letter are significantly different at p<0.05 (mean±SD; n=3).

The efficiency of phytoextraction of Cd and Ni

Bioconcentration factors (BCF) and translocation factors (TF) were used to examine how soil-Cd and Ni levels affect *Z. mays* phytoextraction efficiency (Figures 5 and 6). Root BCF ranged from 0.79 to 3.15 for Cd and 0.97 to 4.41 for Ni. The shoot-Cd and Ni-BCF values were 0.17–0.95 and 0.27–1.26, respectively. Also, adding EDTA to polluted soil increased *Z. mays*' ability to absorb Cd and Ni. The CAP1 treatment had the highest root-and-shoot BCF values. The root BCF values for Cd and Ni in CAP1 treatments were 3.15 and 4.41, while the shoots had 0.95 and 1.26, respectively. As soil Cd and Ni concentrations increased, root and shoot BCF and TF levels decreased. The highest TF value (0.87) was found in the CT (control) treatment and P4 and CAP4 had the lowest.

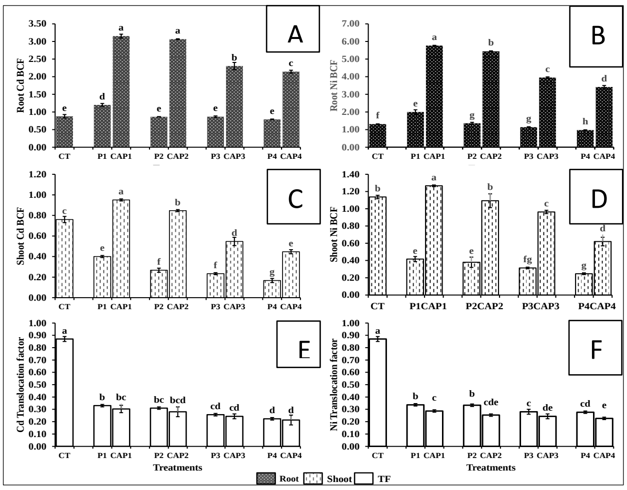

Figure 5 (A-F): Cadmium and nickel bioconcentration and translocation factors in root and shoot of Zea mays cultivated in Cd + Ni and Cd + Ni + EDTA soil. Mean values without a common letter are significantly different at p<0.05 (mean±SD; n=3).

DISCUSSION

This type of tropical soil environment, characterized by low levels of native nitrogen and organic carbon, is classified as having low fertility. The growth of *Z. mays* can be assessed through reliable indicators such as shoot length and biomass (dry weight) when exposed to heavy metal toxicity.[25] In the current study, treatment with high concentrations of Cd + Ni alone or in combination with EDTA resulted in a significant decrease in *Z. mays* shoot length compared to non-contaminated treatment. This reduction in plant growth due to heavy metal toxicity, particularly Cd and Ni, is commonly associated with the disruption of essential physiological processes in plants, including nutrient uptake, photosynthesis, and enzyme activity. Studies by Tipu et al and Liu et al have also reported similar findings regarding the negative impact of heavy metals on maize growth.[6,26]

Effects of HMs and EDTA exposure on biomass of Z. mays

A notable decline in the dry root biomass of Z. mays was observed upon exposure to Cd + Ni either alone or in conjunction with EDTA. The decrease ranged from 22.40% to 72.17.0% and 28.44% to 81.35%, respectively. These outcomes align with prior research conducted by Tipu et al, which similarly documented a reduction in maize root and shoot dry weight following treatment with Ni alone or in combination with EDTA.[5] The establishment of plant biomass may encounter hindrances under heavy metal stress conditions, leading to disruptions in metabolic processes, diminished photosynthetic activity, and impaired uptake of vital soil nutrients.[4,25] Phytoremediation plants are sought after for their rapid growth, extensive root system development, remarkable biomass accumulation, and capacity to sequester substantial quantities of heavy metals.[8,22] The findings of our investigation suggest that *Z. mays* exhibit rapid growth and a certain degree of resilience, indicating promise for implementation in phytoremediation efforts.

Cadmium and nickel uptake and accumulation in Z. mays tissues

Our research revealed that the P4 and CAP treatments exhibited the highest metal concentrations in both plant roots and shoots. Specifically, the levels of Cd in *Z. mays* roots and shoots significantly increased by 4.98 and 1.24 times, respectively, in the P4 treatment compared to the control group ($p<0.05$). with the addition of EDTA (i.e., CAP 4 treatment), the increment was even more pronounced, with 7.43 and 1.80 times increase in roots and shoots, respectively, compared to the control group ($p<0.05$). This finding is consistent with prior studies. For example, Tipu et al found that the combination of EDTA and Ni was the most effective treatment for enhancing Ni accumulation in maize, surpassing Ni alone, EDTA alone, and the control group.[5] Similarly, Anwar et al observed that the application of EDTA with Cd was the most effective treatment for increasing Cd accumulation in maize compared to other treatments.[27] The trend of increased metal concentrations in plant tissues with rising soil metal concentrations as found in our study has been reported by other researchers as well.[27-29] The use of exogenous EDTA treatment in heavy metal-treated *B. juncea* seedlings has been suggested to enhance heavy metal bioavailability for phytoextraction.[30] Our study also demonstrated enhanced metal uptake in Z. mays tissues cultivated in soils treated with Cd, Ni and EDTA. Additionally, higher quantities of cadmium and nickel were detected in *Z. mays* roots compared to shoots across all treatments, indicating a preference for root accumulation. This observation aligns with previous research indicating that maize roots contain higher levels of certain metals (Zn, Cu, and Pb) compared to stems and leaves.[31] In conclusion, *Z. mays* shows promise as a plant species for absorbing and accumulating cadmium and nickel in plant tissues, particularly roots, with the potential for acceleration through the use of a metal chelator.

Phytoremediation efficiency

The study found that certain root BCF levels in soils treated with EDTA were higher than previously reported values by other researchers, but still lower than those reported by Hegedusová et al for Cd and by Abiya et al for Ni.[32,33] These differences may be due to various factors such as the specific treatments used for Cd and Ni, the type and concentration of chelating agents employed, and how maize cultivars respond to metal-induced stress.[32,34,35] The research also showed a significant accumulation of Ni in the shoots at certain soil co-contamination levels (5 and 15 mg/kg Cd and 15 and 30 mg/kg Ni, i.e., P1, P2, CAP1, and CAP2). Additionally, the study indicated that BCF values in shoots were generally lower than in roots, suggesting a natural limitation on the transport of Cd and Ni from roots to shoots, resulting in higher metal concentrations being retained in the roots. The data suggests that as soil Cd and Ni concentrations increase, the capacity for accumulation decreases (Figure 5). *Zea mays* was found to primarily use phytostabilization as a mechanism to remediate Cd and Ni, with BCF values exceeding 1 for most treatments and Translocation Factor (TF) values remaining below 1.[36] This aligns with previous research showing reduced TF values with higher metal concentrations, indicating a decreased ability of plants to translocate metals as soil metal levels increase. Overall, the study suggests that *Zea mays* can be considered a phytostabilizer plant species for Cd and Ni, with phytostabilization efficiency improving with higher levels of Cd and Ni in the soil.

CONCLUSION

The research investigated the potential of maize plants to help remediate soil contaminated with cadmium and nickel. It was discovered that as the levels of these metals in the soil increased, the roots and shoots of maize plants absorbed higher quantities. The addition of EDTA to the contaminated soil resulted in increased absorption of these metals by the maize plants. Despite the negative impact of cadmium and nickel on the growth and shoot length of maize plants, the study suggested that the presence of EDTA in soil could enhance phytoextraction, particularly for nickel. Furthermore, the study revealed that maize plants primarily remediate Cd-Ni-contaminated soils through phytostabilization, as shown by low translocation factors (TF <1) and higher concentrations of cadmium and nickel in the roots compared to the shoots in all treatments.

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