



Phytoremediation efficacy of *Nerium oleander* L. for removal of Cd, Ni, and Pb-contaminated soil

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Abstract

A pot experiment was conducted to assess the effectiveness of *Nerium oleander* L. in phytoremediation of cadmium (Cd), nickel (Ni), and lead (Pb)-contaminated soil. This evaluation included the application of ethylene diamine tetra-acetic acid (EDTA) and a nutrient solution containing NPK. The results revealed that the inclusion of EDTA and NPK, in combination with varying metal concentrations, significantly increased the dry weight of the plants. The results demonstrated that oleander, when aided by EDTA and NPK, exhibited a high capacity to efficiently absorb and accumulate Pb without any amendments. The addition of EDTA increased Pb concentrations in both aerial parts and roots (84.9 mg kg⁻¹ and 80.2 mg kg⁻¹, respectively). For Nickel, in the presence of NPK, the concentration in roots was 80.2 mg kg⁻¹. Cd accumulation was highest in the treatment with 100 mg kg⁻¹ EDTA, reaching 72.9 mg kg⁻¹ in the aerial parts. Particularly, NPK reduced Ni levels in aerial parts, while EDTA enhanced root metal uptake. The Translocation Factor (TF) was greater than 1 for Cd and Ni treatments, indicating oleander's remarkable ability to absorb and accumulate these heavy elements. The treatment involving Pb50+EDTA displayed the highest Bioaccumulation Factor (BAF) value at 1.7. Furthermore, for Pb50, Ni50 and Cd50, the NPK treatment exhibited the highest Bioconcentration Factor (BCF) values, ranging from 0.3 to 1.69. *Nerium oleander* L. exhibits promise for both Ni phytoextraction and Cd and Pb phytostabilization, suggesting its potential as an effective phytoremediation agent. Future research should focus on optimizing metal uptake conditions and investigating long-term plant health and soil impacts to refine sustainable strategies for mitigating metal pollution in contaminated environments.

Keywords: Enhanced phytoremediation; metal accumulation; soil contamination; phytoextraction; environmental remediation; heavy metal uptake

Introduction

Industrial development, urbanization, and population explosion in the last two decades have resulted in the generation of massive quantities of toxic wastes in many parts of the world. This hazardous waste includes a variety of organic and inorganic pollutants which pose serious threats to ecosystems (Chandra and Kumar, 2017). Heavy metals are the main inorganic contaminants and a considerable large

area of land is contaminated with them due to use of sludge or municipal compost, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, residues from metalliferous mines, and smelting industries. The most common metals/metalloids found at contaminated sites, are lead (Pb), chromium (Cr), arsenic (As), cadmium (Cd), zinc (Zn), copper (Cu), nickel (Ni), and mercury (Hg). Their presence in the soil can pose risks to humans and the ecosystem and inhibit the biodegradation of organic

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contaminants (Mitra *et al.*, 2022). Toxic metals are eliminated into the environment due to the increasing growth of the agricultural and chemical industries and the disposal of waste, fertilizers and pesticides. These pollutants enter the environment and affect biological and physiological functions, while other minerals accumulate in one or more organs in the human body, causing several serious diseases such as cancer (Briffa *et al.*, 2020).

Physical, chemical, and biological processes can be applied to remove these heavy metals from the contaminated environment. However, they all have some disadvantages in relation to their cost effectiveness or failure of in situ treatment. Heavy metals, and natural and even synthetic chemicals can be accumulated in plants, extracted, and valued. The technology is cost-effective and environmentally friendly. Phytoremediation is an innovative technology for the use of plants to remove or degrade toxic elements from the soil and water. Phytoremediation is a new technology for the removal of metals from soils. This technology can be subdivided into five distinct sub-technologies including phytodegradation, phytoextraction, phytostabilization, phytovolatilization and rhizofiltration. For inorganics, mechanisms can be further categorized into phytostabilization and phytoextraction (Babu *et al.*, 2021). However, the proper selection of plant species for phytoremediation is one of the key factors in phytoremediation research (Fischerova' *et al.*, 2005). There are several diverse groups of plant species according to their trace element accumulation capability. In particular, candidate plants to remove heavy metals must be resistant and able to translocate toxic metals (Fischerova' *et al.*, 2005).

The term "hyperaccumulator" has been first used by Baker and Brooks (1989) to describe plants that have the capability of taking up large amounts of metals. At present, about 400 species are known as hyperaccumulator plants. This species, therefore, has considerable potential for use Phytoremediation uptake model of heavy metals (Ibrahim and El Afandi, 2020). Phytoextraction, a common method of phytoremediation, includes uptake of the contaminant by plant roots with subsequent accumulation in the above ground plant parts, followed generally by harvest and then disposal of plant biomass (Jabeen *et al.*, 2009). There are two basic strategies for phytoremediation, the first is use of hyperaccumulator plants, and the second is chemical chelate-assisted phytoextraction (Jabeen *et al.*, 2009).

Assisted phytoextraction tolerates the use of high biomass producing species with low uptake ability of toxic elements into the roots and slow translocation to shoots as a difference to hyperaccumulators. The addition of chelator

agents to contaminated soils can increase the levels of the toxic metals in the soil solution and their consequent uptake and transport to shoot while this technique should be used carefully to minimize the risk of leaching of toxic pollutants. Chelating agents are added to solubilize metals that have a low solubility in the soil solution. However, the choice of the chelator could determine the success of this process (Jabeen *et al.*, 2009). More recently, EDTA (ethylenediaminetetraacetic acid) has been the most widely studied of the chelates used in studies of phytoextraction because it is highly effective in mobilizing metals in soils (Hart *et al.*, 2022). Although many earlier studies have suggested that the application of chelating agents has shown positive effects in increasing the solubility of heavy metals in soil and therefore in enhancing phytoextraction (Hart *et al.*, 2022).

Nerium oleander L. was chosen for the present study to examine its ability of metal extraction from soil. This plant is a perennial evergreen flowering shrub that belongs to the dogbane family and has previously been used to study the distribution of heavy metals in soils from different countries (Monaci *et al.*, 2020). *N. oleander* has been reported to be a useful biomonitor of lead and other heavy metals pollution in Mediterranean environments (Seridou *et al.*, 2022). In Algeria, few studies are underway on the phytoremediation of contaminated soil.

The purpose of this study was to investigate the potential of *N. oleander* L. for phytoremediation of Cd, Ni, and Pb contaminated soil and to determine the distribution of toxic metals in different plant parts. The study was conducted in the Mascara region, where metal-tolerant flora is poorly documented. The primary objective of the study was to evaluate the phytoremediation potential of *N. oleander* L. and contribute to the knowledge of metal-tolerant flora in the region. Additionally, the study examined the effect of different amendments, namely NPK as fertilizer and EDTA as a chelating agent on the uptake and accumulation of metals in different parts of the plant. The study aimed to understand the ability of *N. oleander* L. to tolerate and accumulate metals in the presence of different amendments, which could potentially increase its efficiency as a phytoremediation agent. This study aimed to contribute to the knowledge of metal-tolerant flora in the Mascara region and evaluate the phytoremediation potential of *N. oleander* L. The study also examined the effect of amendments on the uptake and accumulation of metals in different parts of the plant. By conducting this research, the study aimed to contribute to the development of effective and sustainable phytoremediation strategies for heavy metal-contaminated soils.



Materials and Methods

Soil sources and characterization

At the non-polluted site of Mustapha Stambouli University in Mascara, Northwest Algeria (35.414399, 0.126441), soil samples were taken from a depth of 0–20 cm. The samples were air-dried, crushed, and sieved to 2 mm, and then placed into plastic pots of 4.5 litters.

The soil samples were analysed for various parameters, including pH (measured in a 1:2.5 soil to water ratio), organic matter (determined using the loss on ignition method), and soil electrical conductivity (using a soil to solution ratio of 1:2.5). The hydrometer method was used to determine the soil texture, while X-ray fluorescence (CubiX XRF/PANalytical) was used to study the mineral content. Standard procedures of Wilke (2005) were followed to determine the soil physicochemical characteristics, which are listed in Table 1. Based on the analysis, the soil was classified as Loamy Fine Sand and considered as a control.

Plants were grown in pots for 90 days under greenhouse conditions in laboratory. After 01 week from transfer into pots, *N. oleander* L. plants were watered with different treatments of metals concentration and amendments. At the beginning of the experiment, pots were also divided into 3 experimental groups: The first group of pots contained contaminated soil without amendments Cd₅₀ (50 mg of Cd added to 1 kg of dry soil), Cd₁₀₀ (100 mg of Cd added to 1 kg of dry soil), Ni₅₀ (50 mg of Cd added to 1 kg of dry soil), Ni₁₀₀ (100 mg of Cd added to 1 kg of dry soil), Pb₅₀ (50 mg of Pb added to 1 kg of dry soil), and Pb₁₀₀ (100 mg of Pb added to 1 kg of dry soil). Amendments were applied for the second group with a nutrient solution containing commercial inorganic fertilizer NPK (15:15:15) at 2 g L⁻¹ (Al-Menaie *et al.*, 2012).

However, for the third group after 15 days of growth in pots with 0.265 g L⁻¹ of EDTA (0.708 mM) to approximate a concentration of 0.1 mg kg⁻¹ in soil applications. Each treatment was performed in triplicate. In order to compare with other experiments in literature, Cd, Ni, and Pb were applied as nitrates:

Table 1. Physical and chemical characteristics of soil

characteristic	%	Mineral	%
Clay	2.33	SiO ₂	83.87
Silt	16.14	Al ₂ O ₃	6.67
Sand	80.87	Fe ₂ O ₃	2.84
pH	8.03	K ₂ O	1.27
Organic matter	1.32	CaO	2.78
Conductivity(mS cm ⁻¹)	52.8	MgO	0.23
Cd (mg kg ⁻¹)	1.7		
Ni (mg kg ⁻¹)	0.8		
Pb (mg kg ⁻¹)	0.3		
Texture	Loamy Fine Sand		

Plant material

For this study, *N. oleander* L. was used as a candidate of phytoremediation in pot experiments, being planted in soils with different concentrations of Pb, Ni, and Cd. *N. oleander* L. seeds were collected in 2018 autumn from wild plants on nonpolluted farmland soil near Mascara city, Algeria. After a month of germination, seedlings with the first pair of true leaves were replanted in pots, at four seedlings per pot. The pots were polystyrene (22 cm diameter, 15 cm high) containing 3 kg of soil. During the experiment, pots were watered every 3 days with deionized water. Pots were kept for three months in laboratory conditions.

Experimental design

cadmium as Cd(NO₃)₂·4H₂O, nickel as Ni(NO₃)₂·6H₂O and lead as Pb(NO₃)₂. The pH of the metal solutions was adjusted to 6.0–6.5.

Analyses and Measurements

Plant growth measurements

The study analysed the growth parameters of *N. oleander* L., which encompassed plant height, root length, and dry biomass weight. The experiment was concluded in February to May 2020, after which the *N. oleander* L. plants were harvested. After washing the plants with deionized water, they were separated into their roots, stems, and leaves. Subsequently, each part of the plant was subjected to oven-drying at 70°C for three days and weighed using a precision balance to determine their dry biomass weight.



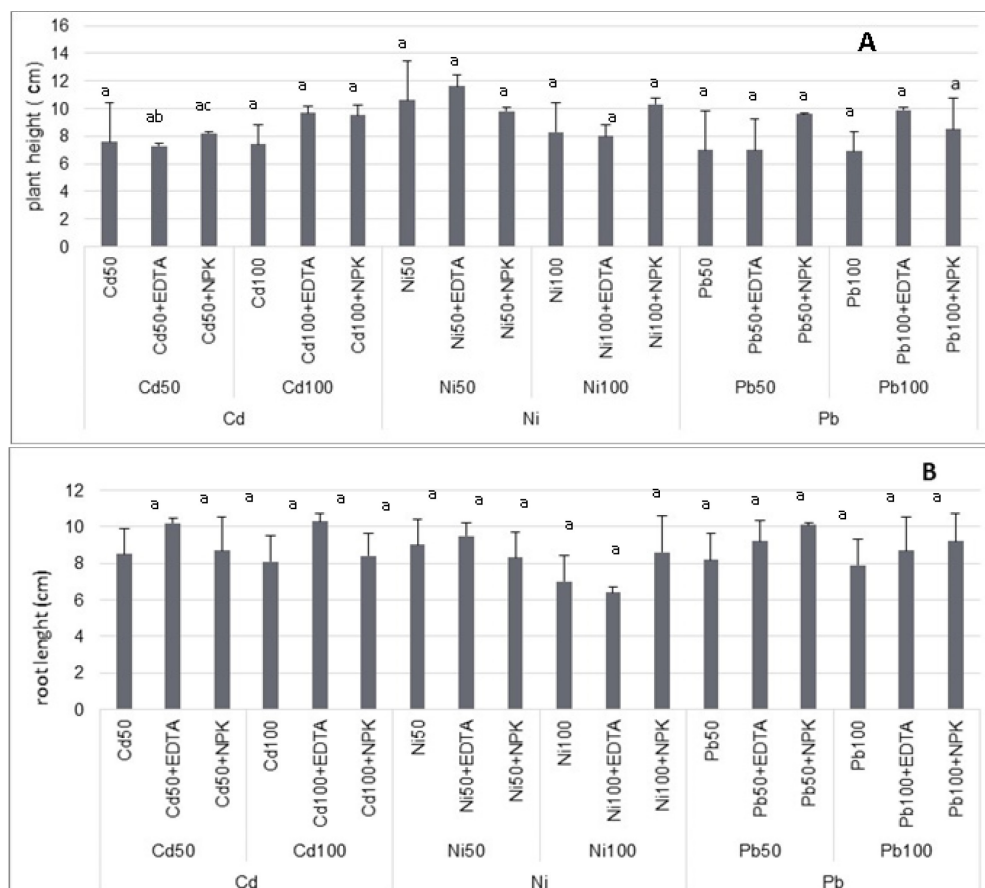


Figure 1: Effect of different treatments Cd, Ni, Pb, EDTA, and NPK on plant height (A), root length (B). Error bars represent sample standard deviation (n = 3). Different letters in the same group indicate a significant difference at $p \leq 0.05$ according to the Student's t test

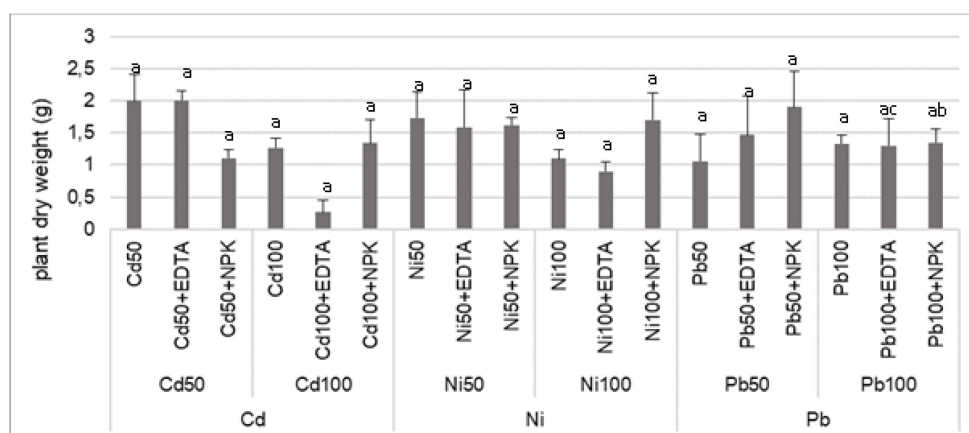


Figure 2. Treatment effects on plant dry weight. Error bars represent sample standard deviation (n = 3). Different letters in the same group indicate a significant difference at $p \leq 0.05$ according to the student's t test

Metal uptake, bioaccumulation, and translocation in plants

The plant parts were washed with deionized water to remove soil particles and dried in an oven at 105°C until a constant weight was achieved. Then, 1g of dried and crushed samples from each organ (aerial part and roots) were digested using a wet digestion method. The mineralization vessel was used to weigh the previously dried sample and 1 ml of concentrated sulfuric acid, 3 mL of concentrated nitric acid, and 3 ml of 30% hydrogen peroxide were added as reagents. The mixture was boiled according to the method outlined by Hoenig (2001). For the soil samples, they were dried in an oven at 70°C and then 1g of the sample was digested on a hot plate with 15 mL of nitric acid and 10 mL of hydrogen peroxide. The digests were then diluted with deionized distilled water to a volume of 50 mL, following the method of Hoenig (2001). The mineralized samples were then transferred quantitatively into a calibrated flask, completed to volume, and analysed using flame atomic absorption spectroscopy (FAAS) with a Shimadzu AA6600 instrument. The ability of plants to extract or stabilize metals in the soil can be determined using three parameters: BF (bioaccumulation factor), BCF (bioconcentration factor), and TF (translocation factor). Bioaccumulation factor (BF) was defined as the ratio of the metal concentration in the aboveground biomass to the metal concentration in the soil. BCF was defined as the ratio of metal concentration in plant roots to the metal in the soil, while the TF was defined as the ratio of metal concentration in the aerial part of the plant to the metal concentration in the plant's root.

Bioaccumulation factor (BF), bioconcentration factor (BCF), and translocation factor (TF) were calculated as follows (Siyar *et al.*, 2022).

$$BCF = C_{\text{root}}/C_{\text{soil}}; BF = C_{\text{aerial}}/C_{\text{soil}}; TF_{\text{Aerial}} = C_{\text{Aerial}}/C_{\text{root}}$$

Statistical Analysis

All the data obtained from this study were subjected to statistical analysis of variance (ANOVA) using Microsoft Office Excel 2010. Differences at the $p < 0.05$ level were considered significant using Student's t-test.

Results

Growth and biomass measurements

During 90 days of this experiment, all plants of all treatments showed no toxic symptoms. The data presented show the plant high (Figure 1) root length (Figure 2.) and dry biomass (Figure 3) of *N. oleander* L. plants growing in Ni and Cd contaminated soil at varied concentrations (0, 50 and 100 mg L⁻¹) and Pb (0, 50 and 100 mg L⁻¹) exposed to different treatments:

metal, metal + NPK and metal + EDTA. Results obtained showed that medium concentrations of Ni50+EDTA enhanced the growth (Figure1a) however, at higher concentrations in Pb50, the growth of the plant was reduced. Root length was influenced by Cd100+EDTA addition (Figure1b).

Treatments had significant effects on plant high ($p \leq 0.001$), it was significantly enhanced by Cd addition and highest result was obtained from 10 to 11 cm at the Ni +EDTA (Figure 1.a). *N. oleander* L. root length (Figure1b) was not statistically significant in all treatments ($p \geq 0.05$). Root length (Figure 2.b) was lowest in the Ni₁₀₀+EDTA treatments than those in the Cd and Pb treatments, although this effect was not statistically significant ($p \geq 0.05$). Results showed that the addition of EDTA and NPK in the presence of different concentrations of metals, enhanced dry weight (Figure 2) in the plants as compared to those cultivated in the absence of this chelator and fertilizer for 90 days.

Nonetheless, the highest values of dry weight occurred in Cd100+EDTA supplied treatment. Plant dry weight, Cd50 and Cd50+EDTA were 2g, and 2.1 g, respectively were various in order 0.26 g and 0.9 g in EDTA treatments with 100 mg kg⁻¹ of Ni and Cd. However, the results indicated that there was significant reduction of dry weight biomass of *N. oleander* L. plants with increased Pb concentration in EDTA and NPK treatments.

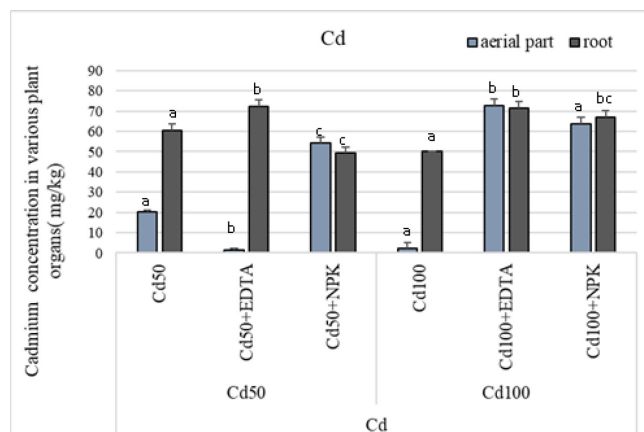


Figure 3. Cadmium concentrations (mg kg⁻¹ dry weight) in different parts of *Nerium oleander* L. plants grown in the Cd, Cd + NPK and Cd + EDTA

The accumulation of metals in various plant parts

The results of concentration in different plant parts grown in contaminated and chelated soil are presented in (Figure 3) for Cd (Figure 4) for Ni and (Figure 5) for Pb.



The maximum concentration in roots was 89.7 mg kg⁻¹ at Pb100 supplied content (Figure 5). However, the minimum concentration was at Cd100 (2.3 mg kg⁻¹) in aerial part (Figure 3). For the treatments without additions, the metal was not statically significant. Overall, the total concentrations of Pb in plants was greater than Ni (Figure 4).

Although, metal content decreased by increasing the metal concentration in soil, the accumulation pattern in various plant organs was as follows:

Pb>Cd>Ni for various treatments.

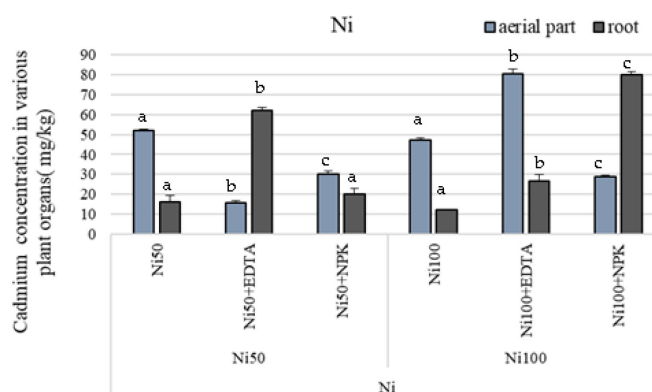


Figure 4. Nickel concentrations (mg kg⁻¹ dry weight) in different parts of *Nerium oleander* L. plants grown in the NiNi+NPK, and Ni+EDTA amended soil

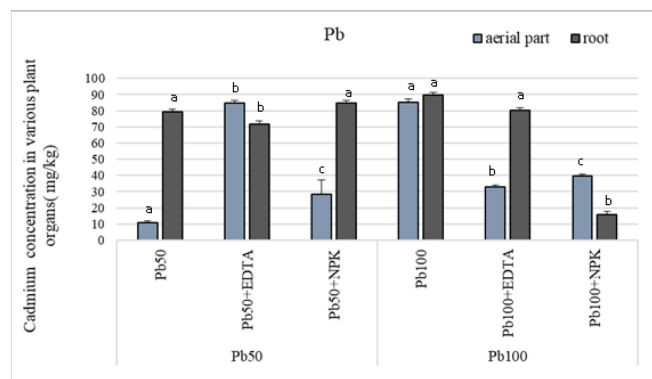


Figure 5. Lead concentrations (mg kg⁻¹ dry weight) in different parts of *Nerium oleander* L. plants grown in the Pb, Pb+NPK and Pb+EDTA amended soil

The effect of chelating agents on heavy metal accumulation

Accumulation metal element in aerial part was higher than in root in the presence of chelating agent. Maximum

concentration in roots was 80.2 mg kg⁻¹ at Pb100+EDTA. For aerial part, the maximum uptake occurred at Pb50+EDTA, which was 84.9 mg kg⁻¹. The Cd concentration at 50 mg was 1.6 mg kg⁻¹. Pb concentration in root increased with an increase in supplied Pb in soil and contrary, in the Aerial part the uptake of Pb decreased in soil. However, the comparison between three metals (Cd in Figure 3, Ni in Figure 4 and Pb in Figure 5) shows a direct correlation between the total concentration of metals in plant for all irrigation solutions with EDTA. The accumulation pattern of metals in plant was as follows: Pb>Cd>Ni for various treatments.

The effect of fertiliser NPK on heavy metal accumulation

At higher supplied Pb (100 mg kg⁻¹ of soil) the concentration was, minimum in roots (16.01 mg kg⁻¹ dry weight) and maximum concentration in roots was 84.9 mg kg⁻¹ at Pb50 supplied content (Figure 5). For aerial part, Ni content decreased by increasing the Ni concentration in soil (Figure 4). The maximum uptake occurred at Cd100+NPK treatment which was (63.8 mg kg⁻¹ dry weight). The total Ni concentration in the plant was in the range of 50.27 to 108.9 mg kg⁻¹ dry weight for various supplied Ni concentrations (Figure 4). Unlike EDTA treatment and metal treatment without amendment, the accumulation pattern in plant was as follows: Cd>Pb>Ni.

The impact of EDTA and NPK on the ability of metal accumulation and translocation in plants

The ability of metal translocation in plants can be assessed which estimates the efficiency of a plant to transfer the metal from roots to aerial part for plants grown in contaminated soil (table 2) with the presence or not of NPK and EDTA.

Metal translocation in *N. oleander* L.

The values of TF for Ni treatment were mostly superior to 1 except for Ni50+EDTA and Ni100+NPK. The Maximum TF for Ni was for Ni100 with 3.87. Only in the assay with amendments in Pb and Cd treatment, TF obtained were superior to one (>1). TF values for Cd were recorded at Cd50+ NPK and Cd100+EDTA with 1.09 to 1.02 and the lowest value was TF =0.02 at Cd50+EDTA treatment. However, all the concentrations of Pb treatment showed TF values<1 except Pb50+ADTA, Pb100+NPK with values of TF 1.18 and 2.48, respectively.

Bioconcentration factor (BCF)

For both treatments, all values of BCF were<1 in high concentration of metal and were at a low level: 0.12 to 0.89(table 2). BCF values did not exceed a critical value of 1,

which proves a very low ability of *N. oleander* L. to accumulate metals in high concentration. At all experiment with EDTA, BCF was great than one (<1) at 50 mg kg⁻¹ for Ni, Cd and 50 mg kg⁻¹ of soil for Pb. Maximum BCF was

increased metal concentrations with all applications. At all experiment, BF was much lower than one (<1) at all treatments except Cd50+NPK, Ni50 and Pb50+EDTA.

Table 2. Bioaccumulation factor (BF), bioconcentration factor (BCF), and translocation factor (TF) for *Nerium oleander* L. grown in different treatment. Values > 1 are in bold font

Cadmium						
Experiment	treatment	Amendement	BCF	BF	TF	
Cd	Cd50	Cd50	1,212	0,404	0,33	
		Cd50+EDTA	1,444	0,03	0,021	
		Cd50+NPK	1	1,084	1,09	
	Cd100	Cd100	0,5	0,023	0,046	
		Cd100+EDTA	0,716	0,729	1,02	
		Cd100+NPK	0,67	0,638	0,95	
Nickel						
Experiment	treatment	Amendement	BCF	BF	TF	
Ni	Ni50	Ni50	0,3236	1,036	3,20	
		Ni50+EDTA	1,238	0,312	0,25	
		Ni50+NPK	0,4	0,6054	1,51	
	Ni100	Ni100	0,122	0,472	3,87	
		Ni100+EDTA	0,266	0,803	3,02	
		Ni100+NPK	0,801	0,288	0,36	
Lead						
Experiment	treatment	Amendement	BCF	BF	TF	
Pb	Pb50	Pb50	1,586	0,218	0,14	
		Pb50+EDTA	1,438	1,7	1,18	
		Pb50+NPK	1,698	0,568	0,33	
	Pb100	Pb100	0,897	0,853	0,95	
		Pb100+EDTA	0,802	0,331	0,41	
		Pb100+NPK	0,16	0,397	2,48	

obtained for Pb50 application. However, metal+ EDTA was accumulated more effectively in comparison with metal+NPK.

Metal bioaccumulation in *N. oleander* L.

Bioaccumulation factor, which was defined as the ratio of metal concentration in the aboveground to the metal concentration in the soil, was calculated in *N. oleander* L. plant. Maximum bioaccumulation factor (BF= 1.7) was observed in the soil treated with Pb EDTA at concentration of 50 mg kg⁻¹. It was observed that BF decreased with

Discussion

Phytoremediation is a green technology that uses plants to reduce the concentrations of harmful toxic elements on the environment (Bhat *et al.*, 2022). Heavy metal uptake through phytoremediation technology includes processes like phytostabilization, rhizodegradation, phytoextraction, phytodegradation, phytoaccumulation and phytovolatilization (Mahmood *et al.*, 2015). Specific plants for phytoremediation have the potential to accumulate, tolerate and extract high levels of heavy metals and are



adapted to different environments (Antoniadis *et al.*, 2021). In many cases, plants cannot execute well and required assistances to enhance phytoremediation like Ethylene Diamine Tetra Acetic Acid (EDTA) (Hasan *et al.*, 2019) and NPK (Al-Menaie *et al.*, 2012). The capability of *N. oleander* L. to accumulate Cd, Ni, Pb was estimated by Ibrahim and El Afandi (2020).

Here, this study was designed to determine the impacts of EDTA and NPK fertilizer with different concentrations on the plant biomass, Cd, Ni and Pb uptake and accumulation potential of *N. oleander* L. Besides, the results indicated that exposure to metals inhibited plant growth impacting both heights, root length and weights of plant. Results showed that the addition of EDTA and NPK in the presence of different concentration of metals, enhanced dry weight. The same behaviour has been widely reported in the literature (Vázquez *et al.*, 2016). An increase in Pb concentration in EDTA and NPK treatments led to a decrease in the dry weight biomass of *N. oleander* L. plants. It was demonstrated by Saifullah and Rabbani (2009) that plants exposed to high levels of both free Pb and EDTA produced low biomass. Applying EDTA in a single dose immediately after germination can severely inhibit growth in the plant through EDTA itself or indirectly due to enhanced solubility of the mineral. Addition of EDTA when plants have attained sufficient biomass, can minimize its adverse effects on plant growth. However, EDTA-enhanced bioavailability of heavy metals including Pb may inhibit plant growth. It was observed and in accordance with the findings of Adamu (2019) who reported that the NPK application enhanced the biomass production of *N. oleander* L. . This may be as a result of the plants receiving more nutrients from the soil with the application of NPK.

N. Oleander L. has received considerable attention as a possible bio monitor of heavy metal pollution (Seridou *et al.*, 2022) and showed a good tolerance against multi-metals (Bortoloti and Baron, 2022). Experimental results showed that in the case of natural phytoremediation, the highest determined concentrations were in the root of *N. oleander* L. with Pb treatment. Similarly, Trigueros *et al.*, (2012) found higher accumulation of Pb in roots in comparison with storage in stem and leaves.

In the case of Nickel, the aerial part of *N. oleander* L. contained 3.8 times more of Ni than the storage roots which is in accordance with other observations (Franco *et al.*, 2013). The concentration of Cd was greater in roots than in aerial parts in accordance with the results of Ibrahim *et al.* (2020) who found that, in the roots, toxic metals required to cell walls and vacuoles, or by extracellular precipitation, which prevented it from entering the cytoplasm avoiding the toxic

effects of heavy metals on the cytosol, then metals became slightly accumulated in roots, but very low content of it transfers to above ground plant parts. However, the plant capacity to transport the heavy metals above the ground parts depends on the plant efficient transport system, physiological condition, antioxidative defence system and vacuolar compartmentalization (Yaashikaa *et al.*, 2022). Heavy metal phytoremediation by plants is typically limited by the availability of heavy metals in soils. The application of chemical enhanced technology is one of the predominant approaches, which aids the uptake and translocation of heavy metals in the aerial parts of plants.

In recent years, several chemical amendments including ethylenediamine tetra acetic acid (EDTA) are widely used for soil remediation applications (Hasan *et al.*, 2019). It is also demonstrated in this study that the presence of EDTA has an impact on the ability to increase the toxic elements bioavailability, induce greater absorption, and translocation from the roots to the stem and leaves of the plants. The results indicate that lead accumulation in the aerial parts was higher than in stem and leaves in Pb50+ EDTA and Cd100+EDTA treatment. The addition of EDTA to the treatment increased plant uptake of Pb and Cd with total content was 156.8 mg kg⁻¹ and 144 mg kg⁻¹, respectively.

According to Hasan *et al.* (2019), EDTA is an effective organic ligand for increasing the dissolution, absorption, and translocation of Pb because it forms a highly soluble and stable metal-EDTA complex. It is well known that EDTA is known to be the most efficient organic ligand that can increase metal uptake, solubilization, and translocation as it can form highly stable and soluble metal-EDTA complexes (Hasan *et al.*, 2019). The work of Meighan *et al.* (2011) indicated that the translocation factor increased up to 100 times by applying a threefold excess of EDTA, which resulted in high concentrations of Cd in plant leaves. Moreover, in the study by Liphadzi and Kirkham (2006), it was indicated that the concentrations of heavy metals, such as Cd, Pb, and Cr increased in shoots of *T. latifolia* when EDTA was used. Previous studies have shown the majority of metals to be retained in the roots in EDTA assisted phytoremediation. The work of Li *et al.* (2020) indicated that the effects of EDTA on the metal content in ryegrass roots and shoots were also elemental dependent, with more accumulation of Cd, Ni and Pb in roots and more accumulation of Zn and Cu in shoots. Another reason should be the addition of EDTA, we found an increase of recovery of metals with the application of EDTA in the study.

However, some of the negative effects of EDTA that have been reported are that EDTA can contribute to



environmental damage produced by leaching of metals into groundwater, and also the plant, chelator source and level will make a difference in metal uptake. Recently, Ponce-Hernández *et al.* (2022). indicate that among the main advantages accessible by the use of chemical amendments in plant remediation is the decontamination of metals in plants, and prevention of oxidative stress by toxic elements, modification of phytochemical behaviour of plant, also increase the translocation and the availability of influence of amendments NPK as fertilizer on metal accumulation in plants exposed to extreme metal concentrations. This study, in particular, intended to see if the presence of Cd, Ni and Pb stimulated the growth of *N. oleander* L. and whether this could be related to the ability of growth on contaminated soils and to quantify the amount of metal taken up by different organs. Similar studies also have been carried out on this plant (Trigueros, 2012). An interesting feature of the present study was the association between accumulation of Ni, fertilization by NPK and metal complexation by EDTA. The success of the plant extraction technique is determined by the amount of biomass, and the concentration of toxic metals in plant tissues. The obtained data revealed that the maximum concentration of plant grown in soil amended with Ni50+NPK fertiliser was in roots with 80.1 mg kg^{-1} and a total content was 108.9 mg kg^{-1} .

However, total content of metal in plant in Cd100+NPK treatment was the greater one with 130 mg kg^{-1} . According to Carbonell *et al.* (2011) Roots presented a higher metal concentration than shoots in the plants (*Zea mays* L.) growing in the NPK fertilizer-amended soils. Root system acts as a barrier for Cr, Ni, Pb, Hg and Cd (Wang *et al.*, 2020), so metals uptake was poor and causes very low concentrations of these metals in aerial plant parts. Similar to our study, Adamu (2019) reported that there was an improvement in metal accumulation in *N. oleander* L. plants with NPK fertilizer compared to those in non-amended soils. The results further revealed that plant rarely take up Ni from the soil and transported it to the shoots in presence of NPK. The possible reason for lower concentrations was explained by the use of basic soil and loss of the metal-chelate complex due to the leaching occurred in the experiment pots (Beqaj *et al.*, 2016).

Bioconcentration factor (BCF) and Translocation factor (TF) are the two important factors required to evaluate the efficacy of metal extraction in plants. The BCF values > 1 indicates the potential of a plant species for remediation of metal polluted soils. The TF values of < 1 suggest the limited translocation of metal from roots to shoots at lower Cd concentrations in the soil. From the BCF and TF values > 1 at

50Cd, it is strongly suggested that *N. oleander* L. has the basic characteristics of a Cd-hyperaccumulator and is potentially employed for remedying Cd-contaminated soils (Sidhu *et al.*, 2017).

We can compare the ability of different plants to absorb metals from the soil and transfer them to the buds by comparing the bioconcentration coefficient and the translocation coefficient. Plants tend to restrict soil-root, and root-shoot tolerant transfers, and therefore have much less accumulation in their biomass, while hyperaccumulators uptake and translocate metals into their aerial biomass. Plants exhibiting TF and particularly BCF values less than one are unsuitable for phytoextraction. Few plants were capable of accumulating heavy metals in the roots, but most of them had low TF and BCF values, which means limited ability of heavy metal accumulation and translocation by the plants (Yoon *et al.*, 2006). The BF values > 1 registered in this study were observed for the NPK treatment at low Cd50+NPK (BF=1.08), Ni50 (BF=1.036) and Pb50+EDTA (BF=1.7) in all other cases were lower than one.

In this study, NPK addition showed ability of bioaccumulation and translocation. A BCF value > 1 indicates high efficacy in the phytostabilization of metal. Plant with high TF values were considered suitable for phytoextraction generally requiring translocation of heavy metals in aboveground plant parts. The results without amendment indicated that this plant was identified as no - hyperaccumulator because metal accumulated was less than 100 mg kg^{-1} according to Baker and Brooks (1989). Our investigation indicated that NPK and EDTA addition enhance the potential of phytoremediation of metal contaminated soil and at Cd, Ni and Pb concentrations, the content of Cd in total plants were more than 100 mg kg^{-1} , the critical value of a Cd, Ni, Pb-hyperaccumulator and has high BCF and TF values. According to Yoon *et al.* (2006) and Sharma *et al.* (2022), plants with BCF >1 and TF <1 are reported to demonstrate the possibility of phytostabilization. Furthermore, plants with BCF <1 and TF above one and BCF and TF <1 also exhibit potential for phytostabilization. Plants with these combinations lack mechanisms to regulate the intake of soil-metal content and the metal content, keeping them at low levels. Although plants with BCF and TF >1 are suitable for phytoextraction and are considered accumulators.

Among the three metals tested, the plants growing on the site were most efficient in taking up and translocating of Ni. Low translocation of Pb indicates that plants were unwilling to transfer Pb from their roots to shoots possibly due to Pb toxicity. Although, *N. oleander* L. is a Pb-tolerant with high



BCF and low TF and can be used for phytostabilization of contaminated site. However, the ability of these plant to tolerate and accumulate heavy metals may be useful for phytostabilization of Pb and Cd. Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation of Ni.

Conclusion

The present study evidently demonstrates the metal (cadmium, nickel and lead) accumulation potential of *N. oleander* L. During this study, this plant has appeared as a good stabiliser of Cd and Pb and extractor of Ni, as evidenced by high metal accumulation in both roots and aerial parts. Overall, our results revealed that plant did not show any symptom of phytotoxic elements that makes *N. oleander* the ideal candidate to encourage main colonization of mine tailings, contaminated soils and other wastes. Based on the soil amendments, NPK and EDTA can enhance the biomass to some level and BF and TF values were greater than one, which confirmed its suitability for phytoremediation of contaminated soil. We can propose that the studied plant can be considered as accumulators and useful plant for phytostabilization in the case of chelator-assisted phytoextraction. This plant was not intended to human or animal feeding. Therefore, can be used for decoration and for treatment of vast contaminated soils and water. *N. oleander* L. has been shown to be effective in enhancing phytoremediation of soil contaminated with heavy metals such as Cd, Ni, and Pb. Phytoremediation is a process that utilizes plants to remove, contain, or degrade pollutants from soil or water, and *N. oleander* L. has demonstrated a strong ability to absorb and accumulate these heavy metals in its tissues, thereby reducing the number of contaminants in the soil. This makes *N. oleander* L. a promising candidate for use in remediation efforts aimed at addressing the growing problem of heavy metal contamination in soils around the world.

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