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Effect of EDTA on nickel (Ni) phytoremediation by rapeseed (*Brassica napus* L.)

Parisa Aghaei-Gharachorlou[1*], Safar Nasrollahzade[1], Kazem Ghassemi-Golezani[1], Shahin Oustan[2]

[1]Department of Eco-physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2]Department of Soil Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
*Corresponding Author: Parisa Aghaei-Gharachorlou, Email: p.aghaei_90@yahoo.com

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ABSTRACT

Heavy metals should be removed in soil for the safety of the environment, and phytoremediation, which is the use of plants to remove contaminants from the environment, can be useful in rehabilitating polluted sites. Chelating agents such as ethylenediamine tetraacetic acid (EDTA) have been used in different situations in phytoremediation to enhance the extraction of heavy metals by plants from soil. A pot experiment was conducted in 2018 in a greenhouse at the University of Tabriz, Iran, to evaluate the role of EDTA (0, 0.5 and 1 mmol kg^{-1} soil) on the phytoremediation potential of rapeseed (*Brassica napus* L.) under Ni stress (0, 50, 100 and 200 mg kg^{-1} soil). Increasing Ni and EDTA concentration significantly decreased the root and shoot dry weight and grain yield of rapeseed. Ni concentration in different parts of rapeseed increased significantly due to high concentration of Ni. The Ni concentration was higher in root, shoot and grain of EDTA-treated plants as compared to untreated plants. The translocation factor and bio-concentration factor calculated for the EDTA-treated plants were increased compared to the non-treated plants. These results suggested that EDTA application might be a useful strategy for increasing phytoremediation of Ni from contaminated soils.

Keywords: *Brassica napus*, chelate, nickel, phytoremediation, translocation factor.

INTRODUCTION

Metals are essential to plants as they are required micronutrients. When metals are present in excess, however, they can act as toxicants, reducing the success of plant life. Among the metals that are most toxic to plant and animal life are those that displace essential metal ions in biological processes, these include Cd, Zn, Pb, Cu and Ni (Guo *et al.*, 2019). Ni is one of the most abundant heavy metal contaminants of the environment due to its release during mining and smelting practices and it has been the target of many cleanup strategies (Ali *et al*., 2009). During the last few years, Ni is becoming the serious concern due to its increasing concentration in agricultural soils which reached up to 26,000 ppm and 20-30-folds higher than unpolluted soils (Matraszek *et al*., 2016). Such higher concentration can easily enter and accumulate in human body through food consumption (Sainger *et al*., 2014).

Pollution of the environment by heavy metals poses a threat to surface water and groundwater, which are used as the main sources of drinking water by many people in the world. Heavy metals in the soil may enter the human food web through plants. Phytoremediation, which is the use of green plants to remove metals from the soil or environment, is an environmentally sustainable approach to remediate moderate, diffuse, and shallow metal contamination (Lago-Vila *et al*., 2019). However, the efficiency of plants to uptake and translocate heavy metals towards harvestable parts may vary among plant species, soil types, and environmental conditions (Gill *et al*., 2015). Many plant species including Brassica have been used for phytoremediation of heavy metals such as Cd, Pb, Cr and Ni etc (Ehsan *et al*., 2014). Recently, different Brassica species have been evaluated for the phytoremediation of heavy metals (Ali *et al*., 2009; Ehsan *et al*., 2014). Among Brassica species, rapeseed (*Brassica napus* L.) has been found relatively more tolerant to excessive Ni concentrations than other Brassica species possibly due to its specific physiological and biochemical activities (Ali *et al*., 2009). Thus, it can be an ideal candidate for the phytoremediation of Ni from contaminated soils (Marchiol *et al*., 2004).

Success of phytoremediation depends upon the metal solubility and availability in soil for root uptake. Metal bioavailability mainly depends upon soil characteristics such as clay content, pH, cation exchange capacity and soil organic matter (Lago-Vila *et al*., 2019). Most of the heavy metals have low bioavailability in the soil solution and consequently different chelating agents are applied to increase the metal bioavailability (Sun *et al*. 2016). Ethylenediamine tetraacetic acid (EDTA) is the most effective chelating agent used for phytoremediation because it has a strong chelating ability for different metals and it also increases the bioavailability and plant uptake of the metals in the soil (Bloem *et al*., 2017). About 80% of the total soil metal is solubilized and becomes available for phytoremediation when EDTA is applied (Han *et al*., 2018).

However, limited literature is available on increased Ni phytoremediation by EDTA application (Han *et al*., 2018). In addition, there is a need to study the growth response of rapeseed towards Ni and EDTA application for developing Ni-remediation protocols. In this study, we attempted to (1) explore the potential of EDTA for increasing phytoremediation of Ni contaminated soils and to (2) investigate the effect of Ni on growth and performance of Ni-stress rapeseed plants.

MATERIALS AND METHODS

Experimental set-up

A pot experiment was conducted in 2018 in a greenhouse at the University of Tabriz, Iran. In present research, the soil was collected from agricultural field being utilized for crop cultivation. First of all at room temperature 22–25 °C, the soil was dried, homogenized, and then passed through a mesh of 2 mm. Soil physicochemical characteristics are given in Table 1.

Table 1. Physicochemical characteristics of the soil

Parameter	Value
Clay (%)	14
Silt (%)	23
Sand (%)	63
FC (%)	18.2
Organic carbon (%)	1.54
Total N (%)	0.15
Available Ni (mg kg^{-1})	0.38
Available P (mg kg^{-1})	21.1
Available K (mg kg^{-1})	318
Available Zn (mg kg^{-1})	2.88
Available Cu (mg kg^{-1})	1.24
Available Fe (mg kg^{-1})	7.36
Available Mn (mg kg^{-1})	2.98

New plastic pots were filled with 4 kg of soil and contaminated with different concentrations of Ni. Appropriate amounts of sulphate salt of Ni were added to the soil so as to maintain the required levels of Ni (50, 100 and 200 mg kg^{-1} soil). The soil in the pots was mixed thoroughly several times in order to ensure uniform mixing of the metal salts. A set of pots containing uncontaminated soil was kept as control. Mature and healthy seeds of *B. napus* L. var. RGS003, were received from Karaj Seed and Plant Improvement Institute. The surface of seeds was decontaminated by soaking for 5 min in 3% H_2O_2 and then thoroughly washed with deionized water. Seeds were sown 1 cm deep in each pot. Pots were then placed in the glass greenhouse under natural light and temperature around 25±2 °C. After emergence, seedlings were thinned to keep 4 plants in each pot. During plant growth and development, the pots were weighed and the water loss was compensated by irrigation with distilled water. The tetrasodium salt of EDTA, at two doses (0.5 and 1 mmol kg^{-1} soil), was dissolved in deionized water and added to the pots at flowering stage. The experiment was conducted following completely randomized design (CRD) with three replications.

Measurements

The plant shoots, cut at the soil surface, were harvested at maturity. The soil was then broken up and roots were harvested by hand. The roots were washed in tap water until free of soil particles. The shoots and roots were further washed with deionized water, oven-dried at 70 ◦C for 24 h, weighed, and then ground and passed through a 1.0 mm sieve. Plant samples were turned into ash by using muffle furnace at 600 °C for 6 h. The ash samples were dissolved in concentrated solution of HNO_3 and HCl (3:1). The samples were then filtered in volumetric flask and distilled water added to make volume up to 50ml. Concentration of Ni was determined by Atomic Absorption Spectrophotometer (Model Shimadzu 6300).

Two indicators were calculated to evaluate plant's phytoremediation efficiency. The translocation factor (TF = Cshoots/Croots) was calculated from the compartment concentrations of heavy metals to evaluate the plant's ability to translocate heavy metals from roots to the harvestable aerial part (Marchiol *et al*., 2004). The bioconcentration factor (BF = Cshoots/Csoils), defined as the ratio of metal concentration in plant shoots to metal concentration in soil, is a measure of the ability of a plant to take up and transport metals to the shoots (Evangelou *et al*., 2007).

Statistical analyses

Statistical analysis of the data was performed by MSTAT-C software. Duncan multiple range test was applied to compare means of each trait at 5% probability. All figures were drawn by Excel software.

RESULTS AND DISCUSSION

Statistical analysis of the data showed that the effects of Ni toxicity and EDTA application on shoot dry weight were significant (Table 2). Shoot dry weight significantly decreased with increasing Ni concentration in the soil, so the highest (13.7 g/plant) and the lowest (6.01 g/plant) shoot dry weight were recorded for control and 200 mg Ni kg^{-1} soil, respectively (Table 3). Mean shoot dry weight significantly decreased by EDTA application of 1 mmol kg^{-1} EDTA, compared with other treatments (Table 3). The mechanism of Ni toxicity is not completely understood yet; however, as per the pertaining literature, it can alter the uptake of minerals by plants (Gajewska and Skodowska, 2010) and inhibit the stomatal opening, transpiration, photosynthesis, and nitrogen metabolism in plants (Sengar *et al*., 2008; Gajewska and Skodowska, 2010). Ruley *et al.* (2006) reported depressed photosynthetic activity of *Sesbania drummondii* seedlings exposed to EDTA in solution culture. This EDTA-enhanced toxicity of metals is due to elevated metal uptake (Han *et al*., 2018). There are also typical phytotoxic effects of EDTA, which may be due in response to the increased uptake of metals by plants (Ruley *et al*., 2006).

Morphological and biochemical characterization of bacteria isolates from the analyzed leachate sample

Table 1 presents the result of morphological and biochemical characterization of bacteria isolates from the analyzed leachate samples. It revealed that the bacteria genera were identified as *Pseudomonas, Bacillus, Citrobacter, Yersinia, Enterobacter, Serratia* and *Shigella*.

Ni stress and EDTA treatments significantly influenced root dry weight of rapeseed (Table 2). Maximum root dry weight per plant was obtained under control, which was decreased with increasing Ni toxicity (Table 3). Application of EDTA at rates of 0.5 and 1 mmol kg^{-1} soil decreased the root dry weight of rapeseed by about 7.4% and 17.1%, respectively (Table 3). Like other toxic metals, Ni at higher concentrations has also been reported to decrease fresh and dry weights of

Table 2. Analysis of variance for the studied traits in rapeseed affected by Ni stress and EDTA application treatments.

		Mean Square							
S.O.V	df	Shoot dry weight	Root dry weight	Grain yield	Root Ni concentration	Shoot Ni concentration	Grain Ni concentration	Bio-concentration factor	Translocation factor
Replication	2	0.399	0.004	0.001	1.719	168.44	0.575	0.009	0.0002
Nickel (A)	3	119.29 **	3.501 **	4.195 **	7276900.5 **	2269813.8 **	513.42 **	79.929 **	0.0473 **
EDTA (B)	2	5.77 **	0.179 **	0.101 **	369599.2 **	66029.11 **	23.087 *	5.971 **	0.0122 *
A × B	6	0.39	0.003	0.003	60240.1 **	12805.52 *	2.952	0.446	0.0057
Error	22	0.452	0.013	0.008	2610.54	3901.16	5.205	0.181	0.0024

* and **: Significant at 5% and 1% probability level, respectively.

Table 3. Mean ± SE of dry weight of different parts of rapeseed, grain Ni concentration and translocation factor and bioconcentration factor indices affected by Ni and EDTA applications.

Treatments	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Grain dry weight (g/plant)	Grain Ni concentration (mg/kg)	Bio-concentration factor	Translocation factor
Ni application level (mg kg^{-1})						
0	13.70 ± 0.31 a	1.97 ± 0.05 a	2.87 ± 0.02 a	6.14 ± 0.53 c	0.85 ± 0.08 c	0.66 ± 0.01 a
50	13.08 ± 0.29 a	1.76 ± 0.05 b	2.74 ± 0.03 b	12.02 ± 0.24 b	5.46 ± 0.25 b	0.56 ± 0.01 b
100	8.87 ± 0.28 b	1.04 ± 0.04 c	2.07 ± 0.03 c	13.42 ± 0.76 b	7.97 ± 0.34 a	0.68 ± 0.02 a
200	6.01 ± 0.22 c	0.63 ± 0.03 d	1.38 ± 0.03 d	24.25 ± 1.25 a	5.62 ± 0.26 b	0.54 ± 0.02 b
EDTA dose (mmol kg^{-1})						
0	11.14 ± 0.97 a	1.47 ± 0.16 a	2.36 ± 0.17 a	12.55 ± 1.61 b	4.24 ± 0.68 c	0.58 ± 0.02 b
0.5	10.34 ± 0.95 b	1.36 ± 0.16 b	2.26 ± 0.18 b	14.01 ± 1.27 ab	5.04 ± 0.79 b	0.61 ± 0.02 ab
1	9.76 ± 0.95 c	1.22 ± 0.16 c	2.18 ± 0.18 c	15.33 ± 1.33 a	5.64 ± 0.87 a	0.64 ± 0.02 a

Different letters in each column for each treatment indicate significant difference at $p \leq 0.05$.

various plant parts during different growth stages (Sengar *et al*., 2008; Sainger *et al*., 2014; Matraszek *et al*., 2016). Nutrient uptake, water relations, assimilatory enzymes, and photosynthesis, which are directly related to growth and productivity, have been reported to be inhibited by Ni toxicity (Yusuf *et al*., 2011; Rehman *et al*., 2016). EDTA application could have a toxic effect on soil bacteria and fungi (Guo *et al*., 2019) and plants (Awokunmi *et al*., 2012). Several other authors also reported similar results of reduced plant biomass in the presence of EDTA (Xu *et al*., 2009).

Grain yield was significantly affected by Ni and EDTA treatments. The interaction of Ni × EDTA on this trait was not significant (Table 2). In comparison with control, three Ni toxicity treatments showed significantly lower grain yield. Application of 50, 100 and 200 mg Ni kg^{-1} soil decreased grain yield by 4.5, 27.8 and 51.9%, respectively, compared with control (Table 3). The reduction of grain yield under 0.5 and 1 mmol kg^{-1} EDTA was about 4.2% and 7.6%, respectively (Table 3). It is also suggested that Ni-induced reduction of plant growth may be attributed to inhibition of photosynthesis and diversion of nitrogen for the synthesis of stress metabolites in place of growth-related proteins (Sainger *et al*., 2014). The observed EDTA toxicity symptom induced in *Brassica juncea* and *Lolium perenne* was a significant decrease in plant biomass (Johnson *et al*., 2010). For *Typha angustifolia*, a significant decrease of plant height and biomass, such as stunted plant growth, was also recorded (Muhammad *et al*., 2009).

Effects of Ni and EDTA treatments and also the interaction of these factors were significant for Ni concentration in root ($p \leq 0.01$) (Table 2). EDTA significantly improved concentration of Ni in rapeseed roots under low (50 mg Ni kg^{-1}), moderate (100 mg Ni kg^{-1}) and severe (200 mg Ni kg^{-1}) Ni toxicity treatments. The highest Ni concentration in roots was recorded for severe Ni toxicity treatment under application of 1 mmol kg^{-1} EDTA (Fig. 1). The overall Ni uptake by plant mainly depends upon the Ni concentration in soil, acidity of soil, plant metabolism, soil organic matter, and presence of other heavy metals (Sainger *et al*., 2014). Khaliq *et al.* (2016) also observed a higher level of Ni in roots and stems of cotton seedlings exposed to Ni stress. EDTA forms complex with Ni, and plant roots easily uptake the whole complex and translocated to higher parts (Liphadzi and Kirkham, 2006).

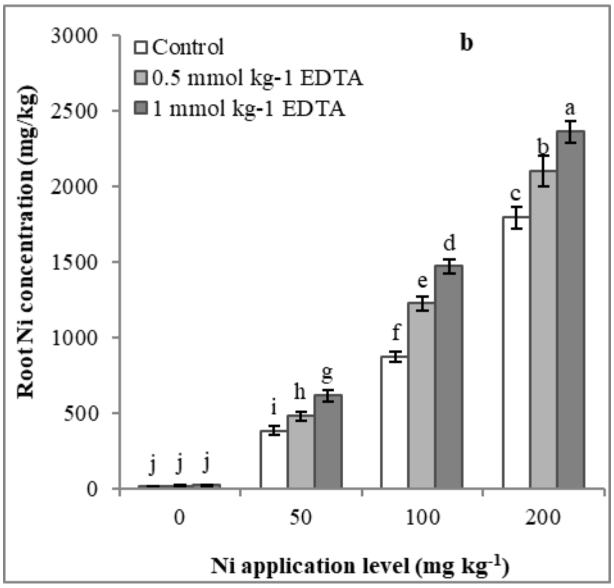

Fig. 1. Mean Ni content of rapeseed root under different Ni and EDTA application levels. Different letters indicate significant difference at $p \leq 0.05$.

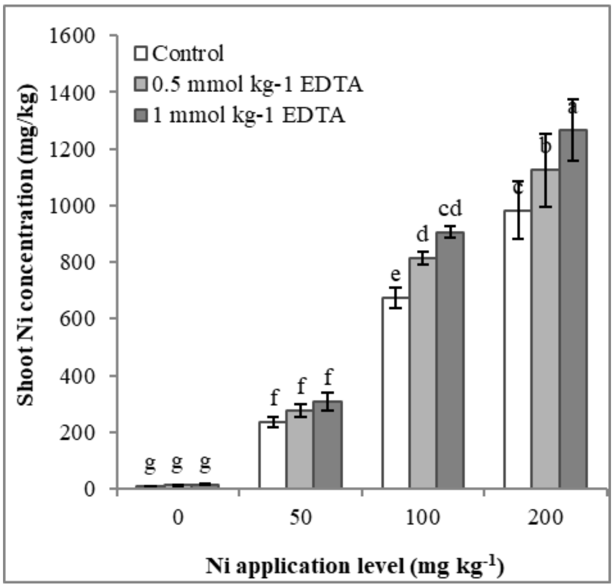

Fig. 2. Mean Ni content of rapeseed shoot under different Ni and EDTA application levels. Different letters indicate significant difference at p ≤ 0.05.

Ni toxicity and EDTA treatments significantly influenced shoot Ni concentration. The interaction of these factors on shoot Ni concentration was also significant ($p \leq 0.01$) (Table 2). Changes in shoot Ni concentration of rapeseed plants from untreated and 0.5 and 1 mmol kg^{-1} EDTA treated under control and low Ni toxicity (50 mg Ni kg^{-1}) were statistically similar, but it was considerably increased under moderate and severe toxicity treatments (Fig. 2). Increasing Ni concentration in soil significantly enhanced the Ni uptake and accumulation by the rapeseed both in EDTA-treated and untreated plants as compared to controls (Fig. 2). Kacalkova *et al.* (2014) also found higher concentration of Ni 5.04 mg kg^{-1} in the root of herbs grown on Ni contaminated soils. Rapeseed has already proved hyper-accumulator of different heavy metals such as Zn, Cu, Ni, Cr, Cd, As, and Pb (Gill *et al*., 2015; Farid *et al*., 2015; Lago-Vila *et al*., 2019). The addition of EDTA further increased the accumulation and translocation of Ni from root to shoots of rapeseed (Table 3). Chelating role of EDTA helps plant roots to uptake and concentrates Ni in the plant tissues (Han *et al*., 2018).

Statistical analysis of the data showed that the effects of Ni toxicity and EDTA application on grain Ni concentration were significant (Table 2). Grain Ni concentration significantly increased with increasing Ni concentration in the soil, so the highest (24.2 mg kg^{-1} grain) and the lowest (6.1 mg kg^{-1} grain) grain Ni concentration were recorded for 200 mg Ni kg^{-1} soil and control, respectively (Table 3). Mean grain Ni concentration significantly increased by EDTA application of 1 mmol kg^{-1} EDTA, compared with other treatments (Table 3). Similarly, Ali *et al*. (2009) reported higher Ni uptake and accumulation by different *B. napus* L. cultivars. The EDTA-treated plants showed higher uptake and accumulation of Ni in all parts of plants as compared to untreated and controls. This increase in uptake and accumulation has already been observed for Brassica plant species grown on metal contaminated soils (Ali *et al*., 2009).

Bio-concentration factor was significantly affected by Ni and EDTA treatments. The interaction of Ni × EDTA on this trait was not significant (Table 2). In comparison with control, three Ni toxicity treatments showed significantly higher bio-concentration factor. The application of 200 mg Ni kg^{-1} soil increased the bio-concentration factor by approximately 7-fold, compared with control (Table 3). Bio-concentration factor was generally increased under 0.5 and 1 mmol EDTA kg^{-1} soil (Table 3). The results indicated that the efficiency of phytoremediation for rapeseed was enhanced after the plant was treated with EDTA. The bio-concentration factor for Ni in different Ni concentrations was more than 1. One of the important factors affecting the success of phytoremediation of Ni-polluted soils is the availability of high biomass plants with the ability to concentrate Ni to high levels within their shoots (Matraszek *et al*., 2016). Therefore, the plant treated with EDTA is a promising plant for the extraction of Ni and possibly other heavy metals from soils. The metal entrance to plant roots involves: (1) transport of soluble metals towards plant root zone through diffusion or mass flow (Degryse *et al*., 2006); (2) adsorption on plant roots; and (3) binding to functional groups of rhizoderm cell surface (Seregin and Ivanov, 2001). In the presence of EDTA, the formation of metal-EDTA complex affects almost all of the above-mentioned steps of metal entrance into plant roots. First, EDTA facilitates the diffusion of metals towards plant roots by (1) desorbing metals from soil and subsequently increasing their concentration in soil solution; and by (2) decreasing the apparent diffusion coefficient of the metal under the metal-EDTA form (Degryse *et al*., 2006). Secondly, due to neutral charge, metal-EDTA complex are not blocked or attached by carboxyl groups or polysaccharides of rhizoderm cell surface (Liphadzi and Kirkham, 2006). In this way, EDTA causes the metal to enter directly to the plant roots.

Ni stress and EDTA treatments significantly influenced translocation factor (Table 2). Maximum translocation factor was obtained under control, which was decreased with increasing Ni toxicity (Table 3). Application of EDTA at rates of 0.5 and 1 mmol kg^{-1} soil increased the translocation factor by about 5.1% and 10.3%, respectively (Table 3). The EDTA treated plants grown in the Ni contaminated soils showed the most efficient results for extracting Ni. Therefore, the efficiency of phytoremediation was improved. Some recent studies reported that binding of metals by EDTA reduces their accumulation in roots and enhances plants metal translocation to aerial parts (Zhivotovsky *et al*., 2011). It has also been shown that application of EDTA causes several-fold stimulated translocation of metals from plant roots to aerial parts. Recent findings suggest that metals are transported towards plant shoots as chelated by EDTA (metal-EDTA complex) and thus increase their concentration in shoots (Awokunmi *et al*., 2012).

CONCLUSION

This study indicates that rapeseed is reasonably tolerant to Ni toxicity and it has a significant potential to extract Ni from the contaminated agricultural soils. In general the growth of the rapeseed plants decreased significantly by increasing the levels of Ni in soil. Application of EDTA to enhance the accumulation of Ni resulted in reduction of dry mater production of rapeseed. The findings, therefore, revealed that application of EDTA increases the efficiency of phytoremediation by enhancing Ni accumulation in plant tissues. In contaminated soil, the present study show that application of EDTA could be regarded as an efficient chelate candidate for the simultaneous phytoremediation of Ni.

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