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Chelate-Induced Phytoextraction Potential of *Brassica rapa* for Soil Contaminated with Nickel

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Abstract

The aim of present study is to induce for phytoextraction of Ni by Brassica rapa from contaminated soil by application of EDTA. Brassica rapa seeds were planted in pots with Ni concentrations ranging from 0 to 2000 mg/kg in the absence or presence of 10 mg/kg EDTA. After 60 days of growth, Ni concentration of plants were observed. Brassica rapa showed the remarkable resistance to Ni toxicity with no visual toxic symptoms as chlorosis and necrosis. The addition of 10 mg/kg EDTA significantly increased both the plant growth and the Ni concentration, compared with the control. Especially the addition of 10 mg/kg EDTA and 500 mg/kg Ni produced fertilizer effect and maximum dry matter achieved to 1.96 mg/plant from 0.82 mg/plant. While Brassica rapa accumulated 3763 mg/kg Ni in the absence of EDTA, the addition of 10 mg/kg EDTA increased Ni accumulation to 3942 mg/kg Ni at Ni application dose of 2000 mg/kg. Experimental results indicated that Brassica rapa is Ni hyperaccumulator plant (>1000 mg/kg in shoots) both in the absence or presence of EDTA. The bioaccumulation coefficient (BAC) for Ni by Brassica rapa was greater than 1, providing further evidence for the transport of Ni from Ni contaminated soils.

Key words

Brassica rapa, EDTA, Hyperaccumulator, Nickel, Phytoextraction

1. INTRODUCTION

There is a great worldwide interest surrounding issues of soil contamination including heavy metals [1]. Soil contamination with Ni has become a worldwide problem [2]. Nickel (Ni) is an important heavy metal in soil and is an essential element required for nitrogen (N) metabolism. Nickel plays a role in the structure and activity of the urease and hidrogenaz enzyme in plants [3]. In recent years, Ni pollution has gained importance due to negative potential impact on agriculture and human health. Nickel contamination mainly results from volcanic eruptions, land fill, forest fire, bubble bursting and gas exchange in ocean, weathering of soils and geological materials, effluent disposal from mining and smelting, fossil fuel burning, vehicle emissions, disposal of household, municipal and industrial wastes, fertilizer application and organic manures [4-6].

Phytoextraction is a non-destructive, cost-effective and safe alternative to conventional clean up techniques of contaminated soils with heavy metals such as Ni [7].

Phytoextraction is either a continuous (natural) process (using metal hyperaccumulating plants, or fast growing plants), or an induced process (using chemicals to increase the bioavailability of metals in the soil) [8].

The success of phytoextraction, either natural or chemically assisted, is largely determined by plant biomass, metal concentration in the plant tissue, and the phytoavailable fraction of metals in the rooting medium [9]. Researchers initially applied hyperaccumulator plants to clean metal polluted soils. However, many such plants have limited utility for phytoremediation, because of their slow growth, difficult propagation, seasonal growth, and low biomass [2]. Then, chelates such as EDTA, hydroxy ethylethylenediaminetriacetic acid (HEDTA), and ethylenebis-2(o-hydroxyphenyl) glycine (EDDHA) are used to enhance the phytoextraction of a number of metal contaminants including cadmium (Cd), chromium (Cr), cooper (Cu), lead (Pb), zinc (Zn) and Ni.

This chelate-assisted accumulation of toxic quantities of metal in a non-accumulator species is termed "chelate-induced hyperaccumulation" [10]. Chelate-induced phytoextraction is an innovative technique for cleaning metal contaminated soil [11]. Ethylenediaminetetraacetic acid is the most effective chelating agent for chelate-induced hyperaccumulation because it has a strong chelating ability for different metals and increases the bioavailability and uptake of metals in the plant from soil [11-12]. Several researchers have screened on chelate-induced hyperaccumulation of some fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots [13]. Huang *et al.* [10] reported a 1000-fold increase of Pb in in agronomic crops such as corn (*Zea mays* L.) and pea (*Pisum sativum* L.) after HEDTA application in comparison to soil solution of a control (no HEDTA addition). Under these conditions Pb concentrations in the shoots of corn and pea increases from less than 500 mg/kg to more than 10.000 mg/kg within one week after HEDTA application.

Brassica species are well known as metal accumulators and are being used for phytoremediation of contaminated soils. However, the metal tolerance mechanism in the plant still remains unclear [14]. Purakayastha *et al.* [15] reported that in a pot culture experiment, five different species of Brassica (*Brassica juncea, Brassica campestris, Brassica carinata, Brassica napus, and Brassica nigra*) were grown for screening possible accumulators of heavy metals, viz. Zn, Cu, Ni, and Pb. Among all species, *Brassica carinata* showed the highest concentration (mg/kg) as well as uptake (μg/pot) of Ni and Pb at maturity. Grčman *et al.* [16] studied the effect on the uptake of Pb, Zn and Cd by *Brassica rapa* and found that the concentrations of Pb, Zn and Cd in shoots were detected, up to 104.6, 3.2 and 2.3-times as much as that in the control.

In a study performed by Putnik-Delic *et al.* [17] had been determined the biggest concentration of Ni (300 ppm), both in leaf and in stem, was in *B. rapa*, with 64.25 times increase in concentration compared with the control group, and 66.5 times in stem in comparision with the respective control group.

In this study, Ni was choosen as target metal because it is widespread on serpentine rocks and the area in which the study was performed. It is known that serpentine soils are high in several heavy metals (e.g. nickel, cobalt and chromium) and these high heavy metal concentrations are thought, in part, to lead to varying levels of plant adaptation and soil affinities (i.e. endemic vs. non-endemic plant species) [18]. Koleli *et al.* [19] defined common soil formations distinguished in this area as follows: brown forest soils, reddish Mediterranean soils and brown calcareous soils reported that the maximum concentrations of metals in 11 soil samples collected from Mersin-Findikpinari (as dry mass) were 909 mg/kg Cr, 3615 mg/kg Ni, 246 mg/kg Cu, 467 mg/kg Zn, 8.2 mg/kg Cd and 111 mg/kg Pb. As it can be seen from the results, Ni concentration is always higher than the other metals. Because of that reason in this study investigated only nickel.

The objectives of this study were to evaluate the effect of EDTA application on Ni phytoextraction by *Brassica rapa* growing in artificially Ni contaminated soil, and to evaluate EDTA application effects on toxicity symptom, dry matter production, Ni concentration, Ni content and BAC of the plant. *Brassica rapa* is preferred because of its high yield (1.5 tons- 4.0 tons per ha) and high oil content of 42-46% [20], resist to soil and climate conditions and efffective root depth is ~1-1. 20 cm.

2. MATERIAL AND METHODS

2.1. Soil Sample and Analysis

Surface soil sample (0-30 cm) was collected from the experimental farm on General Directorate of Agricultural Research, Tarsus-Mersin, Turkey. After collecting soil from the surface (0–30 cm), it was brought to a laboratory, air dried, ground to pass through a 2-mm sieve, and stored in plastic bags for analyses and pot experiments. The some initial physical and chemical properties of the soil used in pot experiment were measured with routine analytical methods. Soil organic matter was determined using the Walkley and Black method [21]. Soil particle size distribution was estimated by the hydrometer method after pre-treating soil with H_2O_2 and dispersing overnight in sodium hexametaphosphate by the hydrometer method [22]. The carbonate content was determined by a calcimeter method, and the soil pH was measured at 1:1 soil:water ratio [23]. The concentrations of diethylenetriamine pentaacetic acid (DTPA)-extractable Ni were estimated using the method described by [24]. Total Ni concentrations in soil sample were digested according to [25] Anonymous (1995) with aqua regia method using inductively coupled plasma mass spectrophotometer (ICP-MS, Agillent 7500ce). Certified reference material (CRM 7003) was also analyzed in order to control the data quality. All results were given in terms of mg Ni per kg soil. All tests were performed in triplicates.

2.2. Artificial Soil Contamination

Two kg air-dried soils were weighed and loaded into a plastic bags and were contaminated by nickel nitrate [$Ni(NO_3)_2 \cdot 6H_2O$] salt solution containing 0, 500, 1000, 1500 and 2000 mg Ni kg^{-1} onto it and spread uniformly on plastic sheet. Then, soil was thoroughly mixed to achieve uniformity with respect to metal spiking.

After contamination and mixing, soil was left on plastic sheet and allowed to equilibrate at room temperature for almost three months with frequent and thorough mixing and by adding distilled water to maintain water content in soil at 60% of water-holding capacity and to periodic alternating wetting and air-drying cycles.

2.3. Greenhouse Pot Experiments

Total 4 seeds were planted in plastic pots, each containing 2 kg soil supplemented with increasing supply of Ni (0, 500, 1000, 1500 and 2000 mg/kg soil) in the absence or presence of 10 mg EDTA per kg soil. Ethylendiaminetetraacetic acid was added in the form of Na_2EDTA ($C_{10}H_{14}N_2Na_2O_8.2H_2O$).

A basal treatment of 200 mg/kg N as $Ca(NO_3)_2$ and 100 mg/kg P as KH_2PO_4 was applied to all pots. After germination, the seedlings were thinned to 2 plants per pot. During this 60 days, soils in the pots were kept humid (~80% water holding capacity). After 60 days of growth in the greenhouse, only the shoots of the plants were harvested, and dried at 70 ^oC for the determination of dry matter production.

2.4. Plant Sampling and Analysis

The oven-dried shoot samples were first ground and digested using 2 mL 30% H_2O_2 and 5 mL 65% HNO_3 in sealed vessels of a microwave (MarsXpress) apparatus. The digested samples were then analyzed with ICP-MS for Ni. Certified reference materials (*SRM 1573A, SRM 1547*) were also used in order to check the accuracy of the extraction technique used in the study. All analyses were carried out in triplicates, and the results were presented in terms of mg Ni per kg biomass (DW) or µg Ni per plant biomass. In this study, Ni was choosen as target metal because phytoextraction efficiency is related to both plant metal concentration and dry matter yield. Thus, the ideal plant species to remediate a contaminated site should be a high yielding crop that can both tolerate and accumulate the target contaminants [26].

3. RESULTS AND DISCUSSION

3.1. Some Initial Physical and Chemical Properties of the Soil Used in Pot Experiment

Some physical and chemical properties of the soil used in pot experiment are summarized in Table 1. The greenhouse pot experiments were performed using unpolluted agricultural soils with a clayey loam texture, a pH of 8.1, 26.2% $CaCO_3$ and 1.3% organic matter contents. The analysis of soils for total and DTPA-extractable Ni contents indicates Ni concentrations of 57.0 and 2.2 mg/kg soil, respectively.

In serpentine soils, Ni is not strongly held by clay and Fe-oxyhydroxide surfaces relative to other transition elements; consequently, Ni was more mobile than other metals in the serpentinitic landscape surrounding the study area. The soils derived from serpentine, displayed strong chemical fertility limitations due to a very low Ca/Mg ratio and limited available phosphorus [27]. Therefore, the application rate for 200 mg/kg N as $Ca(NO_3)_2$ and 100 mg/kg P as KH_2PO_4 amended in the study soil.

The total metal contents are much lower than the typical concentration ranges observed for unpolluted soils as set by the "Turkish Soil Contamination and Control Legislation", and were under the critical value (100 mg/kg) according to KabataPendias [28].

Table 1. *Some initial physical and chemical properties of the soil used in pot experiment*

Parameters	**Tarsus Soil**
pH (1:2)	8.1
Organic Matter (%)	1.3
$CaCO_3$ (%)	26.2
Particle Size Distribution	
Sand (%)	41
Silt (%)	36
Clay (%)	23
Texture Class	Clay Loam (CL)
Total Ni (mg/kg)	57
DTPA Extractable Ni (mg/kg)	2.2

3.2. Effect of EDTA on Ni Toxicity and Plant Growth

Following Ni application, visual symptoms were also monitored throughout the experiments. The toxicity effects were observed based on plant growth, chlorosis, and necrosis symptoms of the plant. Although *Brassica rapa* did not exhibit any significant symptoms as observed in a typical Ni toxicity including chlorosis and necrosis, increasing Ni application led to a visual reduction in the growth of plants, especially in absence of EDTA. Toxic effects of high concentrations of Ni in plants have been frequently reported as reductions in plant growth and adverse effects on fruit yield and quality ([2], [29]). Plants are known to tolerate metals to some extent, but high concentrations of available metals affect and induce disorders in the plant metabolism [30]. Plants can absorb and distribute metals internally in many different ways and may localize selected metals mostly in leaves and roots [31]. As a mechanism of metal tolerance or accumulation in plants, the response to metal stress is observed in both the leaves and roots. This response and accumulation of metals is more dependent on the type of metal rather than metal concentration [31].Fig. 1 shows the effects of Ni and EDTA application on dry matter production (shoot) after 60 days of growth. Application of 2000 mg/kg Ni decreased dry matter production of *Brassica rapa*. But the addition of 10 mg/kg EDTA significantly increased the plant growth compared with the control. The addition of EDTA promoted an increase in DW by *Brassica rapa*, up to 74% in the shoots. Especially the addition of 10 mg/kg EDTA and 500 mg/kg Ni produced fertilizer effect and maximum dry matter achieved to 1.96 mg/plant from 0.82 mg/plant. EDTA is a synthetic chelator. In this study, it has been studied in fairly low concentration against the possibility of damaging the soil structure. Increases in the doses of EDTA and DTPA applied increased the mobilization of heavy metals; however, environmental risk associated with synthetic chelators is consequently increased regardless of the EDTA and DTPA application rate under such saturation [27]. According to Chaturvedi *et al.* [32] considering the overall plant growth as it is highly essential for efficient phytoextraction we conclude that the low molecular weight organic acids (LMWOAs), such as citric acid, are a better and environmentally compatible alternative to synthetic chelators such as EDTA [32]. When our results are evaluated, we can conclude that EDTA is also used as a kind of plant micronutrient fertilizers**.** Because pot experiments data showing the effect of 10 mg/kg EDTA on soluble nickel levels at each of the soil concentrations would be benefical. A two-sided t-test with *p*-values less than 0.05 confirmed that the EDTA application had a positive effect on plant growth. The findings obtained were in agreement with the results obtained by Alam *et al.* [33]-[35]. This reduction in yield parameters might be attributed to poor plant development and reduced photosynthesis as a consequence of reduction in photosynthetic pigments in the leaves of the Ni-treated plants which resulted in suppressed supply of nutrients and photosynthates to the reproductive parts that ultimately affected yield [36].

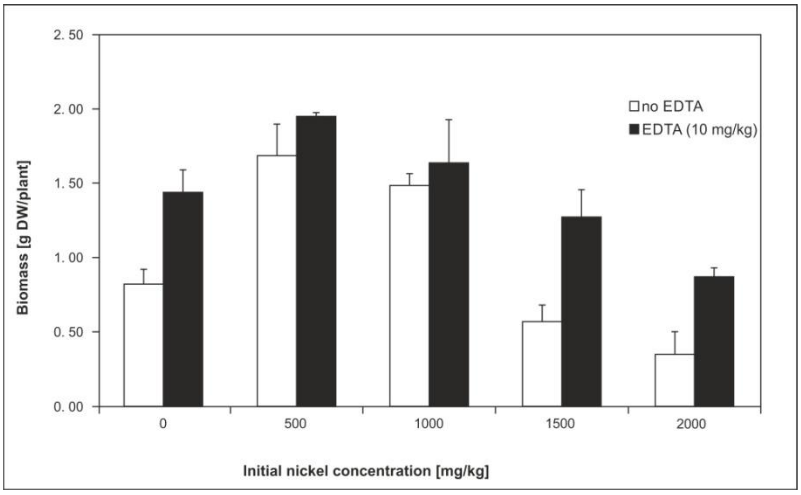

Fig. 1. *Effect of Ni and EDTA on dry matter production (shoot) of Brassica rapa.*

3.3. Effect of EDTA on Ni Concentration in Plant Shoots

The added EDTA remained most effective in increasing Ni accumulation in plants shoots and thus enhanced the phytoextraction [37]. The synthetic chelators EDTA was the most efficient amendments for increasing concentrations of Ni in soil solutions. In the present study, the concentrations of Ni in the soil solutions treated with EDTA were markedly higher than in the control soil.

As seen Figure 2 plots the Ni concentration in shoots in the control treatment and following the application of 0 and 10 mg/kg EDTA. Concentrations of Ni in the shoots of the control *Brassica rapa* varied between 23 and 61 mg/kg. This concentration according to Kabata and Pendias [28] was the pyhototoxic range of Ni (30 and 100 mg/kg). Experimental results indicate that the accumulation of Ni was significantly greater than those of control plants and the strong dependence of Ni concentration on initial Ni dose and EDTA concentration. *Brassica rapa* accumulated more Ni in the presence of EDTA. While *Brassica rapa* accumulated 3763 mg/kg Ni in the absence of EDTA, the addition of 10 mg/kg EDTA increased Ni accumulation to 3942 mg/kg at Ni application dose of 1500 mg/kg. (Fig.2) Experimental results indicated that applying 10 mg/kg EDTA could significantly increase the concentrations of Ni in the soil solution and remarkably enhance Ni accumulation in the shoots of *Brassica rapa*.

The addition of 10 mg/kg EDTA increased Ni uptake in the shoots from 61 mg/kg in the control to 3942 mg/kg. Shoots of *Brassica rapa* exceeded the Ni hyperaccumulation threshold value (>1000 mg/kg) according to the criteria described by [38]. Experimental results indicated that *Brassica rapa* is Ni hyperaccumulator plant (>1000 mg/kg in shoots) (Fig. 2). Nickel concentrations are >1000 mg/kg in shoots both the absence and presence of EDTA which these result confirm Ni hyperaccumulator of *Brassica rapa.* This result was higher than value reported Wenzel *et al.* [39] for *Brassica rapa*. Authors found that chelate effects were significant in the order EDDS > EDTA, control for *Brassica rapa and* EDDS enhanced only Ni content up to 10 mg/kg in *Brassica rapa* [40].

Chelating agents such as EDTA had positive effects on the enhancement of the bioavailability of heavy metals in soils, thereby increasing the amount of metals accumulated in the plants [41-43].

The amounts of metal removed from the soil via phytoremediation are affected by different factors. One important factor controlling the amount of Ni removed through phytoremediation is distribution of chemical forms of this metal in the soil. As the proportion of Ni in insoluble forms increased, the amount of Ni removed via phytoremediation decrease [44]. The increase in Ni accumulation by plants can be explained through the formation of highly soluble and less toxic Ni-EDTA complexes. This finding is in agreement with the results obtained by Barona *et al*. Panwar *et al*. [45-46]. Nascimento *et al.* [47] reported that EDTA increased Ni concentration in plant shoots to maximum and is confirmed by high concentration of AB-DTPA-extractable Ni in the soil in EDTA treatment; this could be due to greater stability constants of Ni-EDTA.

Some investigations have used the *Brassica* species for phytoremediation of sites contaminated with heavy metals [11,14, 48]. But they have not examined the use of EDTA, to increase plant phytoextraction capacity. Kumar et al. [48] tested many fast growing Brassicas for their ability to tolerate and accumulate metals, including Indian mustard (*Brassica juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L). Although all *Brassicas* accumulated metal, *Brassica juncea* showed a strong ability to accumulate and translocate Cu, Cr (VI), Cd, Ni, Pb, and Zn to the shoots. Kos *et al.* [49] studied that the Pb, Zn and Cd phytoextraction potential of 14 different plants including *Brassica rapa* was assessed in a chelate (as EDTA and ethylenediamine-disuccinic acid (EDDS) induced phytoextraction experiment. But they no study has been performed on this plant species to show whether the EDTA could efficiently improve its phytoextraction in Ni contaminated soil [6] (Muhammad *et al.* 2009). Statistical analysis (two-sided t-test) (p < 0.05) indicates that the differences obtained for Ni concentration in the absence and presence of EDTA are statistically significant.

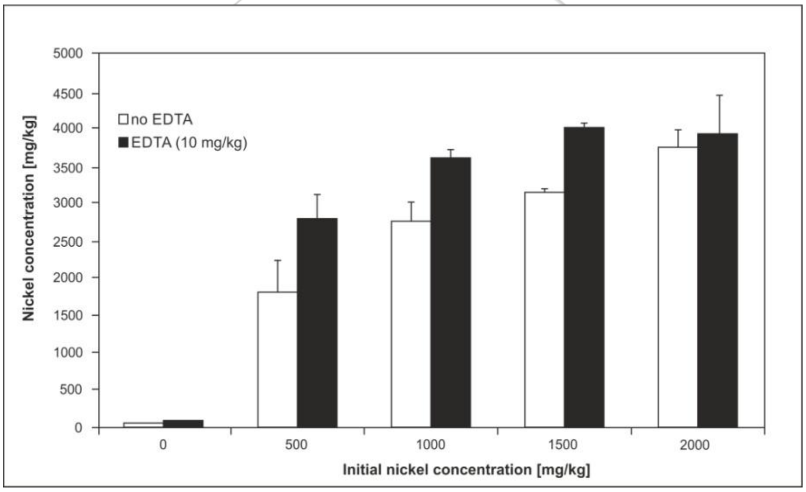

Fig. 2. *Effect of Ni and EDTA on shoot Ni concentration (mg/kg) of Brassica rapa.*

3.4. Effect of EDTA on Ni Content in Plant Shoots

Plant Ni content (μg/plant) was calculated as dry weight (mg/plant) multiplied by Ni concentration (mg/kg). The maximum Ni content by *Brassica rapa* was 4111 μg per plant in the absence of EDTA, the addition of 10 mg/kg EDTA increased Ni uptake to 5878 μg per plant at an initial Ni application of 1000 mg/kg. Wenzel *et al.* [39] (2003) reported that overall extracted mass per plant varied between 7and 50 μg in the control, between 29 and 135 μg in the EDTA treated pots, which were not consistent with the our study. The enhanced Ni accumulation in the presence of EDTA indicates the formation of less toxic Ni-EDTA complexes, which led to an increase in plant growth. Nickel content by plants reached a maximum at an initial Ni application of 1000 mg/kg, and then decreased sharply with increasing initial Ni level due to the fact that excess Ni reduced the biomass of plant (Fig. 1), thereby decreasing the amount of Ni accumulation. EDTA at selected concentration (10 mg/kg) increased uptake of the heavy metals from the soils it also resulted in low plant biomass. Greater biomass production is a desired parameter for plants being used for phytoextraction so that these plants can extract more amounts of heavy metals from the soil. A decrease in biomass production might be due to increased metal uptake because of the destruction of the physiological barrier by these acids in roots, which controls the uptake of solutes [37] (Sabir *et al.* 2014). In a study carried out Wu *et al.* [26] has shown EDTA increased shoot Cu and Pb concentrations in Indian mustard (*Brassica juncea*) plants growing in the soil. There were no visible symptoms of heavy metal toxicity in Indian mustard during germination and growth. However, 2–4 days after EDTA addition into the soil there were numerous brown dots on the leaves, and the whole leaf became yellow and died slowly, indicating phytotoxicity of EDTA metals [26].

These findings are consistent with the results of present study. In our study, after Ni content in plants reached 1000 mg/kg, it was observed that biomass of plant was decreasing sharply with increasing initial Ni concentration.

The correlation analyses were performed between Ni content and the plant growth and correlation was much stronger ($r > 0.95$, $p < 0.01$). This strong correlation can be explained through much higher affinity of *Brassica rapa* to accumulate Ni species as shown in Fig. 3. The enhanced Ni accumulation in the presence of EDTA indicates the formation of less toxic Ni-EDTA complexes, which led to an increase in plant growth (Fig. 1), thereby increasing both Ni tolerance and capacity for Ni transport to the shoot [50].

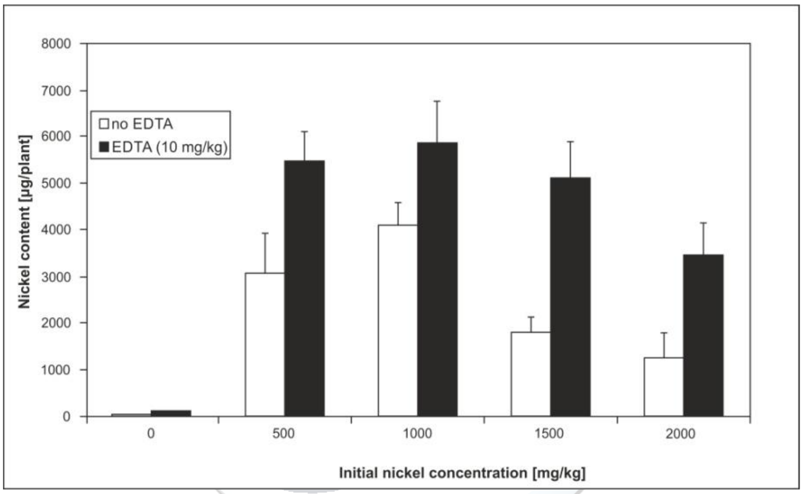

Fig. 3. *Effect of Ni and EDTA on Ni uptake by Brassica rapa plotted in terms of initial nickel dose versus shoot Ni content (µg/plant).*

According to Laurie *et al*. [51] and Molas and Baran [35], there are mainly two different patways for the uptake of metal ions from contaminated soils. The first path involves the transport of free metal ion (M^{2+}) to cell root across plasmalemma following the dissociation of metal-ligand complex in the diffuse layer. The second pathway, on the other hand, involves the absorption of metal-ligand complex by root cell membrane where the metal-ligand complex is either transported to root cells across plasmalemma, or dissociated in the cell membrane; free metal is then transported to cell, and the ligand goes back to solution [52] (Wang *et al.* 2007). Here, the increase in Ni uptake in the presence of EDTA may be explained through a mechanism similar to the second pathway mentioned above. Molas and Baran [35] suggests that Ni is absorbed by plants in the form of a free ion rather than a Ni-ligand complex.

3.5. Biological accumulation coefficient (BAC)

In addition to total Ni concentration and content, the BAC needs to be considered while evaluating hyperaccumulators. The BAC commonly used commonly to evaluate metal accumulating capacity of plants relative to the degree of soil contamination [53]. According to the criteria described by Brooks [38] to define hyperaccumulators, bioaccumulation coefficient (ratio of metal concentration in plant to soil) is greater than 1. Biological accumulation coefficient (BAC) was defined as the concentration of heavy metals in plant shoots divided by the heavy metal concentration in soil [$BAC = [Metal]_{shoot} / [Metal]_{soil}$] [54] and indicate the ability of plants to tolerate and accumulate heavy metals.

The bioaccumulation coefficients of Ni in *Brassica rapa* growing in a artifically contaminated soil are shown in Table 2. *Brassica rapa* was between 1.9 and 5.6 for Ni, providing further evidence for the transport of Ni from Ni contaminated soils to plant shoots. Adding 10 mg/kg EDTA significantly increased the bioaccumulation coefficient of Ni in *Brassica rapa* shoots (Table 2). This information confirm also Ni hyperaccumulation of *Brassica rapa.*

Table 2. *Bioaccumulation coefficients of Ni in Brassica rapa growing in a artifically contaminated soil*

Application (mg/kg)		**Bioaccumulation Coefficient**
Ni	**EDTA**	
500	0	3.6
1000	0	2.8
1500	0	2.1
2000	0	1.9
500	10	5.6
1000	10	3.6
1500	10	2.7
2000	10	2.0

4. CONCLUSION

Greenhouse experiment was carried out to evaluate the potential for phytoextraction of Ni, with or without with the use of EDTA. Nickel hyperaccumulation was defined by [38] Brooks *et al.* (1989) as the accumulation of at least 1000 mg/kg Ni in the dry biomass of plants grown on a natural substrate. As indicated in the results, the pot experiment conducted with contaminated soils indicated that *Brassica rapa* had the capability to accumulate high levels of Ni. Ethylenediaminetetraacetic acid is a good chelating agent for enhancing phytoextraction of Ni by *Brassica rapa*, especially when 10 mg/kg EDTA is applied, because it exceeded the threshold value (1000 mg/kg) as a Ni hyperaccumulator [11, 55-56] (Baker and Brooks 1989; Alkorta *et al.* 2004; Gupta *et al.* 2011). Moreover, the BAC value were 1.9 and 5.7, respectively, which were higher than 1 with or without with the use of EDTA. Therefore, *Brassica rapa* might be useful for the remediation of soil contaminated with Ni. Since *Brassica rapa* is fast and easy growing a agronomic crop plant. In recent years, *Brassica rapa* is seen as a key and strategic crop for raw material supply in the biodiesel (green diesel) industry throughout the world and Turkey [20,57] (Baydar 2005; Mahasi and Kamundia, 2007). Therefore, the distribution of the accumulated Ni within the plant and whether the transport to *Brassica rapa* seeds of Ni should be also study in the future.

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