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# Phytoremediation of nickel (Ni) from agricultural soils using canola (*Brassica napus* L.)

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ABSTRACT

The aim of this research is to propound an innovative method to remediate the nickel (Ni) pollution in agricultural soils by using canola (*Brassica napus* L.) plant. For this purpose, a field experiment was conducted on the campus of Namık Kemal University, namely its Faculty of Agriculture Research and Experimental Area, during 2011. Nickel (100 mg/kg) as $NiSO_4$ forms was applied to each parcel. Then ethylene diamine tetra acetic acid (EDTA) chelate was applied to each parcel at four doses (0, 5, 10, and 15 mmol/kg) during the blossom period of the plants. The plants were harvested four months after planting. The soil in the samples was classified with neutral pH, low lime, and insufficient organic matter. The available phosphorus content and the exchangeable potassium content of research area were both found to be sufficient. The amount of available iron, manganese, and copper of the experimental soil was sufficient, but the available zinc amount of the soil was determined at a deficient level. The experimental area soil was classified to be in clay texture. According to the field experiment results, root and shoot yield of plants decreased with increasing EDTA doses. On the other hand, nickel amount of root and shoot of the plants increased with increasing EDTA application to the plants. These increases and decreases were found to be statistically significant at the level of 1%. According to the results of the experiment, heavy metal pollution of the soil of Tekirdağ province can be remediated by the phytoremediation method.

*Keywords:* Phytoremediation; Soil pollution; Nickel; Canola; Tekirdağ

## 1. Introduction

In recent years, soil pollution caused by heavy metals has become a point of interest in scientific research all over the world. The issue of soil pollution, which has gradually become a universal problem promotes, among other things, the idea that the best solution would certainly be taking preventive mitigation strategies. Phytoremediation improves the biological and physical properties of the soils. One of the advantages of phytoremediation is its economic process costs. In a study on the remediation of a

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polluted by Ni 0.4 ha area, estimated costs for 30 years are as follows: Removal by improvement cost is 12,000,000 US dollars, soil washing is 6,300,000 dollars, capping costs is 600,000 dollars, and phytoextraction is 200,000 dollars [1].

The plants left over on agricultural soils after having been harvested can be isolated in ash form by being burnt, or getting dried, or getting decayed so as to make composites, or by recycling of the biological metal materials [2]. The use of chemicals at home, in agriculture and in medicine increased in the last 50–100 years. The accumulation of heavy metals in the soil has adverse affects not only on the fertility and ecosystem of the soil but also on human health. The heavy metal pollution in soil has become a global problem as a result of increasing industrial and mining activities and the use of contaminated water in irrigation as well as the wide spread use of treatment sludge in agriculture.

There are also some health problems of the farmers that have not yet been researched and that can result from contacting the heavy metal contaminated soil, inhalation of contaminated soil dusts or vapors of heavy metals.

According to some researchers, the best plant to be used against nickel pollution with phytoremediation method for removal of nickel from agricultural soil is canola (*Brassica napus* L.) plant [1,2].

According to Bryan [3], concerning the toxicity of the metals, the most toxic metals are mercury, silver, copper, cadmium, zinc, lead, chrome, nickel, and cobalt. All heavy metals in high concentration are potentially toxic.

Nickel is a silver-like metal, which melts at 1,453˚C. If nickel is separated into particles, it can react with air and can spontaneously conflagrate. The inorganic nickel compounds are solid matters which are easily soluble in water, like such substances as nickel sulfate, nickel acetate, and nickel nitrate [4].

Geochemically, the distribution of Ni, Co, and Fe is similar on the surface of earth. The total tolerable amount of nickel is 50 mg/kg in soil [5,6]; and the extractable tolerable amount of nickel is 10 mg/kg [5]. In another research [7], the plants which are tolerable to nickel contain 11–30 mg/kg for dry weight of plant and a higher value of nickel than this is toxic for plants.

If the nickel amount is above the limit in the plant, it has an adverse affect on chlorophyll syntheses and on oil and protein metabolism, and creates an antagonist affect with other elements in the roots of plants. A research on bean plant indicated that 0.1, 0.3, and 0.5 mM doses of nickel decreased the plant's chlorophyll a, chlorophyll b, carotenoids, total pigment I, and total pigment II uptake [8,9].

As a result of nickel toxicity, the roots of the plants are damaged, slightly yellow strips appear on the leaves of the cereals, and then the leaves turn white. With higher levels of nickel toxicity, the leaves necroses burn [10]. The more the distance of the field from the motorways, the less is the nickel amount in the soil, and this is explained by the nickel being contained in the fuel consumption in the motor vehicles [11].

A large part of the nickel in soil formed from nickel-rich rocks belongs to the silicate, and therefore, it is not in an available form [12]. It is indicated that the nickel amount of igneous rocks changes between 2 and 3,400 mg/kg and in sedimentary rocks is between 26 and 1,000 mg/kg. It is stated that the nickel amount of the soil depends on the properties of the rocks, for example, the usual nickel amount in the soil is 20 mg/kg, whereas, this content reaches 7,000 mg/kg in the soils formed from antimonite rock. It is stated in the research that the nickel amount of the plants cultivated soils with normal level of nickel is between 0.1 and 5 mg/kg. However, according to some other researchers' reports [11], the nickel amount in the plants are generally 20–100 mg/kg in antigorite rock-composed fields and some of the hyper accumulator plants (*Sebertia acuminate*) are more than 1,000 mg/kg nickel.

The average nickel amount in the rain water is 2.5 μg/L, in ground water is 1–6 μg/L, in running water is 4–14 μg/L, and in sea water is 1–6 μg/L. Regarding the air, the nickel amount is 6 ng/m$^3$ in the countryside, 17–25 ng/m$^3$ in urban areas, and 150 ng/m$^3$ in the industrial regions [13].

The aim of this research is to propound an innovative method to remediate the Nickel pollution in agricultural soils by using canola (*Brassica napus* L.) plant.

## 2. Materials and methods

The research was conducted at the Faculty of Agriculture Experiment and Research Area, Namık Kemal University in Tekirdağ province in 2011, between the coordinates of 40º59´43.63´´N and 27º35´39.75´´E. 100 mg/kg $NiSO_4$ was used as pollutant in the research area. ethylene diamine tetra acetic acid (EDTA) was used to increase the penetration of nickel in the soil. As testing plant, the canola (*Brassica napus* L.) plant, which is the most suitable hyper accumulator plant, was used [14]. 100 mg/kg nickel as $NiSO_4$ form was applied in the experimental area. After the $NiSO_4$ was applied to the experimental area, the incubation lasted for 30 d to allow the creation of the pollution in the soil and the absorption in the soil of the particles, and then some soil samples were analyzed for the extractable amount of the nickel. In order to hinder the pollutants contamination, in each parcel of soil some PVC lysimeters were placed in a depth of 30 cm. Canola seeds were sowed and then some 0, 5, 10, and 15 mmol/kg EDTA have been applied to each parcel before the blossom of the plants in order to activate the heavy metal absorption by plants.

### 2.1. Methods of analysis

Then, the soil samples were analyzed for pH, lime, organic matter, and available phosphorus [15]. The extractable nickel amount of the soil samples were analyzed according to [16] in ICP-OES. Nickel amounts of the plant samples were analyzed by the method of wet digestion and with ICP-OES (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794 (USA). The dry matter amount of the plant was obtained after being dried at 70˚C for 48 h [17].

### 2.2. Statistical analysis

The data obtained from the research were transferred to computer for analysis to be made by using the PASW® Statistics 18 for windows software. Testing the differences between the groups, the data were subjected to ANOVA variance analysis and for the important mean data, the Duncan multiple comparison tests was applied. The analyses were performed after the harvest of the plant.

## 3. Experimental results

The soil samples have neutral pH, lime low, and insufficient organic matters. The available phosphorus content is sufficient and exchangeable potassium content is also sufficient. The available iron, copper, and manganese amounts are sufficient and the available zinc amount is deficient, and the soil on the experiment area and texture class are clay obtained [18,19]. Some of the physical and chemical properties of the soil samples are given in Table 1. The extractable nickel amount of the research area soil was determined as 0.95 mg/kg for experiment before and as 6.20 mg/kg after 30 d of incubation.

During the experimental period, the temperature and rainfall data in experimental area in Tekirdağ are given in Fig. 1.

The variance analysis results of the effects of EDTA application on the dry matter yield of canola plant are given in Tables 2 and 3.

Table 1
Some physical and chemical properties of the experimental soils

| Soil properties | Unit | Value |
|---|---|---|
| pH | (1:2.5 soil:water) | 6.81 |
| Electrical conductivity | dS/m | 128.3 |
| $CaCO_3$ | % | 2.40 |
| Organic matter content | % | 1.88 |
| $P_2O_5$ | kg/da | 11.42 |
| $K_2O$ | kg/da | 25.32 |
| Fe | mg/kg | 3.46 |
| Cu | mg/kg | 0.63 |
| Zn | mg/kg | 0.40 |
| Mn | mg/kg | 5.72 |
| Clay | % | 42.98 |
| Silt | % | 25.44 |
| Sand | % | 31.58 |

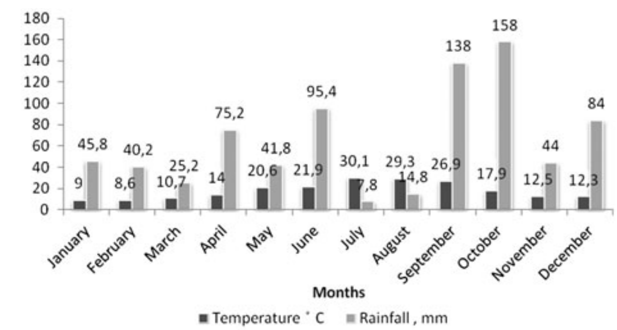


Fig. 1. The temperature and rainfall data of experimental area in Tekirdağ.

According to Table 2, the effect of EDTA application on the dry matter yield of canola plant are found significant statistically at the level of 1%.

When focusing on the heavy metal amount of nickel in Table 3, the highest nickel amount in the root and shoot of the plant, after the control parcels, is found as 0 mmol/kg EDTA applicated parcels, and the obtained nickel amount is 640 kg/da for the roots and 850 kg/da for the shoots. The lowest dry matter yield is obtained from 15 mmol/kg EDTA applied parcels, and the values are of 430 and 590 kg/da for the root and shoot, respectively. The effects of EDTA application on the dry matter yield of canola plant are found statistically at the level of 1%. According to the Duncan multiple comparison test, the nickel amounts in the root and shoot of canola plant are ranked to different groups. The effects of EDTA application on nickel amount of canola plant and variance analysis results are given in Tables 4 and 5.

Table 2
Variance analysis of EDTA applications on dry matter yield of canola plant with nickel-polluted soils

| SV | df | SS root | MS root | *F* value root | SS shoot | MS shoot | *F* value shoot |
|---|---|---|---|---|---|---|---|
| General | 14 | 121382.0 | | | 252170.0 | | |
| EDTA dose | 4 | 121,260 | 30315.0 | 0.00** | 251940.0 | 62985.0 | 0.00** |
| Error | 10 | 122.0 | 12.2 | | 230.0 | 23.0 | |

Notes: SV: Source of variation, df: degree of freedom, SS: Sum of squares, MS: Mean of squares, *F*: *F* value, **: Significant at 1%, ns: Non-significant.

Table 3
Mean values and significance groups of EDTA applications on dry matter yield of canola plant with nickel polluted soils, kg/da[a]

| | Nickel (Ni) | |
|---|---|---|
| EDTA applications | Root | Shoot |
| Control | 680 ± 3.21 e | 975 ± 3.21 e |
| 0 mmol/kg | 640 ± 1.00 d | 850 ± 2.51 d |
| 5 mmol/kg | 585 ± 1.52 c | 790 ± 2.08 c |
| 10 mmol/kg | 510 ± 2.08 b | 710 ± 2.88 b |
| 15 mmol/kg | 430 ± 1.52 a | 590 ± 3.00 a |

[a]The values mean of three replications and root and shoot were evaluated individually.

Table 5
Mean values and significance groups of EDTA applications on nickel content of canola plant with nickel polluted soils, mg/kg[a]

| | Nickel (Ni) | |
|---|---|---|
| EDTA Applications | Root | Shoot |
| Control | 4.76 ± 0.11 a | 5.83 ± 0.63 a |
| 0 mmol/kg | 24.43 ± 0.13 b | 13.12 ± 0.97 b |
| 5 mmol/kg | 49.65 ± 0.34 c | 37.60 ± 0.56 c |
| 10 mmol/kg | 77.80 ± 0.60 d | 61.40 ± 0.28 d |
| 15 mmol/kg | 85.30 ± 1.01 e | 65.10 ± 0.057 e |

[a]The values mean of three replications and root and shoot were evaluated individually.

According to Table 4, the effect of EDTA application on the nickel content of root and shoot yield of canola plant is found to be statistically significant at the level of 1%.

According to Table 5, the nickel amount of the plants has increased with increasing EDTA applications. The reason for this result could be that the EDTA application increased the solubility of nickel in the soil, and thus the absorption of this heavy metal by the plant was made easier. The lowest nickel amounts of plant for root and shoot were determined as 0 mmol/kg EDTA applied parcels. The highest nickel amount in plant for roots and shoots were obtained with 15 mmol/kg EDTA applied to parcels. These results were earlier obtained by some other researchers. The researchers concluded that the EDTA application increased the solubility of the heavy metals, and consequently the absorbability of the heavy metals from the soil [20–25]. The effects of EDTA application on nickel amount of canola plant are given in Fig. 2.

The nickel amount in root and shoot of the canola plant increased with increasing the EDTA application. The increasing of nickel amount in the root and shoot of the canola plant was found significant at a level of 1% and different groups were formed in Duncan multiple comparison test.

Some of the physical and chemical properties of the research area soils have not been determined in our experimental study, because these research values are revelatory only for 2011. Therefore, this research will continue in the future until the removal of the nickel pollution from the research area.

Table 4
Variance analysis of EDTA applications on nickel content of canola plant with nickel polluted soils

| SV | df | SS root | MS root | *F* value root | SS shoot | MS shoot | *F* value shoot |
|---|---|---|---|---|---|---|---|
| General | 14 | 14128.9 | | | 8790.6 | | |
| EDTA dose | 4 | 14119.6 | 3529.9 | 0.00** | 8780.1 | 2195.0 | 0.00** |
| Error | 10 | 9.3 | 0.9 | | 10.4 | 1.0 | |

Notes: SV: Source of variation, df: degree of freedom, SS: Sum of squares, MS: Mean of squares, *F*: *F* value, **: Significant at 1%, ns: Non-significant.

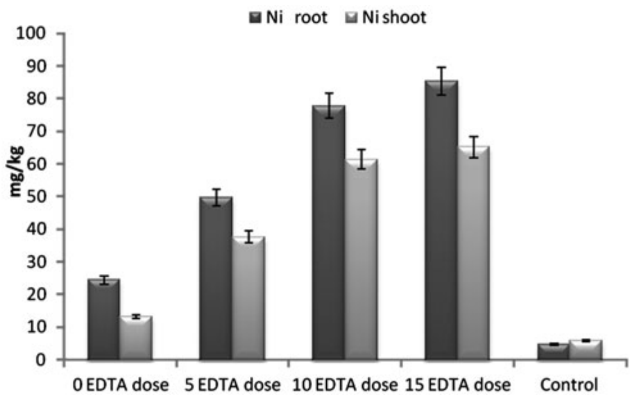


Fig. 2. The effect of EDTA applications on nickel amount of canola plant.

## 4. Conclusion

According to the Baker theory, which is used to assess the performances of the plants in phytoremediation method, plants are classified into two groups as good accumulators and as root collect. According to the Baker theory, good accumulator plants accumulate heavy metal parts above the surface. If the ratio of the elements in the parts above the surface is more than one compared to the elements in the root, this plant is considered to be a good accumulator. If this ratio is lower than one, this plant is classified as a root collect and this plant cannot convey the elements taken from the soil to the other parts of the plant. According to the Baker theory, good accumulators are used in phytoextraction method and root collected are used in phytostabilization method [26]. In our research, this ratio is lower than one. As a result, both phytoremediation and phytoextraction, which are types of the fitoremediation method, are used to reduce the heavy metal pollution in the experimental soil.

According to our research results, the phytoremediation method can be used for the removal of heavy metal pollution from the soil of the Trakya region. On the other hand, the heavy metal pollution is increasing with developing industry day by day in this region. The present research is the first one in Trakya region, and therefore it may serve as a guide and help for other researchers.

The heavy metals like Cr, Co, Ni, and Pb, which are released during various industrial activities, contaminate the soil of Trakya region. To remove these heavy metals from the soil by using the classical physicochemical methods is expensive and the applicability of these methods is limited. For this reason, heavy metals like Cr, Co, Ni, and Pb, which are immobile in the soil, can be removed from the soil in natural ways by increasing their movement through the use of EDTA and hyperaccumator plants like canola. This method is natural, easily applicable, and cheap in comparison with other chemical methods for removing the heavy metals from the soil, and it is getting increasingly important for Trakya region and its agricultural soils.

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