

Absorbability and translocation of Nickel from soil using the sunflower plant (*Helianthus annuus*)

Maryam Rafati^{1,✉}, Nikoo Siahpoor², Maryam Mohammadi Roozbahani³, Masomeh Heidari⁴

1. Young Researchers and Elite Club, North Tehran Branch, Islamic Azad University, Tehran, Iran
Corresponding author
2. M.Sc of environmental pollution, Department of the Environmental pollution, Ahvaz branch, Islamic Azad University, Ahvaz, Iran
3. Department of the Environmental pollution, Ahvaz branch, Islamic Azad University, Ahvaz, Iran
4. Department of the Environmental science, School of Agriculture and Natural Resources, Sanandaj branch, Islamic Azad University, Sanandaj, Iran

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ABSTRACT

Today, soil pollution with heavy metals is a major environmental concern across the world. Phytoremediation is defined as a technique through which plants are able to absorb contaminants and potentially recover the soil that is polluted by heavy metals. The present study aimed to investigate the level of nickel concentration in the roots, stems, and leaves of sunflower, as well as the mobility of this heavy metal in the organs of the plant. Various concentrations of nickel nitrate (50, 100, and 200 mg/kg) were added to the soil in the form of solutions. After the growing season, samples of the plant organs and corresponding soils were collected in order to measure the total concentration of nickel. According to the results, the highest concentration of nickel at the treatments of 0, 50, and 100 was detected in the stem, while the highest concentration in 200 mg/kg was detected in the roots. The lowest concentration of nickel was observed in the leaves in all the treatments. In addition, the measurement of the nickel mobility in various layers of the soil samples indicated that this index was above one only in the soil to the root layer (200 mg/kg) and root to the stem in the other treatments, which denoted the high translocation of this metal in the mentioned layers. Considering the risk of nickel toxicity, it seems that sunflower accumulates the highest level of nickel in the root and sending the lowest one to the shoot at high concentrations in soil.

Keywords: Phytoremediation, Nickel, Mobility, Translocation, Accumulation

Introduction

Heavy metals are metallic elements with an atomic density of more than six grams per cubic centimeters. Currently, heavy metal contamination represents a growing environmental concern across the world. Heavy metals are ubiquitous, highly persistent, non-biodegradable compounds, which tend to bioaccumulate in the food chain.^{1,2} The concentration of heavy metals increases as a result of the natural weathering of rocks, waste disposal, mining operations, smelting of metal ores, use of fertilizers and pesticides, and

industrial effluents.³ To remediate the sites that are contaminated with heavy metals, conventional methods such as landfilling, excavation, and extraction have proven efficient. However, these methods have a limited scope due to their high energy input and may not be economically feasible due to their high costs, particularly when used for the removal of heavy metals at low concentrations (<100 mg/L).^{1,2,4}

Some heavy metals are not needed for plants and have no beneficial function. On the other hand, heavy metals such as nickel (Ni) improve the structure of some plant enzymes, and their low concentrations are essential to the growth of plants and sustainability of agroecosystems. It is notable that high concentrations of nickel are associated with adverse health effects in humans, including

✉ Maryam Rafati
m.rafati.env@gmail.com

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cancer, pulmonary disorders, and skin sensitivity.⁵

Phytoremediation is the process through which green plants extract, sequester, and detoxify pollutants. This cost-efficient technique has attracted the attention of researchers over the years. Phytoremediation could be accomplished in-situ, while it is also an environmentally friendly approach for the removal of heavy metals from the soil by the use of appropriate plants for this purpose.⁶

Sunflower (*Helianthus annuus*) is a proper option for phytoremediation.⁵ Sunflower grows in tropical and mid-tropical areas depending on the type of the hybrid. It is cultivatable in a wide range of climatic conditions. The sustainability of sunflower is caused by its various morphological and physiological features, including the rapid growth (90-150 days depending on the level of environmental factors), having developed roots, resistance to soil salinity, passiveness to the length of the day, and no need for highly fertilized soil to provide satisfactory products.^{7,8}

Accumulation of heavy metals in the sunflower plant has been investigated in various studies. For instance, Sharma and Dubey have reported that the root of sunflower could contain high levels of lead.⁹ In a research by Zabetakis, variable levels of Cr (VI) and Ni (II) were added to the soil (0 µg/L (control) to 10.000 µg/L), and the findings indicated a significant increase in the levels of these elements in all the plant organs compared to the controls.¹⁰

After the meticulous analysis of sunflower, Fulekar concluded that this plant is able to absorb cadmium and zinc from contaminated soil, accumulating them in its leaves.¹¹ On the other hand, Adesodun reported that in the soil with a high level of zinc pollution, sunflower could concentrate half of the soil zinc in its shoots.¹² In this regard, Mohammadzadeh compared the phytoremediation potential of the soil contaminated with nickel by sunflower and sorghum, claiming that the absorbed concentrations of nickel by sunflower roots were mostly translocated to the shoots, while sorghum concentrates were mostly stored in the roots.⁷ In another study, Nikseresht compared

sunflower with other plants, such as *Trifolium pratense* and *Amaranthus retroflexus*, reporting that sunflower was able to absorb and accumulate zinc in its leaves.⁸

The present study aimed to investigate nickel concentration in various organs of *Helianthus sp.*, including the roots, stems, and leaves, as well as the mobility potential from soil to different layers of soil to the root, root to the stem, and stem to the leaves.

Materials and Methods

This pot experiment was performed to investigate the absorbability of sunflower with various levels of nickel in Ahvaz, located in Khuzestan province, Iran. Sufficient amounts of sunflower seeds were provided from Alborz Seed and Plant Improvement Institute. The seeds were compatible with the climatic condition of Khuzestan province and resistant to pests.

The seeds were planted in pots, and each pot was filled with seven kilograms of proper soil. The soil was a well-blended combination of clay, sand, and animal manure with a ratio of 3:1:1 (v/v/v). This particular soil combination is prepared through a lengthy process by local farmers. The same instructions were followed in the present study in order to obtain the proper combination in a greenhouse in Ahvaz. Additionally, five samples of the soil were collected before planting and analyzed in terms of the physicochemical properties. Afterwards, the pots were placed outdoors in natural conditions, irrigated with tap water twice per week, and partially covered for protection against rainfall.

Two months after planting when the leaves of the plants budded, various concentrations of nickel (50, 100, and 200 mg/kg) were added to the pots using NiN₂O₆. 6H₂O in the form of solutions over four weeks (1/4 of the total solution each week instead of one irrigation turn) (Figure 1). During irrigation, there was no water drainage from the bottom of the pots.¹³ The treatments selected for the present study were mainly within the range of the values used in the previous studies in this regard.^{5,7,8}



Fig. 1. Sunflower Plant Growth Treated in Pots

After the complete growth of the plants, the entire structures of all the plants were dug out, and sampling was performed. At this stage, samples were provided from the leaves, roots, and stems of the plants. In addition, one soil sample was obtained from the depth of 0-25 centimeters (root zone) per each plant. All the samples were placed in polythene bags, labeled, and transferred to the laboratory for further analysis.

The soil samples from each pot were homogenized and air-dried in an oven at the temperature of 30 °C overnight to a constant weight and filtered through a two-millimeter sieve before analysis. Approximately 0.25 gram of the soil samples was digested with six milliliters of H₂SO₄:15 ml H₂O₂ in a closed Digesdahl system (Hach Co., USA) at the temperature of 440 °C to obtain the total extraction of nickel. Following that, the samples were filtered and diluted with deionized water to

50 milliliters.^{13,14} Total concentrations of nickel were determined using inductively coupled optical plasma emission (ICP-OEC) spectroscopy (GBC, Australia).

All the plant parts (leaves, stems, and roots) were washed with tap water to remove the residual soil or dust and dried in an oven at the temperature of 70 °C for 48 hours. Afterwards, they were ground and sieved to <1 millimeter. The resulting sample (0.5 g) was digested using a mixture containing four milliliters of H₂SO₄:13 ml H₂O₂ in a closed Digesdahl system at the temperature of 440 °C (Hach Co., USA). At the next stage, the samples were filtered and diluted with deionized water to 50 milliliters. Finally, the solutions were analyzed in terms of nickel concentrations using ICP-OEC.^{14,15} This research was completely random, and the measurements were carried out with three independent replicates for nickel concentrations. Data analysis was performed in SPSS version 21.0 using the Games-Howell test for mean comparisons at the significance level of P≤0.05.

Results and Discussion

Table 1 shows the main features of the soil that was initially provided for plantation before treatment. The soil had a loamy texture, with the average electrical conductivity and cation exchange capacity (CEC) of 3.70 ds/cm and 16 meq¹/100 g, respectively. In addition, the soil was slightly alkaline (pH=7.22) and categorized as salty soil, with proper acidity for plant growth.

Table 1. Physiochemical Features of Primary Soil

Ni (mg/kg)	O.C %	CEC (meq/100gr)	EC (ds/cm)	pH	Sand %	Silt %	Clay %	Soil Texture
2.95	2.48	16.1	3.70	7.22	46	38	16	Loamy

Figures 2-5 depict the total concentrations of nickel in different organs of sunflower. As can be seen in these figures and the treatments, the highest concentration of nickel was detected in the roots of sunflower (treatment: 200 mg/kg), showing a significant difference

compared to the stems and leaves of the plant. In the sunflower stems, the estimated concentrations of nickel were 38.17, 38.32, 39.52, and 95.86 mg/kg in the treatments of 0, 50, 100, and 200 mg/kg, respectively. Moreover, the lowest concentration of nickel

¹ Milliequivalent

was observed in the leaves with a significant difference with the roots and stems.

According to the hypothesis proposed by Kabata-Pendias, the nickel concentrations of 10-100 mg/kg are poisonous to plant leaves.¹⁴ In the current research, the nickel concentrations in the leaves of sunflower (30, 28.29, 28.19, and 27.52 mg/kg in the treatments of 0, 50, 100, and 200 mg/kg, respectively) remained within the lower range in different plant organs, they again locate in the poisonous areas in the leaves.

Our findings are consistent with the results obtained by Dubey and Sharma, which denoted the highest accumulation of lead in the roots of sunflower.⁹ However, the results of the present study are inconsistent with some of the previous studies in this regard. For instance, Mohammadzadeh and Khosravi identified the highest accumulation of cadmium and nickel in the shoots of sunflower through individual researches.^{7,16} In the study by Khosravi, increasing the concentration of nickel in the treatments from zero to 200 mg/kg was associated with the higher accumulation of nickel in the roots and the shoots of the plant, while the concentration was reported to be higher in the shoots compared to the roots.

On the other hand, the findings of Fulekar and Salimi showed that sunflower is capable of absorbing cadmium and zinc from contaminated soils and cadmium from municipal compost and sewage sludge, accumulating these metals in its organs.^{11,17} In addition to the type and age of plant species and type of heavy metals, the uptake of pollutants from the soil mostly depends on the properties of the soil, such as the pH, CEC, organic carbon, oxidation conditions, and recovery. Correspondingly, even similar plants may behave differently toward heavy metals,¹⁴ which is a clear finding in the aforementioned studies.

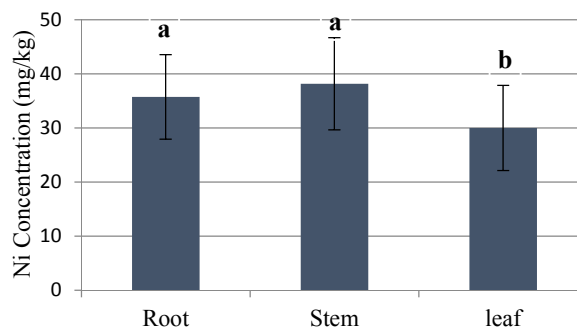


Fig. 2. Mean Ni Concentration in Treatment of 0 mg/kg (control)

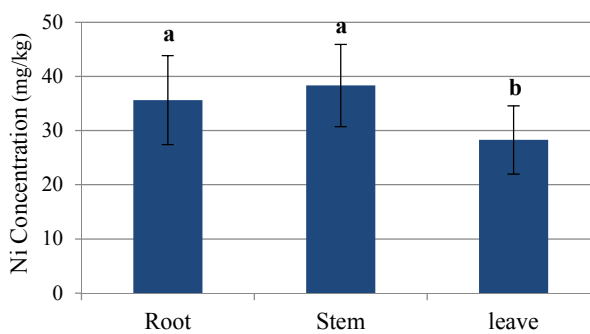


Fig. 3. Mean Ni Concentration in Treatment of 50 mg/kg

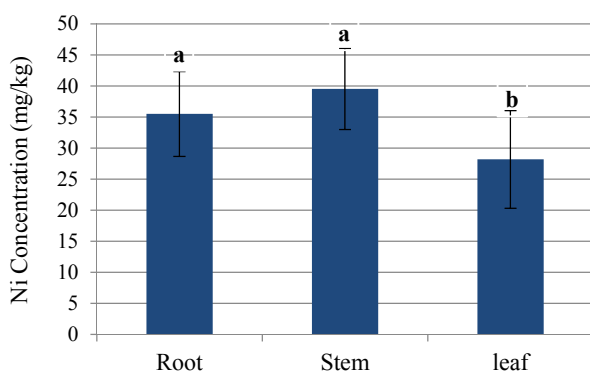


Fig. 4. Mean Ni Concentration in Treatment of 100 mg/kg

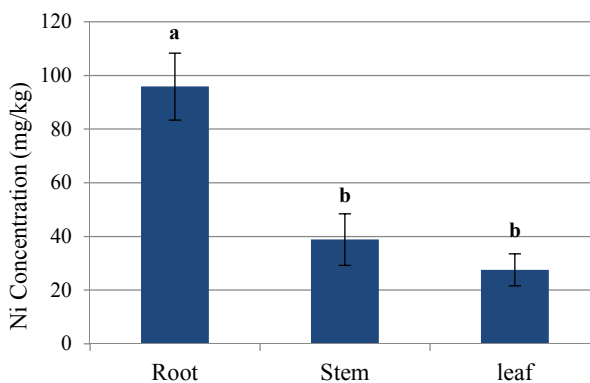


Fig. 5. Mean Ni Concentration in Treatment of 200 mg/kg

Data in the figures are expressed in mean and standard deviation ($n=3$). In addition, the mean values in the diagrams that are marked by letters (a-c) show the significant differences ($P<0.05$).

Various levels of the nickel mobility index in different treatments are presented in Table 2. The index was obtained through dividing the metal concentration by the receiver layer to the base layer,¹⁸ which was calculated in the soil to the root, root to the stem, and stem to the leaves layers in the current research.

Table 2. Ni Mobility Index in Sunflower

Treatment (mg/kg)	Mobility Index (MI)		
	Soil-Root	Root-Stem	Stem-Leaves
0	0.62	1	0.82
50	0.53	1.11	0.75
100	0.46	1.08	0.73
200	1.26	0.41	0.7

The calculated mobility index was an average of three replicates for every treatment. According to the information in Table 2, the mobility index was above one in the soil to the root layer only in the treatment of 200 mg/kg and in the root to the stem layer in the treatments of 0, 50, and 100 mg/kg. The index exceeded one in none of the treatments in the stem to leaves layer. The amount of this index in the treated levels indicated that with approaching from 0 to 50 in the treatment of 100 mg/kg, the amount of the mobility index in all the layers gradually decreased until reaching below one in the treatment of 200 mg/kg in the roots to the stem layer ($P=0.41$). However, this gradual reduction in the soil to the root layer in the treatment of 200 mg/kg remarkably increased and exceeded one (Figure 4). This finding could suggest nickel stabilization in the roots of sunflower in the treatment of 200 mg/kg. In other words, although nickel had lower translocation capability in the soil to the root layer in the treatments of 0, 50, and 100 mg/kg, while this accumulated amount in the roots of sunflower in these treatments could translocate to the stems with force and the coefficient of more than one. However, the lower mobility index than one in the surface of the leaves in all the treatments was indicative of its lower

accumulation in the leaves of sunflower (Figures 1-4).

In a study in this regard, Nikseresht reported that the translocation amount of zinc from the roots to the shoots of sunflower was above one in the soil containing 120 mg/kg of this element.⁸ Moreover, another research by Motesharezadeh regarding sunflower demonstrated that cadmium had a remarkable capability in translocation from the roots to the shoots. By increasing the concentration of cadmium in the soil, its accumulation in the roots and shoots could increase significantly.¹⁹

It is also noteworthy that the studies in this regard are mainly focused on the translocation factor, which differs from the mobility index. The translocation factor is obtained by dividing the concentration of the heavy metal by the shoots (leaves, stems, branches, flowers, and even fruits) to the roots,⁸ while the mobility index is more accurate and could individually show translocation on every level. In the current research, we were not able to retrieve the studies that investigated the mobility index in sunflower.

Conclusion

In the current research, the high concentration of nickel in the stems (treatments: 0, 50 and, 100 mg/kg) and roots (treatment: 200 mg/kg) confirmed the possibility of nickel phytoremediation. Nickel is a nutritious substance for plants. At the high concentrations of nickel in the soil, it seems that sunflower accumulates the highest concentration of this element in its roots due to the toxicity of this heavy metal, sending the lowest concentration to the shoots. Under such circumstances, the threshold level of nickel accumulation in the stems or roots was observed in the treatments of 100 and 200 mg/kg, demonstrating that further investigation is needed to determine its highest concentration in the roots with the treatments of more than 200 mg/kg. On the other hand, with the entry of nickel from the roots to the stems of sunflower in all the treatments, the low translocation coefficient from the stems to the leaves indicated its low entry into the leaves and possibly the flower and seeds of the plant.

Considering that sunflower seeds are among important food sources, this finding confirms the lower accumulation of nickel in the seeds, assuring its safety for human and animal consumption, even in the sites that are polluted with high levels of nickel. It is recommended that further investigation be conducted in this regard.

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