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# Investigating the Interaction of Silica and Earthworm on Biological Refining of Heavy Metal Nickel in Dwarf Lilyturf (Ophiopogon Japonicus)

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**Abstract**

A pot trial was conducted in 2016 for investigating the use of earthworm and silica element on the biological refining of heavy metal nickel in the dwarf lilyturf (Ophiopogon japonicus). The testing was designed in a factorial based on a complete randomized design. That first factor nickel was in three levels (0, 100, 200 mg per kg soil), Consume (0 and 200 mg/kg silica) as a second factor and third factor considered (0 and 5 earthworms).Some characteristics were measured like: root dry weight, shoot dry weight, soil nickel content, root nickel content, shoot nickel content and soil silica content. The results showed that with increasing of contamination, root and shoot dry weight decreased drastically. Application of earthworm resulted in increased root and shoot dry weight, This is mainly due to the effect of earthworms on increasing organic carbon and increasing the cation exchange capacity on one side, and on the other hand, increasing the absorption of heavy metals in nickel and accumulation in the tissue. Silica is also able to reduce soil toxicity and increase the nickel transfer coefficient from soil to root.

**Keywords**: Biological refining, Earthworm, Heavy metal, Nickel, Silica

## 1. Introduction

Environmental stresses are important factors yield limitation and it always brings about a significant reduction in agricultural products. Soils are contaminated by many pollutants with different concentrations and combinations. These days, soil contamination with heavy metal is one of the major environmental problems in human societies. In addition to harmful effects on soil fauna, flora and groundwater pollution through leaching and caused to reduce plant growth yield and product quality and ultimately endangers the health of individuals and other living organisms. Heavy elements (Cadmium, Chromium, Lead, Arsenic, Nickel, Cobalt and Zinc) produced as a result of major urban and industrial activities and have caused pollution of vast areas of the world [2]. Soil is the main source of mineral nutrition of plants. Plants are the first organisms that react to changing soil conditions. Hence, plants as biological markers reflect the adverse changes in the soil. In bioremediation, it is very important to use resistant plants with high biomass, strong root systems with high elemental transfer coefficients. Some metals such as cadmium, lead, mercury, chromium and cobalt are very harmful and toxic because of the widespread use of industrial processes for the food chain. Chrome is a toxic heavy metal for microorganisms, animals and plants which has become a serious environmental pollutant over the last decade due to extensive industrial use. Bioremediation is a pollution elimination technology in which a biological system is used to degrade or deform a harmful chemical. The importance of applying earthworms can increase the efficiency of these methods and use them to refine soils with a higher percentage of contamination. These earthworms cause to inter air to different parts of the soil and its layers, and increase the amount of nutrients and available materials for plants and

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microorganisms. Research shows that earthworms can tolerate contamination in soils contaminated with heavy metal and alter their distribution [12]. Environmental pollution by heavy metals is a serious and complex problem, which the world today focuses on it. Cadmium, lead, cobalt, copper, mercury, nickel, selenium and zinc are the most polluting metals that are most abundant, Pollution of agricultural ecosystems to heavy metals and other pollutants and consequently, the transfer of these materials to the food chain and ultimately humans, is a global problem [18]. These materials have caused many health problems and can have a devastating effect on the environment and on herbal and animal creatures. Soil contamination with heavy metals causes a significant reduction in the production of the product, so there is a serious risk to agriculture [7]. Plants that can tolerate high concentrations of heavy metals open new ways to the repair of contaminated soils. In many studies, grains are useful for Phytoremediation that have high biomass yield and are known as heavy metal and pollutants adsorbents.

Heavy metals can add in crops or plants and cause the harm and changes in humans and animals' physiological roles during the food chain [13; 5; 8]. Previous studies have shown human exposure to the risk of accumulation of heavy metals and their addition in the tissues full of fat of the mankind corpse [1]. It may impact the central nervous system or be settled in circulatory system and interrupt the normal task of inner organs [18; 4].

## 2. Materials and methods

For investigating the use of earthworm and silica element on the biological refining of heavy metal nickel in the dwarf lilyturf (Ophiopogon japonicus) a pot experiment was carried out in Nowshahr, Mazandaran province, Iran, 2016. This area based on climatic divisions, it is part of humid and semi-humid areas. Dwarf lilyturf (Ophiopogon japonicus) were planted in 12 pots of 20×20 cm and 30 kg capacity. First of all, the pots filled with 25 kg of soil, then, by adding livestock manure to a certain proportion of the pots, they were ready to apply the treatments. The desired metals (nickel and silica) were measured for different treatments with precision scale and the desired treatments were added to the pots. Soil analysis was considered for total nitrogen, phosphorus and potassium absorption, pH, EC, organic matter and CEC. In this study, a factorial experiment was carried out in a randomized completely design (RCD) with three repeats. Factors were as follows:

1. Silica element in two levels ($Si_1$: 0 and $Si_2$: 200 mg/kg)
2. Nickel element in three levels ($Ni_1$: 0, $Ni_2$: 100 mg and $Ni_3$: 200 mg/ kg)
3. Earthworm in two levels ($W_1$: 0 and $W_2$: 5earthwormsper kg)

*2.1 Measure the amount of silica and nickel in the plant:*

At the harvesting stage, the crops were carefully uprooted from the soil and separated into the root and leaf portions, to determine the absorption of silica and nickel. By wet digestion have measured amount of silica and nickel in the plant. Put 2 g of sample in oven at $450^0$C for 24 hours to dry. The dried samples were placed in a furnace at 550 ° C for 3 hours and turned into ashes. The ash was placed in a sample into a glass tube numbered. Then add nitric acid to each of the samples and place it at 80 ° C for one hour Distilled water added to each volume to reach 100 cc then it was extracted and finally, the amount of silica and nickel in the samples was read by atomic absorption set.

*2.2 Measuring the amount of silica and nickel absorbed in the soil:*

The amount of silica and nickel absorbed by the soil was calculated via the Lindsay and Norvell (1978) method after harvest. First, the soil is passed through a sieve of 0.5 mm Add 50 ml of water to the soil to saturate and after 24 hours, the solution was smooth and read by atomic absorption device.

*2.3 Statistical Analysis:*

In order to analyze the statistics, the data was first entered into Excel software. Then, the normalization of the data was done by Kolmogorov-Smirnov method using SPSS (version 16). Some data conversion traits were performed. Then SAS version 9.1 was used for analysis of variance and the mean comparison was based on the least significant difference (LSD) test at 5% probability level. Charts were also drawn by using Excel software.

## 3. Results and Discussion

*3.1 Soil Nickel:*

The soil nickel was statistically influenced by earthworm, silica and the interaction of earthworms on soil nickel levels at 5% probability level (Table 1).

By soil nickel levels increasing, soil nickel content increased. The minimum amount of nickel was obtained in the treatment of no nickel (17.5 mg/kg) and the highest amount found in $Ni_3$ (200 mg/kg) treatment (115.3 mg/kg). The application silica and earthworms also led to the reduction of soil nickel (Table 1). This specify earthworm role in nickel absorption and inactivation of nickel by the use of silica. Silica can increase the nickel transfer coefficient from soil to plant roots. High amounts of Ni can slow down cell separation at root meristems in non-tolerant crops and reduce crop development [14].

*3.2 Soil Silica:*

Soil silica was statistically influenced by the simple effect of silica treatment at a probability level of 1% and other treatments did not (Table 1). The utmost quantity of silica observed in $Si_2$ (200 mg/kg) treatment (96.2 mg/kg) and the least quantity seen in $Si_1$ (0 mg/kg) treatment (28.7 mg/kg). By soil silica levels increasing, soil silica increased significantly. Silica application can decrease metal toxicity, salinity, drought and temperature stresses [9].

*3.3 Root dry weight:*

Root dry weight was affected by nickel levels and application of earthworm at 5% probability level (Table 1). By increasing of nickel levels, root dry weight decreased significantly. The lowest root dry weight (5.58 g) shown in the treatment of nickel ($Ni_3$: 200 mg/ kg) and the highest root dry weight was obtained in no use of nickel (85.4 g). Application of earthworm increased root dry weight in two ways; first, the earthworm can accumulate large amounts of nickel in the body and thus reduce soil toxicity and on the other hand, creating an appropriate plant growth environment by increasing the cation exchange capacity (C.E.C), carbon (C) and soil organic matter (SOM).

In excluder species, which accumulate Ni mostly in their roots, root development is reserved more heavily than the growth of shoots [16; 15] and consequently the root analysis is broadly used for evaluating the toxicity of different agents, containing heavy metals [19].

*3.4 Aerial dry weight:*

The dry weight of the aerial was significant by the simple effect of nickel and earthworms apply at a 5% probability level (Table 1). The consumption of nickel in the soil led to a reduction in aerial dry weight and by increasing nickel levels decreased significantly aerial dry weight. Minimum aerial dry weight (23.5 g) was found in nickel treatment ($Ni_3$: 200 mg/kg) and maximum dry weight of aerial was gained in utilize of earthworm (Table 4). The importance of earthworm role in relieving soil pollution by nickel is due to the absorption and tolerance of heavy metals that cause to soil fertility. By increasing nickel levels, photosynthetic structure will damaged which decreases aerial dry weight. The inhibition mechanisms of crop enlargement and expansion by Ni2+ are in adequately explained. In adding to common metabolic disarray, heavy metal are recognized to drop off the flexibility of cell wall, almost certainly by straight binding to pectins and by supporting peroxidase movement in the cell walls and intercellular space; these peroxidases are necessary for lignifications and connection involving extensin and polysaccharides including ferulic acid [19].

Previous researches demonstrated that addition of Ni critically influences the yield of crops, drastically declining the numbers of seeds/pod, 100-seed weight and seed yield per crop [17].

*3.5 Nickel amount in earthworm body:*

The amount of nickel in earthworm was affected by nickel levels and silica and the interaction between nickel levels with silica at 5% probability level (Table 1). By increasing nickel levels in the soil, the accumulation of nickel has increased in earthworm body. The amount of nickel in earthworm (3.5 mg) was by without use of nickel ($Ni_1$: 0) treatment and by use of nickel the amount measured (37.3 mg/kg) in earthworm body (Table 2).Silica reduced the amount of nickel in the soil and silica consumption has also been affected by the accumulation of nickel in the body of the earthworm. Use of silica in the soil was effective on the absorption and transfer of nickel from soil to root of the plant (Table 3). The dual interaction of nickel levels in silica (Table 6) illustrated that the amount of nickel accumulation in the earthworm has reduced by raising the levels of silica. Maximum accumulation of nickel was founded in interaction between nickel treatment ($Ni_3$: 200 mg/kg) and silica treatment ($Si_1$: 0 mg/kg) and minimum of it between nickel treatment ($Ni_1$: 0 mg/kg) and silica treatment ($Si_2$: 200 mg/kg).

More and Patole (2012) proved that, significantly decrease in heavy metal concentration especially Cd, Pb and Ni indicates the capacity of earthworm to excrete heavy metal in surrounding soil or vermincompost. Similarly, most of heavy metals are accumulated in their body tissues.

### *3.6 Nickel accumulation in Aerial part:*

This character was statistically influenced by the simple effects of silica, earthworms and nickel levels, and the interaction between nickel levels with earthworm at 5% probability level (Table 1). The accumulation of nickel in the aerial demonstrated a linear raise with growing levels of nickel from 0 to 200 mg/kg. The highest accumulation (95.3 mg/kg) was in nickel treatment ($Ni_3$: 200 mg/kg) but silica treatment has reduced nickel accumulation. Nickel has accumulated in the roots by use of silica and has prevented its transfer to the aerial (Table 2). The earthworms has led to a decrease in the accumulation of nickel in the plant aerial parts by nickel absorption in its body and the inactivation of nickel in the soil by the formation of Chelate compounds and bonding to nickel. This is clearly reflected in the dual interaction effects between nickel levels with use or no use of earthworms (Table 5).

Numerous structures of Nickel are in soils that contain: adsorbed or compound on organic cation surfaces or on inorganic cation replace surfaces, inorganic crystalline minerals or precipitates, water soluble, free-ion or chelated metal composite in soil solution [6; 3].

**Table 1.** Analysis of variation (Squares Mean) for root and aerial dry weight and Nickel and Silica content under the influence of experimental treatments

| Source of variation | df | Root Dry Weight | Aerial Dry Weight | Soil Ni content | Earthworm Ni content | Aerial Ni content | Soil Si content |
|---|---|---|---|---|---|---|---|
| Nickel (Ni) | 2 | 53.4* | 44.2* | 216.3* | 17.48* | 19.4* | 65.4 |
| Silica (Si) | 1 | 12.7 | 14.7 | 38.66* | 0.47* | 5.23* | 15.7 |
| Earthworm (W) | 2 | 21.4* | 18.7* | 18.87* | 7.82 | 6.4* | 27.4 |
| Ni×Si | 5 | 17.87 | 19.72 | 13.54* | 6.48 | 3.9 | 23.59 |
| Ni×W | 5 | 6.45 | 16.45 | 4.49 | 9.4 | 4.8* | 7.87 |
| Si×W | 3 | 4.71 | 9.72 | 4.45 | 3.85 | 7.25 | 3.94 |
| Ni×Si×W | 11 | 8.12 | 9.15 | 1.95 | 9.12 | 9.4 | 10.21 |
| Error | 17 | 3.94 | 1.89 | 2.9 | 9.17 | 3.12 | 4.32 |
| CV (%) | - | 13.80 | 11.40 | 2.29 | 8.75 | 6.43 | 4.75 |

*. **: In the order of significance, the probability level is 5% and 1% respectively

**Table 2.** Mean Comparison root and aerial dry weight in dwarf lilyturf and the amount of soil nickel, Earthworms and aerial parts under the influence of Nickel treatment

| Nickel treatments mg/kg | Root Dry Weight | Aerial Dry Weight | Soil Ni content | Earthworm Ni content | Aerial Ni content |
|---|---|---|---|---|---|
| $0Ni_1$: | a85/4 | c38/5 | c27/5 | c3/5 | c12/1 |
| $100Ni_2$: | b61/2 | b27/9 | b63/2 | b12/8 | b32/4 |
| $200Ni_3$: | c50/48 | b23/5 | a115/3 | a37/3 | a95/3 |

In each column, same letter meanings do not differ significantly from each other.

**Table 3**. Mean Comparison of Soil Ni Content, earthworm and Aerial parts of dwarf lilyturf under the influence silica treatment

| Silica treatments mg/kg | Soil Ni content | Earthworm Ni content | Aerial Ni content |
|---|---|---|---|
| $0Si_1$: | a46/1 | b15/7 | a24/7 |
| $200Si_2$: | b17/5 | a28/1 | b12/4 |

In each column, same letter meanings do not differ significantly from each other.

**Table 4**. Mean Comparison of Aerial Dry Weight, Soil Ni Content and Aerial parts of dwarf lilyturf under the influence of earthworm treatment

| Earthworm Treatments | Aerial Dry Weight | Soil Ni content | Aerial Ni content |
|---|---|---|---|
| $W_1$: 0 | b27/4 | a52/7 | a32/4 |
| $W_2$: 5 | a38/1 | b12/8 | b13/8 |

In each column, same letter meanings do not differ significantly from each other.

**Table 5.** Mean Comparison of the interactions between nickel levels and silica on the amount of nickel in soil

| Treatments | Soil Ni content | Aerial Ni content |
|---|---|---|
| $Ni_1$*$W_1$ | 22/4c | 14/1d |
| $Ni_1$*$w_2$ | 15/1c | 7/2d |
| $Ni_2$*$w_1$ | 74/6b | 35/4bc |
| $Ni_2$*$W_2$ | 39/4c | 25/8c |
| $Ni_3$*$W_1$ | 130/4a | 94/1a |
| $Ni_3$*$W_2$ | 74/8b | 43/7c |

In each column, same letter meanings do not differ significantly from each other.

**Table 6.** Mean Comparison of the interactions between earthworm and nickel levels on the treatments

| Treatments | Soil Ni content |
|---|---|
| $Ni_1$*$Si_1$ | 23/4d |
| $Ni_1$*$Si_2$ | 16/7d |
| $Ni_2$*$Si_1$ | 63/7b |
| $Ni_2$*$Si_2$ | 34/5c |
| $Ni_3$*$Si_1$ | 121/4a |
| $Ni_3$*$Si_2$ | 57/1b |

Same letter meanings do not differ significantly from each other.

## 4. Conclusion

The results of this study showed that with increasing nickel levels, the root and aerial dry weight decreased. Also, the use of silica has led to a reduction in the accumulation of heavy metal in the shoots. Earthworms have led to an increase in the dry weight of the roots and aerial. Soil toxicity is reduced by collecting heavy metal in earthworm tissue. The toxic effects reduce with interaction between silica * nickel and earthworm * nickel. They can be used effectively in contaminated areas of this metal to reduce toxicity. Therefore, similar experiments can be very successful with other metals and plants in large industrial cities where pollution of heavy metals caused by cars traffic and industrial wastewater.

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