Phytoremediation capability of nickel and manganese polluted soil by *Sorghum biocilor* L.

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Abstract

A pot experiment was conducted based on a factorial study and complete random block design with three replicates to investigate the phytoremediation potential of *Sorghum biocilor* L. in soils polluted with nickel and manganese during 2017-2018. The first factor was nickel nitrate (0, 60, and 120 mg kg^{-1} soil) and the second factor comprised of manganese sulphate (0, 50, and 100 µM). The characteristics under investigation were shoot height, root length, shoot dry weight, root dry weight, nickel concentration of shoots and roots, manganese concentration of shoots and roots, and nickel and manganese translocation factors. Findings suggested that under nickel and manganese stress, shoot height, root length, shoot dry weight, and root dry weight reduced. Also, with an increase in the soil nickel and manganese, so did the concentration of these heavy metals in roots and shoots of the plants under study. Maximum effect of nickel and manganese were recorded in the treatments comprising 120 mg Kg^{-1} Nickel and 100 µM manganese sulphate, respectively while compared with nickel, manganese caused less damage to the plants under study. Application of nickel to the soil increased the translocation factor while there was no difference between the two levels of nickel applied. On the other hand, application of manganese did not affect the translocation factor compared to the control and the translocation factors for both elements were less than 1. Therefore, based on the findings of the study, sorghum showed a relatively good tolerance to nickel and manganese, the absorbed nickel and manganese were mostly concentrated in the plant root, and *Sorghum biocilor* L. is not recommended for phytoremediation.

Keywords: translocation factor; heavy metals; sorghum; manganese sulphate; nickel nitrate

Naeini, J. and M. Yousefi Rad. 2016. 'Phytoremediation capability of nickel and manganese polluted soil by *Sorghum biocilor* L.'. *Iranian Journal of Plant Physiology* 8 (3), 2427-2435.

Introduction

A serious ecological problem is the pollution with heavy metals which has negative influence on human health and agricultural lands (Naees et al., 2011). Heavy metal pollution is increasingly spreading in the environment and negatively affecting the life of organisms (Dinakar et al., 2008). Unlike water and air, soil pollution is not easy to analyze chemically and as a result of developmental projects and pollution of the soil with heavy metals, the soil structure is intoxicated and harmful for the growth and development of plants which in turn disturbs the biodiversity of the soil (Maradanpour and Mehrabi, 2008). Numerous techniques have been developed to

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Received: October, 2017
Accepted: March, 2018

clean the heavy metal pollutants from the soil. Phytoremediation is recognized as an effective, cheap, and environment friendly method of purification of soils in which suitable plants are used to remove pollutants (Gardea-Torresdey et al., 2005). Plants are valuable tools for redemption of polluted soils through the process of phytoremediation. This technology improves the quality of the soil, adjusting its quality and therefore, reestablishing its biological activities (Rahmanian et al., 2011).

A heavy metal, nickel plays an important role in plants. While it has no toxic effect on plants at low concentrations, nickel is poisonous for plants at high concentrations (Baycu et al., 2006). Nickel causes chlorosis and necrosis in cereals with white strips in leaves (Seregin, 2008). Excessive nickel may disturb electron transport chain during photosynthesis and prevent electron establishment and stomatal transactions (Chen et al., 2009). In plants under nickel stress, uptake of minerals, root growth, cell metabolism, photosynthesis, and respiration are heavily disturbed (Liamas et al., 2008). Reduced biomass is also reported in plants such as wheat and Jatropha curcas under high concentrations of nickel (Yang et al., 2008; Gajewska et al., 2006). There are many studies suggesting that plants can redeem nickel from the environment and accumulate it in their roots and shoots (Ruley et al., 2006).

Manganese is an essential trace element in plants' nutrition which because of the possibility of interchanges in its oxidation forms, plays an important role in redox (oxidation and reduction). It is toxic for plants at high concentrations and in some soils such as acid and volcanic soils, excessive reduction of this element results in manganese toxicity in many agricultural and rangeland soils (Rezai and Farbodnia, 2008).

Symptoms of manganese toxicity are first observed in leaves and in many plants such as barley, green pea, sunflower, and beans, these symptoms include dark brown spots on old leaves (Demirevska et al., 2004). Excessive concentration of manganese is reported to reduce yield, yield components, and photosynthesis in plants (Pospisilova, 2003).

As an element of the biosphere, soil plays an important role in production of food and stability of the environment. Increase in population and the growth in science and technical knowledge and the resulting expansion in the industry without consideration of the environmental issues and standards have led to the pollution of the environment and disturbance of the equilibrium in the ecosystem. Therefore, an awareness of the soil pollutants and the subsequent attention to the appropriate strategies to reduce pollution are of critical importance.

Sorghum biocilor L. is a cereal plant capable of adaptation to various soils which is tolerant against the toxicity of elements and various stress conditions (Saadat and Homaee, 2015). This study was an attempt to investigate the phytoremediation potential of *Sorghum biocilor* L. in soils polluted with various concentrations of nickel and manganese.

Materials and Methods

The present pot experiment was conducted based on a factorial study and complete random block design with three replicates in the Research Greenhouse of the Faculty of Agriculture, in Islamic Azad University, Saveh Branch during 2017-2018. The first factor was nickel nitrate (0, 60, and 120 mg kg^{-1} soil) and the second factor comprised of manganese sulphate (0, 50, and 100 µM). Eight kilogram pots were used in the study. Nickel nitrate and manganese sulphate solutions in water were used to pollute the soils in each pot. For this purpose, nickel nitrate and manganese sulphate were dissolved in distilled water to achieve the moisture needed for field capacity and were sprayed on soils after passing through a 0.45 µm filter. The polluted soil for each pot was kept in plastic bags for two weeks to keep the moisture and stabilize. Then the soil from each plastic bag was put in a pot.

Seeds (sorghum var. *Kimia*) were obtained from Karaj Seed and Plant Improvement Institute. The seeds were sterilized in hypochlorite sodium 2% solution for 5 minutes and then were washed three times with distilled water. The germinated seeds were then used for sowing in the pots. Six seeds were sown in each pot and after 20 days, the weak plants were removed and only 4 plants were kept in each pot. The pots were irrigated up to 75% of the field capacity using distilled water. The growth period was 4 month and then the plants were harvested. The shoots were then cut at the soil surface with a special blade. The roots were also removed using a sieve and repeated movement in water.

In order to assay the dry weight of shoot and root, plant organs were kept in an oven set at 70 °C for 48 hours before they were weighed by a digital scale. Atomic absorption method (Lozak and Soltyk, 2002) was used to assay concentrations of nickel and manganese in roots and shoots. Ten mL nitric acid 80% was added to 0.5 g dried plant tissue and the solution was kept for 24 h under laboratory condition o that the plant samples were quite digested in the acid. The solution was then warmed to remove acidic vapors from the solute. Then distilled water was added to the solution to increase the volume to 50 mL before passing through paper filter. The resulting solution was submitted to spectrophotometry using an atomic absorption spectrophotometer (Spectra AA 220, USA) for assaying the elements. Nickel and manganese contents of roots and shoots were calculated by multiplying their concentrations in roots and shoots by their relevant dry weights expressed in mg per plant. Also, translocation factor was calculated by the ratio of concentrations of these elements in shoots to those of roots (Cui et al., 2007). Analyses of the obtained data were carried out using SAS and the means were compared using Duncan's multiple Range Test (p≤0.05).

Results

Shoot height

Analysis of variance showed that the effects of nickel and manganese on shoots were significant (p≤0.01) while interaction of effects of nickel and manganese was not significant (Table 1). The presence of nickel reduced shoot height compared with the control, so that the maximum shoot height (47.72 cm) was recorded in the control plats. On the other hand, minimum shoot height (31.16 cm) was recorded for the treatment involving 120 mg Kg^{-1} soil nickel suggesting 35% reduction in shoot height (Table 2).

Application of manganese also reduced shoot height in comparison with the control plants. Maximum and minimum shoot heights were observed in control (46.89 cm) and the treatment with 100 µM manganese (31.42), respectively, showing 33% reduction in the shoot height (Table 3).

Root length

Analysis of variance revealed that the effects of nickel and manganese on root length were significant (p≤0.01) while no significant effect was observed for the interaction of the effects of nickel and manganese on root length (Table 1). Application of nickel reduced root length in comparison with the control plants and the maximum and minimum root lengths were

Table 1
Analysis of variance of the phytoremediation capability of sorghum for nickel and manganese

Source of Variations	df	Shoot Height	Root Length	Shoot Dry Weight	Root Dry Weight	Shoot Nickel Concentration	Root Nickel Concentration	Shoot Manganese Concentration	Root Manganese Concentration	Nickel Translocation Factor	Manganese Translocation Factor
block	2	1.33** 64	2.92ns 7	0.14ns	1.15**	0.19ns	1.6**	0.44ns	1.22*	0.05ns	0.001ns 0
Nickel (a)	2	61.6** 9	2.47** 37	2.51**	4.95**	10.91**	15.7**	0.99*	3.79**	0.15**	0.02ns
Manganese (b)	2	15.48** 38	1.43** 16	0.67*	3.08**	0.62*	3.7**	9.58**	29.79**	0.005ns 0	0.05**
a*b	4	3.72ns 4	9.16ns	0.04ns	0.21ns	0.1ns	0.79*	0.26ns	0.86*	0.005ns 0	0.007ns 0
error	16	19.43	14.43	0.14	0.16	0.14	0.2	0.16	0.29	0.02	0.008
CV%		11.26	18.82	16.07	15.55	17.65	11.45	22.44	17.31	23.95	14.95

* and **: significant at p≤0.05 and p≤0.01, respectively; ns: not significant

recorded in the control plants and those treated with 120 mg Kg^{-1} nickel, 25.4 cm and 15.14 cm, respectively. This means 40% decrease in root length of the plants treated with 120 mg Kg^{-1} nickel (Table 2).

Application of manganese reduced root length compared with the control and the maximum (24.06 cm) and minimum (16.95 cm) root lengths were recorded in the control plants and the treatment with 100 µM manganese sulphate, respectively showing 30% reduction as compared with the control. On the other hand, no significant difference in root length was observed between 50 and 100 µM manganese treatments (Table 3).

Shoot dry weight

Analysis of variance showed that the effects of nickel and manganese on shoot dry weight were significant at $p \leq 0.01$ and $p \leq 0.05$, respectively while the interaction of effects of these two metals on shoot dry weight was not significant (Table 1). Application of nickel reduced shoot dry weight and the maximum (8.71 g) and minimum (3.58 g) shoot dry weights were observed in control plants and the plants treated with 120 mg Kg^{-1}, respectively which suggests 59% decrease (Table 2). Treatment with manganese sulphate reduced shoot dry weight compared with the control and the maximum (7.12 g) and minimum (4.59 g) shoot dry weights were recorded in the control and 100 µM manganese sulphate treatment, suggesting 36% decrease. On the other hand, no significant difference was observed between the control plants and those treated with 50 µM manganese (Table 3). Therefore, the damage caused by nickel stress was more than manganese on the dry weight of sorghum shoots in this study.

Root dry weight

Results of analysis of variance revealed that the effects of nickel and manganese on root dry weight were significant at $p \leq 0.01$ (Table 1) whereas the interaction effects of nickel and manganese sulphate on root dry weight were not significant. Just like shoot dry weight, with an increase in the nickel concentration of the soil, root dry weight decreased. While the highest root dry weight (3.31 g) was observed in the control plants, the lowest dry weight was recorded in the roots of the plants treated with 120 mg Kg^{-1} nickel (1.83 g), which suggests 45% decrease compared with the control (Table 2).

Also, treatments involving manganese reduced root dry weight and the maximum and

Table 2
Comparison of mean effects of nickel on the sorghum

Nickel (mg/Kg)	Shoot Height (cm)	Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot Nickel Concentration (ppm)	Shoot Mangaese Concentration (ppm)	Nickel Translocation Factor
0	47.72^{a}	25.4^{a}	8.71^{a}	3.31^{a}	1.19^{c}	2.48^{b}	0.16^{b}
60	38.55^{b}	20.03^{b}	5.33^{b}	2.58^{b}	4.48^{b}	4.62^{ab}	0.38^{a}
120	31.16^{c}	15.13^{c}	3.58^{c}	1.83^{c}	11.1^{a}	5.33^{a}	0.43^{a}

Similar letters show no significant difference based on Duncan's test at $p \leq 0.05$.

Table 3
Comparison of mean effects of manganese on the sorghum

Manganese Sulphate (µM)	Shoot Height (cm)	Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Shoot Nickel Concentration (ppm)	Shoot Manganese Concentration (ppm)	Manganese Translocation Factor
0	46.89^{a}	24.06^{a}	7.12^{a}	3.19^{a}	4.17^{b}	0.53^{c}	0.45^{a}
50	39.11^{b}	19.54^{b}	5.91^{ab}	2.5^{b}	5.72^{ab}	3.65^{b}	0.28^{b}
100	31.42^{c}	16.95^{b}	4.59^{b}	2.03^{c}	6.88^{a}	8.26^{a}	0.37^{ab}

Similar letters show no significant difference based on Duncan's test at $p \leq 0.05$.

minimum root dry weights were recorded under control and 100 µM manganese treatments, 3.19 g and 2.03 g, respectively, showing 37% reduction compared with the control (Table 3).

Shoot nickel concentration

Analysis of variance showed that the effects of nickel and manganese on the shoot nickel concentration were significant at $p \leq 0.01$ and $p \leq 0.05$, respectively. On the other hand, interaction of effects of nickel and manganese was not statistically significant (Table 1). With an increase in soil nickel content, so did the concentration of this element in shoots so that the highest shoot nickel concentration (11.1 ppm) was recorded in the treatment with 120 mg Kg^{-1} nickel (Table 2). Comparison of mean shoot nickel concentrations revealed that under manganese treatment, the highest nickel concentration (6.88 ppm) belonged to the shoots of the plants treated with 100 µM manganese sulphate while no significant difference was found between control and 50 µM treatment (Table 3). Therefore, reduction in the dry weight due to application of 100 µM nickel probably led to the increased concentration of manganese in the plants under study.

Root nickel concentration

Results of analysis of variance showed that the effects of nickel and manganese sulphate on root nickel concentration at ($p \leq 0.01$) and interaction of effects of nickel and manganese on root nickel concentration at ($p \leq 0.05$) was significant (Table 1). Comparison of mean concentrations of nickel in roots under nickel and manganese sulphate showed that the highest nickel concentration (44.45 ppm) was recorded under the combined treatment of 120 mg Kg^{-1} nickel and 100 µM manganese sulphate. Findings also suggest that increasing the concentration of nickel in soils led to an increase in the concentration of this metal in roots of the plants under study while variation in the level of manganese applied had no significant effect on root nickel concentrations at any nickel treatments of the soils. The effects of manganese content of the soil on root nickel concentration seems to be more due to the reducing effect of manganese at 100 µM on root dry weight (Table 4).

Shoot manganese concentration

Results of the analysis of variance showed that while the effects of nickel and manganese on shoot manganese concentration were significant ($p \leq 0.01$), the interaction of these factors had no significant effect on shoot manganese concentration (Table 1). Comparison of mean manganese concentrations of shoots under nickel treatment (Table 2) showed that highest level of manganese (5.33 ppm) was recorded under 120 mg Kg^{-1} nickel treatment and there was no significant difference between control and the treatment with 60 mg Kg^{-1} nickel. The highest concentration of manganese sulphate in shoots

Table 4
Comparison of mean phytoremediation capability of sorghum for nickel and manganese

Nickel (mg/kg)	Manganese Sulphate (µM)	Root Nickel Concentration	Root Manganese Concentration
0	0	5.64^{e}	1.08^{d}
0	50	7.71^{de}	8.86^{c}
0	100	9.53^{cde}	13.39^{bc}
60	0	1.1^{cde} 0	1.1^{d}
60	50	1.36^{cd} 3	11.99^{bc}
60	100	15.3^{cd}	19.48^{b}
120	0	17.8^{bc}	1.45^{d}
120	50	27.43^{b}	19.43^{b}
120	100	44.45^{a}	13.64^{a}

Similar letters show no significant difference based on Duncan's test at $p \leq 0.05$.

(8.26 ppm) was observed under 100 µM manganese sulphate treatment (Table 3).

Root manganese concentration

Analysis of variance showed that the effects of nickel and manganese sulphate at p≤0.01 and interaction of effects of these treatments at p≤0.05 were significant on concentrations of manganese in roots (Table 1). The highest manganese concentration of roots (13.64 ppm) was recorded under the treatment with 120 mg Kg^{-1} nickel and 100 µM manganese sulphate. At all levels of soil nickel, increase in soil manganese content led to an increase in concentration of manganese in roots; however, no significant difference was observed between application of 0 and 60 mg Kg^{-1} nickel under any level of manganese (Table 4). Therefore, concentration of nickel in soil had no effect on the capability of the plants to redeem manganese and with a decrease in in the dry weight of the plant, concentration of manganese increased in them.

Nickel translocation factor

Translocation factor is of particular importance in the remediation technique for plants since shoots are harvested through this technique. Translocation factor in plant species and cultivars must be higher than 1 for the remediation technique. In other words, concentration of heavy metals in shoots must be higher than in roots. Results of analysis of variance showed that the effect of nickel on the translocation factor was significant at p≤0.01 (Table 1). Analysis of mean translocation factor for nickel under the effect of nickel (Table 2) showed that highest translocation factor for nickel was related to the treatments with 60 and 120 mg Kg^{-1} nickel, 0.38 and 0.43, respectively.

Manganese translocation factor

Results of analysis of variance revealed that the effect of manganese sulphate on manganese translocation factor was significant at p≤0.01. However, no significant effect was detected for the treatment with nickel as well as combined treatment of nickel and manganese sulphate (Table 1). Comparison of mean manganese translocation factor under manganese sulphate treatment (Table 3) suggested no significant difference between control and 100 µM manganese in translocation factor. Similarly, the difference between manganese translocation factor under the first and third level of manganese was not significant. This means that increase in manganese content of the soil did not make a noticeable change in the translocation of manganese from roots to shoots of the sorghum plants under study.

Discussion

Treatments of the soils with nickel and manganese in this study resulted in a reduction in root length, and root and shoot dry weights. Also, In the presence of these two metals, their concentrations in roots and shoots increased. Heavy metal stress including that of nickel and manganese is one the most important limiting factors for root growth which is related to the reduction in cell division and elongation (Molas, 2002). Heavy metals stop plant growth in various ways. On the one hand, heavy metals reduce cell division and control its growth by reducing cell turgescence (Baccouch et al., 2001). On the other hand through accumulation on cell walls and entering cytoplasm and then disturbing normal metabolism in cells, they reduce the growth in them (Molassiotis et al., 2005).

High levels of nickel reduced the growth in plant shoots. It is likely that heavy metals limit the growth in plant roots and stems directly by controlling cell division or elongation or a combination of both (Wang et al., 2010). Reduced biomass is probably due to the changes in the metabolic processes under influence of nickel (Tripathi, 1973) and reduced water content of the plant (Vijayarengan and Dhanavel, 2005). Reduced weights of roots and shoots under influence of nickel is already reported by other researchers (Khatib et al., 2008; Fuentes et al., 2006; Mohammadzadeh et al., 2017). As a result of the reduction in root growth, the uptake of water and mineral ions is reduced with a consequence of reduction in the plants' general growth (Rashid Shomali et al., 2012).

Reduced shoot growth due to increased toxicity of manganese is probably a consequence of increased activity of indole acetic acid oxidase. Reduced growth under manganese toxicity seems to be related to deficit in auxin, though the mechanism through which manganese activates oxidases is not identified yet (Qinghua and Zhujun, 2008). Another reason which can be attributed to the reduction in shoot growth is reduced Zn uptake (Venkatesan et al., 2007). Limitation in root growth under manganese is due to a disturbance in osmotic relation of the plant (Qinghua and Zhujun, 2008). Previous studies have suggested that the concentration of heavy metals in plants is directly related with their concentration in soils and the environment and with an increase in their concentration in the environment, their concentration in plant organs also increases (Davis and Boyd, 2000; Peralta-Videa et al., 2002). Yousefi Rad and Ghasemi (2015) reported that increasing the nickel content of soils increased the concentration of this heavy metal in roots and shoots of Persian clover (*Trifolium resupinatum* L.) although unlike the findings of the present study, the concentration of nickel in roots was less than in shoots. Also, similar to the present study, the translocation factors for nickel in sunflower and sorghum were less than 1 and these plants are reported as suitable for stabilization of heavy metals but they are not recommended for phytoremediation (Mohammadzadeh et al., 2017). Also, phytoremediation of nickel in corns was reported to be probably through phytostabilization (Shafigh et al., 2017).

Conclusion

Based on the findings of this study, sorghum is better resistant to manganese compared with nickel. Sorghum also did not show a suitable capability for phytoremediation of manganese and nickel in the polluted soils since the translocation factors were not more than 1 and the concentrations of these heavy metals in roots were more than in shoots. This means that, under normal condition, the plant is not able to translocate the absorbed heavy metals in roots to shoots. In fact, sorghum was not suitable for phytoremediation while it was suitable for phytostabilization of nickel and manganese in the polluted soils. Overall, sorghum was more successful in translocation of nickel than that of manganese to shoots. Therefore, this plant performs phytoremediation through stabilization of nickel and manganese.

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