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Research article

Accumulation potential of Indian mustard (*Brassica juncea* var. *arawali*) and fenugreek (*Trigonella foenum-graecum* L.) planted on Lead and Nickel contaminated soil

Leela Kaur

Department of Environmental Science, Maharaja Ganga Singh University, Bikaner, Rajasthan, India

***Corresponding Author:** leela.kaur@gmail.com [Accepted: 26 July 2018]

Abstract: The aim of the present study was to examine the accumulation potential of Indian mustard (*Brassica juncea* var. *arawali*) and fenugreek (*Trigonella foenum-graecum*) plants in lead and nickel contaminated soil. Plants were exposed to higher concentrations of Pb and Ni (800 mg l^{-1}). EDTA and SA were amended at 2.4 mM concentrations. Plants were harvested and oven-dried. The accumulation of Pb and Ni in plants was determined and their effects on plant growth were evaluated for both species. Seed germination rate of *T. foenum-graecum* plants was higher as compared to *B. juncea arawali* plants. In conclusion, the accumulation capacity of fenugreek was found more than Indian mustard in Pb and Ni contaminated soil.
Keywords: Accumulation - Lead - Nickel - Indian mustard - Fenugreek.

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INTRODUCTION

The contamination of soil by toxic heavy metals is a global environmental problem. These heavy metals can go into soil by natural sources and anthropogenic sources (Ali *et al.* 2013). Anthropogenic sources of heavy metal from agriculture, mining, smelting, electroplating, and other industrial activities have resulted in the deposition of undesirable concentration of metal such as As, Cd, Cr, Cu, Ni, Pb and Zn in soil (Ensley 2000). A number of techniques such as soil excavation, extraction, immobilization and phytoremediation have been used to remove the toxic metals from soil. All these methods have advantages and limitations. Phytoremediation process has been extensively used to remove heavy metals from contaminated soil using plants. Phytoextraction is one of the types of phytoremediation where contaminated soil is treated by using high biomass containing crops and natural hyperaccumulating plants. Metal hyperaccumulators are generally slow growing plant species having low biomass and metal specific too. To enhance the uptake and accumulation of metals in above-ground parts of plant, chelating agents were introduced. Ethlenediaminetetraacetic acid (EDTA) helps in enhancement of metal solubility and translocation from roots to shoots. EDTA-assisted phytoextraction has been done by many studies (Saifullah *et al.* 2009, Barrutia *et al.* 2010, Zaier *et al.* 2010, Lee & Sung 2014). EDTA has drawback of its persistence in environment due to which another chelating agents have been used such as the use of organic acids or more degradable aminopolycarboxylic acids. Salicylic acid (SA) has also been used as chelating agents for phytoextraction of heavy metals (Meng *et al.* 2009, Duman *et al.* 2010, Khan *et al.* 2015, Elhassan *et al.* 2016).

Recent phytoextraction research is focused on identification of metal hyperaccumulator plants and selection of suitable chelants for heavy metal binding (Anwar *et al.* 2016). The known metal hyper accumulators are species of Thalspi, Brassica and Alyssum (Sarma 2011). Many studies have been done to determine heavy metals phytoextraction potential of plants by investigating bioconcentration factor and translocation factor (Yang *et al.* 2016, Zhou *et al.* 2016, Hosman *et al.* 2017, Lee *et al.* 2017, Tabasi *et al.* 2018, Tawatchai *et al.* 2018).

Lead and nickel metals were chosen for the present study as their concentrations are increasing in soil due to industrial development and both are considered carcinogenic (Beyersmann & Hartwig 2008). Lead is one of the

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toxic heavy metal which is harmful to human health. Its retention time in soil is about 150 to 5000 years (Shaw 1990). While, Ni is an essential element but its excess concentration have detrimental effects.

Indian mustard and Fenugreek were taken for the present work as both are food crops and their growth is affected by heavy metal contaminated soil. However, Indian mustard is a known metal hyperaccumulator (Mourato *et al.* 2015) and fenugreek have been explored slightly for remediation potential.

The objective of this study was to evaluate the performance of two plants Indian mustard (*Brassica juncea* var. *arawali*) and fenugreek (*Trigonella foenum-graecum* L.) in phytoremediation of lead and nickel with and without chelants (EDTA and SA). The present work will further help in investigating a comparison of phytoremediation capacity of both plants.

MATERIALS AND METHODS

Experimental site

A pot experiment was carried out under field conditions to examine the accumulation potential of Indian mustard and fenugreek in lead and nickel contaminated soil. Experiments were done at the Micromodel experimental site of the Indian Institute of Technology, Delhi. It is located at 77.09º E longitude and 20.45º N latitude, and 28 m altitude above sea level. The mean maximum and minimum temperature during the study period were 18–43 ºC and 3–15 ºC, respectively. Different physical and chemical soil characteristics are summarised in table 1.

Table 1. Various soil characteristics.

Parameter	Unit	Value
Texture	-	Sandy loam
Clay	%	16.30
Silt	%	14.23
Sandy	%	69.47
Electrical Conductivity	mS cm^{-1}	0.28
pH	-	7.5
Cation Exchange Capacity	Cmol kg^{-1}	18.4
Organic Carbon	%	0.72
Available N	kg ha^{-1}	272
Available P	kg ha^{-1}	9.0
Available K	kg ha^{-1}	200.7
Total Pb	mg kg^{-1}	0.02
Total Ni	mg kg^{-1}	4.0

Pot experiment

The seeds of *Brassica juncea* var. *arawali* and *Trigonella foenum-graecum* L. were obtained from the National seeds Corporation Ltd., Beej Bhawan, Pusa, New Delhi. About 20 seeds were sown in 11×11 cm pots containing unsterilized field soil, farm yard manure (organic carbon 12.2 %, total N 0.55 %, total P 0.75 %, total K 2.30 % and pH 7.2) and sand in a 2:2:1 ratio in October 2008. In chemical treatment, Pb, Ni, EDTA and SA were added as per the designed treatment. The designed treatment for mustard and fenugreek plants was as follows: (T1) Pb, (T2) Ni, (T3) Pb + Ni, (T4) Pb + Ni + 2.4 mM EDTA, (T5) Pb + Ni + 2.4 mM SA, and (T0) Control or untreated plants. The concentrations of lead and nickel (supplied as $Pb(NO_3)_2$ and $Ni(NO_3)_2$) were 800 mg l^{-1} in all treatments. Dipotassium salt of EDTA was supplied in the treatment. Plants were watered daily using tap water to maintain optimal growth conditions. Tap water was analysed for lead and nickel concentration which were found negligible. Seed germination started after the seventh day of sowing and plants were thinned to 3 plants per pot after 30 days of sowing. Samples of plants were taken one day before and after chelant treatment and monthly after the treatment. At the end of the experiment the plants were harvested and then washed accurately, the aerial part was divided from the roots and the two parts were analysed separately to determine the metal content.

Soil and plant analysis

The organic C, N, P and K were estimated by the methods of Walkley and Black, Micro-kjeldahl, Olsen and Flame photometer, respectively, in soil and farm yard manure as descried by Rowell (1994). Cation exchange capacity was carried out using the $BaCl_2$ method (Hendershot & Duquette 1986). Metals concentration in soil was determined using the aqua regia extraction method (ISO 12914 2012). Plant samples were washed with tap water and dried at 70ºC for 48 hours. The dried material was digested with aqua regia. All determinations were performed in triplicate. Metal concentrations in solutions were analysed by ICP-OES (Varian Vista-MPX CCD

Simultaneous ICP-OES, Varian Australia Pty. Ltd) with a Ni detection limit of 0.007 mg l^{-1} and a Pb detection limit of 0.01 mg l^{-1}.

Translocation factor

Translocation factor (TF) was calculated to evaluate the potential of *B. juncea* var. *arawali* and *T. foenum-graecum* L. for phytoextraction. This ratio is an indication of the ability of the plant to translocate metals from the roots to the aerial parts of the plant (Marchiol *et al.* 2004). It is represented by the ratio:

$$\text{Translocation Factor} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in roots}}$$

Bioaccumulation Factor (BAF)

Bioaccumulation Factor can be employed to quantify toxic element accumulation efficiency in plants by comparing the concentration in plant and soil (Baker 1981, Ma *et al.* 2001).

$$\text{Bioaccumulation Factor} = \frac{\text{Metal concentration in aerial parts}}{\text{Metal concentration in soil}}$$

Statistical analysis

The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following analysis of variance (ANOVA); when the ANOVA was significant the means were separated using least significant difference at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Plant growth

Effect of treatments on seed germination and dry weight of plants is represented in table 2. Application of SA with heavy metal (Pb^{2+} and Ni^{2+}) treatment helped in reducing the inhibitory effects of these metals on seed germination along with increased dry weight. Our results are supported by Mishra & Choudhuri (1997) and Elhassan *et al.* (2016). Elhassan *et al.* (2016) also found that salicylic acid enhanced lead uptake as well as all growth parameters of maize (*Zea mays* L.).

Table 2. Effect of treatments on seed germination and dry weight in *Brassica juncea* var. *arawali* and *Trigonella foenum-graecum* L.

Treatment	Seed germination (%)		Dry weight (g)	
	B. juncea* var. *arawali	***T. foenum-graecum***	***B. juncea* var. *arawali***	***T. foenum-graecum***
T0	100±0.8	100±0.5	6.2±0.70	3.8±0.31
T1	80±1.3	100±0.6	3.42±0.28	2.7±0.16
T2	85±1.8	95±1.4	2.28±0.30	1.7±0.48
T3	65±2.2	90±1.9	1.3±0.06	1.2±0.19
T4	75±1.5	85±1.2	1.5±0.42	1.4±0.09
T5	90±2.8	95±1.6	2.2±0.60	2.3±0.25

Note: Values represents mean ± SD (n=3).

Rate of seed germination was higher in fenugreek plants as compared to mustard plants in all treatments. A potential decrease in total plant dry weight was observed in plants treated with combined Pb + Ni. Dry weight was higher in SA treated plants as compared to Pb + Ni treatment (T3). Alyemeni *et al.* (2014) also found a significant increase of dry mass of *Cicer arietinum* plants with the application of 10^{-5} M salicylic acid.

Metal concentrations in plant roots and shoots

Accumulation of Pb and Ni in root and shoot part of mustard and fenugreek plants are shown in table 3 and 4. Lead bioaccumulation in roots and shoots of plants was significantly different. Pb and Ni concentration in root organs of the plants was higher than that in shoot organs in all treatments. Pb concentration increased in both organs of the plant with EDTA treatment, but it decreased with SA treatment (except in the shoot part of fenugreek). Applying EDTA increased Pb concentration in roots of mustard plants significantly ($P < 0.05$) from 370 mg kg^{-1} in T3 up to 1010 mg kg^{-1} in the case of application of EDTA soil at T4. Zaier *et al.* (2010) found that EDTA addition increases the ability of *Brassica napus* to accumulate heavy metals (Zn, Mn and Pb) from sludge-amended soil. Lee & Sung (2014) also investigated the effect of EDTA on phytoremediation of soils contaminated with Cd, Cu, Pb, Zn, and Ni by *Brassica juncea* and found that EDTA was most effective for heavy metal uptake, but had significant effects on plant biomass. Our findings are corroborated with Gill *et al.* (2015) and Kanwar *et al.* (2015) who studied Cr stress on *Brassica* species and suggested that inhibited growth of these plants is directly interrelated with its accumulation.

Table 3. Accumulation of Pb and Ni in shoots and roots of *Brassica juncea* var. *arawali*.

Treatment	Shoot concentration (mg kg^{-1} DW)		Root concentration (mg kg^{-1} DW)	
	Pb	Ni	Pb	Ni
T0	43±12	79±11	96±16	123±41
T1	280±201	65±15	502±182	119±33
T2	36±9	91±36	83±15	187±121
T3	370±176	217±134	525±246	303±202
T4	1010±425	52±27	16395±989	2876±541
T5	517±228	69±28	3828±782	610±348

Note: Values represents mean ± SD (n=3).

Table 4. Accumulation of Pb and Ni in shoots and roots of *Trigonella foenum-graecum* L.

Treatment	Shoot concentration (mg kg^{-1} DW)		Root concentration (mg kg^{-1} DW)	
	Pb	Ni	Pb	Ni
T0	23±8	47±11	68±22	124±25
T1	429±218	58±13	684±250	106±20
T2	31±12	216±135	53±17	634±241
T3	17624±985	5322±576	43140±969	15291±586
T4	401±252	170±112	2888±755	1187±481
T5	512±214	230±98	1653±682	871±364

Note: Values represents mean ± SD (n=3).

Bioaccumulation factor and Translocation factor

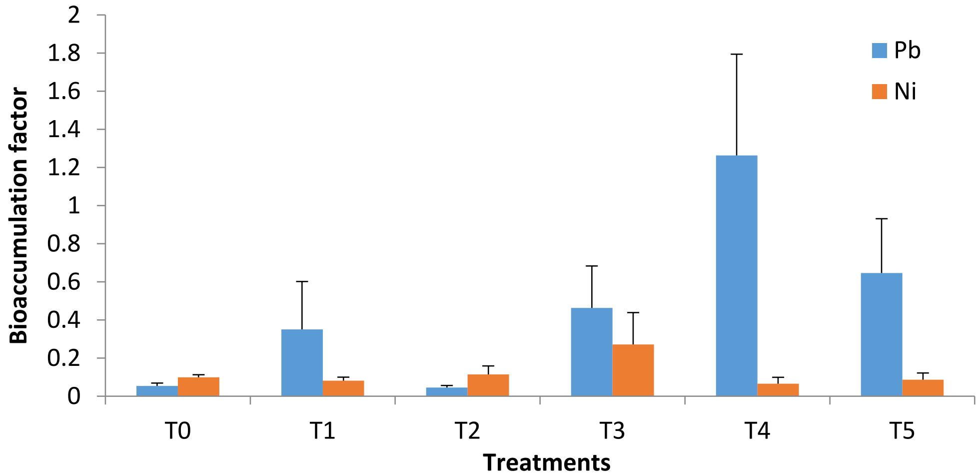

Figure 1. Bioaccumulation factor of *Brassica juncea* var. *arawali* treated with lead and nickel.

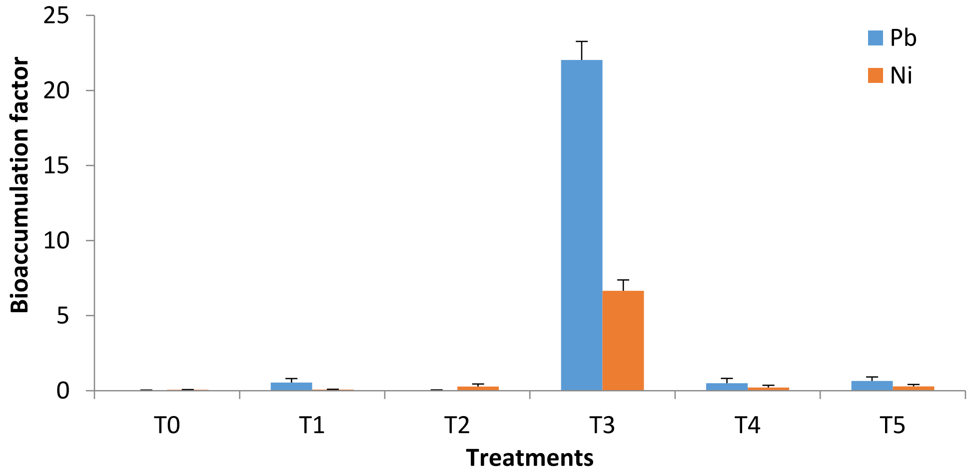

Figure 2. Bioaccumulation factor of *Trigonella foenum-graecum* L. treated with lead and nickel.

Figure 1 and 2 represents the bioaccumulation factor of *B. juncea* var. *arawali* and *T. foenum-graecum* treated with lead and nickel respectively. Bioaccumulation factor is the measure of the capability of plants to transport the metals to its aerial organs from the medium. Bioaccumulation factor mainly depends on environmental efficacy, metals particularity, species-specific characteristics and disposal route. If the value of bioaccumulation factor is greater than 1.0, it indicates that plants are able to extract metals and these plants can be selected for phytoremediation of the contaminated soil (Zhao *et al.* 2003, Luoma & Rainbow 2005, Kamari *et al.* 2012). Bioaccumulation of Pb and Ni were highest in fenugreek plants treated with T3 (22.03±1.23 and 6.65±0.72 respectively). The results show that Pb and Ni bioaccumulation by *T. foenum-graecum* was greater than *B. juncea* var. *arawali*.

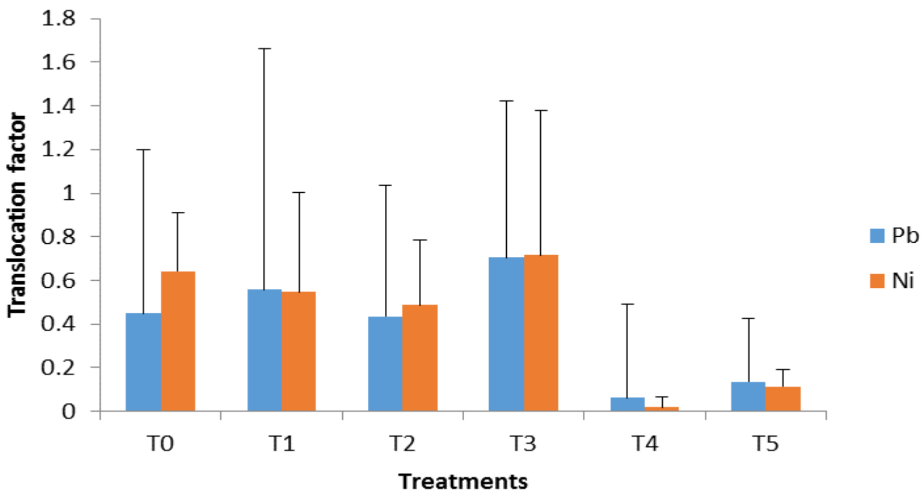

Figure 3. Translocation factor of *Brassica juncea* var. *arawali* treated with lead and nickel.

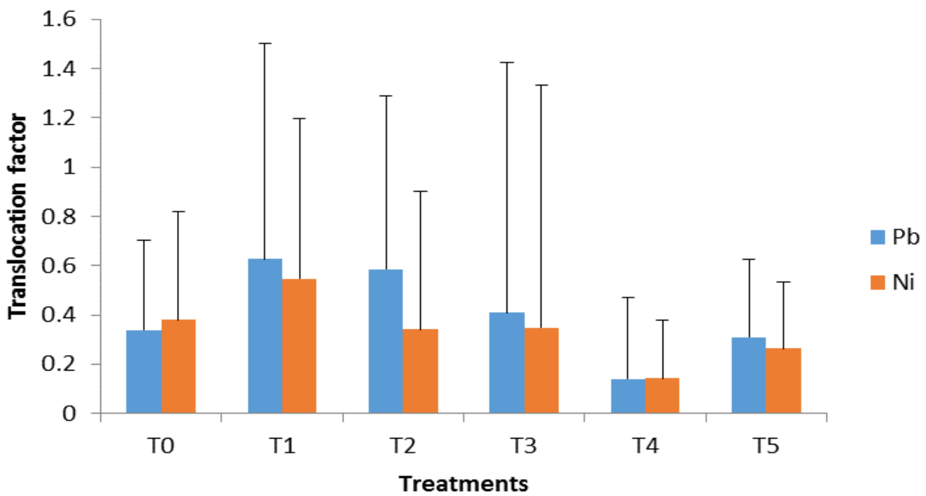

Figure 4. Translocation factor of *Trigonella foenum-graecum* L. treated with lead and nickel.

Translocation factor (TF) is an effective measure for determining the metal phytoextraction from soils (Kamari *et al.* 2012). There are three categories of plants based on their translocation factor namely accumulator (TF>1), excluder (TF<<1), and indicator (TF near 1) (Baker 1981, Ghosh & Singh 2005). Translocation factor of *B. juncea* var. *arawali* and *T. foenum-graecum* treated with lead and nickel are shown in figure 3 and 4 respectively. Our result revealed less than 1 value of translocation factor which signifies that Pb and Ni could not be effectively translocated from the roots to the shoots. Overall, fenugreek showed better Pb-translocation as compared to mustard. On the contrary, mustard was superior in Ni-translocation as compared to fenugreek. However, translocation factor was less than 1.0 for both plant species. This study revealed that both the plant species have high accumulation of Pb and Ni in roots and low translocation in shoots, nevertheless they could be used for phytoremediation of Pb and Ni contaminated soils.

CONCLUSION

Brassica juncea var. *arawali* and *Trigonella foenum-graecum* L. showed reduced seed germination and plant growth under Pb and Ni contaminated soil. Furthermore, both plant species were able to accumulate Pb and Ni in root and shoot parts. However, *B. juncea* var. *arawali* and *T. foenum-graecum* may be used as an alternative for phytoremediation of Pb and Ni contaminated soils.

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