Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2019, Volume 10, Issue 5, Page 463-469

http://www.jmaterenvironsci.com



# Phytoremediation Potential of *Brassica juncea* for removal of selected heavy metals in urban soil amended with cow dung

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*Received 20Feb 2018,*
*Revised 15 April 2019*
*Accepted 18 April 2019*

*Keywords*
- ✓ *Metal extraction ratio,*
- ✓ *Translocation factor,*
- ✓ *Phytoavailability,*
- ✓ *Hyperaccumulator,*
- ✓ *Phytoremediation.*

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## Abstract
Soil is a necessary and integrated system for the right growth of the plant. Due to various anthropogenic activities the soils get contaminated by both organic and inorganic ions specifically heavy metals. Increase in the concentration of heavy metals in soils is due to various industrial activities, indiscriminate usage of fertilizer, pesticides, benthic sludge and petro chemicals spillages, etc. Phytoremediation is an important and economically viable remediation technique which supports the heavy metal removal from soil, which is the objective of the current study. The study was carried out through pot experiments for a period of 81 days, analysis was carried out both in soils and plants. Physico-chemical parameters like pH, Electrical Conductivity, Organic Matter and Organic Carbon were analyzed in soil on 0d, 21d, 51d and 81d. Heavy metal analysis were performed through ICP-MS in both plant and soil samples. Translocation Factor and Metal Extraction Ratio were calculated to assess the uptake of metals by *Brassica juncea*. Results revealed that uptake of Lead has been greater than other heavy metals and based on Translocation Factor, ***Brassica juncea*** act as hyperaccumulator.

## 1. Introduction
Soil is an integrated biological system and it consists of various organic and inorganic materials which are essential for proper nutrition and growth of the plant. Several anthropogenic activities have constantly influenced the contamination of soil particularly with heavy metals. Activities like smelting of ores, utilization of fertilizers, benthic sludge and chemicals, mining, dumping and application of municipal wastes to soil, etc; elevated the concentration of heavy metals in soil apart from natural activities like rock weathering **[1 - 6]**. Heavy metals are of severe concern due to their accumulation and persistent nature in the natural environment, as they are not biodegradable **[7, 8]** and tend to persist for longer periods. They may enter into the food chain consequently, which are carcinogenic and can cause behavioral and neurological disorders especially in children **[9]**. To overcome the metal contamination in soil, remediation is of much importance **[10, 11]** and there are many expensive remediation methods like soil flushing, stabilization, vapor extraction, solidification, land filling, leaching and soil fixation **[12, 13]**. But in the recent years a cost effective and environmentally sound emerging technology which has gained much prominence is "Phytoremediation" **[14]**. Phytoremediation is one of the emerging eco-sustainable techniques which can remediate heavy metals in soil, in addition to other toxic contaminants **[15, 16]**. By the combined use of soil amendments, phytoremediation helps in the natural uptake of metals from contaminated soils through roots of the plant and accumulate them into the harvestable parts of the plant, which together with bio-accumulation, translocation and ability of degradation by the plant body. Such type of plants are considered as Hyperaccumulators**[17, 18]**, with translocation factor more than one. The efficiency of uptake of metals by plant is also influenced by many factors like type of species, soil properties, etc.**[19]**. Compost application is necessary to maintain a suitable soil structure and besides supporting the organic matter lost due to the extensive agricultural practices. There is a need to examine the presence of heavy metals in soils amended with compost which are applied to plants. The average content of heavy metals

in composts are significantly low, contamination of soils is possibly due to pedological or geological origins, which are also responsible for the limit violation of metal levels due to anthropogenic activities **[20]**.

Characterization of soil helps in understanding the bio-availability and speciation of heavy metals. Remediation studies on contaminated soils would provide knowledge on contamination sources, environmental risks and health effects associated with heavy metals **[10]**. Application of compost or addition of organic amends enhances the process of Phytoremediation **[21]**. Availability of metals in soil depends on a number of factors like the association between the metal and soil environment, its concentration in both soils and compost and the capability of a certain plant for the uptake of a certain element **[22]**. After the application of compost is ended, increase in the content of metal into available form was not observed. However, experiments reveal that compost contributes to lower the metal availability in amended soils compared to other techniques. Hence, the risks associated with metals towards human health, crop yield, quality and fertility of amended soils is negligible **[22]**.

The current study objective is to determine the content of heavy metals in the urban soil amended with cow dung and to assess the rate of removal efficiency by using a plant *Brassica juncea.*

## 2. Material and Methods

### *2.1. Collection of soil samples.*

Red soil samples were collected from the urban areas of Visakhapatnam exposed to traffic and industrial activities. Collected soil samples were air dried and also sieved using a 2 mm sieve. Air dried - powdered cow dung is used as compost and was mixed in 1:1 ratio into the soil. It was used as an amendment to enhance the fertility and productivity.

### *2.2. Pot experiments.*

Pots with capacity of 10 kg were taken and the soil amended with compost was added. The growth period of the plants was analyzed over a period of 81d, i.e on 0d, 21d, 51d, and 81d. pH, Electrical Conductivity, Organic Carbon and Organic Matter experiments were performed along with plant growth parameters. After 81d, the plant samples were harvested, dried and digested by the process of acid digestion for analysis of Heavy metals, which was performed through ICP-MS and the removal of metal efficiency was determined.

In the present study, the seeds of Indian mustard **(*Brassica Juncea*)** (Varun T-59) were used, which belong to the family of *Brassicaceae*. The advantages in usage of ***Brassica juncea*** is it produces high biomass and suitable for remediation studies. Continuous Phytoextraction is the procedure followed throughout its life cycle.

### *2.3. Plant sampling and Analysis.*

Plant samples of different replicates were collected and washed thoroughly using distilled water. Root and shoot were separated from the plant and dried in hot air oven at $60^{O}$ C until get a constant weight. 0.5 g of plant tissue was measured and transferred into a Teflon vessel. 5 ml $HNO_3$ and 2ml of $H_2O_2$ were added and digested. After digestion, the remnants of digest were transferred to a volumetric flask and analysis of heavy metals was performed.

Then the samples were aspired through Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The mass of the metals were calculated by multiplying the concentration of metal average by their volumes **[23]**. To assess the likely usage of *Brassica juncea* as a hyperaccumulator during the growth period, accumulation of metal and biomass in the root and shoots were measured **[24]**.

### *2.4. Soil sampling and analysis.*

Soil samples were collected and analyzed for heavy metals on initial day of experiment (0d) and on final harvest day i.e. 81d. Soils were air dried and digested with 5 ml $HNO_3$, 2ml of $H_2O_2$ on a hot plate at 150 – $180^{O}$ C till the completion of digestion. Digested sample residue were dissolved in distilled water and transferred into a volumetric flask. For heavy metal analysis samples were aspired through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin-Elmer 3110). Mass balance of the metals were calculated by multiplying the metal average concentration by their volume **[23]**.

Physico-chemical characteristics of soil were analyzed on different phenological stages of the plant i.e 0d – initial day, 21d – vegetative period, 51d – flowering period and 81d – seed maturation period. Parameters such as pH & Electrical Conductivity were measured by electrometric Method, organic carbon and organic matter of soils were estimated by Walkley-Black method. Analytical grade of chemicals, reagents and double distilled water (de-ionized) were used during the analysis. Six replicates were analyzed for all parameters in throughout the experiment.

### *2.5. Heavy metal removal efficiency and Translocation Factor.*

Metal efficiency removal was calculated to assess the efficiency of ***Brassica juncea*** in phytoextraction**[25]**. The efficiency removal is calculated by using the following formula:

$$\text{Efficiency of removal} = [C\ (\text{shoot}) + C\ (\text{roots})/C\ (\text{soil})] * 100$$

The Translocation factor is the ability of plant to phytoextract metals from soil to its harvestable plant parts **[26, 27]**.

$$T.F = \text{Concentration (root)} / \text{Concentration (Shoot)}$$

Obtained data was verified statistically through linear regression and correlation coefficient analysis **[28]**.

## 3. Results and discussion

### A. Soil Characteristics

Pot soils of six replicates were analyzed on 0d, 21d, 51d and 81d synchronizing with the plant phenological stages. The parameters selected for analysis include pH, Electrical Conductivity, Organic carbon and Organic matter. Metals are one of the constituents of soil. They are categorized into two types based on their role in physiological activities. Metals such as Zn, Ni, Cu, Mn, and Fe are essential for the growth of the plants as micronutrients **[29, 30]** and are also constituents of enzymes and proteins which can be accumulated easily from the soil solution **[31].** Metals which are not essential for plant growth (Cr, Cd, As, Hg, Pb, etc) also accumulated in various parts of the plant body **[32]**. Higher contents of both the groups may tend to inhibit the growth and can lead to symptoms of toxicity in plants, animals and humans **[30, 33-36]**.

**pH:** An increasing trend of pH has been observed overall from 0d to 81 d. The value of pH ranged from 5.98 to 7.08 **(Table 1)**. Initially the soil was observed to be in acidic medium and during the growth stages of the plant, the soil gradually turned neutral. Phytoavailability of metals is influenced by pH directly, as acidity of the soils regulate the solubility and ability of the metal movement in soil solution, at lower pH bioavailability increases and large number of metals are released into the soil solution **[31]**. At high pH, precipitation of cations take place or they adsorb to mineral surfaces. At neutral pH, metals are not in available form to plant uptake. Thus metal availability is most active under acidic conditions **[31]**.

**Electrical Conductivity:** Electrical Conductivity has observed to decrease from 0d to 51d from 1.10µS/cm to 0.79µS/cm and a slight increase has been observed on 81d (0.82µS/cm) **(Table 1)**. The decrease in the conductance is due to the absorption of the ions including heavy metals by plants during the growth period.

**Organic Carbon and Organic Matter (%):** Both Organic Carbon and Organic Matter were found to be initially high during the early growth stages due to amendment with cow dung, but after 21d it has been observed that there is a decrease in the amount of Organic Carbon and Organic Matter till the end of crop growth i.e. 81d **(Table 1)**. Organic matter in soil influences on metal availability to plants and soils amended with compost also reduce the bioavailability of metals, as sorption takes place **[37, 31]**. Higher amounts of organic matter helps in minimizing the absorption or uptake of metals, whereas lower amount of organic carbon in soil is liable to contamination by heavy metals **[38]**.

**Table 1:** pH, Electrical Conductivity, Organic carbon and Organic matter in soil amended with cow dung.

| Parameter | 0d | 21d | 51d | 81d |
|---|---|---|---|---|
| **pH** | 6.43 ± 0.57 | 5.98 ± 0.49 | 6.23 ± 0.25 | 7.08 ± 0.11 |
| **Electrical Conductivity (µS/cm)** | 1.10 ± 0.22 | 0.89 ± 0.18 | 0.79 ± 0.08 | 0.82 ± 0.39 |
| **Organic carbon (%)** | 1.83 ± 0.02 | 0.80 ± 0.07 | 0.55 ± 0.17 | 0.40 ± 0.14 |
| **Organic matter (%)** | 3.16 ± 0.05 | 1.37 ± 0.11 | 0.94 ± 0.30 | 0.69 ± 0.24 |

Studies revealed that, pH has a negative correlation with Electrical Conductivity, Organic Carbon and Organic Matter. It shows that pH has direct influence on the ability of plant uptake and also controls other parameters mentioned above. During initial stages the pH was more or less acidic, much of the activity have been observed under acidic conditions and the electrical conductivity was recorded high. As the pH increased from initial to final stages, the utilization of compost along with the uptake of metals took place which is responsible for the decrease in electrical conductivity and the content of Organic Carbon, Organic Matter. Thus, Electrical Conductivity has positive correlation with both of them. Reduction of Organic Matter has been observed as 56%, 69% and 78% respectively on 21d, 51d and 81d **(Table 2)**.

**Table 2:** Correlation coefficient analysis between soil parameters

| ***Parameter*** | ***pH*** | ***E.C (µS/cm)*** | ***O.C (%)*** | ***O.M (%)*** |
|---|---|---|---|---|
| **pH** | 1 | | | |
| ***E.C*(µS/cm)** | -0.12 | 1 | | |
| ***O.C (%)*** | -0.23 | **0.98*** | 1 | |
| ***O.M (%)*** | -0.23 | **0.98*** | **1.00*** | 1 |

*** Significant at (< 0.01 level)**
**(E.C – Electrical Conductivity, O.C – Organic Carbon, O.M – Organic Matter)**

**Metal content in soil and % of reduction:**

The concentrations of metals vary in each soil depending upon the physical conditions and the availability. In the present study, among all the metals Lead (Pb) has achieved highest percentage of reduction (73%). The initial concentration was reported as 132.3 ppm and was reduced to 35.63 ppm on the final harvest day. Copper (Cu) and Chromium (Cr) were reduced by 12.7% and 12.1% respectively. Nickel (Ni) and Zinc (Zn) were reportedly least reduced with 11% and 10.4% respectively **(Table 3)**. The reason for the greater reduction of Lead in soil could be due to its ready available form. Lead forms several complexes with soil constituents and is phytoavailable from the soil solution. As Lead doesn't support any essential functions in plants, it is easily absorbed from the soil solution through roots. The behavior of lead in soil and its uptake by plants are controlled by several factors like soil pH, particle size of the soil, Cation Exchange Capacity (C.E.C), surface area of root, etc. **[39]**.

**Table 3:** Metal content absorbed from soil amended with cow dung.

| **Parameter(ppm)** | **0d** | **81d** | **% of reduction** | **Metal absorbed from soil in ppm** |
|---|---|---|---|---|
| **Cr** | 91.22 ± 12.34 | 80.11 ± 14.06 | 12.18 | 83.36 |
| **Cu** | 38.70 ± 3.83 | 33.78 ± 7.5 | 12.73 | 36.96 |
| **Ni** | 61.16 ± 6.88 | 54.41 ± 14.04 | 11.04 | 50.63 |
| **Pb** | 132.39 ± 8.63 | 35.63 ± 8.36 | 73.09 | 725.68 |
| **Zn** | 135.16 25.63 | 121.01 ± 28.9 | 10.47 | 106.09 |

**Heavy Metal uptake by *Brassica juncea*:**

It can be described as the percentage of recovery from soil to plant parts. It was calculated on the basis of the absorption of the metal by the plant in each pot (ppm/pot). The highest metal absorbed by the plant is Lead (151.4 ppm) followed by Zinc (55 ppm) < Copper (15.4 ppm) < Chromium (9.6 ppm) < Nickel (3.1 ppm). % of recovery was greater in Zinc (51.8%) < Copper (41.6%) < Lead (20.8%) < Chromium (11.5%) < Nickel (6.1%) **(Table 4)**.

**Table 4:** % of metal recovery by ***Brassica juncea*** in soils amended with cow dung.

| Parameter | Heavy metal concentration in whole plant on 81d (ppm) | Metal absorption by plant/pot (ppm) | % recovery of heavy metal by plant |
|---|---|---|---|
| **Cr** | 0.117 | 9.611 | 11.529 |
| **Cu** | 0.188 | 15.410 | 41.693 |
| **Ni** | 0.038 | 3.121 | 6.165 |
| **Pb** | 1.848 | 151.496 | 20.876 |
| **Zn** | 0.671 | 55.005 | 51.849 |

**Concentration vs. Uptake:**

Linear Regression analysis was performed to assess the uptake of metal by the plant from soil. On the basis of $R^2$ value the overall strength between the metal concentration in soil and metal uptake by the plant can be assessed. In the present case, the highest $R^2$ is 0.88 and 0.73 recorded for Pb and Zn and lowest $R^2$ is recorded for Cr. This explains that the uptake of Pb, Zn and Cr by the plant is able to explain 88%, 73% and 47% variation in the soil metal concentration. Upon all the metals analyzed Lead has shown highest uptake followed by Zinc, Copper, Chromiumand Nickel. All metals uptake is statistically significant at <0.01 level **(Table 5)**. Rhizosphere is the place where modification of soil properties takes place and can influence the phytoavailability of metals. Plant enzymes from roots play a vital role in the chemical speciation and transformation of metals to ease the uptake by plant **[36]**. Biological degradation of heavy metals is not possible, they can only be transformed from one oxidation state to another or from one organic complex to other **[40]**.

**Table 5:** Linear regression between plant metal uptakeversus soil concentration.

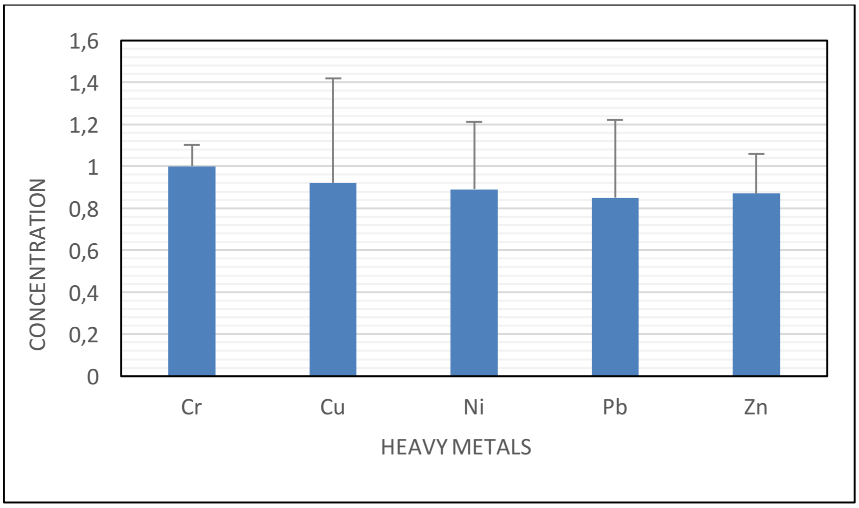
| Parameter | y | $R^2$ | r |
|---|---|---|---|
| **Cr** | -0.0004x + 0.1515 | 0.4782 | 0.69* |
| **Cu** | -0.0112x + 0.5647 | 0.6203 | 0.78* |
| **Ni** | -0.0004x + 0.0607 | 0.5117 | 0.72* |
| **Pb** | 0.037x + 0.5291 | 0.8813 | 0.94* |
| **Zn** | -0.0032x + 1.0605 | 0.7381 | 0.86* |

*** Significant at 0.01 level**

**Translocation factor (T.F) and Metal extraction ratio (M.E.R):**

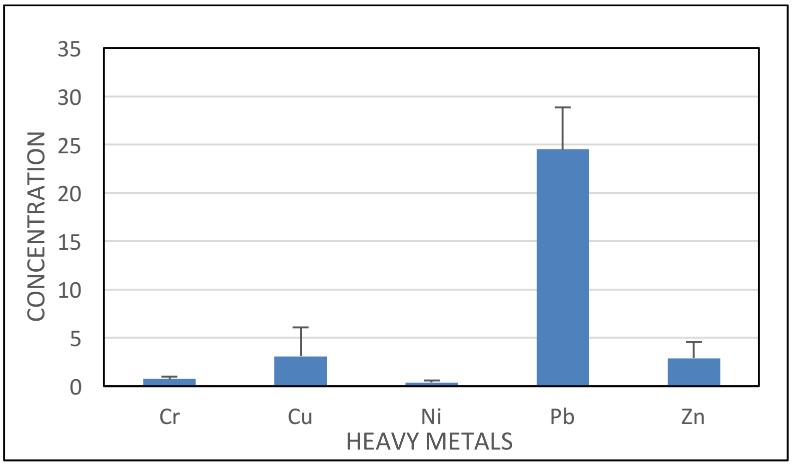
Translocation factor is the ability of translocating metals from roots to its upper parts of the plant. The highest translocation was observed in Chromium with 1.0, followed by Copper (0.92), Nickel (0.89), Zinc (0.87) and Lead (0.85) **(Figure 1)**. When Translocation Factor is close to 1, the plants can be considered as hyperaccumulators. Translocation Factor (TF) is the ability to tolerate and accumulate heavy metals inside the upper physiological parts (leaves and stems). Translocation factor greater than one (TF > 1) indicates that the plant suitable as a hyperaccumulator. **[26, 27]**.

**Figure 1:** Translocation Factor from root to shoot ratio in ***Brassica juncea***




The Metal extraction ratio is the capability of a hyperaccumulator to produce higher biomass content and collect contaminants in to the plants, and the efficiency is calculated **[25]**. The highest efficiency was recorded in Lead (24.52), followed by Copper (3.08), Zinc (2.89), Chromium (0.71) and Nickel (0.37) **(Figure 2)**. The metal extraction ratio is dependent on the ability of uptake of contaminants by plants. The absorption of metals or contaminants is possible only, if they are in phytoavailable form. Phytoavailability plays a vital factor which regulates the plant uptake of metals **[17]**.

**Figure 2:** Metal Extraction Ratio from soil to plant upper parts in ***Brassica juncea***




## Conclusion

The study was conducted to assess the uptake efficiency and phytoremediation potential of *Brassica juncea* in soils amended with cow dung. It has been observed that pH has regulated the phytoavailability of metals. Other parameters like Electrical Conductivity, Organic Matter and Organic Carbon has also played a significant role throughout the growth period and substantial reduction of Organic Matter and Organic Carbon has been observed which represents the regular activity of uptake of heavy metals by *Brassica juncea*. Through Metal Extraction Ratio, it has been observed that the uptake of Lead has been greater than the other heavy metals. Translocation factor indicates that *Brassica juncea* is a hyperaccumulator in the present study.

**Acknowledgements:** Authors are thankful to Department of Environmental Studies, Institute of Science, GITAM (Deemed to be University) for providing the necessary facilities to carry out the experiments and lab work during the study period. We sincerely thank National Geophysical Research Institute (NGRI) for providing required facilities to carry out analysis on ICP-MS and providing standards of NIST.

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