STUDY OF PHYTOREMEDIATION POTENTIAL OF *IPOMEA CARNEA* JACQ. FOR THE SOIL CONTAMINATED WITH HEAVY METALS Cr, Fe, Ni, Cu AND Zn

Abstract

The research involved assessing the phytoremediation capabilities of Ipomea carnea Jacq. for five heavy metals—chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), and zinc (Zn)—in both in-situ and ex-situ conditions. The samples of soil and plant material were collected from Thane-Belapur Industrial Area, Navi Mumbai, India. Further laboratory pot experiment was conducted using the same plants and each pot was treated with selected five metals in three different doses 25 ppm, 50 ppm and 100 ppm. The plants were harvested and separated into soil, roots and shoots and analyzed for the presence of heavy metals by using ICP-AES. The Bioaccumulation Factor (BCF) and The Translocation Factor (TF) was calculated to study its efficiency for heavy metal bioaccumulation. The result showed that *I. carnea* accumulates Fe more than 10,000 mg/kg; therefore it is hyperaccumulator of it. It also bioaccumulated other heavy metals in varying concentration but 25 ppm and 50 ppm are most suitable dose for translocation of these metals from root to shoots. It is clear that *Ipomea carnea* Jacq. have ability to withstand such high concentration of heavy metals and can be used for restabilization of disturb habitat.

Keywords: Heavy metals, Bioaccumulation, *Ipomea carnea* Jacq., phytoremediation

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I. INTRODUCTION

The concentration of heavy metals has reached toxic levels even though they are natural components of the Earth's crust (Abii, 2012). There are fifty three elements which fall into the category of heavy metal and defined as the group of elements whose densities are higher than 5 g cm^{-3} and recognized as ubiquitous environmental contaminants (Massa et al., 2010). Many of these elements are essential to the body of organisms in very low concentrations but are toxic at high concentration. Heavy metal in soil may enter into food chain and ultimately consumed by human and causing adverse health effects (Bordajandi et al., 2003). The physical and chemical remediation tends to be expensive and disruptive to the surrounding. The phytoremediation method offers sustainable remediation technique by overcoming the conventional chemical and physical technologies (Salt *et al*., 1995 and Chaney *et al*., 1995). This idea of phytoremediation comes from the discovery of different wild plants that accumulated high concentration of metals in their foliage (Baker, 1987 and Raskin et al., 1997).

The categories of phytoremediation includes phytoextraction (the use of plants to remove contaminants from the soil through plant roots and translocate them to aerial part), phytovolatilization (plants take up contaminant from soil, transforming them into volatile form and transpire them into environment), rhizofiltration (the use of plant roots to remove contaminants from flowing water), phytostabilization (the use of plants to transform soil metals to less toxic forms, but not remove the metals from the soil) and Phytotransformation (the use of plants to transform toxic metals into nontoxic form) (Ghosh and Singh, 2005). Discovery of hyper accumulator plant species increase the interest of most of the scientists in phytoremediation. Researchers have screened out a number of plants species called hyperaccumulator (Kramer, 2010) which do not only grow well in heavy metals contaminated soil but also accumulate extraordinary heavy metals in their harvestable parts. Hyperaccumulator must accumulate at least 100 mg/g (0.01% dry wt.) Cd, As and some other trace metals, 1000 mg/g (0.1% dry wt.) Co, Cu, Cr, Ni & Pb, and 10,000 mg/g (1% dry wt.) Mn and Ni (Reeves and Baker, 2000; Wantanabe, 1997). The normal heavy metal contents of terrestrial plants growing in uncontaminated soils were found to be in range of 0.2-8.4 mg kg-1 for Cr, 0.1 to 3.7 mg kg-1 for Ni, 0.4-45.8 mg kg-1 for Cu and 1-160 mg kg-1 for Zn, (Kabata-Pendias and Kabata, 1984). Therefore, heavy metal concentration above these limits in plant body may be helpful to decide the capabilities of plants to remediate contaminated soil.

Ipomea carnea Jacq. is popularly known as Besharam, Behaya in India and Morning glory in English. It is common and aggressive weed occurring in India and world. Many researchers such as Ghosh and Singh (2005), Pandey et al.,(2016), Kavitha and Jegadeesan (2014), Das et al., (2013), etc. conducted pot experiment to study the phytoremediation capacity of *Ipomea carnea* Jacq. to remediate soil contaminated with Cr, Mn, Fe, Pb, Cu Hg, Cd & Mn and found that this species is very effective in remediation of heavy metals. Some of these authors studied the phytoremediation at on-site and some studies at off-site in pot experiment but no one study both the aspects.

It can grow in varied type of soil including polluted soil with heavy metals and can be one of the potential species to restore the contaminated soil and thus check soil erosion (Bhalerao and Chaphekar, 2009; Bhalerao, 2010).

Therefore this plant was selected for the study because it found growing luxuriantly in not only local area but also in area with normal and contaminated soil. The roots of plant can plunge deeply into soil so that it can translocate large amount of heavy metals. This plant was chosen due to its wide distribution, fast-growing, easy maintenance, and high rate of propagation and importantly non-edible. The objectives of the study are (i) To determine the concentration of heavy metals and presence of the plant species in study area. (ii) To determine the bioaccumulation and translocation of metals from soil to roots and shoots. (iii) To study the effects of metal concentration on the metal uptake and suitability of plant to remediate selected heavy metals.

II. MATERIAL AND METHODS

1. **Collection of Samples from Study Area:** The Plants along with soil samples were collected from the Thane-Belapur industrial area, Navi Mumbai, Maharashtra state, India, which is referred in MIDC document as the TTC (Trans-Thane Creek), is one of the most industrialized area containing near about 2000 industrial units. It covers an area of 2,546 hectares at 19004’22.52”N and 73001’08.40”E, and lies on east of the Thane creek, Thane- Belapur road between the urban centers of Thane and Nerul. These industries discharge polluted water into canals, rivers, creeks and sea. This area is highly polluted with heavy metals such as Fe, Cu, Cr, Zn, Ni and Co (Gajbhiye and Bhalerao, 2016). The collected plant materials were washed thoroughly in 1% detergent, tap water, cleaned with distilled water and then separated into roots and shoots. All plant parts were oven dried at 72°C for 72 h, ground to powders and analyzed for heavy metals contents.

2. **Laboratory Scale Pot Experiments:** The pot culture experiments were conducted using plastic pots. The garden soil used for experiment was obtained from the nursery. The soil was alluvium with sandy loam texture. Before experiments, the soil samples were air dried, sieved through a 2 mm mesh, homogenized and analyzed for some physico-chemical characters. The soil was mixed with vermicompost in 2:1 ratio as this composition was found to suitable for proper growth plant after trial and error (Jadia and Fulekar, 2008). Each pot was filled with 2 kg potting mixture contain garden soil and vermicompost. The cuttings of plant species were collected from Thane-Belapur Industrial area. The four cuttings from each plant species were sown at equidistance in each pot. The plant cuttings were allowed to acclimatize and grow in the pot for 4 weeks.

3. **Maintenance of Environmental Condition:** To prevent leaching of nutrients and trace elements out of pots, plastic plates were placed in each pot. The pots were regularly watered by weight to about 60% of its water holding capacity with distilled water (Gupta, 2000). The prepared pots were placed in field conditions to grow the plants in natural environment.

4. **Selection and Treatment of Metals:** The heavy metals were selected on the basis of their common occurrence in the industrial area. After 6 weeks, the pots were treated with heavy metals in interval of two days at the concentration of 25 ppm, 50 ppm and 100 ppm which were prepared from stock solution of 1000 ppm. The solutions of metals were prepared by dissolving salts $K_2Cr_2O_7$ For Cr, $FeSO_4.7H_2O$ For Fe, Ni $(NO_3)_2.6H_2O$ for Ni, $CuSO_4.5H_2O$ for Cu and $ZnSO_4.7H_2O$ for Zn in double distilled water (DDW) based

on earlier research (Gardea-Torresdey et al., 2004; Odjegba and Fasidi, 2004; Ahalya et al., 2005). The plant without heavy metal treatment served as control.

5. **Harvesting and Heavy Metal Analysis:** The plants were harvested after 60 days (period of October and November 2015). The plant and soil from each pot were collected. The plants were thoroughly washed with tap water and then distilled water, separated in root and shoot and dried in oven at 70^0C while soil samples were dried directly in oven. The oven dried sample were ground to fine powder and stored in polythene bags for analysis of heavy metals. For analysis of heavy metals from soil and plant samples were prepared as per guidelines given by SAIF, IIT, Mumbai, where the samples were analyzed. 0.5 gm of soil samples were taken in a Teflon beaker and 10 ml nitric acid and perchloric acid (2:1) and 5 ml Hydrofluoric acid were added to it. The solution is heated on a hot plate to dryness. 10 ml of aqua-regia was added to the dry mass and heated till everything dissolves. 1.0 g of plant samples in 10 ml of nitric acid were heated on a hot plate and perchloric acid were added drop wise till all organic matter were destroyed and solution became clear. The solution was diluted to standard volume, 100 ml with distilled water. The analysis was done by Inductively Coupled Plasma- Atomic Emission Spectrometer (ICP-AES) model ARCOS.

6. **Estimation *of Phytoremediation Indices***

- **The Bioconcentration Factor (BCF) (Determination of the movement of metals from soil to plant):** The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metals that is absorbed by plant from soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005) and is calculated using the formula:

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue (whole plant/portal)}}{\text{Initial concentration of metal in substrate (Soil)}}$$

- **Translocation Factor (TF) (Determination of the movement of metals from Root to plants (Shoot)):** To evaluate the potential of plants for Phytoextraction the Translocation Factor (TF) was used. This ratio is an indication of the ability of plant to translocate metals from root to the aerial parts of the plants (Marchiol et al., 2004). It is calculated using the formula:

$$\text{TF} = \frac{\text{Metal concentration (Stem + leaves)}}{\text{Metal concentration in roots}}$$

Descriptive statistics (Mean, standard deviation) of the data obtained were done by software Microsoft excel and SPSS 17.0 version.

III. RESULTS

1. **Metal concentration accumulated in I. carnea collected from contaminated soil (In-situ):** The result of metals present in soil and accumulation in roots and shoots along with bioaccumulation factor (BCF) and translocation factor (TF) for samples collected from

contaminated soil (*In-situ)* is given in Table 1. The Fe has the highest concentration in soil followed by Zn, Ni, Cr, and Cu. This sequence of heavy metal present in the study area is due to the geographical location and industrial composition (Gajbhiye and Bhalerao, 2016). The concentration of metals absorb in root shows the slightly different trends as compared to metal concentration present in the soil. The roots show highest Fe accumulation more than the amount present in soil. The sequence of metals accumulation in root is Fe> Zn>Cu>Cr > Ni. Although, Ni found to be at high concentration in soil, it is not absorbed by the plant roots in that extent. The same trend of metal translocation into shoot observed as in root. The sequence of metal translocation in the shoot is Fe> Zn> Cu>Cr>Ni.

2. **Metal concentration accumulated in I. carnea from pot experiment (Ex-situ):** The value for metal available in soil and accumulation in roots and shoots by I. carnea is given in Table 2. As shown in the table, the highest concentrations of available heavy metals are present in 50 ppm except for Fe (100ppm) and Zn (25ppm). The highest accumulation of Cr is found to be 264.2±5.04 mg/kg at 100 ppm in roots and 33.2±4.55 mg/kg at control in shoots respectively. The highest concentration of Fe accumulated in roots is 17271.2±19.45 mg/kg at 50 ppm while that in shoots 1561.7±9.14 mg/kg in at 25 ppm respectively. The highest concentration of Ni accumulated in roots is 34.6±5.66 at 100 ppm while that in shoots is 10.7±1.96 mg/kg in at 50 ppm respectively. The highest concentration of Cu accumulated in roots is 116.6±1.68 mg/kg at 100 ppm while that in shoots is 366.1±2.20 mg/kg in at 25 ppm respectively. The concentration of Zn accumulated in roots is 386.3±0.09 mg/kg while that in shoots is 363.5±0.15 mg/kg at 25 ppm respectively.

3. **Bioconcentration factor (BCF) and Translocation factor (TF):** Bioconcentration factor (BCF) and Translocation factor (TF) is given in Table 1 for in-situ and table 3 for ex-situ study respectively. It has been recognized that high biomass and high bioaccumulation factor (BCF) are two key factors for successful phytoextraction (Zhao et al., 2003**).** From the in-situ study, only Fe is metal which having value of BCF greater than one (1.780) indication high accumulation of Fe in the plant tissue while other metals show values less than one indicate less accumulation of metals as compared to soil. Only Ni show high value for TF indicates that even it accumulates less concentration of metals in its root tissues but aerial translocation is high. In case of ex-situ study (pot experiment), Cu has the highest value (10.775) for BCF indicating high accumulation in plant tissues from the soil in all concentration. After Cu, Zn shows very good values of BCF at all concentration while Fe, Ni and Cr (except 2.145 at 100 ppm) show all values less than one. Cu shows a high value of TF at 25 ppm (20.027) and at 50 ppm (4.201) while other metals show all values less than one at all concentration.

IV. DISCUSSION

From both the in-situ analysis and ex-situ, it was found that roots accumulate more all the studied metals than shoots except Ni (Table 1), Cu and Zn (Table 2). The sequence of metal accumulation is Fe>Zn>Cu>Cr>Ni and available metal in the soil also follows more or less the same sequence except for Ni. The concentration of Fe is found to be more in root than in soil (Table1). The high concentration of Fe is due to the fact that it is one of the earth metal and part of the soil. The same trend is also observed in the pot experiment (Table 2)

where its concentration is more than 10,000 mg/kg. Fe is essential for the growth of organisms and this high accumulation of Fe due to the ability of roots to reduce Fe^{+3} to Fe^{+2} is believed to be fundamental in the absorption of this cation by most plants. The similar findings by Nematian and Kazemeini in 2013 who were reported Fe concentration 93266.00 mg/kg in roots and 35722.80 mg/kg in shoots from *Centurea iberica* and *Carthamus oxyacantha* respectively. Hence *I. carnea* may be considered as hyperaccumulator of Fe. Cr is a non-essential and toxic metal to plant growth and it may be possible that plants do not have any specific mechanism of transport of Cr (Shankar et al. 2005). From both in-situ and ex-situ, it is clear that Cr even present in high concentration in soil do not accumulate and translocated in roots and shoots respectively. The concentration of Cr is higher in roots than shoots and this may due to sequestration in vacuoles of roots cells, which have identified mechanisms of detoxification (Shanker et al., 2005). Badr et al., (2012) reported the value of Cr 528 mg/kg attained in roots of *Phragmite australis. I. carnea* can be used for phytoextraction of chromium.

It is a well-known fact that Ni is an essential micronutrient for plant growth in low concentration (Taiz and Zeiger, 1998). Thus the study shows less amount of Ni accumulate in roots even though it is available in high concentration in soil. Ni can interact with iron found in haemoglobin and helps in oxygen transport, stimulate the metabolism as well as being regarded as a key metal in several plants and animals enzyme systems. However, at higher concentrations, Ni can be toxic (Jadia and Fulekar, 2009). This observation found true as in the plant, the concentration of nickel is higher in shoots than roots (table1) but less in case of pot experiment (table 2). Copper (Cu) is an essential element for plants and animals. However, excessive concentrations of this metal are considered to be highly toxic (Badr et al., 2012). In the present study, the accumulation of Cu in roots is very less except at 100 ppm (116.6±1.68 mg/kg) where it is more than the soil sample (table 2). The translocation of Cu from roots to shoots should be less but it is found more in case of 25 ppm and 50 ppm (table 2). This is maybe due to fact that it contributes to several physiological processes in plants including photosynthesis, respiration, carbohydrate distribution, nitrogen and cell wall metabolism, seed production including also disease resistance (Kabata-Pendias and Pendias, 2001). Robson and Reuter (1981) explained that there are different tolerance ranges for plants but a critical toxic level of Cu is in the range of 20-30 ppm for most plants. The result shows that the concentration of Cu in the studies plant is much higher than the critical toxic level.

Zn is an essential trace element for organisms which serves as structural ions in transcription factors and transferred in metallothionein. It is the only metal represented in all six enzymes classes (Webb, 1992). 100 mg/kg of Zn in a plant are toxic (Nematian and Kazemeini, 2013). In the present study, the available Zn in the soil at both in-situ and ex-situ were high and quite high amount of Zn accumulates in both roots and shoots which were higher than toxic levels. In general, Zn metal is compartmentalized in the vacuole of hyperaccumulator species (Ma et al., 2001) and leaf vacuoles are found to be the primary site for Zn sequestration in *T. caerulescens* (Küpper et al., 1999). Although 100 ppm concentration is toxic level for most of the plant, *I. carnea* sustain and absorb most of the studied metals.

V. CONCLUSION

The present study shows that concentration of Cr, Fe, Ni, Cu and Zn in the studied native *I. carnea* was higher than the normal plant and thereby indicating that this plant has a strong ability to tolerate heavy metals. From an analysis of heavy metals from the field as well as laboratory experiment it is clear that *Ipomea carnea* Jacq. have the ability to withstand such a high concentration of heavy metals. The present research work showed that the plant species can be a suitable option for phytoextraction and phytostabilization, and also can be used to construct artificial wetland in an industrial area to treat effluent so as to remove or detoxify heavy metals. But there is still a need for field trial experiments which have been realistic and help to incorporate the knowledge such as chelating agents on metal uptake, transfer and distribution.

Table 1: Metal concentration (mg/kg) in *Ipomea carnea Jacq.* collected From contaminated sites (n=3)

Metals	Conc of metals in mg/kg			Bioaccumulation Factor (BCF)	Translocation Factor (TF)
	Soil	Roots	shoots		
Cr	516.67±0.79	56.83±2.87	15.6±8.17	0.140	0.274
Fe	2087.67±3.09	2592.0±12.08	1126.04±24.98	**1.780**	0.435
Ni	904.00±0.46	6.23±0.54	8.82±1.56	0.016	**1.415**
Cu	298.50±0.79	105.3±6.54	19.26±5.76	0.170	0.182
Zn	1684.0±0.61	284.62±9.13	135.46±8.45	0.249	0.472

Table 2: Metal concentration (mg/kg) in *Ipomea carnea Jacq.* in pot Culture (n=3)

	Cr			**Fe**			**Ni**		
Doses	Soil	Roots	Shoots	**Soil**	**Roots**	**Shoots**	**Soil**	**Roots**	**Shoots**
Control	73.6±5.21	48.5±0.94	33.2±4.55	6306.5±16.23	1908.3±1.55	1314.4±5.47	64.3±1.76	11.7±3.28	2.9±0.34
25 ppm	107.4±1.02	74.8±1.005	3.9±0.08	144893±11.21	12110.8±15.41	1561.7±9.14	129.4±21.27	27.1±6.07	6.0±0.52
50 ppm	163.6±2.22	134±1.0	4.5±0.06	62254.6±19.56	17271.2±19.45	1144.3±4.58	244±30.74	18.8±1.07	10.7±1.96
100 ppm	125.4±3.45	264.2±5.04	4.8±0.14	187515±18.47	2551.7±13.48	1094.5±3.14	79.0±5.22	34.6±5.66	6.1±0.43

	Cu			**Zn**			
Doses	**Soil**	**roots**	**Shoots**	**Soil**	**Roots**	**Shoots**	
Control	54.5±15.47	144.0±1.59	144.0±1.59	144.0±1.59	15.4±0.03	4.7±0.02	
25 ppm	36.6±11.04	672.0±4.66	672.0±4.66	672.0±4.66	18.28±0.02	366.1±2.02	
50 ppm	188.6±14.65	166.8±5.48	166.8±5.48	166.8±5.48	46.6±0.85	195.8±0.91	
100 ppm	41.6±17.66	218.4±6.30	218.4±6.30	218.4±6.30	116.6±1.68	17.6±0.03	

Table 3: Bioaccumulation Factor (BCF) and Translocation Factor (TF) for metals in *Ipomea carnea Jacq.* in pot Culture(n=3)

	Bioaccumulation Factor (BCF)					**Translocation Factor (TF)**				
Metal	**Cr**	**Fe**	**Ni**	**Cu**	**Zn**	**Cr**	**Fe**	**Ni**	**Cu**	**Zn**
25 ppm	0.732	0.0943	0.255	10.775	1.115	0.052	0.066	0.221	20.027	0.94
50 ppm	0.846	0.295	0.1209	1.285	2.589	0.033	0.428	0.569	4.201	0.501
100 ppm	2.145	0.0194	0.514	7.625	2.835	0.018	0.128	0.176	0.15	0.646

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