Metal uptake Potential of wild plant*Ipomoea carnea*growing in Contaminated Site

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Abstract

Wide varieties of plants have exhibiteda specific ability to accumulate metals from soil and waste water into the roots, stems, leaves, flower and seeds. This unique characteristic of plants is being exploited extensively to remove toxic metals from contaminated sites by employing locally growing wild plants that have high adaptation capabilities without extra care. This is an eco-friendly safe, cheap, effective and green technology in cleaning the ecosystem. This work is carried out to evaluate the metal uptake potential of the wild plant *Ipomoea carnea*, (Convolvulaceae) growing on contaminated site for Cd, Cr, Cu, Fe, Ni, Pb and Zn. The bio-concentration factor (BCF) and translocation factor (TF) are also evaluated to assess the bio-concentration of metals from soils in the roots, stem and leaves of the plants. Most of the BCF values were more than unity, except BCF_{Root} forCr and BCF_{Leaf} for Zn, Cr and Cd. The order of BCF_{Root} was Fe (4.38) > Zn (2.51) > Cu (2.44) > Pb (1.70)>Ni (1.38)> Cd (1.35)> Cr(0.52). The order of BCF_{Stem} was Fe (5.47)> Cu (3.30) > Ni (2.80) > Cd (2.46) >Zn (1.43) > Pb (1.31) > Cr (1.19), however the order of BCF_{Leaves} was Cu (3.53)> Fe (1.36) >Pb (1.24)>Ni (1.14)> Zn 90.76) > Cr(0.63) > Cd (0.27).The observed order of Translocation of metal ions from *roots to the stem* of *Ipomoea* was found to be Cr (2.29) >Cd (1.97) > Cu (1.35) > Fe (1.27) Ni (1.22) >Pb (0.77) >Zn (0.57), and the order of translocation from *stem to leaves* was Cu (1.06) > Pb (0.95) > Zn (0.54) > Cr (0.53) > Ni (0.41) > Fe (0.25) > Cd (0.10). These results indicated that *Ipomoea carnea* exhibited considerable metal uptake potential for Cd, Cr, Cu, Fe, Ni, Pb and Zn in its roots as well as translocated these to above ground parts.

Key Words: Metal uptake potential, Contaminated Site, *Ipomoea carne*,BCF, TF.

I. INTRODUCTION

Minerals are key factors for human health and body function. Many metals in certain very small quantities needed for the proper growth, development and to regulate hundreds of bio-chemical reactions to sustain life [1]. Dietary mineral or trace elements are indispensably required by the body for certain specific functions that normally needed in small amounts ranging from micrograms to milligrams. Important minerals necessary for survival are Iron, zinc, cobalt, copper, manganese, molybdenum, iodine, selenium, sulfur, chloride, boron, silicon, vanadium, nickel, arsenic, chromium, but may be toxic at higher levels[2].Some of these are trace elements e.g., iron, copper, manganese and zinc, while metals such as Zn, Mn, Ni and Cu are termed as essential micronutrients. Some metals have structural roles and are essential for bio-mineralization for making a hard structure (Ca). Few metals are essential for stabilizing proteins in its natural conformation [3]. Magnesium metal has important role in balancing phosphate ions. Many metals play active role in electron transport system and cellular energy production. Some are essential as charge carrier in fast information transfer, catalyst for metabolic reactions and redox pairs e.g., Fe (II) / Fe (III) etc. Many metals act as catalyst in metabolic reactions and degradation of bio-molecules [4]. Some are important for fixation of molecular nitrogen and its conversion to ammonia and few metal ions are essential for blood coagulation, osmotic regulation and fluid balance. Many metals are important constituents of essential bio-molecules e.g. vitamin B_{12}, hemoglobin, insulin, thiamine, thyroxin, etc. [5].

Toxic metals have always been present in the Earth's ecosystem, but since the modern progress achieved in mining and metallurgical fields, while exploiting Nation's mineral resources at optimum level, globally minerals from deep crust brought to the upper surface of the earth (upper soil) that supports various agricultural activities. The toxic metals have a great tendency of bioaccumulation through which they enter the food chain and bring adverse effects on human beings, other living organisms and plants [1]. Toxic metals travel to soil and surface water mainly due to weathering of rocks, mining waste, finishing of metal and metal products, battery manufacturing, electroplating,paint-distemper - chemicals, coal- fields- power plants, manufacturing and use of fertilizers, automobile emission, burning of coal and fossil fuel, foundries- refectories, steel - cement - tile industries, effluents from hospitals-Schools- College- University laboratories - Scientific R & D laboratories, etc.[6]Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel, and zinc can cause deleterious health effects in humans[7].No metal is degradableby human body and plants therefore these can be carried long distance and spread by water and air, once entered through food chain these have chance to be deposited in tissues and body parts, affecting adversely the survival of man and plants[1].

Most of the plants accumulate essential micronutrients and few other metals as per their metabolic needs generally at ppm level (<10 ppm), yet in addition to absorbing these micronutrients, certain plants have a characteristic ability to selectively bio-concentrate exceptionally high amounts of metals (thousands of ppm)from contaminated soil and waste water [2]. Such plants are called metal hyper-accumulators species. Metal bio-concentrationis a green technology called 'Phyto-remediation' that selectively accumulates the metal ions and contaminants from contaminated soil, dump sites and waste water, into the plant roots and translocate them to above ground parts of the plant. The success of this unique technique depends on the metal uptake, bioaccumulation and translocation potential of plant root systems. Several locally abundantly growing plants have proved their capability of bio-accumulating toxic metals effectively in the roots and other plant parts and thereby cleaning of contaminated soil and waste water up to a considerable extent. Usually, local wild plants have fast growing near the contaminated sites and producing considerably high bio-mass offer good metal accumulating potential because of their ability to survive under local conditions. Globally, several groups of workers are engaged in the vibrant field of soils- plant- metals uptake interactions to investigate the deep understanding of all the aspects of metal uptake mechanisms and related applied knowledge [2, 8-15]

This communication is focused on the evaluation of the metal uptake potential of a robust wild plant *Ipomoea carnea* growing in contaminated site for by determining the concentration of these metals in the roots, stem and leaves of the plant and by finding Bio-concentration factors and Translocation factors. The published work on metal uptake by Ipomoea carne are by Ghosh and Singh (Cd, Cr and Pb)[8,9], Adhikari et al.[16], 2010 (pot culture study, Pb), Kavitha et al,[17]2014 (pot culture study, Hg and Cd uptake), Sarangam et al,[18] 2016 (hydroponic study, Cr) and Pandey et al,[19] 2016 (uptake potential of Cu, Cr, Cd, Mn, Fe, Ni and Pb by Ipomoea growing on fly ash dump site).

II EXPERIMENTAL:

2.1: Selection of Plant for Study

The selection of plant species for bio-accumulation study depends upon the site, growing conditions, type of contaminants, metal uptake and tolerance and ability to withstand of the plant species under study. The abundantly wildly growing shrub *Ipomoea carnea* **(**Convolvulaceae**)** is commonly known as 'morning glory' which grows in aquatic as well as in terrestrial environment [20]. This is spread all over the world including India, American tropics, Argentina, Brazil and Bolivia, Pakistan and Srilanka [21].It grows 1 - 3 m, with an erect or ascending habit when growing in open positions; in shady positions it is more likely to adopt a climbing habit with twining stems up to 5 m long. The roots are lateral, the stem is thick, develops into a solid trunk erect, woody, and more or less cylindrical in shape and greenish in color. It had alternate leaves. The plant blooms in clusters of pale rose, pink or light violet pink flowers throughout the year, except during winter [21]. The plant also serves as living fences when grown along the boundaries of agricultural fields, to prevent livestocks from consuming and destroying the growing crops. The plant has medicinal values and leaves are slightly purgative and are toxic to livestock and hence non-grazed [19]. Traditionally, the plant extract has immense potential as anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, anti-convulsant, anti-diabetic, anti-inflammatory, sedative and has healing activities [21].The plant is selected for bio-accumulation studies because of its fast growing, rampant spreading and invasive nature with high biomass yield[8,9], enough metal tolerance ability, high bio- accumulation cum translocation potential and high adaptive potential for survival in diverse conditions. Latex in the plant is used to treat skin problems. It contains several bioactive compounds such as glycosides, alkaloids, reducing sugars, flavonoids, fatty acid, esters, alcohol and tannins [21].

Fig. 1: Photograph of *Ipomoea carnea*(Convolvulaceae) plant.

2.2. Collection of soil samples

The selected site is a somewhat low lying land to the south-east of the undergraduate Chemistry laboratory that remained submerged in rainy season. The untreated laboratory effluents containing varieties of toxic and non-toxic chemicals, reagents and metal salts were being discharged directly to the selected site for last several decades. During high flow season from nearby elevated locality of the city, the selected site gets over flow of urban and agri-runoff. The representative soil samples were collected randomly during October 2015, from five locations of the selected site from depth of 15- 20 cm. The samples were air dried for 8-10 days then thoroughly mixed and oven dried at 80^{0}C for 8 hours. The dry samples were finely grinded to obtain homogenized fine particles and stored in clean and dry plastic bags.

2.3. Collection of plant samples

Ipomoea plants were rooted out in January 2016 from the soil, cleaned with tap water carefully to remove soil particles from the plant including roots, then washed twice using distilled water and then kept over filter paper sheets making moisture free. The moisture free plants were separated into roots, stem and leaves and placed on the separate clean sheets for air dry for 10 days in dust free chamber, then oven dried for 5-6 hours at 75- 80 ^{0}C. The dried root, stem and leaves were weighed and grinded to powder and kept separately in sample bottles and labeled.

2.4. Treatment of plants, and soil Samples

To evaluate the accumulated metals in *Ipomoea carnea*, 1.0 g of the grinded samples of roots, stem or leaves were subjected to Nitric acid - Perchloric acid (5:1, v/v) digestion following the standard methods by APHA [22], for several hours to get a transparent light colored liquid thus obtained was filtered in a 100mL volumetric flasks and make up to the mark with double distilled water. Three replicates of each sample were analysed for metal ion concentration. To estimate the concentration of metals present in soil 1.0 g of the finely grinded soil samples were digested separately with HNO_3 –HCl–$HClO_4$ mixture(5:1:1) for several hours to get transparent extracts, which was filtered and diluted to a volume of 100 mL with double distilled water[23,24].

2.5. Estimation of Metals in soil and plant parts

The concentration (mg/kg Dry Weight) of Cd ,Co , Cr , Fe , Ni , Zn , Mn ,Cu and Pb (mg/L) in the samples of soil and root, stem and leaves of Ipomoea were determined by using a Perkin Elmer Model 200 Atomic Absorption Spectrometer, using an air-acetylene flame in accordance to Standard Methods for the Examination of Water and Wastewaters, IPHA [22, 23]. Various A.A.S. standard stock solutions of metal ions used were from Sigma Aldrich, which were diluted to required concentrations to prepare working standards. Other reagents, chemicals, and solvents used were of Analytical grade. Doubly- distilled water is used for all purposes. All the estimations were run in triplicate.

III RESULTS & DISCUSSION

The observed concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn found in the Soil Samples, Root, Stem and Leaves of *Ipomoea carnea* are presented in Table- 1 All the results shown are mean of triplicate determinations.

Table-1: Concentration (mg /Kg DW) of Metal ions in Soil, root, stem and leaves of *Ipomoea carnea*

Metal	Concentration in Soil (mg/Kg, DW ± SD)	WHO Limits (Mg/Kg)	Concentration in (mg/L ±SD)			WHO Limits in Plants, mg/L
			Root	Stem	Leaves	
Cadmium	1.23±0.12	1.0	1.66 ±.02	2.28±0.66	0.33±0.02	0.02
Copper	1.31±0.15	20.0	3.20±0.04	4.32±0.21	4.62±0.42	10.0
Chromium	2.82±0.21	50.0	1.46±0.02	3.35±0.58	1.78±0.02	1.30
Iron	5.38± 0.33	20.0	23.26±2.12	29.44±4.2	7.32±0.38	20.0
Nickel	1.17 ±0.11	20.0	2.68±0.03	3.28±0.42	1.34±0.03	10.0
Lead	2.85 ±0.28	10.0	4.84±0.08	3.72±0.62	3.55±0.21	2.0
Zinc	13.82 ±1.43	250.0	34.69±2.36	19.84±2.4	10.46±1.08	50.0

3.1. Uptake and accumulation of Metals in plants

Plants have unique, inbuilt mechanisms of roots that are supported by specific transporting proteins, to accumulate and translocate certain micronutrients and water from soil, that are essentially required for proper growth and development. Uptake and transport of metals across root cellular membrane is an important process which initiates metal absorption into plant tissues [10]. Translocation of metal-containing sap from the root to the shoot is primarily controlled by root pressure and leaf transpiration [25].While, crossing selectively the biological membranes, the intracellular concentration of metal ions is maintained up to the physiological range. The structure of biological membrane permits the movement of a particular ions type through it. Selective metal uptake from soil by plants may be either active transport or passive, the active transport crosses the plasma membrane of root epidermal cells while passive plant involves mass flow of water into the roots [26]. Due to convection, soluble metal ions move from soil solids to root surface. From the rhizosphere, water is absorbed by roots to replace water transpired by leaves. Water uptake from rhizosphere creates a hydraulic gradient directed from the bulk soil to the root surface. Some ions are absorbed by roots faster than the rate of supply via mass flow. Thus, a depleted zone is created in soil immediately adjacent to the root. This generates a concentration gradient directed from the bulk soil solution and soil particles holding the adsorbed elements, to the solution in contact with the root surface. This concentration gradient drives the diffusion of ions toward the depleted layer surrounding the roots [25].The metal bio-accumulation depends on its bio-availability in soil solution around the plant root system. Soil pH affects metal bio-availability and also the metal uptake into roots, the effect is metal specific [24].

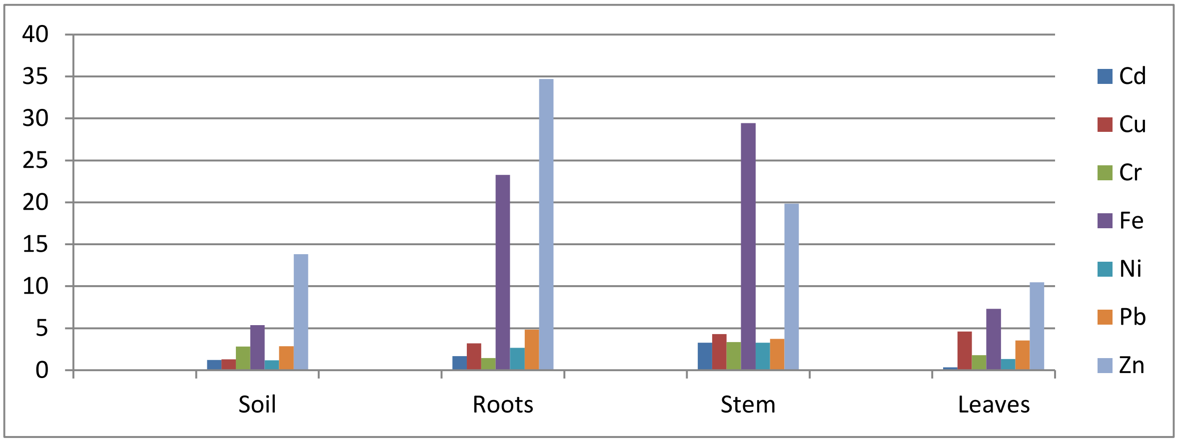

Fig. 2: **Concentration (mg /Kg DW) of Metal ions in Soil, root, stem and leaves of *Ipomoea carnea***

The order of observed metal concentrations in soil (Table-1, Fig. 2) was Zn (13.82 ±1.43) > Fe (5.38 ± 0.33)> Pb (2.85 ±0.28) > Cr (2.82±0.28) > Cu (1.31 ±0.15) > Cd (1.23 ±0.12) > Ni (1.17 ±0.11 mg/Kg, DW). Ipomoea plant behaved differently to accumulate metals under study in different plant- parts. Thus, in Ipomoea the order of metals uptake by root system was found to be Zn (34.69± 2.36) > Fe (23.26±2.12)> Pb (4.84±0.08) > Cu (3.20±0.04) > Ni (2.68±0.03) > Cd (1.66 ±.02) > Cr (1.46±0.02mg/L). The order of metals uptake in stem was Fe (29.44±4.2) >Zn (19.84±2.4) > Cu (4.32±0.21) > Pb (3.72±0.62) > Cr (3.35±0.58) >Ni (3.28±0.42) > Cd (2.28±0.66 mg/L), however the order of metals uptake by ipomoea leaves was Zn (10.46±1.08)>Fe (7.32±0.38) > Cu (4.62±0.42) > Pb (3.55±0.21) > Cr (1.78±0.02) > Ni (1.34±0.03) > Cd (0.33±0.02 mg/L). The accumulation of Zn and Pb were found higher in Ipomoea roots than in stem and leaves, while Cd, Cr, Cu, Ni and Fe exhibited higher accumulation in stems and leaves than in roots, similar to the earlier findings [19].

3.2. Bio-concentration factors (BCF)

Bio-concentration factors are the ratio of concentrations of metals found in plant roots, stems, or leaves and concentration of metals in soil in which the concerned plant is growing. BCF is not a constant; instead it is a variable depending on different environmental and biological conditions. BCF values give an idea about the bio-magnification of metals [27].BCF is inversely dependent on metal concentrations in the soil or water [28]. Metals uptake by root depends on soil and plant factors such as soil pH, organic matter, plant species, the redox conditions, plant age etc.[19].The observed results revealed that most of theBCF values were more than unity, except BCF_{Root} of Cr (0.52mg/Kg), and BCF_{Leaves} of Zn (0.76mg/Kg), Cr (0.63mg/Kg) and Cd (0.27 mg/Kg).The order of BCF_{Root} was Fe (4.38)>Zn(2.51) >Cu (2.44)>Pb (1.70) >Ni (1.38)> Cd(1.35) > Cr (0.52).The order of BCF_{Stem} was Fe(5.47) > Cu (3.30)> Ni (2.80) > Cd (1.85) >Zn (1.43) > Pb (1.31) > Cr (1.19), however the order of BCF_{Leaves} was Cu (3.53)> Fe (1.36) >Pb (1.24) >Ni(1.14)> Zn (0.76)> Cr (0.63) >Cd (0.27).The increasing orders of BCF_{Root} do not necessarily follow the same order of concentration of metals in soil. These observed results have shown almost same metal uptake trends as reported earlier [8, 16-19]. To understand the exact metal uptake potential of Ipomoea, monitoring of various physiological parameters of a plant species such as photosynthesis, respiration, chlorophyll content, and different enzyme activities including nitrate reductase (NR), peroxidase (POD), and succinate dehydrogenase (SD) etc. are useful [16].

Table-2: Bio Concentration Factors and Translocation Factors of various Metal ions in *Ipomoea carnea*

Ipomoea carnea	**Cd**	**Cu**	**Cr**	**Fe**	**Ni**	**Pb**	**Zn**
	Bio Concentration Factors (BCF)						
BCF_{Root}	1.35	2.44	0.52	4.38	1.38	1.70	2.51
BCF_{Stem}	1.85	3.30	1.19	5.47	2.80	1.31	1.43
BCF_{Leaf}	0.27	3.53	0.63	1.36	1.14	1.24	0.76
	Translocation Factors (TF)						
TF_{Stem}	1.97	1.35	2.29	1.27	1.22	0.77	0.57
TF_{Leaf}	0.10	1.06	0.53	0.25	0.41	0.95	0.54

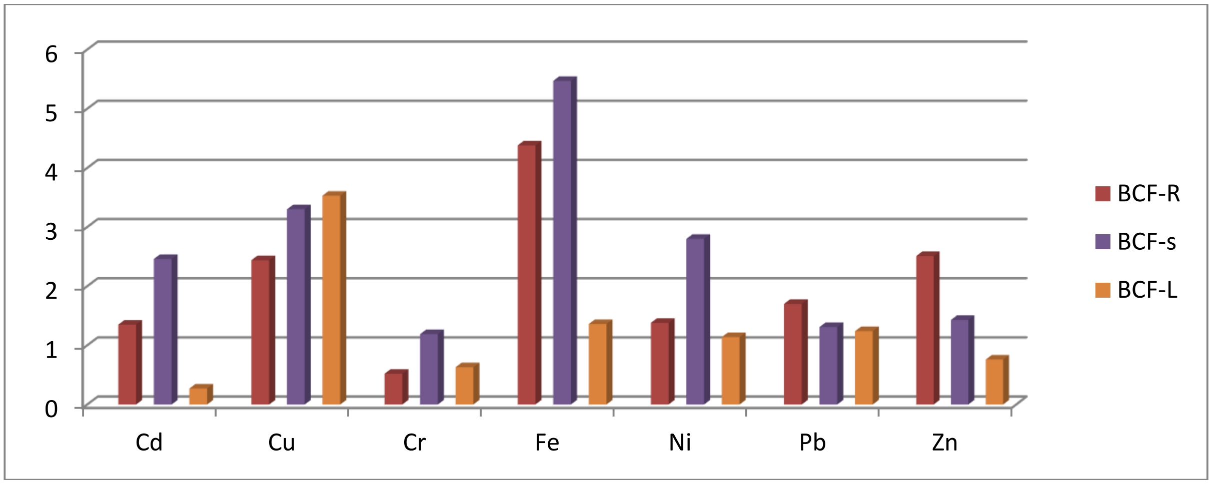

Fig. 3: **Bio Concentration Factors of various Metal ions in *Ipomoea carnea***

3.3. Translocation factor (TF)

Movement of metal-containing sap from the root to the shoot is termed translocation. Translocation factor (TF) is an important tool used to assess a plant's potential for phytoremediation purposes. TF is the ability of a plant to translocate metals from the roots to the plant parts or shoots.TF is the measure of translocation of a metal from soil to a particular part of the plant. TF is calculated from the ratio of the metal's concentration in the plant parts, stem or leaf to that in the plant roots [27] using the equation, TF = Metal $_{(stem)}$ / Metal $_{root)}$.

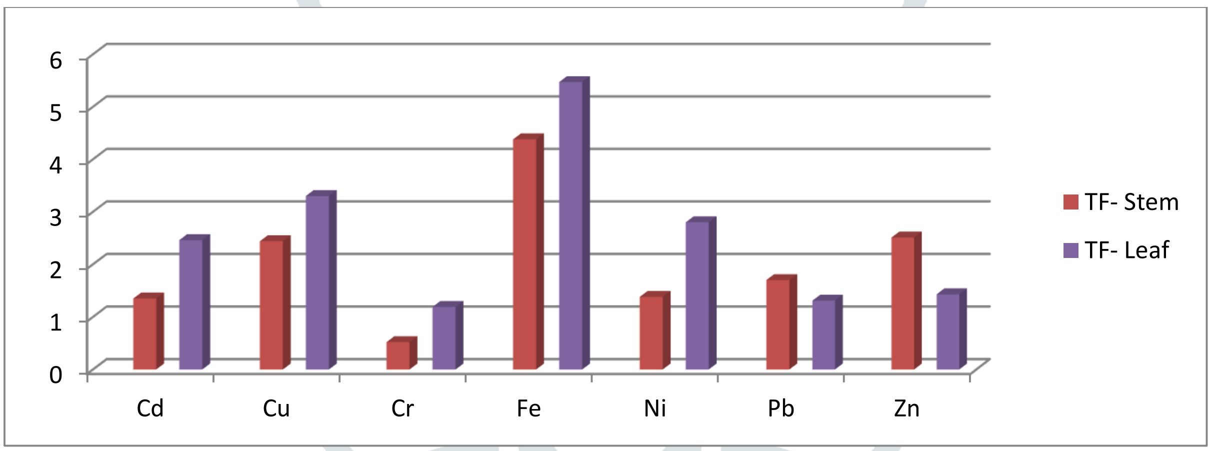

Fig. 4: Translocation Factors (TF $_{Stem}$ and TF $_{Leaf}$) of various Metal ions in *Ipomoea carnea*

The observed order of Translocation [Table-2 , Fig:4] of metal ions from *roots to the stem* of Ipomoea was found to be Cr (2.29) >Cd (1.97) > Cu (1.35) > Fe (1.27) Ni (1.22) >Pb (0.70) >Zn (0.57), while the order of translocation from *stem to leaves* was Cu (1.06) > Pb (0.95)> Zn (0.54) > Cr (0.53) > Ni (0.41) > Fe (0.25) > Cd (0.10). Thus, Cr, Cd, Cu, Fe and Ni have shown good translocation from roots to the stem in Ipomoea plants, while; only Cu and Pb have exhibited good translocation up to the leaves of the plant.

Conclusion:

The metal uptake potential of a plant species is a complex interaction that depends on soil composition and other properties, hydrogeology and climate of selected area. The experimental results of BCF_R revealed that Ipomoea *carnea* has enough metal uptake potential for Fe (4.38), Zn (2.51), Cu (2.44), Pb (1.70), Ni (1.38), Cd (1.35) and Cr (0.52). Similarly all the BCF_{Stem} values were more than unity, Fe (5.47), Cu (3.30), Ni (2.80), Cd (2.46), Zn (1.43), Pb (1.31), Cr (1.19), while the BCF $_{Leaf}$ values for Cu (3.53), Fe (1.36), Pb (1.24) and Ni (1.14) were more than unity. The bio-concentration factors and observed appreciable values of Translocation Factors (Table-2) revealed that *Ipomoea* has considerable uptake potential for most of the metals under study. Thus, *Ipomoea carnea* that produces the huge biomass and has much high growth rate with immense stress tolerance characteristics, also exhibited better accumulation and translocation potential for toxic metals such as Cd, Cr, Pb and Ni, from roots to the above ground parts- stem and leaves, therefore the plant can be used for the phytoremediation of metals from contaminate sites, as an environmentally friendly Green method with carbon neutral approach.

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