Bio- accumulation potential of wild plant *Cassia Occidentalis* L. grown on contaminated soil for Cd, Cr, Cu, Ni and Pb.

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ABSTRACT

The contamination of ecosystem by toxic metals has proved hazardous to both plant and man. Due to in-built bio-accumulation mechanism several plants exhibited selective accumulation of metals from contaminated soil in roots and above ground parts of the plants. This study was aimed to assess the bio-accumulation potential for Cd, Cr, Cu, Ni and Pb in the root, stem and leaves of *Cassia Occidentalis* L. (Ceasalpiniaceae) grown on waste dump site, by analyzing bi-accumulated levels of studied metals in soil and plant parts. The bio-concentration factors (BCFs) and translocation factors (TFs) for studied metals were evaluated. The order of metal concentrations in soil was found as Cr (5.86± 0.34) > Pb (4.57 ±0.23) >Cu (1.67± 0.16) > Ni (1.58± 0.13) > Cd (0.48± 0.04, mg/Kg). The bio-concentration factors (BCFs) computed for Cd, Cr, Cu, Ni and Pb were 1.06, 0.75, 0.57, 0.51 and 0.86, respectively. The computed translocation factors from root to stem (TF_{Stem}) for tested metals in *Cassia Occidentalis* were, 1.62, 1.5, 1.91, 1.58 and 1.54, respectively. However, the root to leaves translocation factor (TF_{Leaf}) were computed as, 0.56, 0.91, 1.22, 0.75 and 0.91, respectively.

Key Words: Heavy metal bio-accumulation, *Cassia Occidentalis* L., BCF and TF.

I. INTRODUCTION

Multidimensional development during last century has brought social and economic benefits to the society but these advancements have also caused a wide range of environmental problems at both local and global level [1]. Many metals in certain very small concentrations are essential for growth of plants and human life in various ways. When the concentrations of metals in vegetable crops exceed their required levels these may exert toxic effects on plant growth. The non- degradable nature of metals makes them persistent in ecosystem for indefinite period and spread anywhere via water and air. The repeated use of contaminated water for irrigation may elevate toxic metal levels in farm-soil that may reach ultimately to the vegetables and other farm products. On bio-accumulation metals from farm-soil move to the food chain and thereby stored in human internal organs such as liver and kidney [2]. The deposition of metals beyond their recommended safe limits in human organs may cause serious health problems.

Toxic metals have always been present in the ecosystem, but since the industrial revolution there has been a massive redistribution of metals on the surface of the earth [1, 2]. Some metals are potentially toxic for plants resulting in chlorosis, weak plant growth, reduced nutrient uptake and reduced nitrogen fixation in leguminous plants [3]. Normally, plants bios-accumulate essential micronutrients as per their metabolic needs, however, several plants bio-accumulate selectively much higher amounts of metals. The excessive accumulation of heavy metals in soils may result not only in soil contamination, but has concern for food quality and safety [4]. The metal bio-accumulation efficiency of the plant is induced by the solar driven translocation process of the plant used for ascent of sap / nutrients [5]. Plant species showed remarkable difference in levels of metals accumulated in various plant parts [1]**.** Movement of metal-containing sap
from the root to the shoot is primarily controlled by root pressure and leaf transpiration [1]. Besides, bio-concentration through root system, the metals may also enter plant cells via the foliar surface route.

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Plants have shown to be very effective in the cleanup of contaminated soils, called phyto-remediation. It is possible due to unique ability of plant roots to absorb and translocate ionic species to above ground parts of plants, from soil present in the vicinity of the root cells. Phyto-remediation is eco-friendly Green technology, that is a safe and cheap way to remove toxic metals and other contaminants from soil and water, by involving bio-sorption and

bioaccumulation of the soluble fraction of metals from contaminated soil and waste water [6]. Phyto-remediation takes advantage of the selective metal uptake capabilities of plant root systems, together with the translocation and pollutant degradation abilities of the entire plant body [7-8]. This technology generates little to no secondary waste and cause minimum environmental disturbances.

The studies to evaluate metal bio-accumulation capabilities of various native plant species have become a field of global interest, as these native plants have high biomass and can withstand the local conditions of adaptation. This study was focused on the evaluation of the bio-accumulation potential of abundantly growing wild plant *Cassia Occidentalis* L. collected from a waste dump site, for Cd, Cr, Cu, Fe, Ni and Pb, by analyzing levels of studied metals in soil and plant's root, stem and leaves. The bio-concentration factors (BCFs) and translocation factors (TFs) for studied metals were also evaluated.

II. EXPERIMENTAL

Selection of Plant:

For metal bio-accumulation studies, abundantly growing wild plant *Cassia Occidentalis* L, was selected.
Cassia occidentalis L.(Ceasalpiniaceae) *i*s evergreen perennial plant that is also known as Kasundi or *Kasamarda*. It is a perennial abundantly growing robust and evergreen wild plant that grows fast with considerable biomass and ability to withstand against harsh climatic conditions. It possesses expectorant properties and is effective against cough, asthma, and other respiratory ailments so given the name *Kasamarda*. The leaves, roots, and seeds of the plant are purgative [9]. It is a half woody erect shrub that grows up to about 1.5-2.0 m in height. Seed germinates after rain during May –June. Leaves (12.5 x 5 Cm) are about 20 to 25 cm in length. Each pinna has four to seven pairs of leaflets, which are 3 to 9 cm in length and 2 to 4 cm in width and arranged oppositely [10]. Flowering occurs in mid September and fruits (10 x 0.7 x 0.9 cm) appear at the start of leaflets, singly, may be 2, 3, or 4.

Collection of soil samples:

The selected area for this study is a waste dump site along the Nalapani Rao, Raipur Road, Dehradun. Five representative soil samples were collected randomly from the selected site from depth of 0- 20 cm. The collected soil samples air dried for 8-10 days, followed by oven drying at 100^0C for 24 hours. The dried samples were finely grinded separately to obtain homogenized fine particles and stored in clean and dry plastic bags.

Collection of plant samples:

To estimate the bio-accumulation potential of tested metals in *Cassia occidentalis*, plant samples were collected in February 2014. Plants were rooted out from the soil, washed thoroughly with tap water and finally with distilled water and placed over clean filter paper sheets to make samples moisture free in a dust free cabin. The cleaned and moisture free semi-dried plant samples were separated into roots, stem and leaves and collected on separate clean sheets, first dried in air for 8-10 days in dust free environment and then oven dried for few hours at 80^0C to a constant weight. Dried samples of root, stem and leaves were finely grinded to a fine powder and labeled separately.

Treatment of soil and plant samples:

To estimate the accumulated metals 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 100 mL with double distilled water [11]. To estimate the accumulated metals in *Cassia occidentalis*, 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 [12] for several hours to get a transparent light colored liquid, which was filtered in a 100 mL flasks and make up to the mark with double distilled water. Three replicates of each sample were analysed for metal ion concentration.

Estimation of Metals in soil and plant parts:

The estimation of Cd, Cr, Cu, Ni and Pb in the samples of soil and plant parts were done by using atomic absorption spectrometer by following the standard procedure [12], using an air-acetylene flame. Various standard stock solutions of metal ions used were from *Sigma-Aldrich,* which were diluted to

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required concentrations for preparing various working standards. All the estimations were run in triplicate. All chemicals, reagents, and solvents used were of analytical grade. Atomic Absorption Spectrophotometer (Analyst-200, PerkinElmer) was used to estimate toxic metals in the samples of waste water, soil and plant parts. Double distilled water is used for all purposes.

III. RESULTS AND DISCUSSION

The concentrations of Cd, Cr, Cu, Ni, and Pb found in the soil samples and in roots, stem and leaves of *Cassia occidentalis* are presented in Table- 1. All the results are mean of triplicate determinations.

Table-1: Levels of studied metals in Soil and in the Roots, Stem and Leaves of *Cassia occidentalis*

Concentration of Metals (mg/Kg± SD, Dry Weight)		**Cadmium**	**Chromium**	**Copper**	**Nickel**	**Lead**
Metal ions in Soil Samples		0.48± 0.04	5.86± 0.34	1.67± 0.16	1.58± 0.13	4.57 ±0.23
WHO limits for Soil (mg/Kg)		**1.0**	**50.0**	**20.0**	**20.0**	**10.0**
Cassia occidentalis	*Root*	0.16 ±0.02	1.28 ±0.08	0.23±0.04	0.24 ±0.06	1.14 ±0.09
	Stem	0.26 ±0.03	1.92 ±0.16	0.44 ±0.05	0.38 ±0.08	1.76 ±0.16
	Leaf	0.09 ±0.01	1.17 ±0.09	0.28 ±0.03	0.18 ±0.04	1.04 ±0.08
WHO limits for plants (mg/Kg)		0.02	1.30	10.0	10.0	2.0

Metal ions concentration in Plant:

Metals enter to plants primarily through their root system also reaches to the above ground parts. Root hair cells of plants have a large surface area, thin walls and are close to the xylem cells, to absorb the water from soil by osmosis. Once the micronutrients and water reached inside the root cells, these are translocated to the above ground parts of the plant. This unique process of mass flow is facilitated by specifically designated transporting proteins. The ascent of saps containing dissolved nutrients and minerals and some toxic metals, from the root to the shoot through phloem column is partly controlled by leaf transpiration [5]. Uptake of metals by plants from soil and subsequent accumulation along the food chain is a threat to animals and human health [13]. The bioavailability of metals in soil depends on metal solubility in soil solution. In soil solution, metals available for plant uptake are associated either as free metal ions or soluble metal complexes. Metals in soil may also be present as bound to soil organic matter. Soil pH affects not only metal bio-availabilty, but also the metal uptake into roots. Absorption and accumulation of metals in plant tissues depend upon temperature, moisture, organic matter, pH and nutrients availability of soil [14]. Although, the order of metal concentrations in soil was found as Cr (5.86± 0.34) > Pb (4.57 ±0.23) >Cu (1.67± 0.16) > Ni (1.58± 0.13) > Cd (0.48± 0.04), the order of overall metal levels in *Cassia occidentalis* plant parts was Cr (4.37) > Pb (3.94) > Cu (0.95) > Ni (0.80) > Cd (0.51). These findings are much lower than reported earlier on Nigerian soil [9, 15].

Translocation of metals to above ground parts:

Bio-concentration factor (BCF) and translocation factor (TF) of were used to estimate the accumulation of various metals from soils in roots and then translocation to the stem and leaves, thereby phyto-remediation potential for metal ions. Bio-concentration factor is the ability of a plant to accumulate metals from soils in their tissues. Bio-concentration factors are the ratio of metals in root and metals in soil. This
factor gives an idea about the bio-magnification of metals [16]. The metal availability in soil depends upon the chemical speciation of metals and on the soil pH, organic matter and the redox condition mostly. BCF is calculated by the equation:

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$$BCF = \frac{\text{Concentration of Metal found in Plant (mg/ L)}}{\text{Concentration of Metal found in Soil (mg /L)}}$$

Translocation factors (TF) is the measure of translocation of a metal from soil to a particular part of the plant. TF is the ability of a plant to translocate metals from the roots to the shoots, which is the ratio of metal concentration in the shoots to the roots [17]. The translocation factors are highly variable depending upon metals and plant species [18]. It is calculated by using the equations:

$$TF = \frac{\text{Concentration of Metal found in the plant stem (mg /L)}}{\text{Concentration of Metal in soil/ root (mg/ L)}}$$

$$TF_{Stem} = \frac{\text{Concentration of Metal found in the plant stem (mg /L)}}{\text{Concentration of Metal in root (mg/ L)}}$$

$$TF_{Leaf} = \frac{\text{Concentration of Metal found in the plant leaves (mg /L)}}{\text{Concentration of Metal in root (mg/ L)}}$$

The values of BCF and TF_{Stem} and TF_{Leaf} for Cd, Cr, Cu, Ni and Pb evaluated for *Cassia occidentalis* are presented in Table- 2.

Table-2: Bio - concentration factor and Translocation factor of various Metal ions in *Cassia occidentalis*

Cassia occidentalis	Cd	Cr	Cu	Ni	Pb
BCF	1.06	0.75	0.57	0.51	0.86
TF_{Stem}	1.62	1.5	1.91	1.58	1.54
TF_{Leaf}	0.56	0.91	1.22	0.75	0.91

The computed values of bio-concentration factor (Table 2) for cadmium was highest (1.06) and nickel recorded lowest (0.51), while the overall order was, Cd (1.06) > Pb (0.86) > Cr (0.75) > Cu (0.57) > Ni (0.51). This observation suggested that *Cassia occidentalis* can be used for phyto-remediation of cadmium and lead from contaminated soil. Among the translocation from root to stem (TF_{Stem}), the translocation of Cu was highest to the stem, followed Cd, Ni, Pb and Cr. The computed order of TF_{Stem} was Cu (1.91) > Cd (1.62) > Ni (1.58) > Pb (1.54) > Cr (1.50). However, the translocation from root to leaf (TF_{Leaf}) followed the order, Cu (1.22) >Pb (0.91) > Cr (0.91) > Ni (0.75) > Cd (0.56). All these observed results indicated the suitability of the wild plant *Cassia occidentalis* to be tried for phyto-remediation of tested metals from metal contaminated soil.

CONCLUSION:

Due to considerable bio-concentration factor for Cd, Cr, Cu, Ni and Pb, these metals are accumulated from farm-soil in the roots of *Cassia occidentalis* plant. Further, the appreciable translocation factors (TFs) exhibited by the plant for tested metals, indicated that these metals in varying concentrations are translocated to the stem and leaves of the plant. Thus, *Cassia occidentalis* showed moderate absorption of tested metals from the soil and their translocation from roots to the above ground parts (AGP) of the plants. *Cassia occidentalis* which is a perennial abundantly growing evergreen, robust wild plant that grows faster to give considerable biomass and has high ability to withstand against harsh conditions, make the plant suitable species to be tried for phyto-remediation of tested metals from contaminated site.

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