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Bioaccumulation, Translocation and Internal Partitioning of cadmium in *Vigna mungo* and Assessment of its Phytoremediation Potential

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ABSTRACT: Seedlings of *Vigna mungo T-9* were given Cd exposure (0 ppm, 50 ppm, 100 ppm,200 ppm, 300 ppm 500 ppm $CdSO_4$) in pot experiments having metal amended soils. After 45 days plant parts roots, shoot, and leaves were analysed. Cd accumulation in them was measured with the help of inductively coupled plasma – Optical emission spectroscopy (ICP-OES), after digestion with HNO_3 and perchloric acid. Significant accumulation of Cd occurred in root, shoot and leaves of *Vigna mungo*. Chlorophyll estimation as also done at different exposure of Cd. Despite high Cd exposure there was mild decline in chlorophyll content and better Cd accumulation especially in roots (37.5 µg/g at 200 ppm $CdSO_4$) and shoots (20.7 µg/g at 200 ppm $CdSO_4$) of *Vigna mungo T-9* was reported.

Keywords: Bioaccumulation; Chlorophyll; Cadmium; Phytoremediation; translocation; Heavy metals; Optical emission spectroscopy and *Vigna mungo.*

INTRODUCTION: Heavy metals are the most hazardous pollutants as they are non-degradable and get accumulated in soil, also, they are toxic to plants as well as animals. Land and water are precious natural resources on which the sustainability of agriculture and the civilization of mankind depends. Unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities.(Ravichandran *et al.*, 2011).Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add heavy metals to soils and waters are of serious concern due to their persistence in the environment and carcinogenicity to human beings. They cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another (Battaglia*et al*. 2007 and Bolan *et al.,* 2003). Therefore, heavy metal pollution poses a great potential threat to the environment and human health.

Physicochemical approaches have been widely used for remedying polluted soil and water, especially at a small scale.(Zhou *et al.,* 2004) However, they experience more difficulties for a large scale of remediation because of high costs and side effects. The use of plant species for cleaning polluted soils and waters named as phytoremediation has gained increasing attention since last decade, as an emerging cheaper technology. Numerous plant species have been identified and tested for their traits in the uptake and accumulation of cadmium. Mechanisms of metal uptake at whole plant and cellular levels have been investigated.(Wu *et al.,* 2010; Hirve *et al.,* 2013 and Kumari *et al.*, 2011)

Phytoremediation (such as phytoextraction, phytostabilistion and rhizofiltration of soils contaminated by heavy metals has been widely accepted as cost effective and environment friendly cleanup technology, (Ravichandran *et al*. 2011).However this approach can fully exploited only when the mechanisms of tolerance, accumulation and translocation in plants are better understood(Chandra *et al.*, 2009).

Cd is classified under the group I of human carcinogens, with sufficient evidence for carcinogenesis reported in animals as well as in humans. It can lead to renal cancer, and together with arsenic, it can cause lung cancer (Vebrugen *et al*., 2009).Although Cd is a non-essential element for plants, its excessive amounts in water or soil can result in injuries, such as chlorosis and growth inhibition leading to plant death (Yoon *et al*., 2006 and Muneer *et al.,* 2012). This non-redox heavy metal is also known to affect photosynthesis, nitrogen metabolism, water and nutrient uptake (Wangstand *et al*., 2007; Wu *et al.,* 2010 and Rai *et al.*, 2005). Since, Cd is highly mobile therefore it can easily be translocated from the roots to the aerial parts of the plants (Farooq*et al.*, 2008).

The plant species that show heavy metal tolerance at their juvenile stages may produce tolerant adult individuals. Thus, exploration of variation at early growth stages may signify overall potential of a crop for its exploitation on contaminated agriculture lands located in the vicinities of large industries of the country (Becker *et al*., 2008). Keeping in view the increasing Cd toxicity to crop plants and significant importance of pulses as source of low cost vegetable proteins for low income groups in a developing country like India (Hirve and Angoorbala *et al*., 2013). In present study relative Cd tolerance, accumulation and translocation of cadmium, with an aim of assessing phytomediation potential of in *Vigna mungo T-9* species at their early establishment phases was assessed. Accumulation and translocation of cadmium and chlorophyll content in Vigna species after their exposure to different levels of Cd in the soil. The Cd content in plant tissues along with tolerance for root and shoot growths were also investigated to evaluate tolerance and phytoremediation potential of the species to excessive Cd present in the soil.

To assess the phytoremediation potential of Black Gram (*Vigna mungo T-9)*, an experiment has been conducted taking *Vigna mungo* T-9 as a study plant which belongs to family Fabaceae, serves as a main staple food of geographical region of north west Uttar Pradesh. *Vigna mungo*(L) Hepper (Black Gram or Urd Bean) is one of the most widely used pulsecrop in India. It is a highly prized pulse, very rich in phosphoric acid. For this purpose seeds of *Vigna mungo var T-9* were sown in soil amendeds with different concentration of $CdSO_4.7H_2O$ (50, 100, 200, 300, 500 ppm). Plant parts after 45 days of Cd exposer were analysed for Cadmium estimation**.** This pulse crop is being grown in the area adjoining the industries where industrial effluents contaminated with heavy metals is coming to the field so that the present investigation is undertaken to study the phytotoxic effects of cadmium on growth parameters in blackgram and its phytoremediation potential.

MATERIAL AND METHODS: Seeds of *Vigna mung T-9* were procured from Pantnagar Agricultural University, pantnagar and were swon in soil amended with different concentration of $CdSO_4.7H_2O$ (50, 100, 200, 300, 500 ppm). Plant biomass at growth stages of 45 days were analysed for Cadmium estimation. For Cadmium estimation the plant material was carefully washed with distilled water to remove surface contamination, air dried, and wet washed. One gm of powdered sample was digested over night in 10 ml 50%, HNO_3, filtered, brought to a volume with distilled H_2O and then 1 ml 10% $NH_4H_2PO_4$, as matrix modifier, was added. After digestion, Cadmium was measured by an Inductively coupled plasma atomic absorption spectrophotometer (ICP-OES; Spectro analytical Instruments, West Midlands, UK).

For pigment determination, 500 mg of dry leaf were homogenized in 20 ml of 80% acetone using mortar and pastle and centrifuged at 6000×g for 15 minutes finally the supernatant was made up to 20ml and Optical Densities (O.D.) were measured at 480 and 510 nm wavelength for carotenoides and 645 nm and 663 nm for chlorophyll on a UV-VIS spectrophotometer (Systronics Model 119, India). The amount of chlorophyll a and b and carotenoid were calculated by using the formulae give by Machlachan and Zalik (1963) and Duxbury and Yentsch (1956) respectively;

$$Chlorophyll\ a\ \left(\frac{mg}{g}dry\ leaf\right) = \frac{[12.3 \times D_{663}\ - 0.86 \times D_{645}] \times V}{d \times 1000 \times w}$$

$$Chlorophyll\ a\ \left(\frac{mg}{g}dry\ leaf\right) = \frac{[19.3 \times D_{645}\ - 3.6 \times D_{663}] \times V}{d \times 1000 \times w}$$

$$Total\ Chlorophyll\ \left(\frac{mg}{g}dry\ leaf\right) = Chlorophyll\ a\ + Chlorophyll\ b$$

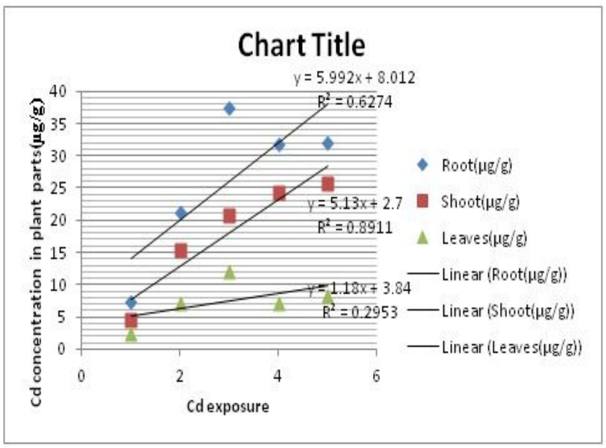
Where, V = Volume of extract (ml), d= length of light path (cm), w = dry weight of leaf.

RESULTS AND DISCUSSION: To study the uptake and accumulation Cadmium content in *Vignamungo T-9* study plants were raised in various level of cadmium (Table 1 and 2). The cadmium content of root and shoots of *Vigna mungo* increased with an increase in cadmium level in the soil. The minimum accumulation in root (7.34 µg/g), shoot (4.45µg/g) and leaf (2.4µg/g) was observed in the soil amended with 50ppm $CdSO_4.7H_2O$. Maximum content was recorded in root (37.5µg/g) at 200 ppm $CdSO_4$ and in shoot (25.7 µg/g) was recorded at 500 ppm cadmium level in the soil in all the plants. Plants treated with 100 ppm of $CdSO_4$ accumulated Cadmium in roots (213.3µg/g), shoots (15.4µg/g) and leaves (7.1 µg/g). Seedlings treated with 200 ppm $CdSO_4$ were found to have Cadmium in shoots (20.7 µg/g and in leaves 12.1 µg/g respectively. At 300 ppm Cadmium concentration was reported as 31.7 µg/g , 24.2 µg/g and 7.1 µg/g in root shoot and leaves respectively. While at 500 ppm $CdSO_4$ concentration *Vignamungo T-9* seedling accumulated Cadmium in the range of 32.1 µg/g, 25.7 µg/g 8.3 µg/g in root shoot and leaves.

Table 1: Accumulation of Cd in different plant parts of *Vigna mungo* T-9, at different concentration of Cd(ppm) in soil.

Plant Parts / Concentration of Cd	Root (μg/g)	Shoot (μg/g)	Leaves (μg/g)
50 ppm	7.34	4.45	2.4
100 ppm	21.3	15.4	7.1
200 ppm	37.5	20.7	12
300 ppm	31.7	24.2	7.1
500 ppm	32.1	25.7	8.3

Figure 1: Relationship between Cd exposure (in ppm×100) to Vigna mungo T-9 and its accumulation in Root, Shoot and Leaves (mg/g).



The absorption mechanism of Cadmium by *Vigna mungo T-9*.can be analysed using Biological accumulation coefficient (BAC), Biological Transfer coefficient (BTC) and bio-concentration factor (BCF) analysis. BAC can be definedas the concentration of heavy metals in plant shootsdivided by the heavy metal concentration in soil (Zu*et al*., 2005), and is given below:

BAC = [Metal] shoot / [Metal] soil

Biological Transfer Coefficient was described as the ratio of heavy metal concentration in plant shoot to that in plant root (Zu*et al*., 2005).

BTC = [Metal] shoot / [Metal] root

Bioconcentation Factor was calculated as ratio of concentration of heavy metal in plant roots to that of soil (Yoon *et al*., 2006).

BCF = [Metal] root / [Metal] soil

Table 2: BAC, BTC, and BCF as reported in Vigna mungo T-9 (45 days old seedlings) at different concentration of Cd exposure.

Concentration of Cd	BAC	BTC	BCF
50 ppm	0.088	0.606	0.14
100 ppm	0.154	0.723	0.23
200 ppm	0.103	0.522	0.16
300 ppm	0.0806	0.763	0.105
500 ppm	0.085	0.800	0.062

Figure 2: Relationship between Cd concentration (ppm) and BAC and BTC of *Vigna mungo* T9 (45 days old seedlings).

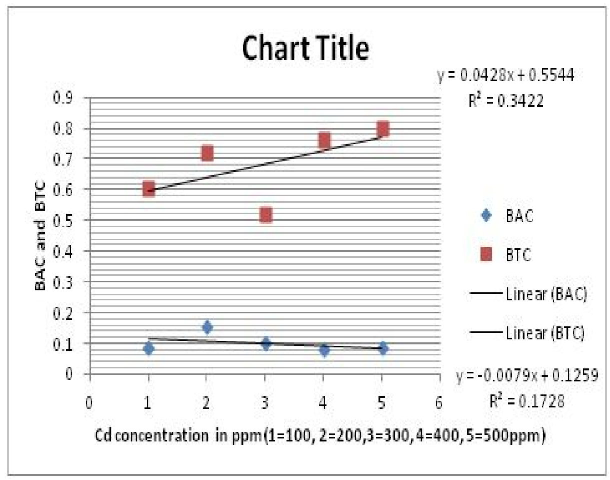


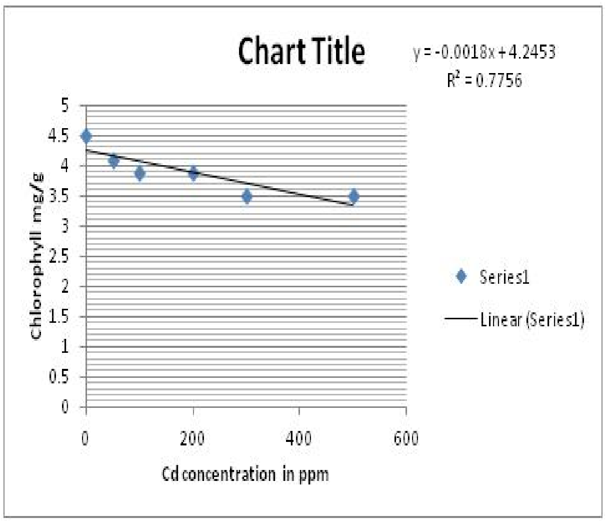
Table-2 shows the BAC, BTC and BCF of metals from soil to other parts of Vigna. BAC was reported as 0.088, 0.154, 0.103, 0.0806, 0.085 at 50, 100, 200 300 and 500 ppm respectively. This shows that BAC was found highest at 100 ppm. BTC was reported as 0.606, 0.723, 0.522, 0.7634, 0.800 at 50, 100 200, 300 and 500ppm. This shows that at 500 ppm concentration BTC was reported highest, while and 200ppm the BTC as reported lowest. BCF was reported as 0.14, 0.23, 0.16, 0.105, 0.062 at 50, 100, 200, 300, 500 ppm respectively. This shows that 100 ppm concentration roots accumulated maximum percentage of Cadmium. This experiment shows the phytoremediation potential of *Vigna mungo T-9*.

This experiment showed increasing cadmium level in the soil, increased the uptake and accumulation of cadmium contents in root and shoot of *Vigna mungo T-9* plants. Similar obsevations in winter wheat, sugarbeet, rice and Solanum nigrum were reported by Wangstand et al., (2007), Pehlivan et al., (2008), Rascio et al., (2008) et. al., (2008) in *Solanum nigrum*, Chopra *et al*., (2013) in *Spinacea.*

Table 3: Chlorophyll content of *Vigna mungo* leaves (45 days old) at different concentration of Cadmium.

Concentration of Cd	*Chlorophyll content in leaves of Vignamungo T9*
Control(0 ppm)	*4.5±.73 mg/g*
50 ppm	*4.1±.65mg/g*
100 ppm	*3.9±.56 mg/g*
200 ppm	*3.9 ±.52mg/g*
300 ppm	*3.5±.45 mg/g*
500 ppm	*3.5±.35 mg/g*

Figure 3: Relationship between Cd concentration(ppm) and Chlorophyll content of *Vigna mungo* T-9.



Chlorophyll estimation of *Vigna mungo T-9* leaves revealed that chlorophyll declined as the Cd exposure increased.Control plants showed chlorophyll content 4.5 mg/g while plants exposed to 50 ppm showed chlorophyll content 4.1 mg/g. Plants exposed to 100 ppm CdSO4 solution had chlorophyll content 3.9 mg/g, while plants exposed to 200 ppm, 300 ppm and 500 ppm were found to have chlorophyll content 3.9 mg/g, 3.9 mg/g and 3.5 mg/g respectively.This shows that though plants were exposed to high concentration of Cd, chlorophyll content did not declined too much, at the same time significant amount of Cd was accumulated in plant parts.

Our results coincides with the findings of (Farooq *et al.*, 2008 and John *et al.*, 2008), who reported that the cadmium accumulation in roots was due to compartmentation of cadmium in the vacuoles.(Malekzadeh *et al.*, 2007). As we know roots are the first organs which contact to the toxic metal ions, and roots usually accumulate significantly higher amounts of metal than shoots. (Zu *et al.*, 2005, Yoon *et al*., 2006).Wu *et al.*, 2006) showed that the cadmium uptake in plants is correlated with the increasing amount of metal in the growing medium or soil.

Accumulation of Cadmium was found to be concentration and time dependent. On prolonged exposure to higher concentration of Cadmium, that is 300 ppm, plant showed decline in accumulation rate of Cadmium. Restriction of heavy metal transport from root to shoot has been thought of as the mechanism of plant tolerance to Cadmium (Verbruggen *et al.*, 2009). Further, the tanslocation of Cadmium from roots to shoots and leaves is an important factor affecting accumulation of Cadmium in aerial tissue of *Vigna mungo var T-9.*Similarly the growth and metabolism of Black gram was adversely affected when the plants were exposed to different concentrations of cadmium. stress causes direct and indirect multiple effects on plant growth and metabolism and also alters some physiological processes (Misra*et al.*, 2008 and Becker *et al.*, 2008).

Results of phytoremediation experiment of Black gram indicate significant recovery of phytotoxicity induced by cadmium in all the parameters studied to a significant level. Growth of Black gram (*Vigna mungo* T-9) plants in artificially contaminated soil was significantly retarded in comparison to plants grown as intercrop with vetiver. AAS studies also confirm that Cd has been accumulated by Khus (Vetiverzizanjoides) where the accumulation is primarily in roots as compared to shoots and leaves and this makes cadmium useful for phytostabilization indicating that Cadmium is accumulated more in below ground parts (roots) and is weakly translocated through vascular system. As a result, phytotoxicity of cadmium on growth parameters has been drastically reduced. Thus *Vigna mungo T9* acts as a powerful phytoremediator for cadmium and makes the soil less toxic.

The mechanisms behind this hyper accumulation and detoxification include chelation to organic acids or proteins (Qureshi *et al.*, 2014; Muneer *et al.*, 2013, Wang *et al.*, 2009 and Heiss *et al.*, 2003) or it may be due to its larger biomass apart from the stronger metal uptake ability. Furthermore, it could yield better covering and benefits. (Chopra *et al.*, 2013,Hussain *et al.*, 2012 and Ravichandran *et al.*, 2011). The fact that *Vigna mungo T-9* has short life span of about 60-80 days. This makes it a promising phytoremediator. Also, this species is an efficient, enduring, low cost and long term remedial option for phytoremediation. Thus, the present study demonstrates that *Vigna mungo* T-9 may be used as potential phytoremediator plant at industrial sites contaminated with Cadmium.

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CONCLUSION:

Since increasing heavy metals poses a ecological risk. Cadmium accumulation studies in Vigna mungo and internal partitioning of it in root shoot and leaves are highly valuable since *Vigna mungo T-9* is prominent pulses of north India. Further, incorporation with one other remedial techniches such as soil amendmends and intercropping system can impove the efficacy of phytoremediation by *Vigna mungo T-9.*

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