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Bioaccumulation of Cadmium in *Capsicum annuum* L

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Abstract
A study was conducted to find out the phytoextraction potential of *Capsicum annuum (C. annuum)* to cadmium under different concentrations (0, 10 mM, 20 mM, 30 mM, 40 mM and 50 mM) and time periods {60 days after germination (DAG), 80 DAG and 100 DAG}. Cadmium content in different plant parts and soil were estimated using Atomic absorption spectrophotometer (AAS) after wet diacid digestion. Bioconcentration factor, translocation factor and enrichment factor were calculated. Statistical analysis of the results revealed that *C.annuum* is not a phytoaccumulator of cadmium as most of the cadmium was accumulated in roots. Also cadmium content in fruits was below detectable limits.

Keywords: phytoextraction, translocation factor, enrichment factor, *C. annuum*

Introduction
Cadmium (Cd) is a heavy metal toxic to both plants and animals due to its high solubility and mobility. Even small concentration of the above produce adverse effects on plant growth and development (Barcelo & Poschenrieder, 1990) [2]. Mining, smelting, electroplating, application of solid wastes in agriculture fields, excessive application of phosphate fertilizers are the main inputs of cadmium to environment by man. Cadmium uptake by higher plants depend upon various factors like soil cadmium concentration, its bioavailability which in turn is depend on pH, organic matter, redox potential and concentration of other elements. It also is a function of various activities like uptake capacity, concentration of chelating molecules, intracellular binding sites, and transport activities (Clemens *et al*.,2002) [5]. Metal uptake into the cell is regulated by the cell membrane.
Cadmium toxicity results in reduction in photosynthetic activity, chlorophyll content, plant growth and induction of oxidative stress. Plants possess the natural ability of hyper tolerance towards particular metals (Chaney *et al.*, 1997) [4]. Although plants absorb toxic metals from soil, their absorption rate is different. Plants which absorb very high concentration of metals in the above ground parts in natural habitat are hyper accumulators (Baker & Brooks, 1989) [1]. Hyper accumulators are plants which can accumulate high amount of heavy metals in their tissues, at concentrations 10 to 100 times higher than tolerated by crop plants (Lasat, 2002) [10]. A hyperaccumulator for Cd has 100 mg/kg Cd in its tissue, compared to a normal level for most plants of 0.1 mg/kg (Brook, 1998) [3]. The bioconcentration factor (BCF) of a particular plant expresses the ability of the plant to accumulate Cd with respect to Cd concentration in the soil solution (Zhu *et al*., 1999). Cadmium is reported to be easily translocated from plant roots to above ground tissues (Sanita di Toppi & Gabbrielli, 1999) [17]. The difference in the ability of plants to accumulate heavy metals also has relation to root
Jarvis (1976) [8] found that the roots of lettuce released much more of their absorbed Cd for translocation to the shoots than other crops such as rye grass and orchard grass. The greater translocation is due to active, transport or lack of metal absorption to fixed or soluble chelators in the root or perhaps due to exchange with the Ca, Mn and Zn moving through the roots (John, 1976) [9]. Moral *et al*. (1994) [13] reported that Cd was easily transported to aerial parts of tomato and was not detected in fruits.
McKenna *et al.,* (1993) [12] reported higher Cd concentration in the older than in younger leaves of lettuce and spinach. The potential accumulation of Cd in old leaves could not be solely due to the transportation rate. Metal-binding peptides were present in older leaves in higher amounts than in younger leaves in tobacco and Cd was transported into the vacuoles as a means of detoxification (Vogeli-Lange & Wagner, 1990) [19].
Phytoextraction is a phytoremediation technique where plants are used to absorb, transport and concentrate metals from the soil into the harvestable above ground parts (Salt *et al*., 1995; Lorestani *et al*., 2011) [15, 11]. Plants which possess bioconcentration factor (BCF), translocation factor (TF) and enrichment factor (EF) greater than one (BCF, TF and EF> 1) are hyper accumulators and they can be used in phytoextraction (Lorestani *et al*. 2011) [11]. Nirmal kumar *et al*. (2009) [14] studied the accumulation and mobility of heavy metals including Cd in vegetable crops in India and found that all the metals are highly mobile (Cd, Co, Cu, Fe, Ni, Pb and Zn) and the mobility gradient was Cd >Pb> Fe > Zn> Co> Ni > Cu.
Uptake and accumulation of heavy metals by plants is important because even low concentrations of toxic metals in soil, end up with adverse effects in plants. The objective of the study was to find out the phytoextraction

potential of *C.annuum* in cadmium contaminated soils with special emphasis on bioconcentration factor, translocation factor and enrichment factor.

Materials and methods

Experimental set up

Seeds of *C. annuum* were washed in sterile distilled water and sown in a pot filled with potting mixture (1:1:1 of soil, cow dung and sand) and seedlings were raised. Uniform sized seedlings (40 DAG) were transplanted in pots filled with 1 kg potting mixture. Control and test plants were raised in triplicates in pots supplied with different concentrations of $CdCl_2$ (10 mM, 20 mM, 30 mM, 40 mM and 50 mM) and designated as T1, T2, T3, T4 and T5 respectively. A control pot was also maintained with the same potting mixture without $CdCl_2$ and designated as T0. Control and test plants were taken out of soil after 60 DAG, 80 DAG and 100 DAG, washed thoroughly in deionized water and allowed to dry in an oven at 70°C. Roots, shoots and leaves were separated and analysed for heavy metal content during the above time interval along with soil samples.

Estimation of heavy metals

Wet diacid digestion method was used for the estimation of heavy metals (Gupta, 1999). The heavy metal content in the digest was determined by atomic absorption spectrophotometer (Analyst 300, Perkin Elmer).

Bioconcentration factor

Plant's metal accumulation ability can be estimated by finding out bioconcentration factor (BCF) which is the ratio of metal concentration in the roots to that in soil $\left(\frac{metal\ (root)}{metal\ (soil)}\right)$.

Translocation factor

TF is the ratio of metal concentration in the shoots to root $\left(\frac{metal\ (shoot)}{metal\ (root)}\right)$.

Enrichment factor

The enrichment factor is calculated as the ratio of plant shoot concentration to soil concentration $\left(\frac{metal\ (shoot)}{metal\ (soil)}\right)$.

Statistical analysis was done using SPSS 17.0. Difference between control and test samples were analysed by one-way ANOVA taking significant level at $p<0.001$. Pair-wise comparison was done using Bonferroni test.

Results

From the result, it was clear that maximum percentage of uptake was from 96.30 % to 98.26 % in 60 DAG plants, from 96.30 % to 98.33 % in 80 DAG plants and from 96.65 % to 98.43 % in 100 DAG plants. In all cases, maximum up take was shown by plants treated with 30 mM concentration (T3). The uptake % increased with increase in Cd concentration up to T3 plants, then in T4 a decrease in % uptake was noticed and again the value increased.

Table 1: Concentration of available Cd in soil (mM / kg) Mean ± SE

Treatment	60DAG	% uptake	80DAG	% uptake	100DAG	% uptake
T0 (control)	BDL	BDL	BDL	BDL	BDL	BDL
T1 (10 mM)	0.160±0.000	96.30	0.158±0.001	96.30	0.143±0.000	96.65
T2 (20 mM)	0.190±0.001	97.77	0.183±0.001	97.85	0.139±0.001	98.37
T3 (30 mM)	0.222±0.001	98.26	0.213±0.000	98.33	0.201±0.001	98.43
T4 (40 mM)	0.445±0.000	97.39	0.436±0.002	97.45	0.414±0.000	97.58
T5 (50 mM)	0.436±0.001	97.95	0.462±0.001	97.83	0.444±0.002	97.79
P value	0.000		0.000		0.000	

Table 2: Cd concentration in different parts of the plant during various time periods

Applied Cd (mg/ 100Ml)	Availble Cd (µg/g)	60DAG (µg/g)				80DAG (µg/g)				100DAG (µg/g)			
		R	S	L	F	R	S	L	F	R	S	L	F
48	16	1	BDL	BDL	BDL	2	1	BDL	BDL	3	2	BDL	BDL
96	21.5	2	BDL	BDL	BDL	3	2	BDL	BDL	4	4	BDL	BDL
144	29.5	5	3	BDL	BDL	6	5	BDL	BDL	6	5	BDL	BDL
192	50.5	4	2	BDL	BDL	5	3	BDL	BDL	6	5	BDL	BDL
240	12	2	BDL	BDL	BDL	3	2	BDL	BDL	4	4	BDL	BDL

Table 3: BCF of Cd (mM) in *C.annum* during 60 DAG, 80DAG and 100DAG

Treatments	BCF 60 (Mean ± SE)	BCF 80 (Mean ± SE)	BCF 100 (Mean ± SE)
T0 (control)	BDL	BDL	BDL
T1 (10 mM)	0.011^{l} ± 0.001	0.022^{g} ± 0.002	0.037^{d} ± 0.000
T2 (20 mM)	0.018^{ij} ± 0.001	0.028^{e} ± 0.001	0.051^{b} ± 0.001
T3 (30 mM)	0.019^{hi} ± 0.010	0.049^{c} ± 0.001	0.061^{a} ± 0.001

T4 (40 mM)	$0.016^{k} \pm 0.000$	$0.020^{gh} \pm 0.002$	$0.025^{f} \pm 0.001$
T5 (50 mM)	$0.008^{m} \pm 0.000$	$0.011^{l} \pm 0.001$	$0.016^{k} \pm 0.001$
P value	0.470	0.000	0.000

There were no significant changes in BCF among 60 DAG plants. However, TF and EF showed significant changes at 60 DAG. BCF values ranged from 0.008 ± 0.00 to 0.019 ± 0.01, TF values from 0.490± 0.017 to 0.590± 0.011 and the EF values from 0.007 ± 0.001 to 0.230 ± 0.017. TF and EF were maximum for plants supplied with 30 mM Cd concentration, there after the value decreased.

Table 4: TF of Cd (mM) in *C.annuum* during 60DAG, 80DAG and 100 DAG

Treatments	TF60 (Mean ± SE)	TF80 (Mean ± SE)	**TF100 (Mean ± SE)**
T0 (control)	BDL	BDL	BDL
T1 (10 mM)	0.000 ± 0.000	$0.485^{g} \pm 0.011$	$0.656^{d} \pm 0.035$
T2 (20 mM)	0.000± 0.000	$0.660^{d} \pm 0.017$	$1.000^{a} \pm 0.000$
T3 (30 mM)	$0.590^{f} \pm 0.011$	$0.830^{b} \pm 0.011$	$0.833^{b} \pm 0.000$
T4 (40 mM)	$0.490^{g} \pm 0.017$	$0.602^{e} \pm 0.057$	$0.830^{b} \pm 0.010$
T5 (50 mM)	0.000± 0.000	$0.660^{d} \pm 0.011$	$1.000^{a} \pm 0.000$
P value	0.000	0.000	0.000

A significant change in the values of BCF, TF and EF was observed among 80 DAG plants. BCF values ranged from 0.011 ± 0.001 to 0.049 ± 0.001; TF values from 0.485 ± 0.011 to 0.830 ± 0.011 and EF values from 0.007 ± 0.001 to 0.041 ± 0.000. BCF, TF and EF values are maximum for plants treated with 30 mM $CdCl_2$ concentration. However, after 30 mM concentration, there was a decline in the values of BCF, TF and EF.

Table 5: EF of Cd (mM) in *C. annuum* during 60DAG, 80DAG and 100 DAG

Treatments	**EF 60 (Mean ± SE)**	**EF 80 (Mean ± SE)**	**EF100 (Mean ± SE)**
T0 (control)	BDL	BDL	BDL
T1 (10 mM)	0.000 ± 0.000	$0.011^{h} \pm 0.000$	$0.020^{e} \pm 0.005$
T2 (20 mM)	0.000 ± 0.000	$0.019^{ef} \pm 0.000$	$0.051^{c} \pm 0.001$
T3 (30 mM)	$0.230^{a} \pm 0.017$	$0.041^{d} \pm 0.000$	$0.061^{b} \pm 0.001$
T4 (40 mM)	$0.007^{i} \pm 0.001$	$0.012^{h} \pm 0.001$	$0.021^{e} \pm 0.001$
T5 (50 mM)	0.000 ± 0.000	$0.007^{i} \pm 0.001$	$0.016^{g} \pm 0.001$
P value	0.000	0.000	0.000

Control and treated plants showed significant differences in BCF, TF and EF values ($p<0.001$) at 100 DAG. BCF value ranged from 0.016 ± 0.001 to 0.061 ± 0.001, TF from 0.656 ± 0.035 to 1.00 ± 0.00 and EF from 0.016 ± 0.001 to 0.061 ± 0.001. TF values are same for 20 mM and 50 mM concentrations which showed that the translocation of Cd from root to shoot was same for the above plants.

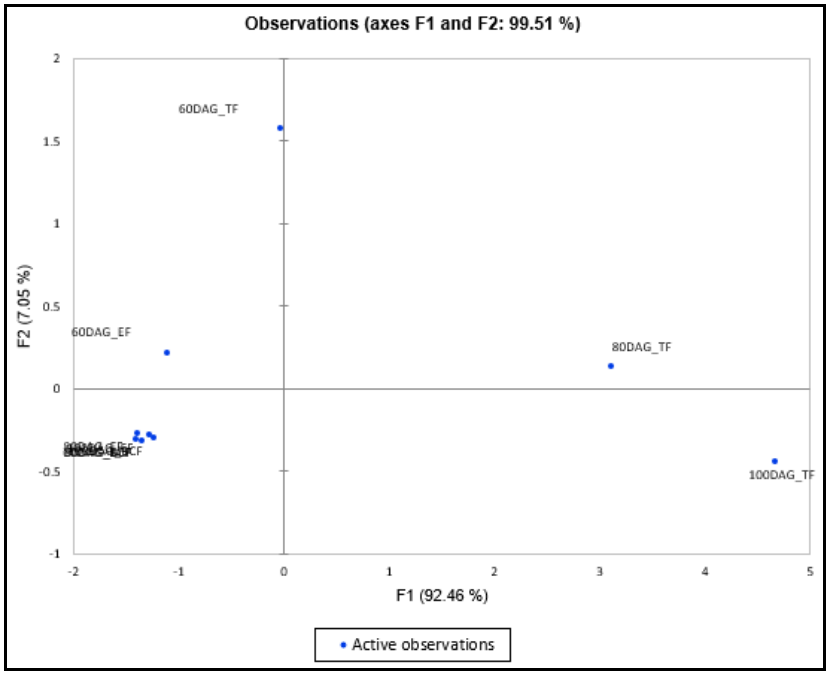

Fig 1

Discussion

Plants differ in their ability of metal absorption and accumulation ability. According to Smical *et al*. (2008) [18], different plant parts show difference in heavy metal quantities, the major quantity in roots and leaves, and the minor in flower buds and fruits. Roots are the organs through which Cd is taken by plants and so higher accumulation of Cd can be seen there (Drazkiewiez *et al*., 2003). Ability of plants to accumulate metals in their plant parts can be estimated by finding out BCF and its ability to translocate metals from the roots to shoots are measured using TF. A hyperaccumulator translocate much of the Cd from root to shoot. A plant having both bioconcentration factor (BCF) and translocation factor (TF) greater than one is considered to be useful for phytoextraction (Yoon *et al*., 2006).

From the result, it was clear that maximum percentage of uptake was from 96.30 % to 98.26 % in 60 DAG plants, from 96.30 % to 98.33 % in 80 DAG plants and from 96.65 % to 98.43 % in 100 DAG plants. In all cases, maximum uptake was shown by plants treated with 30 mM concentration (T3). The results clearly revealed that the uptake % of Cd increased with increase in soil Cd concentration up to T3 plants, then in T4, a decrease in % uptake was noticed and again the value increased. In all cases, rate of Cd uptake was maximum in T3 plants and major portion was located in roots. Greater accumulation of Cd in root might be the complexing of metal with sulph-hydryl group resulted into less translocation of metals to upper parts of the plants (Sinha & Gupta, 2005). The increased uptake of Cd by T3 plant roots might be due to the increased metal mobility and transpiration rate. Various physio-chemical factors also contribute to the above cause. According to Sandalio *et al*., (2001) [16] plants may accumulate Cd in roots in a non- active form and this may reduce its toxicity for roots.

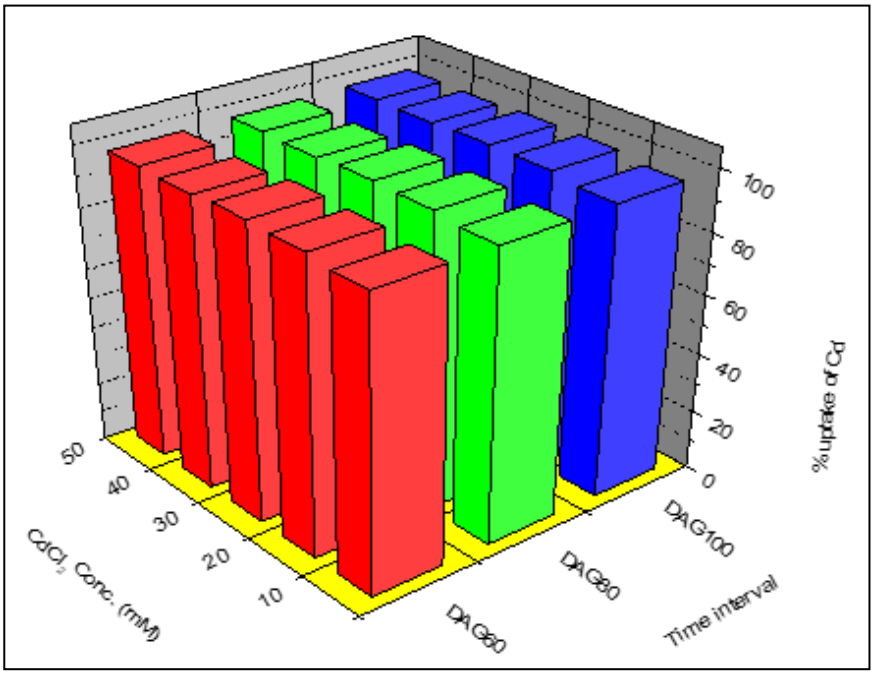

Fig 2: Per cent uptake of Cd on 60 DAG, 80 DAG and 100 DAG

Bioaccumulation was greater in roots than shoots. In leaves and fruits, Cd concentration was below detectable limits. There were no significant changes in BCF among 60 DAG plants. However, TF and EF showed significant changes at 60 DAG. TF and EF were maximum for plants supplied with 30 mM Cd concentration there after the value decreased.

In all the time periods (60, 80 and 100 DAG) BCF, TF and EF showed significant difference (except BCF value of 60 DAG). The value of BCF, TF and EF were less than one (except TF value of T2, T3 and T5 are one) which indicated that the plant *C. annuum* was not a hyper accumulator and not fit for the purpose of phytoextraction. In leaves and fruits, Cd uptake was below detectable limits.

Control and treated plants showed significant differences in BCF, TF and EF values ($p<0.001$) at 100DAG. TF values are same for 20 mM and 50 mM concentrations which showed that the translocation of Cd from root to shoot was same for the above plants.

Conclusion

Phytoextraction potential of *C. annuum* to Cd was studied by analysing the bioconcentration factor, translocation factor and enrichment factor. From the study it was clear that there exists significant differences between the control and test plants in bioaccumulation values except 60 DAG plants. Translocation factor also showed significant variation and maximum TF value was shown by plants supplied with 30 mM $CdCl_2$ solution. However, the TF values obtained were one or below one which indicates that the translocation rate of Cd from root to shoot is less and hence the plant is not a hyper accumulator. The EF value was also less than one. Although *C. annuum* can absorb and accumulate Cd in higher concentrations in root, it is not a hyperaccumulator

because the translocation factor was not greater than one. So *C.annuum* can be classified as a cadmium tolerant plant and the major portion of the accumulated cadmium was in the root. Since the fruit is consumed universally, the result of this study also gives relief to all because fruits contain Cd in below detectable amounts.

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