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HYPER ACCUMULATION OF CADMIUM IN S*OLANUM NIGRUM* L. AND THEIR EFFECTS ON PHYTO-CHEMICALS AND ANTIOXIDANT ENZYMATIC ACTIVITIES

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ABSTRACT: In the pot experiment, *Solanum nigrum* L. treated with different concentration of Cd showed high accumulation of Cd in plant. Growth of plant was not significantly affected up to ≤ 50ppm Cd treated plant, The trends of Cd accumulation in plant were leaf>shoot>root with increasing concentration of Cd and DAS. Plant accumulates 298, 92 and 58 ppm of Cd after 60DAS in leaf>shoot>root respectively, at 50ppm Cd treatment. Solasodine and proline content was increased at ≤ 40 to 50 ppm Cd treated plant, while APX and CAT activity showed the survivability against Cd toxicity up to ≤ 60ppm of Cd. Qualitative phytochemical analysis *viz.* alkaloids, tannins and flavonoids were dominant at early days and unaffected up to ≤30ppm Cd treated plant. BF and TF for was >1.0 for Cd in *Solanum nigrum* L. hence the Cd accumulation capacity of the *Solanum nigrum* L. could make it useful for phytoremediation of the metal.

INTRODUCTION: *Solanum nigrum* L. (Black Nightshade) an herb is a very common species in India and belongs to genus solanum comprises about 1400 species. It is an important medicinal plants and contains solasodine, a steroidal glycoalkaloid, have been reported as chemotherapeutic agent for treatment of cancer especially skin cancer [1]. *Solanum nigrum* L. tolerant to adverse environment, fast growing and with high biomass, under feasible environmental conditions, its biomass could increase rapidly [2] and it has the potential for phytoremediation of metal contaminated soils [3].

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Heavy metal pollution of soils has dramatically increased in recent decades due to the discharge of waste and wastewater from anthropogenic sources (WHO). This situation has become a critical environmental issue owing to the potential adverse ecological effects of the pollutants.

Nearly 400 species of terrestrial plants have been identified as hyper-accumulators of various heavy metals in the world [4], which are capable of accumulating high levels of heavy metals without suffering metal toxicity or cell damage [5]. The exploitation of plants to remove toxic heavy metals from the environment is being given more attention due to considerable commercial interest. So far, numerous studies have focused on the mechanisms of hyper accumulators to tolerate and accumulate metals within their tissues [3].

Hyper accumulators are defined based on the following characteristics:

1. Shoots metal concentrations (threshold values) 100 mg/kg for cadmium [6],
2. Bioconcentration factor (ratio of metal concentration in plant to soil) is greater than 1.0, sometimes reaching 50–100 [7, 8],
3. Translocation factor (ratio of metal concentration in shoots to roots) is greater than 1.0 [9, 10].

Cadmium is not essential to plant growth, and it can interfere with physiological processes including carbon assimilation decrease, chlorophyll synthesis inhibition, oxidative stress generation[11] etc. Cadmium toxicity causes oxidative stress, which can take place possibly by generating reactive oxygen species (ROS). To scavenge ROS and avoid oxidative damage, plants possess the antioxidative enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), glutathione peroxidase, ascorbate peroxidase and glutathione reductase, as well as nonenzyme antioxidants such as ascorbic acid and glutathione [12, 13].

As a non-essential element for living organisms, Cd has a very high mobility in soil-plant systems, with propensity to adversely affect both human health and the functioning of ecosystems [3, 4]. The medicinal plants are either naturally grown or cultivated in metal contaminated regions, there is a danger that the heavy metal accumulation by plants of medicinal value may cause serious health hazards to patients using metal adulterated herbal drugs. The objective of this study was to examine the growth response and uptake, distribution, accumulation of Cd and their effect on solasodine in *Solanum nigrum* L.

MATERIALS AND METHODS:

Set-up of Pot experiment: The soil was collected from an agricultural field of district Jaunpur (25.73°N 82.68°E), UP-India, chemical analysis of the soil showed that organic matter, total N, pH and Cd concentration in this soil were 3.65%, 0.16%, 7.2 and 0.10 mg kg^{-1}, respectively. Surface (0–20 cm) soil samples which were ground to pass through a 4.0mm mesh were used in the pot experiment. The soil samples were air-dried, then mixed with basal fertilizers, at ratios of 100 mgNkg^{-1} dry weight (DW) soil as NH_4NO_3 and 30 mg P kg^{-1} and 80 mg kg^{-1} as K_2HPO_4 according to method [4]. Eleven Cd treatments were applied, namely C (the control) and treatments (Cd concentrations: 10, 20, 30,40,50,60,70,80,90 and 100 mg kg^{-1} DW soil). Each treatment was carried out in six replicates. The tested topsoil samples were mixed thoroughly with $CdCl_2 \cdot 2.5H_2O$ at the above-mentioned concentrations[4], filled into plastic pots (20 cm in diameter, 15 cm in height, 2.5 kg air-dried soil per pot) and equilibrated for 20 days.

Seeds of *S. nigrum* were surface sterilized in 2% (w/v) sodium hypocloride for 3 min, washed several times with sterilized distilled water (SDW), and soaked in SDW overnight. Twenty soaked seeds were sowed directly into the 0.8% agar plate and incubated for three days in dark room. Three similar sizes of sprouted seed were placed in the each treatment pots in the greenhouse with natural light (10–12h; photoperiod) and temperature (20–33^oC). The tested soils were watered to reach 60% of the water-holding capacity and this level was maintained by watering daily throughout the experiment. The plants were harvested after 15, 30, 45 and 60 DAS (days after showing) for further analysis.

Plant growth, Phyto-chemicals, Solasodine and Proline analysis: Root length, shoot length and dry biomass were examined after 15, 30, 45 and 60 DAS of the plant. Qualitative phytochemicals analysis like alkaloid, tannins, flavonoides in crude powder of the *Solanum nigrum* L, was carried out according to the methods earlier described by Trease and Evans [14]. Ten ml of methanol was added in 200 mg of dry plant material and filtered through Whatman filter paper (WFP), 2 ml of filtrate were steamed with 2 ml 1% conc. HCl filtrate and added 5 to 6 drops of Mayer´s reagent/Wagners reagent/ Dragendorffs reagent.

Creamish/brown/ red/orange precipitate indicates the presence of alkaloids. 10 ml SDW were added in 200 mg of dry plant material and filtered through WFP, 2 ml of filtrate were in 2 ml of $FeCl_3$, blue/black precipitate indicate the presence of tannins. 200 mg of dry plant material were dissolved in 10 ml Ethanol and filtered through WFP, conc. HCl and magnesium ribbon were added in 2 ml filtrates, pink/ red color indicate the presence of flavonoids. Solasodin determination was carried out Spectrophoto-metrically at 610 nm

according to Eltayeb [15]. Solasodine contents were expressed as µg/g DW. Proline content (µg mg^{-1} FW) was determined according to Bates [16] in the 0.5 gm fresh weight (FW) of leaf tissue at 520nm.

Antioxidant enzyme activity of the plant: Catalase (CAT; EC 1.11.1.6) and APX activity (EC 1.11.1.11) was analyzed by the method earlier described [13] in fresh leaf tissue (0.5 g) was homogenized with liquid N_2 in QB buffer (100 mm potassium phosphate, pH 7.8, 1 mm EDTA, 1% Triton X-100, 15% glycerol [17]) to assess ascorbic acid peroxidase (APX) and catalase (CAT) activities. Crude homogenate was centrifuged at 15,000 g for 20 min at 4^0C, and the supernatant used for determination of CAT, and APX activity in Umg^{-1} protein. Protein content was determined with the Bradford method using BSA as standard [18].

Plant and soil metal analysis: The plants were immersed in a 0.01M HCl solution to remove any external cadmium and rinsed with deionized (DI) water for 1 min. Subsequently, the plants were separated into three parts: root, stem and leaf. After that, they were dried at 100°C for 10 min, then at 70°C in an oven until completely dry. The plant and soil samples were digested with a solution of 3:1 HNO_3: $HClO_4$ (v/v). The concentrations of Cd were determined using the atomic absorption spectrophotometry [12]. Bioconcentration factor (BF) and translocation factor (TF) of the cadmium was calculated by the method [7, 8, 9, 10]. BF=Metal concentration in plant/Metal concentration in soil and TF=Metal concentration in shoot/Metal concentration in root.

Statistical analysis: The data obtained were subjected to ANOVA, and means were compared with Duncan's multiple range test. All statistical analyses were conducted using SPSS (Version 14; IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION: In the present study, root and shoot growth of *Solanum nigrum* L. in pot experiment decrease with the increasing concentration of cadmium from 50 to 100ppm in pot, while up to 50 ppm growth of plant was unaffected (**Table 1**). Tolerance level of plant was found to be upto 80ppm of Cd treated pot, while at 90 ppm plants were not survive after 30DAS. Plants were not survived at 100ppm in Cd treated pot, even at early DAS. 20 and 28% reduction were found in root length, 11 and 10% shoot length after 30 and 60DAS respectively, over the control at 50ppm of Cd treated pot. Sun and Zhou [19] reported in *solanum nigrum*, no reduction in plant height and shoot dry biomass was noted when the plants were grown at Cd concentration of ≤ 25 mg/kg. While in the present study, dry biomass of the plant was more influenced as compared to the growth under cadmium stress, 33 and 54% of reduction was found after 30DAS and 60DAS respectively over the control at 40ppm (**Table 1**).

Cadmium is an important pollutant with high toxicity towards plants and is expected to negatively affect plant growth[11]. Cadmium decreased water potential and transpiration rate as a consequence of decrease of stomatal conductance and induced inhibition of both photosystems (PS I and PS II), non-cyclic transport of electrons as well as Calvin cycle including activity of Rubisco and PEP-carboxylase [20]. The tolerant mechanisms of Cd tolerant plants have been reported previously[21, 22] with two strategies: exclusion and accumulation [21, 22].

With the accumulation strategy, plants accumulated high amounts of Cd in the tissue, with only a small amount of Cd being stored in the roots and the rest is being all translocated to the shoots. In the present study, alkaloids, tannins and flavonoids were dominant at early days and unaffected up to ≤30ppm Cd treated plant, and was detected up to 80, 70 and 60ppm respectively at both 30 and 60DAS. Gogoi [23] reported alkaloids are act as a defensive mechanism against stress in the plant. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them [24]. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidant cell damage, have strong anticancer activity [25].

In the present study, solasidine content (µg/g DW: dry weight) of the plant was increased and not affected up to 40ppm of Cd treated plant over all DAS (15, 30, 45 and 60) as compared with control (**Fig. 1**). Reduction of solasidine content was found in plant at >40ppm Cd treated plant, and also reduction was observed with increasing DAS at >40ppm Cd treated plant.

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TABLE 1: GROWTH AND QUALITATIVE PHYTOCHEMICAL SCREENING OF *SOLANUM NIGRUM* L. UNDER DIFFERENT CONCENTRATION OF CD AFTER 30 AND 60DAS (DAYS AFTER SHOWING) IN POT- EXPERIMENT

Treatment	Root length (cm)		Shoot Length (cm)		Total Dry biomass (g)		Alkaloid		Tannin		Flavonoids	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
C*	5.3±1.7^{a}	7.3±2.6^{a}	14.2±3.2^{b}	49.3±4.1^{b}	4.60±1.5^{ab}	9.25±3.2^{b}	++++	++++	++	++	++	++
10 ppm	5.2±1.2^{a}	7.2±3.1^{a}	13.7±4.3^{a}	48.0±3.4^{a}	4.10±1.3^{a}	7.12±2.8^{b}	++++	++++	++	++	++	++
20 ppm	5.1±1.6^{a}	7.1±2.8^{a}	13.0±2.7^{a}	47.0±2.8^{a}	3.86±1.3^{ca}	6.26±2.4b^{a}	++++	++++	++	+	++	++
30 ppm	5.0±1.5^{a}	6.9±2.1^{a}	13.0±3.2^{a}	46.2±2.9^{a}	3.21±1.5^{ca}	5.34±2.5^{a}	+++	+++	++	++	++	++
40 ppm	4.3±0.9^{b}	6.1±1.8^{a}	12.6±2.6^{a}	45.6±4.2^{a}	3.06±1.1^{ca}	4.22±2.3^{a}	++	++	+	+	+	+
50 ppm	4.2±1.7^{b}	5.2±1.5^{ab}	12.5±2.7^{a}	45.2±2.8^{ab}	2.52±1.6^{cd}	3.16±2.4^{ca}	++	++	+	+	+	+
60 ppm	3.1±1.2^{bc}	3.7±1.2^{cb}	11.0±2.6^{ab}	31.0±2.5^{ac}	1.55±1.0^{de}	2.18±2.1^{c}	+	++	+	+	+	ND
70 ppm	2.3±1.8^{c}	2.5±1.1^{cde}	10.2±1.8^{cde}	29.0±3.1^{c}	1.43±1.7^{de}	2.05±1.2^{cd}	+	+	+	+	+	ND
80 ppm	1.0±0.5^{cde}	1.0±0.5^{e}	10.0±2.1^{cde}	12.2±1.4^{cd}	1.25±0.8^{de}	1.75±0.7^{d}	+	+	ND	ND	ND	ND
90 ppm	0.6±0.2^{e}	ND	7.2±1.9^{de}	ND	0.67±0.3^{e}	ND	ND	ND	ND	ND	ND	ND
100 ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

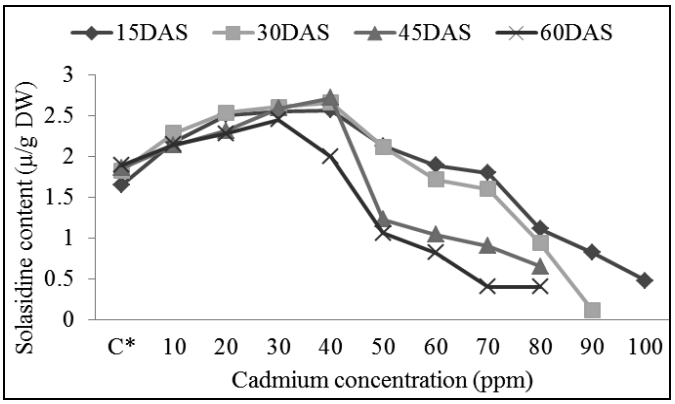
Data are only of 30DAS and 60DAS, data are expressed as mean ± SD. Figures followed by different letters in a same line are significantly different at $P < 0.05$, n= 6. C*= Control (0 ppm cadmium in pot), ++++ = Abundant, +++ = moderately abundant, ++ = moderately presence, + = present and ND=Not detected.

TABLE 2: BIOCONCENTRATION FACTOR AND TRANSLOCATION FACTOR OF CD IN *SOLONUM NIGRUM* AFTER 30 AND 60DAS (DAYS AFTER SHOWING) UNDER DIFFERENT CONCENTRATION (PPM) OF CD TREATMENT IN POT-EXPERIMENT

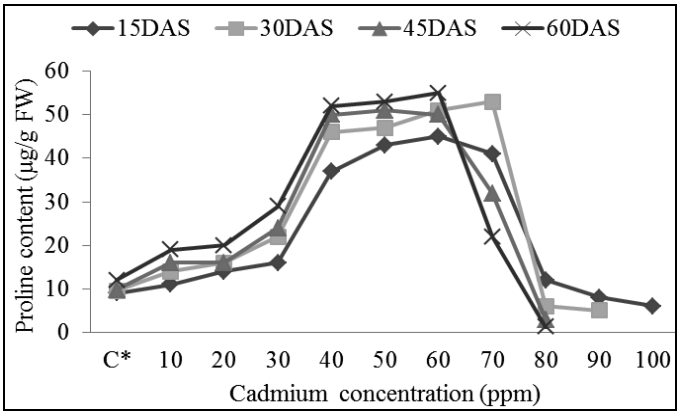
Factor	Cadmium concentration (ppm) in pot											Plant Remark
	C*	10	20	30	40	50	60	70	80	90	100	Hyper- accumulator
BF at 30 DAS	0	2.7	5.35	4.4	6.25	6.68	5.5	6.28	6.05	5.54	0	>1.0
BF at 60 DAS	0	13.4	9.7	9.33	10.17	8.96	7.83	7.88	7.17	0	0	
TF at 30 DAS	0	1.28	1.69	2.14	2.11	1.83	2.06	1.79	1.69	1.92	0	>1.0
TF at 60 DAS	0	1.75	1.77	1.45	1.81	1.58	1.81	2.29	1.98	0	0	

BF= Bioconcentration factor, TF= Translocation factor, C*=Control and DAS=days after showing

Proline contents in leaf (µ/g FW: fresh weight) of the plant was increased with cadmium concentration and DAS, increasing trends was almost 1.5 times of the control up to 30ppm after 15,30,45 and 60DAS, four and five times proline content was increased at 40ppm treated plant after 15 and 45 DAS respectively (**Fig. 1**).



A



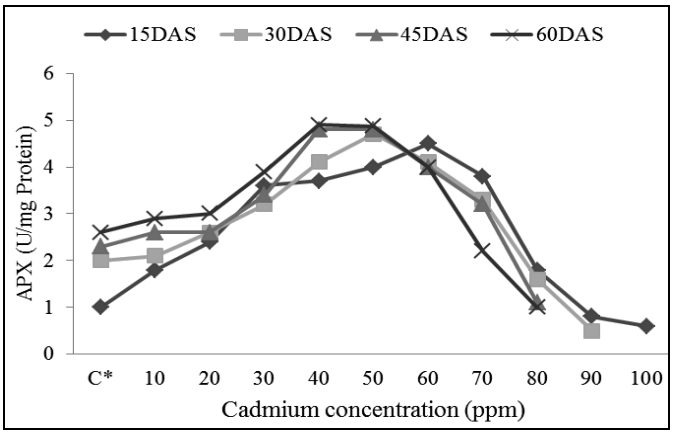
B

FIG. 1: (A) EFFECT ON SOLASIDINE CONTENT (µ/G DW) IN DRY WEIGHT (DW) OF THE PLANT *SOLANUM NIGRUM* L. UNDER DIFFERENT CONCENTRATION OF Cd TREATED POT. (B) PROLINE CONTENT (µG/G FW) IN FRESH WEIGHT (FW) OF PLANT LEAF *SOLANUM NIGRUM* L. UNDER DIFFERENT CONCENTRATION OF Cd TREATED POT. C*=Control, Data are average mean of six replicate after 15, 30, 45 and 60DAS (Days after showing).

Maximum proline content was recorded at 50 to 60ppm Cd treated plant, which was about five to six time more than the control after 15 and 60DAS respectively. The biosynthesis of solasodine starts from acetyl coenzyme (A), later converts to mevalonic acid, via mevalonic acid pathway, in which cholesterol, a key intermediate of solasodine, is synthesized. High salinity seems to enhance in vitro cholesterol and proline production, which in turn increases solasodine in tissues or the enhanced yield, may be due to over expression of genes [26].

Less proline content was recorded at ≤50ppm and ≥50ppm in the Cd treated plant, it might be indicates natural adaptation of plant against Cd at low concentration. Plant proline protect against cadmium toxicity in plant through various mechanism *viz.* functioning as metal chelator, antioxidative properties [27].

Solanum nigrum could naturally survive up to ≤50ppm cadmium treated pot, at that level solasidine content was more and not effected by Cd toxicity, plant cope against Cd toxicity by the production of proline and antioxidant activity. Similar finding was earlier reported [28] that Cd treated plant tissues showed a significant increase in proline as compared to the control samples. APX and CAT activity was increased in fresh leaf of the plant under Cd treated plant up to 40- 50ppm and 50-60ppm respectively, five to seven times of APX and CAT was increased over the control (**Fig. 2**).



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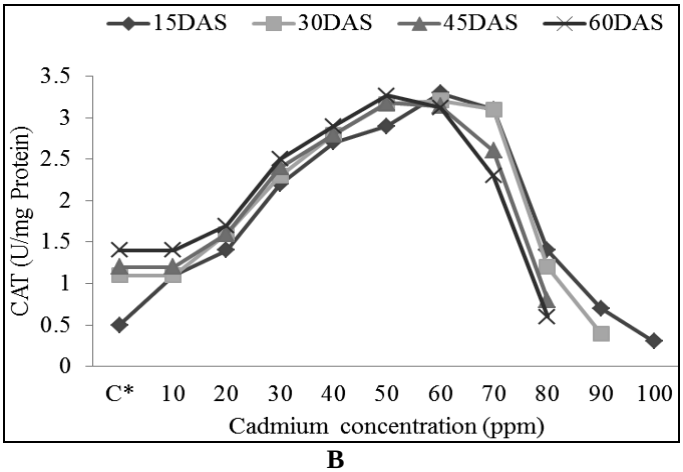

B

FIG. 2: ANTIOXIDANT ENZYME ACTIVITY (U/MG PROTEIN) IN LEAF OF *SOLANUM NIGRUM* L. UNDER DIFFERENT CONCENTRATION OF Cd TREATED PLANT IN POT, C*=CONTROL. Data are average mean of six replicate after 15,30,45 and 60DAS (Days after showing). (a) APX activity and (b) CAT activity.

Maximum APX activity was found at 40ppm after 60DAS, while maximum CAT activity was found at 50ppm after both 15 and 60DAS. APX and CAT both the activity was found at 15DAS at all the Cd treatment, and activity was decrease after 45 or 60DAS at >50ppm and >60ppm Cd treated plant. APX activity was more diverse as compared to CAT activity over the all days. Regulation of antioxidative enzymes can provide plants with an additional protective ability against oxidative stress [12, 13] Under Cd treatment (**Table 2**).

Accordingly, hyper-accumulator should have an effective Cd tolerance strategy related to the expression of antioxidative enzymes under Cd stress [5]. In addition to total metal content, both the bioaccumulation factor (BF) and the translocation factor (TF) need to be considered while evaluating hyper-accumulator. Hyper-accumulating plants are those that have a BF > 1.0 [8]. In the present study, the plant *solonum nigrum* L. showed BF and TF was >1.0. The contents of Cd in the stems increased from 122 to 387 mg/kg with increasing Cd, with the Cd transfer factor and bioaccumulation factor being >1.0. In the pot experiment, plants were almost grown without any effect of Cd toxicity up to 50ppm treated pot. In leaf, shoot and root accumulates 298, 92 and 58 ppm of Cd after 60DAS at 50ppm treated pot, while maximum uptake of cadmium was recorded at 90 ppm treated plant, who survive only up to 30DAS, where in leaf, shoot and root accumulates 352,156 and 81ppm of cadmium. Overall in the plant, maximum cadmium accumulated in leaf followed by shoot and root (**Fig. 3**).

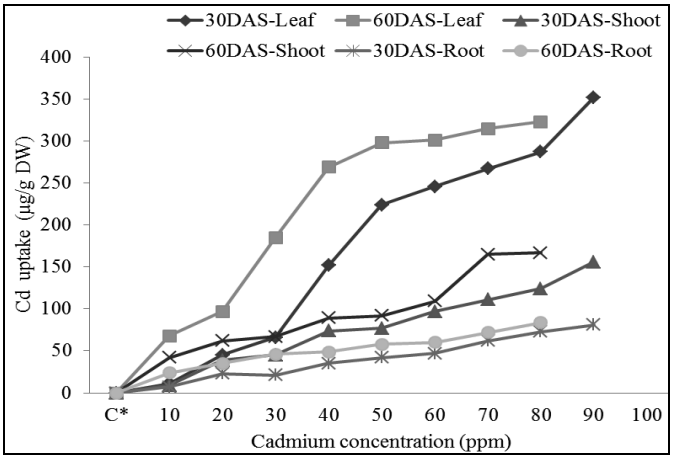

FIG. 3: CADMIUM UPTAKE IN LEAF, SHOOT AND ROOT IN THE PLANT *SOLANUM NIGRUM* L. UNDER DIFFERENT CONCENTRATION OF CD TREATED POT. Data are average mean of six replicate after 30 and 60DAS (Days after showing), C*=Control.

More cadmium accumulation tendency of in the plant leaf was found after 30DAS, while in shoot and root showed no such type of pattern. Therefore, the result indicates that the levels of metals are more than the permissible limit (WHO, 2006)[29] and should not advisable to use plant as a medicinal treatment without metal analysis.

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