Phytotoxicity of Cadmium on Seed germination and Seedling growth of Chick Pea (*Cicer arietinum*)

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Abstract

Heavy metal accumulation in soil has become a worldwide problem, leading to loss of agricultural productivity Cadmium is one of the toxic element of primary importance. Present study aim to evaluate effect of different concentration of Cadmium on seed germination and seedling growth of chick pea (*Cicer arietinum*). Of all the non-essential heavy metals, Cadmium (Cd) perhaps the metal which has attracted the most attention in soil science and plant nutrition due to its potential toxicity to humans and also its relative mobility in the soil plant system. The experiment was performed under the influence of different concentrations of Cadmium. The germination percentage plumule and radical length and number of lateral roots decreased with increase Cadmium concentration (5, 25, 50 and 100 mg/l). Total chlorophyll content declined on 10 day from 1.61 of control to 1.581, 1.573, 1.346 and 1.192 mg/g fresh weight in respective treatments. Similarly on 20 day the decline of total Chlorophyll content was 1.714 control to 1.282, 1.11, 1.07 and 0.96 mg/l fresh weight and on 30 day the decline of total chlorophyll 1.79 of control to 1.10, 0.92, 0.867 and 0.784 mg/g fresh water weight in respective treatments. Likewise fresh weight and moisture contents decreased with increased in Cadmium concentration. The dry weight was increased with increase concentration of Cadmium treatment. The total protein contents were initially decreased on 10 day from 78.96 of control of 62.57, 58.14, 54.15 and 45.84 µg/mg and on 20 days 79.27 to 65.03, 60.13, 51.19 and 44.17 µg/mg and on 30 days 80.13 of control to 67.13, 62.36, 49.71 and 49.79 µg/mg fresh weight of different concentration treatment respectively. Sugar content were significantly decreased all the doses of Cadmium on 10, 20 and 30 days i.e. 5.35 of control to 3.67, 3.09, 3.01 and 2.27 µg/mg in different Cadmium treatments on 10 day and 4.6 of control to 3.51, 2.93, 2.58, 1.86 on 20 days and 4.9 of control to 3.27, 2.79, 2.24 and 1.66 µg/mg on 30 days respectively.

Key words: Cadmium, Heavy metal, *Cicer arietinum*, Phytotoxicity.

Introduction

With the development of industries, mining activities, application of waste water and sewage slug on land phyto-toxicity of heavy metals pollution has serious implications in soil degradation and it may reduce both the quality and efficiency of plants[1]. Although certain metals like Cu, Mn, Fe and Zn are crucial for plants and are used as micronutrients, however at higher concentration they may reveal strong toxicity. Contaminant metals can often accumulate in considerable amounts in the plant tissue and exceed the levels that are toxic to human or animal before may produce visible phytotoxic effects. More Cadmium is being found in soil due to the amendment of chemical fertilizers, sludge and sewage irrigation and atmospheric deposition[2].

Cobalt (Co) is essential for rhizobium which it associates symbiotically with legume roots for N_2 fixation. However it is well known fact that Co at toxic levels inhibits pollen germination, pollen tube growth and inhibits seed germination, causing ultra-structural changes and may cause inhibition in growth of plumale and redicle[3]. The concentration of Cadmium in non-polluted soil solution ranges from 0.04mM to 0.34mM and its concentration in the range of 0.34mM to about 1mM may be categorized as polluted[4].

Present known heavy metals Cd^{+2}, Ni^{+2}, Zn^{+2} are toxic to plants at evaluated levels, were as Cd^{+2} has been observed to cause phyto-toxicity. There are two types of causal relationship existing between the high concentration of heavy metals in the soil and the expression of toxicity symptoms. Heavy metals complete with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plant. On the other hand after the uptake by the plant, it accumulates in plant tissues and cell compartments and hampers the general metabolism of the plant[5].

Accumulation of heavy metals not duly decreased modulation and growth of leguminous plants but also inhibited the growth of micro-organisms present in the soil. More-over they cause metabolic disturbance by altering essential biochemical reactions[6].

Cadmium (Cd) is the element of group-II B in periodic table (A-48). It shows chemical properties similar to the other element of group II B. It has two positive valency and occurs in most of natural aquatic system. The ability of Cd to form complexes with ammonia, amines, halides ions and cyanide indicate similarities with most of the transition metals series ions[7]. It is white lustrous and tamishable relatively volatile element with m.p. 321ºC and b.p. 767ºC. This property makes it susceptible to enter the atmosphere which is a major component of the global Cd cycle. In order to cope with high toxic metals or to maintain the level of essential metals within the physiological range, plants have evolved a variety of complex mechanisms of metals tolerance. Accumulation and detoxification as the main strategies that serve to control the uptake and accumulation to heavy metals[8].

The purpose of this study is to examine the effect of Cadmium on seed germination, growth response of chick pea (*Cicer arietinum*). The possible mechanism of chick pea seedling response to heavy metal stress involving lipid peroxidation and antioxidant changes. Our study will be helpful to identity toxic critical values of Cd based on chick pea response and the changes in bio-chemical parameters; it will provide on reference for eco-toxicity assessment of this heavy metal[8].

Material and Methods

Chick pea (*Cicer arietinum*) seeds were used for the Petri dish experiments. Seeds were surface sterilized with 0.1% $HgCl_2$ for prevention of surface fungal bacterial contamination. The 5, 25, 50 and 100 mg/l Cadmium chloride solution were prepared in pure distilled water in laboratory. After quantification of Cadmium solution as per cent availability in $CdSO_4$, the 5, 25, 50 and 100 mg/l dose of this compound were taken 20 seeds were placed on filter paper in each Petri dish and 10ml solution was used as prepared above. The fresh solutions were applied every alternate day for the prevention of contamination and also for the maintenance of concentration. The nutrient solution were provide once in a week, replacing the Cadmium solution for 20hrs.

The growth parameter like germination, plumule and radical length and number of lateral roots were observed on 10, 20 and 30 day after seedling emergence. The seedlings fresh weight was taken with the help of digital balance and dry weight was measured by placing seedlings at 80±2ºC in hot air oven for 24h till constant weight was recorded. Average the three replicates data are presented in table.

Pigment estimation was done by using the method of Amon as amended by Lichtenthalce. The total protein was estimated by the method of Lowry *et al.*[9], while total sugar determined by the method of Dubais *et al.*[10],

Measurements

Seed germination was recorded daily upto 12 days after the initial day of the experiment. Seeds were considered as germinated when the radical reached a length of 1mm and the germination percentage was calculated as per the following formula:

$$\text{germination percentage} = \frac{\text{No of germinated seeds}}{\text{total number of seeds}} \times 100$$

Root & Shoot Length- It was measured with the help of scale and reading was taken from both treated and control plant.

Leaf area- Leaf area was determined by using standard graph paper method. The leaves outlined and squares covered under the online of leaf were measured under length and width. Average of five leaves was taken per treatment in triplicate for observation.

Root Development- Root development in test plant was determined by counting the number of primary, secondary and tertiary roots.

Biomass production- It was obtained by taking out the plants from the pot, washed under running water, dried on the blotting sheets and weighted. They were dried in hot air over at 60ºC till their weight become constant.

Statistical analysis- Data are expressed on mean ± standard error. Student's t-test was used to dated difference between treatments at 0.05 level of probability.

Results & Discussion

The results obtained in petri dish culture experiment are shown in Table 1 to 4. The results after 10, 20, 30 day of aqueous exposure of Cadmium to (*Cicer arietinum*), showed considerable reduction in seed germination, plumule elongation, radicle elongation and number of lateral roots. An overall inhibition was observed in seed germination and seedling growth as compared to control with increasing Cd concentration. Number of lateral roots increased in 5 mg/l and 25 mg/l of copper treatment and then decreased in 50 and 100 mg/l Cd treatment. The fresh weight, dry weight and moisture contents (biomass production in seedling) slightly decreased with increasing copper concentration on 10 day. But on 30 day dry weight was decreased in 5 mg/l Cd concentration, but it was equal to control in 25 mg/l. Increase was observed in 50 mg/l and 100 mg/l of Cd concentration.

Table-1: Summary of the major anthropogenic inputs of cadmium to soils

Source of cadmium	Concentration in soil (mg/kg)	Input to soil (g $ha^{-1}year^{-1}$)
Wet/Dry deposition, general	-	< 1.10-9.0
Wet/Dry deposition, smelters	-	25.0-1000
Street dust	1.5-13.0	-
Rubber tyre wear	25-90	-
Incenerator fly-ash	3-68	-
Direct application Phosphate fertilizers	0.2-3615 kg/P	0.25-10
By product gypsum	< 6.5	-
Sewage sludge	< 1.0-3620	upto 150
Compost	0.28-12.5	-

Table-2: Effect of Cadmium on seedling growth in *Cicer arietinum*

Day	Observation	Treatment				
		Control	5 mg/l Cd	25 mg/l Cd	50 mg/l Cd	100 mg/l Cd
10	Plumule length (cm)	9.8±0.15	8.30±0.24	6.63±0.29	5.71±0.23	4.84±0.16
	Radicle length (cm)	9.2±0.21	2.41±0.21	1.26±0.06	1.03±0.06	0.64±0.01
	Lateral roots (No.)	6.30±0.12	7.31±0.22	7.65±0.05	5.54±0.12	5.11±0.16
	Fresh weight (g)	0.15±0.002	0.121±0.004	0.121±0.001	0.112±0.002	0.016±0.004
	Dry weight (g)	0.017±0.001	0.0161±0.001	0.0152±0.003	0.0147±0.001	0.0138±0.001
20	Plumule length (cm)	17.04±0.50	14.25±0.50	10.30±0.91	8.71±0.24	7.20±0.22
	Radicle length (cm)	11.16±1.01	3.07±0.10	1.52±0.12	1.28±0.11	0.81±0.01
	Lateral roots (No.)	7.34±0.41	3.80±0.43	7.81±0.52	5.61±0.12	5.32±0.22
	Fresh weight (g)	0.1685±0.002	0.1321±0.002	0.1260±0.006	0.1230±0.007	0.0121±0.002

	Dry weight (g)	0.0192±0.001	0.0180±0.001	0.0191±0.001	0.0196±0.002	0.0204±0.001
30	Plumule length (cm)	26.01±0.52	21.21±0.56	13.91±0.38	11.21±0.22	9.68±0.27
	Radicle length (cm)	13.26±1.03	4.14±0.31	1.82±0.03	1.48±0.12	0.99±0.02
	Lateral roots (No.)	8.72±0.62	2.92±0.34	7.97±0.32	5.69±0.14	5.46±0.26
	Fresh weight (g)	0.1791±0.002	0.1412±0.002	0.1281±0.001	0.1310±0.006	0.0117±0.002
	Dry weight (g)	0.0216±0.001	0.0206±0.001	0.0221±0.002	0.0216±0.002	0.0261±0.002

The various treatments of Cd were found to be toxic at sub optimal (5 mg/l) to supra optimal (25-100 mg/l), concentrations. A significant reducing effect was found in chick pea germination percent, plumule length, radicle length, number of lateral roots, fresh weight, dry weight and moisture with increased Cd concentration. A slight increase was observed in germination percentage in 5 mg/l of Cd concentration. A slight increase was observed in germination percentage in 5 mg/l of Cd concentration. At the supra optimal Cd level (i.e., 100 mg/l, Cd) only 60% seed germination was observed (Table-2). Allaway (1968) reported normal plants to contain Cd higher than 5 to 20 µg/g dry weight. Saravanan *et al*., 2001 observed that supra optimal concentration of Cd drastically inhibited seed germination, growth, biomass and yield soybean crop. Cd was found accumulated in the roots, with restricted transport to foliage in English Oak Quercus roubur seedlings.

Table-3: Effect of Cadmium on Chlorophyll (Total, a & b) and a/b ratio in *Cicer arietinum*

Day	**Observation**	**Treatment**				
		Control	**5 mg/l Cd**	**25 mg/l Cd**	**50 mg/l Cd**	**100 mg/l Cd**
10	Chlorophyll a (mg/gm)	0.92±0.011	0.941±0.003	0.821±0.002	0.767±0.03	0.642±0.021
	Chlorophyll b (mg/gm)	0.55±0.01	0.574±0.008	0.513±0.004	0.489±0.029	0.430±0.017
	Total Chl. (mg/gm)	1.61±0.029	1.581±0.014	1.573±0.015	1.346±0.06	1.192±0.031
	Chl. a/b Ratio	1.66	1.64	1.60	1.568	1.49
20	Chlorophyll a (mg/gm)	0.958±0.021	0.71±0.006	0.569±0.022	0.553±0.003	0.503±0.001
	Chlorophyll b (mg/gm)	0.596±0.012	0.467±0.003	0.448±0.012	0.364±0.005	0.364±0.004
	Total Chl. (mg/gm)	1.714±0.011	1.282±0.008	1.113±0.010	1.073±0.024	0.963±0.003
	Chl. a/b Ratio	1.61	1.52	1.26	1.52	1.38
30	Chlorophyll a (mg/gm)	0.986±0.022	0.562±0.004	0.348±0.011	0.372±0.032	0.391±0.002
	Chlorophyll b (mg/gm)	0.624±0.011	0.386±0.002	0.369±0.012	0.254±0.021	0.286±0.003
	Total Chl. (mg/gm)	1.79±0.012	1.10±0.007	0.921±0.011	0.867±0.021	0.784±0.002
	Chl. a/b Ratio	1.58	1.45	0.94	1.46	1.36

Prolonged exposure of Cd caused gross perturbations of root morphology, and finally reduced root and shoot growth[11]. Cd was relatively more harmful than nickel to both seed germination and seedling of *Raphanus sativus* L. var. Pusa chetki[12,13]. The similar effect of Cd

containing textile, dye and printing industry effluent on germination and growth performance of two Rabi crops namely, wheat and chickpea was studied. Dragun and Baker (1982)[14] concluded that Cd concentrations in the above ground parts of maize are not suitable *C. arietinum* indicator of Cd toxicity. The sensitivity of to the toxicity of the Cd pollutants was in the order of root elongation > shoot elongation > germination rate[15].

Noteworthy decline in pigments with increased Cd exposure was noted in total chlorophyll, chlorophyll a/b ratio (Table-3) and pheophytin contents (total, 'a' and 'b' pheophytin). In leaf tissue significant decline was evident in protein and sugar content in each treatment. Protein and sugar were appreciably reduced at the higher concentration of Cd exposure (Table-4).

Table-4: Effect of Cadmium on protein and sugar content in *Cicer arietinum*

Treatment	10 Days		20 Days		30 Days	
	Protein	**Sugar**	**Protein**	**Sugar**	**Protein**	**Sugar**
Control	78.96±1.40	5.35±0.14	79.27±1.12	4.6±0.04	80.13±0.14	4.09±0.94
5 mg/l (Cd)	62.57±3.24	3.67±0.14	65.03±2.12	3.51±0.12	67.13±2.16	3.27±0.21
25 mg/l (Cd)	58.14±1.23	3.09±0.36	60.13±13	2.93±0.75	62.36±0.63	2.79±0.63
50 mg/l (Cd)	54.15±1.10	3.01±0.04	51.91±4.10	2.58±0.13	49.71±3.60	2.24±0.31
100 mg/l (Cd)	45.84±0.76	2.27±0.10	44.17±4.03	1.86±0.06	42.79±0.12	1.66±0.18

*Protein and sugar content in µg/mg fresh weight tissue. The average value of three replicates ±SE

The poor germination rate and seedling growth in present study seems to be due to the poor break down of starch by low amylase activity as amylase activity in seeds under the influence of different concentrations of Cd solution was found to be decreased in comparison to control.

Our results regarding the inhibition of seed germination and seedling growth in Cd treatments can be correlated to the decreased amylase activity as worked out[16]. Amylase and its role during seed germination through hydrolysis of reserve starch and release of energy have been extensively worked out by Chang,[17] on the role of amylase correlation with the increased seed germination is due to more hydrolysis of starch and release of energy in different test plants. It is well known that catalase and peroxidase play an important role in preventing oxidative stress by catalyzing the reduction of hydrogen peroxide. Devi and Prasad[18] found that catalase and peroxidase activities were increased on copper treatment, suggesting that excess Cd may increase the production of hydrogen (H_2O_2). H_2O_2 is a necessary substrate for the cell wall stiffening process catalyzed by cell wall peroxidase[19,20] which is considered to be one of the mechanisms resulting in growth inhibition. Catalase is an enzyme involved in antioxidant defense that eliminates hydrogen peroxide.

The supra optimal Cd concentration in leaf tissue significantly declined the total of protein and sugars contents in each treatment in all over 10, 20, 30 days of treatment. Protein and sugar were significantly reduced at increased doses of heavy metals. Metal induced inhibition of protein synthesis was also earlier reported by Samantary[3]. The decrease in protein content in *Albizia lebbak* has been interpreted either due to reduced de novo synthesis of proteins or increased decomposition of proteins into amino acids[21].

Presently, increased industrialization in developing countries like India has resulted into enormous deterioration of air, water and soil[22,23]. Liquid effluents are frequently released in water bodies contains a number of pollutants including metals[24]. The agricultural crops irrigated with such water are largely affected by metal Cd toxicity[25,26]. The Cd at lower concentration < 5 mg/l works as nutrient in plants but when its concentration increases in water and soil it causes Cd toxicity[27]. The various parameters like germination, pigment contents, protein and sugar contents were decreased at high concentration[28]. While antioxidants like catalase and peroxidase were increased. Ultimately the crop production is decreased and affects food production and quality of *Cicer arietinum*[29,30].

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