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Cadmium chloride induced alteration in growth and cadmium accumulation in *Triticum aestivum* (l.) var. MP LOK 1

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ABSTRACT

Cadmium is a non-essential heavy metal that can be harmful even at low concentrations in organisms. The present investigation was carried out to determine the extent of deleterious effects of cadmium chloride and tolerance level of wheat with reference to growth and accumulation of cadmium at varied levels of cadmium chloride by Triticum aestivum var. MP LOK1. The plants were treated with increasing concentrations of cadmium chloride i.e. 25, 50 and 75 ppm. Wet and dry biomass of the shoots and roots was decreased linearly with increase in concentrations of cadmium chloride to the rooting medium. It was also observed that cadmium chloride had a clear-cut negative effect on the shoot length of T. aestivum showing perfect negative correlation with the increasing concentration of cadmium chloride to the rooting medium. Similarly, reduction in root length was negatively correlated with the increasing cadmium concentration and might be due to the toxicity of cadmium. It was also accumulated in increasing level in root and shoot parts of wheat seedlings and might be associated with the low molecular weight organic acids secreted by the roots. Linear decrease in root bioconcentration (BCF) might be related with increased linear accumulation by the roots of experimental species while slight linear increase in BCF with respect to shoots of T. aestivum shows lesser transport of Cd^{++} from the soil medium to the shoot parts as compared to roots. The bioconcentration factor above one indicated that the accumulation of Cd^{++} in the root and shoot parts of T. aestivum was more than the suggested values.

Keywords: Cadmium chloride, seed germination, root length, shoot length, bioconcentration factor.

INTRODUCTION

Environmental pollution is one of the most significant problems that the world faces today. There is an increasing body of literature on plant responses to heavy metals, due to the intensified awareness of and concern over pollution of the environment and the increase of population of the world [1]. Mining and smelting of metalliferous ores, use of pesticides, fertilizers, increased liquid and solid waste disposals, landfill leachates, fossil fuel burning, paints, batteries, industrial wastes and land application of industrial or domestic sludge can result in heavy metal contamination of urban and agricultural soils [2]. The threat that heavy metals pose to human and environmental health is aggravated by their long-term persistence in the environment [3]. Additional sources of heavy metals that can cause harm to plants are surface runoff, traffic density, use of oil or fossil fuels for heating, atmospheric dusts, plant protection agents and fertilizers, which could be adsorbed through the leaf blades [4]. Although many metals are essential for cells, all of them are toxic at higher concentrations [1].

One reason, metals may become toxic is that they may cause oxidative stress, especially redox active transition metals, which can take up or give off an electron can give rise to free radicals that cause damage [5]. Another reason why metals may be toxic is that they can replace other essential metals in pigments or enzymes, disrupting the function of these molecules [6]. Metal toxicity is an important factor governing germination and growth of plants. Plants represent the feeding resources for man and most of animals and their contamination with different pollutants determine the propagation of their elements along the tropic chain [7]. For this reason studying the accumulation of different toxic elements of plants is very important, measures being necessary to face out the destruction of the terrestrial and aquatic ecosystems [8]. Test on terrestrial plants has been recognized as an issue of high priority by many governmental agencies around the world [9]. The test methods related with phytotoxicity should be enhanced in assessing the impacts of chemicals on terrestrial ecosystem because vegetation is a functional component of terrestrial ecosystem and crop also serve as an important pathway for human exposure to toxic elements [10]. The problem of farmland heavy metal contamination has raised serious concerns [7]. Heavy metals are absorbed and accumulated by plants thus are absorbed directly or indirectly by human bodies through the food [11].

Cadmium is a highly toxic biologically non-essential heavy metal which can be absorbed readily by crops and accumulated in the human body through the food chain [12]. It is a relatively rare element (0.2 mg kg⁻¹ in the earth crust) and is not found in the pure state in the nature. It occurs mainly in association with the sulphide ores of zinc, lead and copper. Cadmium is particularly a dangerous pollutant due to its high toxicity and great solubility in water [13]. It has anthropogenic effects in activities such as the non-ferrous metal industry, mining, the production, use and disposal of batteries and disposal of metal-contaminated waste [2]. In many respects cadmium has become a vital component of modern technology, with countless applications in the electronics, communications, power generation and aerospace industries [14]. Cadmium toxicity is an important growth limiting factor for plants [15].

The present investigation was carried out to determine the extent of deleterious effects of cadmium chloride and tolerance level of wheat with reference to growth and accumulation of cadmium at varied levels of cadmium chloride by *Triticum aestivum* (L.) var. MP LOK1. The results showed that the cadmium chloride showed drastic effect on the growth of *T. aestivum* and accumulated over a toxic range. The details of the results obtained on root length, shoot length, weight and dry biomass production and accumulation are discussed in the present paper.

EXPERIMENTAL SECTION

2.1 Materials:

The seeds of the *Triticum aestivum* var. MP LOK1 were collected from the market-yard of Pune city. A total of two hundred seeds were sown in earthen pots and allowed to grow naturally for five days and then the cadmium chloride (CdCl₂) treatment was commenced from 6^{th} day to 12^{th} day. The plants were treated with increasing concentrations of cadmium chloride i.e. 25, 50 and 75 ppm. Three different sets of the pots having diameter 15 cm and height 20 cm were used in the present study along with a control set to find out the statistical variation in growth and accumulation of metal in roots and shoots. Every day, they were watered with two litres of cadmium chloride solution to maintain the uniform salt concentration as well as sufficient moisture in the pot soil. This was also done to cope up with the loss of water by evaporation from the soil surface and by transpiration from the plant surface. The metal salt solution was prepared in tap water and twice a day, in the morning and evening was applied to the rooting medium. Fertile soil collected from the agriculture field of Khadkwasala, Pune was used in the present investigation.

2.2. Methods:

2.2.1 Study of the growth characteristics of T. aestivum:

Ten plants of *T. aestivum* from each treatment pot were carefully uprooted and washed thoroughly with water to remove any dirt, metal ions and dust particles on the surface of the plant parts and blotted to surface dry. On each day harvest both the shoots and roots were rinsed in tap water and then deionized water to remove traces of nutrients and Cd ions from the root surface. The growth parameters namely shoot length and root length were studied at 9th, 10th, 11th and 12th day while wet and dry biomass was studied after the completion of treatments. Plants were cut into root and shoot for the measurement of root length and shoot length respectively with the help of plastic scale. At the end of experiment on 12^{th} day, the seedlings with soil bulk were allowed to stay for two hours in the plastic trays full of water and the soil attached to the adventitious roots of wheat was separated with special care. Biomass of the roots and shoots was measured by using Sartorius LA8200S digital weight balance on the basis of wet weight and dry weight. For dry biomass measurement the roots and shoots dried in a forced-air oven for 2 days at 50°C, followed by 3 days at 80°C and overnight at 105°C.

2.2.2 Estimation of cadmium from soil and parts of T. aestivum:

The powdered form of the roots and shoots were used to determine the cadmium (Cd^{++}) concentration. The metal concentration was also estimated from the oven dried treated residual soil of the pots. Residual soil is the soil having cadmium concentration which remained in the treated pots after accumulation by roots and shoots of the total liquid solution poured to the rooting medium. The cadmium content of the poured solution removed from the soil by drainage under the influence of gravity do not comes into the residual soil sample. Residual soil sample was the sample. 0.5 g oven dried powdered form of the sample was acid digested following the standard method by Toth *et al.* [16]. Plant material was taken in a 150 ml clean beaker and to that 10 ml concentrated nitric acid was added. It was covered with a watch glass and kept for an hour till the primary reactions subsided. It was then heated on a hot plate until all the material was completely dissolved. It was allowed to cool to room temperature and then 10 ml of perchloric acid (60%) was added to it and mixed thoroughly. It was then heated strongly on a hot

plate until the solution became colourless and reduced to about 2-3 ml. While heating, the solution was not allowed to dry. After cooling, it was transferred quantitatively to 100 ml capacity volumetric flask, diluted to 100 ml with distilled water and kept overnight. Next day it was filtered through Whatman number 44 filter paper. The filtrate was stored properly and was analyzed for the estimation of cadmium using Perkin-Elmer, 3030-A Atomic Absorption Spectrophotometer.

2.2.3 Determination of bioconcentration factor (BCF):

The bioconcentration factor (BCF) provides an index of the ability of the plant to accumulate the metal with respect to the metal concentration in the substrate i.e. how much in the organism over the how much in the environment. In the present study, the bioconcentration factor (BCF) was calculated as the ratio of Cd^{++} concentration in the plant tissues at harvest (ppm) to the concentration of the element in the external residual environment (ppm) [17]. Following formula was used in the present study.

 $Bioconcentration factor = \frac{Trace element concentration in plant tissue (ppm dry weight)}{Trace element concentration in plant tissue (ppm dry weight)}$

Residual concentration of the metal in soil (ppm dry weight)

The bioconcentration factor is dimensionless. A larger ratio implies better phytoaccumulation capability by the plant. The plant is said to be of high potential if BCF is greater than 1000 and moderate potential if BCF is in the range of 250-1000 while plant is said to be of low potential if the ratio is below 250.

2.2.4 Statistical analysis:

Statistical analysis of the data was carried out by using GraphPad software. Mean, standard deviation and percent variation was calculated. 'One Way Analysis of Variance' (ANOVA) was tested for the parameters in order to see the statistical difference among the means of control values and means treatment values. Tukey-Kramer multiple comparison test of significance was carried out which suggested the variation among the column means is significant or not at different levels of significance. The data was analysed for three different levels of significance based on the 'p' values as * significant (p = 0.01 to 0.05), ** very significant (p = 0.001 to 0.01) and *** extremely significant (p < 0.001).

RESULTS AND DISCUSSION

3.1 Growth characteristics:

3.1.1 Effect of cadmium chloride on biomass of T. aestivum:

Growth is the best indices for evaluating plant response to environmental stress [7]. Bhardwaj [18] reported the reduction in total biomass of *Phaseolus vulgaris* when grown at 1.5, 2.0, 2.5 g Cd kg⁻¹ of soil. They observed that the seedling biomass on fresh weight and dry weight basis declined proportionately with the increasing concentrations of cadmium into the rooting medium. Fresh weight of *P. vulgaris* seedlings decreased by 54.09, 62.07, 82.76% while dry weight by 75.56, 78.89, 81.11% respectively. Oancea [1] also observed the reduction in biomass of tomato due to cadmium chloride treatment. Similar observations have been observed in *T. sativum* [19] and *Lens esculanta* [20]. Muramoto et al. [21] measured the effects of Cd as CdO on wheat and rice grown from seed to maturity to an alluvial soil and reported the reduction in root and shoot biomass.

Cadmium	ium Wet biomass g		Dry bio	mass a
chloride (ppm)	Root	Shoot	Root	Shoot
	14.46	32.56	4.31	4.35
Control	(±1.96)	(±3.97)	(±0.29)	(±0.31)
	100.0 ^x	100.0 ^x	100.0 ^x	100.0 [×]
	13.25 °	28.82	3.82 °	4.05 ^b
25	(±1.67)	(±3.82)	(±0.23)	(±0.26)
	91.63	94.31	88.63	93.09
	12.26 ^a	23.52 ^a	3.74	3.81 ^a
50	(±1.51)	(±3.07)	(±0.21)	(±0.23)
	84.79	76.96	86.77	87.55
	8.91 °	21.54 ^b	3.32 ^a	3.74 °
75	(±1.05)	(±2.77)	(±0.16)	(±0.20)
	61.62	70.48	77.03	85.96

Table 1 Effect of cadmium chloride on wet and dry biomass of T. aestivum

^x The values indicates percentage variation relative to control values. Values are mean of ten determinations with ±SD. Significantly different from the control at ^a P<0.05, ^b P<0.01 and ^c p<0.001 by one-way ANOVA with Tukey-Kramer multiple comparison test. Each value is expressed in g.

In the present investigation root biomass was observed to be decreased linearly from control to 75 ppm of cadmium chloride in the pot soil culture (Table 1). Higher wet biomass of the roots was observed in control set seedlings growing without cadmium chloride as 14.46 g while the minimum wet biomass was observed as 8.91g in the plants treated with 75 ppm of cadmium chloride. Dry biomass of the wheat seedlings was 4.31 g in control sets and was lowest in the pots treated with 75 ppm of cadmium chloride by 3.32 g. It was 77.03% to that of control in the plants treated with 75 ppm of metal salt. Shoot biomass on fresh wet and dry weight basis showed similar linear reduction like that of root biomass. Wet biomass of the shoots was 32.56 g in the pot grown in control sets which decreased to 21.54 g of the seedlings grown in 75 ppm of cadmium chloride. It was 4.35 g in control set and 3.74 g in the plant of 75 ppm cadmium chloride. Our results are in accordance with the results obtained by Bhardwaj [18] and Alsokari [22] and such a decrease in wet and dry biomass of *T. aestivum* might be related with the toxicity of cadmium chloride which limits the normal physiological mechanism because of its toxicity and thereby the biomass.

3.1.2 Effects of cadmium chloride on shoot length of T. aestivum:

Geuns *et al.* [23] suggests that based on changes occurring in growth and physiological attributes, mungbean can be regarded as bioindicator of Cd toxicity. Decrease in shoot length due to the application of cadmium was studied in *Phaseolus vulgaris* [24, 18], *T. aestivum* [25] and in alfalfa by about 16.0% as compared with shoot size of the control group [26]. Muramoto et al. [21] measured the effects of Cd as CdO on wheat and rice grown from seed to maturity to an alluvial soil where they observed the reduction shoot length with increasing concentration of cadmium to the soil medium. In the present investigation shoot length of experimental species was recorded on 9th, 10th, 11th and 12th day (Table 2). It is clear from the result that cadmium chloride had a clear-cut negative effect on the shoot length of *T. aestivum* showing perfect negative correlation with the increasing concentration of cadmium chloride to the rooting medium. It was 15.65 cm on 12^{th} day in the control sets while at the same day the shoot length was observed to be decreased by 9.48 cm in the pot culture treated with 75 ppm of cadmium

chloride. Ouzounidou et al. [19] had also studied the effect of cadmium in nutrient solution on shoot leaf length of wheat where the lowest concentration tested, 29.8 ppm, resulted in a 40% decrease in shoot-leaf length. In the present study reduction in shoot length of the wheat might be due to the impediment in normal physiological changes caused by cadmium and replacement of essential nutrients by the same.

Cadmium	Shoot length in cm			
chloride (ppm)	9 th day	10 th day	11 th day	12 th day
	8.13	10.10	12.61	15.65
Control	(±1.02)	(±1.23)	(±1.59)	(±1.92)
	100.0^{X}	100.0 ^x	100.0 ^x	100.0 ^X
	5.77 ^a	7.62 °	10.26	13.29 ^b
25	(±0.73)	(±0.95)	(±1.31)	(±1.82)
	70.99	75.38	81.32	84.94
	4.85 ^b	6.03 ^b	8.02 °	12.77 ^c
50	(±0.60)	(±0.75)	(±0.98)	(±1.59)
	59.68	59.72	63.57	81.60
	4.60 [°]	5.52°	7.50 ^a	9.48 ^c
75	(±0.54)	(±0.65)	(± 0.88)	(±1.05)
	56.57	54.63	59.47	60.58

Table 2 Effect of cadmium chloride on shoot length of T. aestivum

^x The values indicates percentage variation relative to control values. Values are mean of ten determinations with \pm SD. Significantly different from the control at ^a P<0.05, ^b P<0.01 and ^c p < 0.001 by one-way ANOVA with Tukey-Kramer multiple comparison test. Each value is expressed in cm.

3.1.3 Effects of cadmium chloride on root length of T. aestivum:

Generally, the results of previous studies show that the cadmium affects root growth more severely than shoot growth [27]. Mihalescu et al. [28] were of the opinion that the reduction in root length and height of the plant was due to cadmium accumulation in *Zea mays*.

Table 3 Effect of cadmium chloride on root length of T. aestivum				
Cadmium chloride	Root length cm			
(ppm)	9 th day	10 th day	11 th day	12 th day
	5.52	6.71	9.87	11.19
Control	(±0.70)	(±0.82)	(±1.26)	(±1.36)
	100.0^{X}	100.0^{X}	100.0 ^x	100.0 ^X
	4.20 ^a	5.38°	8.16 ^b	9.48 ^c
25	(±0.53)	(±0.67)	(±1.05	(±1.31)
	76.06	80.30	82.61	84.66
	3.35 °	4.54 ^b	7.05 °	8.76 °
50	(±0.42)	(±0.56)	(±0.88	(±1.07)
	60.76	67.71	71.39	78.30
	3.09 ^c	4.14 °	5.46 °	6.92
75	(±0.36)	(±0.49)	(±0.65	(±0.77)
	55.97	61.80	55.34	61.79

^x The values indicates percentage variation relative to control values. Values are mean of ten determinations with \pm SD. Significantly different from the control at ^a P<0.05, ^b P<0.01 and ^c p < 0.001 by one-way ANOVA with Tukey-Kramer multiple comparison test. Each value is expressed in cm.

They further concluded that such a decrease was directly proportional with the increase of metal concentration in the plant. The reduction in root length under the influence of cadmium was

recorded in tomato [1], *Phaseolus vulgaris* [18], *Solanum melongena* [29] and alfalfa [26]. Cosio et al. [30] reported that at high cadmium concentrations (50 μ M and 100 μ M Cd) roots clearly suffered the effects of Cd toxicity more than shoots. De Knecht et al. [31] found that Cd tolerant plants exhibited a higher Cd root:shoot ratio than sensitive plants of *Silene vulgaris*. Muramoto et al. [21] also measured the effects of Cd as CdO on wheat and rice grown in an alluvial soil from seed to maturity where they observed significant reduction in root length of wheat. Ouzounidou et al. [19] studied the effect of cadmium in nutrient solution on root and shoot leaf length of wheat where the lowest concentration tested (29.8 ppm) resulted in a 53% decrease in root length.

Oxidative stress is the main causes of cellular damage in all organisms exposed to a wide variety of stress conditions [7]. Root morphological changes under varied environmental conditions bring direct influences to the plant population structure, above ground parts and their biomass composition [13]. In the present investigation the root length decreased significantly with increasing concentration of cadmium when compared with control set plants and showed a perfect negative correlation with increased salt treatment. On 9th and 12th day root length was 5.52 and 11.19 cm respectively in the control sets while it was 3.09 and 6.92 cm on 9th and 12th day respectively in the pots treated with 75 ppm of the metal salt (Table 3). The decrease at elevated levels of cadmium chloride might be due to the toxic nature of cadmium chloride. It seems likely that the effect of Cd on shoot and root growth varies depending on the experimental conditions [7]. Our results are in accordance with the results obtained by Ghan [32]; Rout et al. [33] and Chaoui et al. [24] with respect to significant reduction in shoot and root length of the plants under the influence of cadmium in the rooting medium.

3.1.4 Accumulation of cadmium and bioconcentration factor:

Cadmium accumulations in residual soil, root and shoot was investigated in order to see a tolerance level by *T. aestivum* (Table 4). There are two aspects on the interaction of plants and chemical compounds in which chemical compounds show negative effects on plants and plants have their own resistance mechanisms against toxic effects [34]. Bioaccumulation of toxic heavy metals by various crop plants has been reported by number of workers and is a matter of serious health hazard [35]. Plants can withstand heavy metal accumulation until the metal reaches the toxicity threshold in the tissue [36].

Cadmium	Cd ⁺⁺ content (mg kg ⁻¹)			Cd ⁺⁺ content (mg kg ⁻¹) BCF	
Chloride (ppm)	Residual soil	Root	Shoot	Root	Shoot
Control	Ab	Ab	Ab	NA	NA
25	4.21	12.56	5.23	2.98	1.24
	(±0.12)	(±0.26)	(±0.16)	(±0.19)	(±0.24)
50	6.35	15.67	8.19	2.47	1.29
	(±0.18)	(±0.48)	(±0.25)	(±0.14)	(±0.20)
75	8.96	19.82	13.47	2.21	1.50
	(±0.24)	(±0.42)	(±0.12)	(±0.17)	(±0.13)

Table 4 Accumulation of cadmium in the leaves of T	<i>F. aestivum</i> at various treatments and bioconcentration
fa	actor

Ab: Absent NA: Not Applicable BCF: Bioconcentration Factor

^{*X*} The values indicates percentage variation relative to control values. Values are mean of three determinations with $\pm SD$. Each value is expressed in mg kg⁻¹.

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In agricultural soils, cadmium pollution is an increasing problem due to soil amendment and intense use of phosphate fertilizers that contain Cd^{++} alongwith other heavy metals [5]. Both accumulation and distribution of Cd^{++} in the plant differs depending on the species, cultivar and growing conditions [36]. High levels of Cd^{++} in crops are a concern in human diets [34] particularly in cereal grains that represent large portion of human food [37]. Indeed, the major part of Cd^{++} reaching the human body derives from wheat [38]. Threshold levels of cadmium for the optimum plant growth are 0.2 to 9.0 and 1.5 mg kg⁻¹ in hydroponic medium and soil respectively [39].

Present study showed the absence of Cd^{++} in control, soil and root parts, as it was obvious that there were no any external source of cadmium in the control medium. Bioconcentration factor was calculated with respect to root and shoot. Cadmium accumulated in the treatment pots of residual soil, roots and shoots with the linear trend and showed a perfect positive correlation with the increased cadmium chloride to the pot soil culture. Roots as well as shoots of *T. aestivum* exceeded the normal metal level that it can accumulate and might be the main cause for the drastic effect of metal salt on the selected growth characteristics of plants. In the pots treated with 25 ppm of cadmium chloride, residual soil was with 4.21 mg kg⁻¹ cadmium while it was 8.96 mg kg⁻¹ at 75 ppm salt level. It was clearly observed that roots accumulated higher cadmium content than shoot parts with highest 19.82 mg kg⁻¹ of cadmium at 75 ppm of cadmium chloride while at the same level of salt, shoots were with 13.47 mg kg⁻¹ of cadmium. Bioconcentration factor (BCF) greater than one indicates that metal is accumulated in tree relative to the soil or water. Bioconcentration factor was decreased linearly with respects to roots while increased linearly with respect increased concentration of cadmium salt.

The accumulation of Cd^{++} in leaf at high level of soil contamination is noteworthy and leaf thichomes appear to provide a site for the sequestration of Cd^{++} [40]. Stolt et al. [41] suggested that the larger accumulation of Cd^{++} in the grain of durum wheat as compared to bread wheat was associated with a higher total uptake by the plant. Secretion of low molecular weight organic acids may influence root uptake of Cd^{++} [13]. Accumulation of large amounts of Cd^{++} in the root may limit the accumulation of Cd^{++} in edible above-ground portions of the plant [42] and in general, a high amount of Cd^{++} in plants reduces the growth in roots and shoots, and causes leaf rolling and chlorosis [43]. Taken up by roots, Cd^{++} can induce deficiencies and imbalances of mineral nutrients [44]. Thus, from the above discussion it is clear that accumulation of Cd^{++} in shoots and roots of wheat might be associated with the low molecular weight organic acids secreted by the roots. Linear decrease in root BCF might be related with the linear accumulation by the roots of experimental species while slight linear increase in BCF with respect to shoots of *T. aestivum* shows lesser transport of Cd^{++} from the soil medium to the shoot parts as compared to roots.

CONCLUSION

In the present investigation wheat was selected as experimental plant as it is one of the major staple crops of the world having better nutritional importance and thus can act as means of biotransformation of heavy metals towards the superior tropic levels. Generally, an important characteristic of heavy metals is that in excess they are highly phototoxic and our study in relation with effect of cadmium with reference to growth and accumulation reveals this fact. Cadmium produced a significant reduction in biomass, shoot length and in root length. It is also accumulated in increasing quantity in shoots and roots and showed the bioconcentration factor above one indicating the accumulation of Cd^{++} more than suggested normal values. Shoots accumulated lesser Cd^{++} ions as compared to roots of wheat seedlings. We conclude with the need for further study in order to establish the maximum amount of Cd^{++} and the influence of other complex mixture of heavy metals that crop varieties may tolerate. In our opinion, it is very necessary to study the quantity of heavy supplied to crops, their accumulation and threat to the humans after consumption.

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