



## EFFECT OF VARYING CADMIUM STRESS ON CHICKPEA (*Cicer arietinum* L) SEEDLINGS: AN ULTRASTRUCTURAL STUDY

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### ABSTRACT

Seed germination, one of the most important phase in the life cycle of a plant, is highly responsive to the existing environment. High levels of heavy metals significantly change the structural orientation of leaf, root and shoot. This paper reports on the effect of Cd toxicity on seed germination, seedling growth, photosynthetic pigment content and structural orientation of vascular tissues in chickpea (*Cicer arietinum* L) seedlings. Results showed that inhibition of seed germination and root growth started at 0.10 mM cadmium treatment solution and the highest inhibitive effect was found at 50.0 mM concentration. The accumulation of cadmium in plant parts are in the order root > stem > leaf. From the SEM study it was found that normal orientation of the vascular tissues as well as associated tissues are disrupted and the stomatal complexes with guard cells were highly affected in the leaves.

**Keywords:** Cadmium, phytotoxicity, *Cicer arietinum*, stomata, vascular tissue

### Abbreviations

SEM, Scanning electron microscope; %DFC, Percent difference from control; AAS, Atomic absorption spectrophotometry; DMRT, Duncan's multiple range test; TCL, Total chlorophyll; RuBisCo, Ribose-1,5-bisphosphate carboxylase oxygenase.

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### 1. INTRODUCTION

Contamination of agricultural lands caused by heavy metals in and around industrial areas is a serious problem. Such contamination is largely due to injudicious anthropogenic activities such as indiscriminate use of pesticides containing heavy metals in agriculture, discharge of untreated industrial wastes and effluents, faulty waste disposal, high rate of burning of fossil fuels, mining etc. [1-4]. Heavy metal pollutants, among others, are causing concern in contaminating agricultural lands in the district of Burdwan, West Bengal (India), particularly in the vicinity of industrial areas, dumping grounds of industrial wastes and national highways, which are enriched with lead and mercury [5,6]. The presence of excessive amounts of cadmium in soil and water causes a range of plant responses, including leaf chlorosis; stunted growth, reduced photosynthesis, stimulation of stomata opening at lower concentrations, reduced plant fresh and dry mass and stomatal conductance, and even death [7-11]. With the development of modern industry and agriculture, Cd (cadmium) has become one of the most harmful and widespread pollutants in agricultural soils and the soil-plant-environment system, mainly due to industrial emission. Application of Cd-containing sewage sludge and phosphate fertilizers, and municipal waste disposal are culprits [12-14]. As yet, it has been demonstrated that Cd has no biological function in plants [15]. However, it was reported that Cd is accumulated by many cereals, potatoes, pulses, vegetables and fruits and that humans take up at least 70% of the Cd that originates from plant food [16]. Common effects of Cd include affecting the water balance of plants by reducing root growth, limiting water uptake via a reduction in vessel size, and causing partial stomatal closure [17,18]. Again, the presence of excessive amounts of Cd in soil causes many disturbances in mineral nutrition and carbohydrate metabolism [19], and may therefore strongly reduce biomass production. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis in chickpea (*Cicer arietinum* L) [20,21] and photosynthesis in tomato [22]. It is well established that different metabolic processes such as photosynthesis and cell respiration are affected by the presence of Cd [7,23]. Cd basically enters the roots by the mechanism of diffusion [24] and its accumulation is higher in roots compared to shoots [25]. Subsequently, it is associated with cell walls [26] or sequestered in vacuoles [27]. Due to such accumulation of Cd, some significant changes were reported such as leaf structural disorganization,

reduced intercellular air spaces, drastic structural thylakoid alteration in the chloroplast [28-29], stomatal closure, softening of cell wall thickening [30] and decrease in chlorophyll content and efficiency of RuBisCo activity [31]. Cadmium can change RuBisCo sugar levels due to reduced carbon metabolism [32], increase levels of ascorbic acid and proline [33], and also change free amino acid and protein levels [34]. Because Cd ions accumulate at higher concentration in roots than in other plant parts, most research on the phytotoxic effects of Cd focused on the inhibition of photosynthesis [35]. In contrast, there appears to be little information about the effect of Cd on seed germination and ultrastructural deformation. Therefore, the objectives of our present experiment were to evaluate the effects of cadmium stress on growth, phytotoxicity, photosynthetic pigment content, biochemical changes and anatomical modifications of *Cicer arietinum* and to survey the subsequent accumulation of cadmium in different plant parts.

## 2. MATERIALS AND METHODS

### 2.1. Test Substances

The test chemical cadmium chloride ( $\text{CdCl}_2$ ) (CAS 10108-64-2; purity of 99.999%) was obtained from Sigma-Aldrich, Gillingham, UK. A stock solution of  $\text{CdCl}_2$  was prepared in Millipore water. The pH of the stock solution was adjusted to  $7.0 \pm 0.5$  using 1M NaOH to minimize the impact on solution pH prior to starting the toxicity studies. The test chemicals were soluble in water. Intermediate solutions were prepared by diluting the appropriate amount of stock solution with Millipore water. All chemicals other than  $\text{CdCl}_2$  were from Merck.

### 2.2. Experimental Treatment

Laboratory experiments with *C. arietinum* seedlings in petri dishes were conducted in the research laboratory of the Department of Environmental Science, The University of Burdwan, Burdwan, West Bengal, India. Healthy seeds of *C. arietinum* were collected from Kalna farm, Directorate of Agriculture, Govt. of West Bengal. The seeds were kept in airtight packets at room temperature and were used as experimental materials. After collection, seeds were surface sterilized in 0.1%  $\text{HgCl}_2$  solution for 30 seconds, then the seeds were washed several times with tap water followed by distilled water. Fresh, clean, air dried petri dishes (20

cm diameter) were taken and covered with filter paper discs. The filter paper discs were spiked with different treatment solutions ( $T_1$ - control,  $T_2$ - 0.049,  $T_3$ - 0.099,  $T_4$ -0.50,  $T_5$ -0.99,  $T_6$ - 2.00,  $T_7$ -9.99,  $T_8$ -20.0 and  $T_9$ - 50.00 mM Cd). 30 seeds were placed over the filter paper spiked with respective treatment solutions. The entire setup was then kept in a germination cage in a well-ventilated and diffused sunlight mediated room. The ambient temperature of the experimental setup was kept at 22°C, with one hour exposure to sunlight. The experiment was carried out according to the randomized block design with three replicates under laboratory conditions. Each petri dish containing the seeds was sprinkled with the respective treatment solution at 2-day intervals throughout the experimental period (10 days).

### 2.3. Analytical Chemistry

Analytical determination of test chemical concentrations were conducted to confirm the concentration of  $\text{CdCl}_2$  used in *C. arietinum* toxicity studies. Test media analysis was carried out at 0 and 24 hr to determine the concentrations using AAS. Test solutions were analyzed by 100  $\mu\text{g/L}$  of test solution into a 0.4 mm diameter capillary tube at 1  $\text{kg/cm}^2$  air acetylene pressure and 6 mL/minute air flow rate. The analytical measurements demonstrated that at 0 and 24 h, all test concentrations (0.049-50.0 mM) were within 99.6% of the nominal for studies conducted with the *C. arietinum* seeds (Table 1).

**Table 1** Quality control data for Cd solutions

Nominal concentration (mM)	Measured concentration (mM)		
	0 h	24 h	Average
0.05	0.048	0.049	0.049
0.1	0.098	0.099	0.099
0.5	0.50	0.45	0.50
1.0	0.99	1.00	0.99
2.0	2.00	2.01	2.01
10	10.0	10.0	10.0
20	20.0	20.0	20.0
50	50.0	50.0	50.0

### 2.4. Germination and Growth Attributes

After the 5 days of sowing the percentage germination values were considered, and the lengths of shoots and roots were recorded from 10-day old seedlings.

## 2.5. Calculation of Percent Difference from Control

Effects of Cd stress on seed germination were generally described using percentage differences from control (%DFC) [36] calculated as follows (1):

$$\%DFC = \frac{\% \text{ germination of control} - \text{germination of treatment}}{\% \text{ germination of control}} \times 100 \quad (1)$$

## 2.6. Calculation of Percentage Phytotoxicity of Shoot Growth

Effects of cadmium stress on seedling growth were generally described using percentage phytotoxicity of shoot growth (PSG) [37] calculated as follows (2):

$$\% PSG = \frac{\% \text{ Shoot growth in control} - \text{Shoot growth in treatment}}{\text{Shoot growth in control}} \times 100 \quad (2)$$

## 2.7. Calculation of Percentage Phytotoxicity of Root Growth

Effects of cadmium stress on seedling growth were generally described using Percentage phytotoxicity of root growth (PRG) [37] calculated as follows (3):

$$\% PRG = \frac{\% \text{ Root growth in control} - \text{Root growth in treatment}}{\text{Root growth in control}} \times 100 \quad (3)$$

## 2.8. Chlorophyll Assay

Fresh young leaves (0.1g) were selected from plants under each treatment at the last day of the experiment, and washed with deionized water. The leaves were cut into small pieces. Chlorophyll fractions 'a', 'b' and total chlorophyll were determined in the acetone extract (80% v/v) [38] measured in a spectrophotometer at 645, 652 and 663 nm, respectively. The concentrations were expressed as mg chlorophyll g<sup>-1</sup> fresh weight with the following equations (4-6):

$$Chl "a" (\text{mg g}^{-1} \text{fw}) = [12.7 \times D_{663} - 2.69 \times D_{645}] \times \frac{vw}{1000} \quad (4)$$

$$Chl "b" (\text{mg g}^{-1} \text{fw}) = [22.9 \times D_{645} - 4.68 \times D_{663}] \times \frac{vw}{1000} \quad (5)$$

$$\text{TotalChl} (\text{mg g}^{-1} \text{fw}) = D_{652} \times 1000 \times \frac{vw}{1000} \quad (6)$$

Here, D = absorbance, v = final volume of 80% acetone; w = mass of sample; fw = fresh weight of the sample.

## 2.9. Analysis of Biochemical Parameters

**Estimation of Proline.** Proline was extracted from the leaves and estimated by the methods of Bates et al. [39]. Homogenates of the leaf samples were prepared in 3% sulphosalicylic acid. A pink colour was developed by a reaction with glacial acid and ninhydrin. The colour was separated in a toluene layer and the absorbance was measured spectrophotometrically at 529 nm.

**Estimation of Protein.** Protein content of the plants untreated and treated with cadmium was estimated by the method of Lowry et al. [40]. To avoid interference from pigments, the trichloroacetic acid (TCA) precipitate was washed twice with 90% acetone. Bovine serum albumin was used as the standard. Absorbance was recorded spectrophotometrically at 660 nm.

**Estimation of Soluble Sugars.** Soluble sugars were estimated by the method of Montgomery [41]. Plant tissue (0.2 g) was homogenized in 2.0 mL of 80% ethanol (10% homogenate) using a Potter Elvehjem glass homogenizer and centrifuged at 3000 rpm for 20 min. To 0.1 mL supernatant was added 0.9 mL water, 0.1 mL of 80% phenol, and 5.0 mL conc. H<sub>2</sub>SO<sub>4</sub>, and the mixture was allowed to stand at room temperature for 30 min. The absorbance was measured spectrophotometrically at 490 nm.

**Estimation of Ascorbic Acid.** Fresh leaf samples (0.5 g) were homogenized in 20 mL extracting medium of 0.5 g oxalic acid and 0.075 g EDTA in 100 mL distilled water. After centrifuging the homogenate for 15 minutes, 1.0 mL of the homogenate was mixed with 5.0 mL dichlorophenol indophenols (20 µg/mL). After shaking well, its absorbance was measured at 520 nm [42]. A calibration curve was prepared with pure ascorbic acid.

## 2.10. Analysis of Cadmium in Different Plant Parts

For the plant sample preparation, 1g of dry plant material was ashed in a muffle furnace for 16h at 500°C. Ash was dissolved in 20 mL of conc. HNO<sub>3</sub> and kept on a hot plate for evaporation to dryness until all traces of HNO<sub>3</sub> disappeared. After cooling, 5 mL HClO<sub>4</sub> was mixed with it and again kept on a hot plate until dense white fumes of HClO<sub>4</sub> disappeared. After evaporation of acid it was again cooled to room temperature. The volume was made up to 50 mL with distilled water and filtered through Whatman-42 filter paper. The concentrations of cadmium were determined by atomic absorption spectrophotometry (GBC Avanta, Victoria, Australia). The detection limit of the spectrophotometer is 0.1-1.8 µg/mL and the sensitivity range is 0.009

$\mu\text{g/mL}$ . A standard reference material of poplar leaves (GBW07602 (GSV-1) and blanks were carried through the digestion and analyzed as quality control. Standards were always reanalyzed at intervals of 20 samples. The analyses results were only accepted when the measured standard concentrations were within one standard deviation of the certified value. The average recovery of cadmium in certified reference materials was 101.2%.

### 2.11. Scanning Electron Microscopic (SEM) Study

The changes in external morphology of root; shoot and leaf of *C. arietinum* seedlings were studied using a scanning electron microscope (SEM). Root, shoot and leaf specimens were prepared for SEM using the protocol adapted from standard procedures [43]. The fresh root, shoot and leaf samples (5 mm square from similar middle portion) of nine root, shoot and leaves each from the control and Cd treatments) were dissected and immediately fixed in a solution of 2% glutaraldehyde prepared in a 0.1 M sodium phosphate buffer (pH 7.0) for 12 h at room temperature. The specimens were washed three times in 25 mM sodium phosphate buffer (pH 6.8) overnight at 4°C and then dehydrated in absolute ethanol using 10 minutes series samples of 25%, 50%, 75%, 95% and 100% ethanol and then stored at -20°C until examination. The specimens were rinsed, post fixed in 2% osmium tetroxide, critical point dried and sputter coated with gold/palladium before being mounted on aluminum stubs. The specimens were viewed and photographed using a 15 KV scanning electron microscope (LEO Electron Microscope Inc., USA) [44-48].

### 2.12. Statistical Analysis

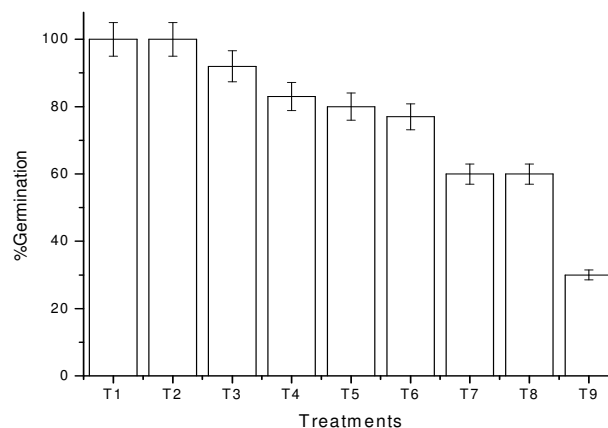
The observed tabular data were analysed statistically by one-way ANOVA analysis and the significant difference between the treatments means were compared through the DMRT test [49,50].

## 3. RESULTS

### 3.1. Percentage of Germination and % Difference from Control

The percentage of germination in the controls and their subsequent decrease in the treatments are depicted in Fig. 1. The highest percentage of germination value was observed at control and the 0.50 mM Cd treated set. At low levels of cadmium concentration germination was

not markedly inhibited.



**Figure 1** Percentage of germination

### 3.2. Shoot and Root Length, Root/Shoot Ratio

The root length and shoot length in the controls and their subsequent decrease in the treatments are listed in Table 2. The results showed that both root length and shoot length were highest at 0.050 mM Cd treated solution but drastically reduced with increasing concentration. The ratio of root to shoot decreased from control to treatment T<sub>6</sub> (2.00 mM) with a slight increment in T<sub>4</sub> (0.50 mM). A steady decrease of root to shoot ratio was recorded after the T<sub>6</sub> treatment (Table 2).

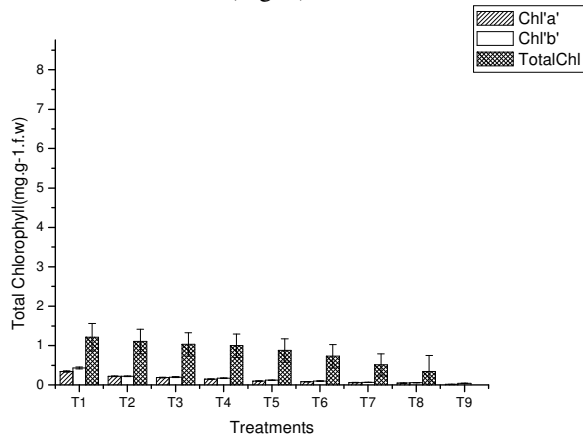
**Table 2** Morpho-physiological attributes of plants and pigment content in leaves

Treat-ment	Shoot length	Root length	Root/Shoot ratio
T <sub>1</sub>	15.8 <sup>a</sup>	8.0 <sup>a</sup>	0.51 <sup>a</sup>
T <sub>2</sub>	14.4 <sup>ab</sup>	7.1 <sup>b</sup>	0.49 <sup>c</sup>
T <sub>3</sub>	14.3 <sup>abc</sup>	6.8 <sup>c</sup>	0.48 <sup>ef</sup>
T <sub>4</sub>	13.3 <sup>d</sup>	6.4 <sup>d</sup>	0.48 <sup>cd</sup>
T <sub>5</sub>	11.9 <sup>e</sup>	5.2 <sup>f</sup>	0.44 <sup>g</sup>
T <sub>6</sub>	10.9 <sup>ef</sup>	4.6 <sup>e</sup>	0.42 <sup>hi</sup>
T <sub>7</sub>	8.2 <sup>g</sup>	4.2 <sup>g</sup>	0.51 <sup>b</sup>
T <sub>8</sub>	7.3 <sup>h</sup>	3.1 <sup>h</sup>	0.43 <sup>h</sup>
T <sub>9</sub>	5.0 <sup>i</sup>	2.4 <sup>i</sup>	0.48 <sup>e</sup>

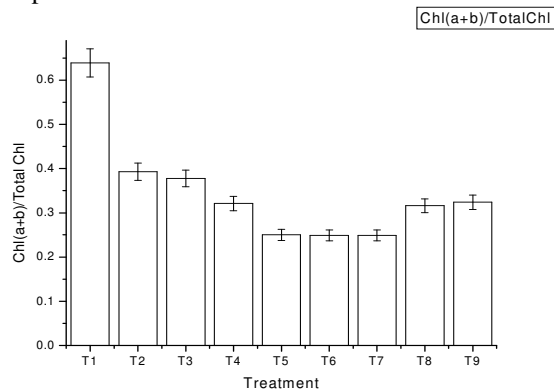
Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT). Means of three replicates are taken.

### 3.3. Chlorophyll

Chlorophyll contents of *C. arietinum* in the controls and their subsequent decrease in the treatments are presented in Fig. 2. Compared to the control, the chlorophyll contents gradually decreased with increase of Cd concentration. Similarly, Chl(a+b)/Total Chl. content gradually decreased with increasing concentrations of cadmium (Fig. 3).



**Figure 2** Variation of Chl'a', Chl'b' and total chl. Values are means  $\pm$  SE (n=3) over two independent experiment

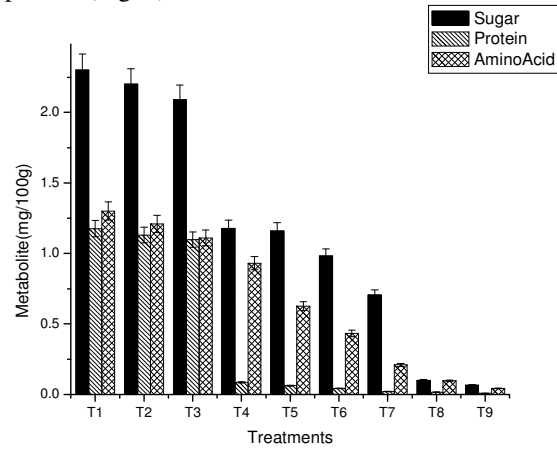


**Figure 3** Changes of Chl(a+b)/Total Chl with different treatments

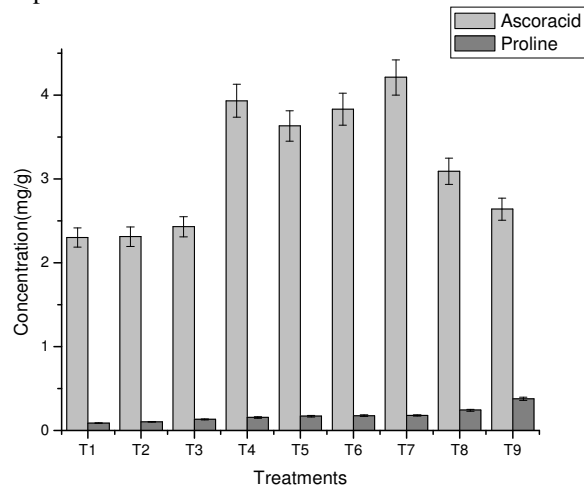
### 3.4. Biochemical Constituents of the Plant Materials

Soluble sugar contents in leaves decreased with increasing concentration of cadmium. The maximum decrease was recorded at higher concentrations with respect to control (Fig. 4). Similar results as in soluble sugar content were found for soluble protein and amino acid content in plants at the highest concentration of cadmium (Fig. 4). Moreover, the results indicate that proline and ascorbic acid accumulation increases with

increasing Cd concentration, but ascorbic acid showed an asymmetrical incremental pattern compared to proline (Fig. 5).



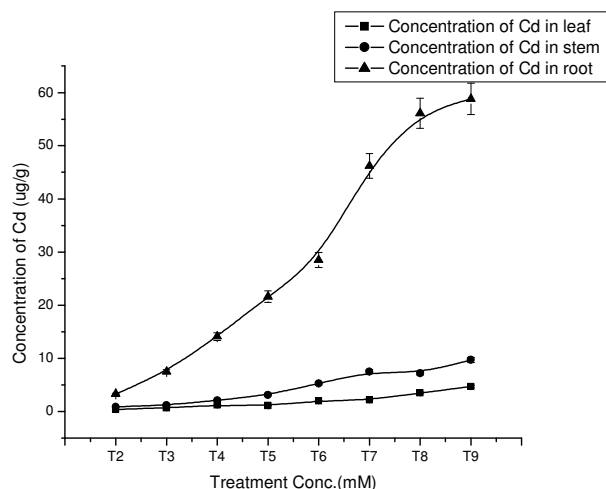
**Figure 4** Variation of sugar, protein and amino acid. Values are means  $\pm$  SE (n=3) over two independent experiments



**Figure 5** Variation of ascorbic acid and proline in treated seedlings

### 3.5. Cadmium Accumulation in Different Plant Parts

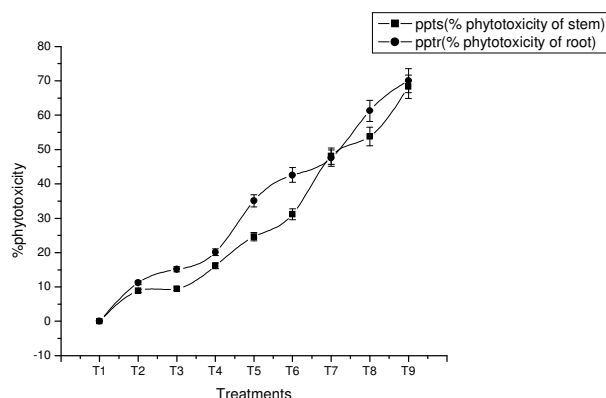
The measured exposure concentration of cadmium in different plant parts under spiked treatments and controls is shown in Fig. 6. The study results revealed that Cd uptake in plant tissue was concentration-dependent. According to Fig. 6, roots accumulated more Cd compared to other parts of the seedling. The accumulation of Cd in lower concentrations (0.05-0.50 ppm) is almost the same in shoots and leaves. However, from 2.00 to 50.0 ppm Cd was higher in roots compared to shoots and leaves.



**Figure 6** shows the amount of Cd ( $\mu\text{g}\cdot\text{g}^{-1}$ ) deposited at root, stem and leaf of different treated plants. Values are means  $\pm$  SE (n=3) over two independent experiments

### 3.6. Percentage Phytotoxicity of Shoot and Root

The percentage phytotoxicity of shoot and root in the controls and their subsequent increase in the treatments are explained in Fig. 7. The highest percentage phytotoxicity of shoot and root value was observed for the 50.0 mM Cd treated set. The percentage phytotoxicity of shoot and root were not greatly affected at lower concentrations of cadmium. At high level treatments, the percentage phytotoxicity of roots and shoots was markedly increased.



**Figure 7** Percent phytotoxicity of shoot and root. Values are means  $\pm$  SE (n=3) over two independent experiments

### 3.7. Scanning Electron Microscopic Studies

SEM images from transverse sections of root samples under control conditions show normal stellar structure

with xylem and phloem tissues for normal conduction of water and photoassimilation, respectively. Gradual degeneration of cellular structure started from low to high levels of Cd treatment. At 0.050 mM Cd, the xylem lumens seem to be wider and the phloem tissues seem to be larger than control samples. At 2.00 mM Cd, the xylem and phloem tissues exhibited structures with subnormal lumens. At 10.0 mM Cd the images were obscure. At 50.0 mM Cd, roots exhibit crimped structures of xylem and phloem elements, indicating impairment of both the conducting systems (Fig. 8.1, 8.4, 8.7, 8.10 and 8.13).

SEM images of transverse sections of shoot samples under control condition show normal structure, with pentagonal/hexagonal structures of xylem and phloem elements that indicate a normal conduction system for transport of water and assimilates. From 0.050 mM onwards there was gradual degeneration of the vascular tissue. At 10.0 mM Cd, transverse sections of the stem exhibited subnormal appearance of xylem and phloem elements in comparison to control samples. At 50.0 mM Cd, a serious impairment of the normal orientation of the vascular tissues along with associated tissues appears. This indicates the toxic effect of Cd leading towards a subdued conduction of water and photosynthates. The magnitude of Cd-induced change is least at 0.050 mM Cd (Fig. 8.2, 8.5, 8.8, 8.11 and 8.14).

Scanning electron microscopic observation of leaf samples of control shows normal stomata with characteristic guard cells. However, the subsidiary cells are not very prominent. Also the frequency of occurrence of stomata is much higher in comparison to treatments. At 10.0 mM Cd, stomatal frequency is minimum and only one stomata is seen with an unusual structure of guard cell: subsidiary cells are almost normal and stomatal pores of both the samples are conspicuous. However, at 0.050 mM Cd the guard cells seem to be comparatively elongated and the pore size is slightly larger than the sample treated with 2.00 mM Cd. At 50.0 mM Cd, a normal stomatal complex with guard cells and subsidiary cells was not encountered and stomatal apertures seem to be closed (Fig. 8.3, 8.6, 8.9, 8.12, 8.15).

## 4. DISCUSSION

Parameters such as percentage of germination control [36], and shoot and root length have been used as an indicator of heavy metal toxicity in plants [51-53]. In the present study, significant reduction in different parameters like length of shoot and root, total

chlorophyll [54], percent phytotoxicity at root and shoot [37] with some anatomical features indicated that Cd concentration produced toxic effects within 7 days (Table 2). The percentage of germination and %DFC clearly indicate the inhibitory effects of Cd on germination of *C. arietinum*. The results indicate that %DFC increase with increasing concentration of metal solutions and higher values of %DFC suggest the greater susceptibility to cadmium at higher concentration.

In low level cadmium concentration treatments, percentages germination were not much inhibited, showing that they were well within the tolerable range of *C. arietinum* seedlings. However, in high level treatments, germination percentages were detrimentally affected, implying that higher concentration of cadmium was not conducive to seed germination. This may be attributed to depression of oxygen uptake and physiological disturbance in mobilization of reserve seed food materials [55].

In low level cadmium concentration treatments the shoot length and root length were not much inhibited, showing that they were well within the tolerable range of *C. arietinum* seedlings. However, in high level treatments, shoot length and root length were detrimentally affected, implying that higher concentration of cadmium was not conducive to vegetative growth. The reduction in the shoot length may be due to direct inhibition of cell division or cell elongation at higher Cd levels as well as retarded root growth and lesser nutrients and water transport to the shoot parts of the plant. The reduction of root growth may be direct interference of Cd in some hydrolytic enzymes, which play a vital role transporting food to the primary root and shoot [56]. In addition to this, Cd transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots, contributing towards significant reduction in plant shoot height as in the present investigation. The root length decreased more than shoot as the Cd concentration increased. Similarly the tip of the root was found curved and brown in color. Heavy metals are found to be more toxic for root growth because they accumulate on roots and retard cell division and cell elongation [57].

The decrease in chlorophyll contents in leaves of *C. arietinum* seedlings with increasing Cd might be due to striking changes in the fine structure of chloroplasts and also destruction of photosynthetic apparatus [58]. Heavy metals stress on chlorophyll levels is well documented [59-62]. The degradation of photosynthetic pigment under heavy metal stress is the result of damage of the PS II reaction centre in the leaf [63-65]. Moreover, the

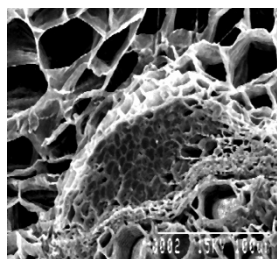
lower Chl(a+b)/Total Chl ratios at high Cd (Fig. 3) also indicate stress and damage to the photosynthetic apparatus [66].

The data suggest that higher concentrations of Cd<sup>2+</sup> progressively decrease soluble sugar, protein, amino acid and protein content in *C. arietinum*. It also causes deficiency of essential metal ions and hampers the growth attributes of plants [67]. Heavy metals interference in protein content in plants is well documented [67,68]. Protein levels in plants affected by heavy metals are due to 1) extensive hydrolysis of protein, which reduces the soluble protein level. 2) catalytic activity of metals and 3) any stress conditions. On the other hand, increasing concentrations of metals in plants support the accumulation of proline, which ameliorates water deficit stress and maintains water balance under such Cd stress [69]. Actually, proline can protect plants through a variety of mechanism like osmoregulation, protection of enzymes against denaturation, and stabilization of protein synthesis [70].

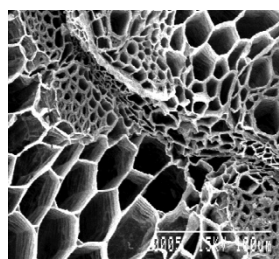
Higher accumulation of Cd was found in roots in all treatments compared to the shoot and leaf (Table 2). Moreover, at 50.0 mM Cd, the decrease of root and shoot length was almost one-fourth with respect to the control. This finding agrees with Aidid and Okamoto [71], who reported that cadmium causes detrimental growth reduction in both roots and stems. This might be due to cadmium adsorption across the plasma membrane of root cells, which is controlled by the electrochemical potential difference between the activity of Cd<sup>+2</sup> in the cytosol and in the root apoplasts [72] and also due to cross-linking of Cd with the carboxyl groups of the cell wall [17] and interaction with thiol residues of soluble proteins [73]. The lesser accumulation of cadmium in stems may be due to retention of cadmium ions in the roots and only small amounts are transported to shoots (Fig. 6) [74].

In low level cadmium concentration treatments, the percentage phytotoxicity of shoots and roots were not significant, showing that they were not phytotoxic towards the *C. arietinum* seedlings. In high level treatments, the percentages phytotoxicity of shoots and roots were detrimental, implying that higher concentrations of cadmium are phytotoxic to plant growth. The % phytotoxicity value of root was greater in comparison to shoots in all Cd-treated plants. This leads to more inhibition of root length in comparison to shoot length and therefore shows the detrimental effect of cadmium on root growth [75].

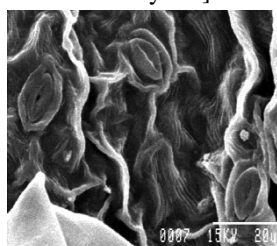
SEM study showed that there is gradual distortion in the cellular structure of different plant parts due to the phytotoxic effect of cadmium at higher treatments.



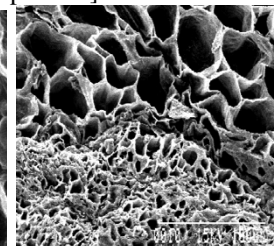
**Figure 8.1** Control-500X Root [normal structure of xylem]



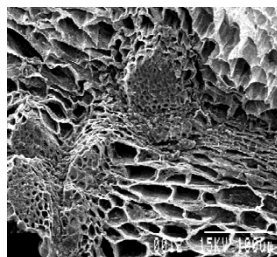
**Figure 8.2** Control-500X Shoot [normal xylem & phloem]



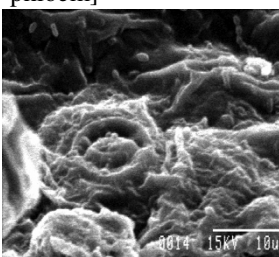
**Figure 8.3** Control-3000X Leaf [normal structure of stomata]



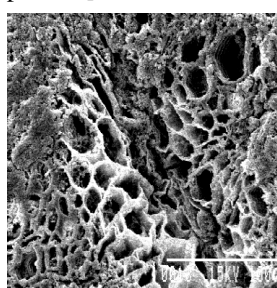
**Figure 8.4** 50.01mM Cd treated-500X Root [deformed structure of phloem]



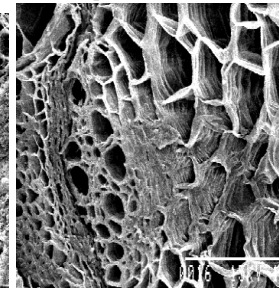
**Figure 8.5** 50.01mM Cd treated-500X Shoot [deformed structure of phloem]



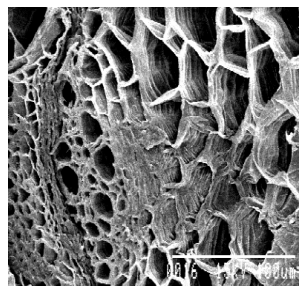
**Figure 8.6** 50.01mM Cd treated-3000X Leaf [deformed structure of stomata]



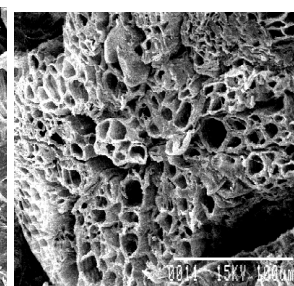
**Figure 8.7** 9.9mM Cd treated-500X Root [deformed of phloem]



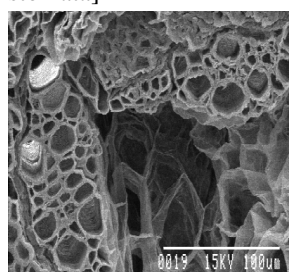
**Figure 8.8** 9.9mM Cd treated-500X Shoot [completely breakdown of phloem]



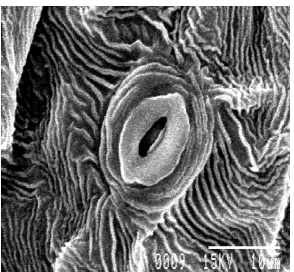
**Figure 8.9** 9.9mM Cd treated-3000X Leaf [maximum opening stomata]



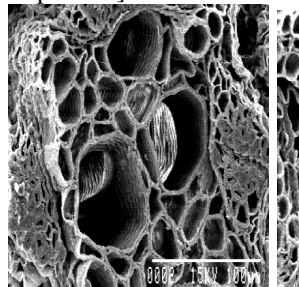
**Figure 8.10** 2.01 mM Cd treated-500X Root [deformed of phloem]



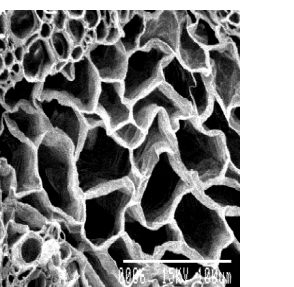
**Figure 8.11** 2.01 mM Cd treated-500X Shoot [completely breakdown of phloem]



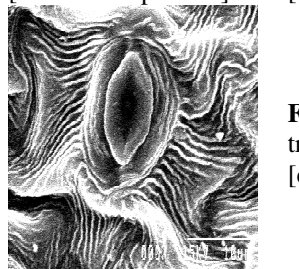
**Figure 8.12** 2.01 mM Cd treated 3000X Leaf [swelling stomata]



**Figure 8.13** 0.049 mM Cd treated 500X Root [Subnormal phloem]



**Figure 8.14** 0.049mM Cd treated 500X Shoot [Subnormal phloem]



**Figure 8.15** 0.049 mM Cd treatment 3000X Leaf [elongated guard cell]

**Figure 8** Histological photos of different parts of *C. arietinum* plant



Leaf samples under various cadmium treatment showed various types of effect, especially on opening of stomata depending upon the concentration of cadmium. It is interesting to note that 10.0 mM treatment shows that the stomata is open, whereas at 0.10 mM and 0.050 mM Cd it is closed. The opening and closing of stomata are mainly regulated by the changes in the turgor pressure of the guard cells. Generally, an increase in the turgor pressure of the guard cells results in the widening of the stomatal aperture [76]. At higher concentration due to the excessive accumulation of cadmium, the swelling of intercellular substance between the guard cells takes places, which results in, the connection between the cells and the guard cells to be split in their median parts, resulting in stomatal opening.

Since the experiments were carried out under laboratory conditions, the phytoavailability of heavy metals and their stress to crops probably would be greater under realistic field situations. This may result in a slight magnification of crop growth inhibition, changes in total chlorophyll levels, heavy metal concentrations and accumulation in different plant parts.

## 5. CONCLUSIONS

In *Cicer arietinum*, Cd phytotoxicity contributed significantly towards reduction in percent seed germination, vegetative growth and also significant distortion of tissue structure of root, shoot and leaf. Root growth was completely inhibited at higher levels of cadmium. Planting crops in cadmium-contaminated soil can produce significant health risks to consumers. Therefore, it is highly recommended that crops with short rooting systems should not be cultivated in cadmium stress areas. Moreover a comprehensive public awareness through media and active participation of local youth is needed for avoiding such cadmium-induced toxicity problems in contaminated areas.

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