Ultrastructural deformation of plant cell under heavy metal stress in Gram seedlings

Uttiya Dey1 and Naba Kumar Mondal1*

Abstract: Nowadays, heavy metal pollution has become a serious environmental problem on global scale. The heavy metals are non-biodegradable in nature, which can easily accumulate in the organisms of lower trophic level, and enter to the human body system through food chain. From this backdrop, the present experiment highlighted the effect of three heavy metals (Cr, Pb, and Mn) in different concentrations (25, 50, and 75 ppm) on Cicer arietinum in terms of growth physiology, metal uptake, biochemistry, and ultrastructural deformation. The results showed that with increasing metals (Cr and Pb) concentrations from 25 ppm to 75 mg/L both root and shoot length decreased along with root and shoot biomass. However, Mn showed little improvement in all growth physiological parameters at 50 ppm concentration. Biochemical parameters also revealed that both Cr and Pb reduced 64.94 and 69.61% total chlorophyll, respectively, with respect to control. Chlorophyll “a” to “b” ratio was highest in Mn followed by Cr and Pb at higher concentration (75 ppm). Metal accumulation pattern indicated that Cr is less accumulated in root shoot and leaf compared to Mn and Pb in all the studied concentrations. However, accumulation of Mn in shoot was always higher compared to Pb in all studied concentrations. Ultrastructural damage was recorded highest for Cr in root, shoot and leaf at both 25 and 50 ppm concentration. However, at 75 ppm Pb showed highest deformation in root and leaf was observed.

Subjects: Bioscience; Earth Sciences; Environment & Agriculture; Food Science & Technology

Keywords: heavy metal; bioaccumulation; biochemistry; growth physiology; ultrastructural deformation

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PUBLIC INTEREST STATEMENT
Heavy metal is a non-degrading substance. However, it can migrated from one segment to another that means soil to plants and ultimately deposited inside the plant body. Present research highlighted that the entire three heavy metals dose did not show same pattern of deposition and toxicity effects. All the three metals have negative impacts on both root and shoot length and biomass of root and shoot. The study also suggested that among the three metals, chromium has adverse effects on ultrastructure of root, shoot, and leaf. Therefore, from the present study it can be suggested that farmers community should be aware that agricultural residue from heavy metal affected area should not be used as food for cattle.
1. Introduction

Nowadays, heavy metal pollution has become a serious environmental problem on global scale. Heavy metal is a member of a loosely defined subset of elements that exhibits metallic properties. It mainly includes the transition metals, some metalloids, lanthanides, and actinides. These metals when present in excess in the environment disturb the balance of the ecosystem. The heavy metals are non-biodegradable, accumulate in the organisms at lower tropic level, and enter to the human body system through food chain resulting in serious threats to human health (Patra, Bhowmik, Bandopadhyay, & Sharma, 2004). Soil and water microbial diversity as well as associated nutrient cycling of water and soil are also adversely affected by heavy metals (Amna et al., 2015). Some heavy metals like chromium, manganese, and lead are exposed to both soil and water resulting in pollution (Meng, Chen, & Yang, 2011).

In the earth crust, chromium is the 21st abundant element (Cervantes et al., 2001). The main source of chromium in the environment is the chromium containing untreated effluent, which is released from various industries, viz. tannery, electroplating industry, plastic, paints, pigments, distilleries, sugar mills, pulp and paper industries, pharmaceuticals, etc. (Sundaramoorthy, Chidambaram, Ganesh, Unnikannan, & Baskaran, 2010). Among these, tannery occupies the major part of chromium pollution. Only a small fraction of the chromium is absorbed in the tanning process and the rest is discharged as effluents without any treatment because of high treatment cost (Shukla, Rai, Singh, Dubey, & Baghel, 2007). The concentration of chromium in the untreated effluent of tannery industry is near about 20,000 ppm (Sundaramoorthy et al., 2010). Manganese generally does not occur as the free metal. It has more than 100 minerals including various sulfides, oxides, carbonates, silicates, phosphates, and borates (Gerber, Léonard, & Hantson, 2002; Howe, Malcolm, & Dobson, 2004). Manganese have three possible oxidation states in soil, namely Mn(II), Mn(III), and Mn(IV). The divalent form is only stable in soil solution, while Mn(III) and Mn(IV) are stable in the solid phase of soil (McBride, 1994). The mobility and solubility of soil manganese are dependent on some soil conditions like, soil pH, wetness, soil organic matter content, biological activity, and redox potential (Kabata-Pendias & Pendias, 2001; McBride, 1994). The principle anthropogenic sources of manganese include municipal wastewater discharges, sewage sludge, mining and mineral processing (particularly nickel), discharge from ferroalloy, steel, and iron production as well as combustion of fossil fuels (Hronec, Vilcek, Thoma, Adamisin, & Huttmanova, 2010). Sometimes manganese contamination of soil is the result of the presence of Mn in the parent material (Yang, Deng, & Lil, 2008). Natural occurrence of lead in soil is very low (Akinci, Akinci, & Yilmaz, 2010). Weathering of parent materials containing lead is one of the major sources of lead pollution of soil and water (Akinci et al., 2010). There are also various anthropogenic sources like mining and smelting of lead-ores, burning of coal, effluents from battery industries, automobile exhausts, metal plating and finishing operations, excessive use of fertilizers and pesticides, pigment additives and gasoline (Eick, Peak, Brady, & Pesek, 1999).

The degree of toxicity of heavy metals depends on the rate of production of reactive oxygen species like superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (OH). Entry of the heavy metals into food chain is the result of consumption of plant materials grown in contaminated soil (Zou, Wang, Jiang, & Liu, 2006). The toxicity of chromium depends upon its oxidation state. Among its various oxidation states, hexavalent chromium ($Cr^{VI}$) is most toxic because of its high solubility, which produces severe oxidative stress (Von Burg & Liu, 1993). Because of its high solubility in water, it enters the cell membrane easily and produces reactive oxygen species (Dixit, Pandey, & Shyam, 2002) which directly interacts with DNA (Beyersmann & Hartwig, 2008) causing DNA degradation and cell cycle damage (Rodriguez, Santos, Lucas, & Pereira, 2011). There is also information that exposure to high level of chromium can inhibit photosynthetic rate in plants by accepting electron during the process of photophosphorylation (Bishnoi, Chugh, & Sawnhney, 1993). Lead uptake by plants depends upon pH, particle size, and cation-exchange capacity of the soils as well as by root exudation and other physicochemical parameters (Akinci et al., 2010). Exposure to lead causes various toxic symptoms in plants like stunted growth, chlorosis, blackening of root system, etc. (Sharma & Dubey, 2005). It also produce oxidative stress by producing reactive oxygen species which in turn causes damage to various biomolecules like membrane lipids, proteins, chloroplast pigments, enzymes, nucleic acids,
etc. (Sharma & Dubey, 2005) and consequently inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status, and affects membrane structure and permeability (Mishra & Singhal, 1992). Manganese is an essential micro nutrient for plants but presence of excess quantity of Mn in the soil can cause toxicity. Accumulation of excess Mn in plant tissues can alter various biochemical processes like enzyme activity, absorption, translocation, and utilization of other mineral elements such as Ca, Mg, Fe, and P and cause oxidative stress (Ducic & Polle, 2005; Lei, Korpe lainen, & Li, 2007). Increase in the level of proline, which is a good stress indicator and decrease in chlorophyll, carotenoid and protein level under heavy metal stress is well documented (Vassilev et al., 2003; Verma & Dubey, 2003; Zengin & Munzuroglu, 2005). There is also evidence that heavy metal stress can cause cell damage in the plants (Mondal, Das, Roy, Datta, & Banerjee, 2013). Keeping in mind the above facts, the objectives of the present experiment are to understand the toxic effect of these metals on plants and their accumulation pattern in different parts of the plant and observation of ultrastructural deformation of xylem and phloem under such heavy metal stress. Numerous works has been done by considering different heavy metals, but use of three heavy metals at a time and their ill effects on ultrastructural deformation is very uncommon.

2. Materials and method
About 100 g dry and healthy seeds of Cicer arietinum were soaked overnight in 250 ml distilled water. After sterilization with 0.1% mercuric chloride for 30 s and thoroughly washing with distilled water, the seeds were transferred to 10 Petri dishes containing moist filter paper, 25 seeds were taken in each Petri dish. All the Petri dishes were divided into four sets, first three replicates were exposed to potassium dichromate of three different concentrations: 25 ppm (Cr1), 50 ppm, (Cr2) and 75 ppm (Cr3). Next three sets of replicates were given the same concentrations of lead chloride (Pb1, Pb2, and Pb3), and the last three sets of replicates were given 25 ppm (Mn1), 50 ppm (Mn2), and 75 ppm (Mn3) of Mn chloride solution. The last one was control with distilled. All the treatments were replicated in three sets. The seeds were treated for seven days and were exposed to sunlight for one hour with one-day interval.

2.1. Physiological parameters
After seven days of treatment, the fresh weight and length of root and shoot of the plants were measured.

2.2. Biochemical parameters
Chlorophyll “a,” “b,” total chlorophyll, and carotenoid were measured by extracting with 80% acetone following Arnon’s (1949) method. The proline from the treated plants was measured by the acid ninhydrin method developed by Bates, Waldren, and Teare (1973).

2.3. Determination of heavy metal
For the determination of heavy metals from different plant parts, root, shoot, and leaf samples from different heavy metal-treated plants were oven dried for 24 h at 60°C temperature. The dried samples were separately grinded to powder and 0.1 gm of the grinded sample was digested with concentrated HNO3: HClO4; (V/V 9:1) for 2 h. Residues were filtered through Whatman filter paper and diluted to 100 ml with double-distilled water. Concentrations of heavy metals from the digested samples were analyzed using Atomic Absorption Spectrophotometer (GBC Avanta) (Bajpai et al., 2011).

2.4. Statistical analysis and graphs
The statistical analysis was done using statistical software Minitab 16. The graphs were drawn using Origin software (version 6.1).

3. Results and discussions
3.1. Physiology of the plants
The first response of plants to any metal stress is growth adjustment (Lei et al., 2007). Under chromium stress severe reduction in the plant biomass was noted (Table 1). Length of root and shoot...
also decreased with increasing chromium concentration. Decrease in plant biomass under chromium stress was reported earlier on different plants (Dixit et al., 2002; Sharma & Sharma, 1996). This can be explained by the fact that under heavy metal stress, availability of water became restricted to all the parts of the plants or due to disturbed carbohydrate and nitrogen metabolisms and reduction in protein synthesis (Sharma & Sharma, 1996). Plants exposed to Cr accelerate the nitrate accumulation in plant tissue and slow down the protein synthesis process (Shanker, Cervantes, Lozatavera, & Avudainayagam, 2005). There is another possible mechanism i.e. Cr\(^{6+}\) interacts with endogenous phytohormones that controls the plant growth (Moya, Ros, & Picazo, 1995). Decrease in seedling vigor (weight and length) of the treated plants is due to interference of Pb with the metabolic and biochemical processes associated with normal growth and development of the plants (Verma & Dubey, 2003). Present finding also highlighted that the growth physiological parameters decreased with increasing Mn concentration. Sometimes excess amount of Mn becomes trapped in various cell organelles like vacuole, endoplasmic reticulum, etc. in inactive form and its toxicity becomes low (Zhao et al., 2012). This phenomenon occurred in case of 50 ppm Mn treatment where all the growth physiological parameters showed higher result even than that of control.

### 3.2. Pigmentation

The pigment content i.e. chlorophyll “a,” chlorophyll “b,” total chlorophyll, and carotenoid in all the treatments declined significantly with increasing treatment concentration. But when 50 ppm Mn was applied, pigment level was observed higher than all other treatments even than that of control (Table 2).

The result of this experiment indicates that there is a gradual decrease in chlorophyll “a,” chlorophyll “b,” and total chlorophyll with increasing chromium concentration. At 25 ppm Cr concentration, chlorophyll “a,” “b” and total chlorophyll were 0.113, 0.090, and 0.211 mg/g, respectively. But when 75 ppm Cr was applied, these content (chlorophyll “a,” “b,” and total chlorophyll) were decreased to 0.074, 0.055, and 0.135 mg/g. Decrease in total chlorophyll content has been well documented under Cr stress (Gupta, Gaumat, & Mishra, 2011; Panda & Choudhury, 2005). This decrease indicates that the chlorophyll synthesis system and chlorophyllase activity were affected by the exposure to high chromium concentrations (Van Assche & Clijsters, 1990). Cr is capable of degrading \(\delta\)-aminolevulinic acid dehydratase, an important enzyme involved in chlorophyll biosynthesis, thereby affecting the \(\delta\)-aminolevulinic acid reducing the chlorophyll content (Vajpayee, Rai, Ali, Tripati, & Singh, 2001). Chromium also reduced carotenoid contents in the treated plants which served as accessory pigments for photosynthesis and also protects plants from photo-oxidation. Similar observations have also been made in other plants exposed to chromium (Vajpayee et al., 2001). On the other hand, increasing dose of lead also decreased the pigment content of the plants. There is very strong inhibitory effect of lead on chlorophyll a and b content in mustard (Fargašová, 2001) and

### Table 1. Physiological parameters

| Metal | Conc. (ppm) | RL (cm) | SL (cm) | RW (g) | SW (g) |
|-------|-------------|---------|---------|--------|--------|
| Cr    | 25          | 2.9 \(\pm\) 0.088 | 7.5 \(\pm\) 0.049 | 0.089 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) | 0.077 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) |
|       | 50          | 2.2 \(\pm\) 0.145 | 4.3 \(\pm\) 0.176 | 0.039 \(\pm\) 0.176 \(\times\) 10\(^{-2}\) | 0.048 \(\pm\) 0.203 \(\times\) 10\(^{-2}\) |
|       | 75          | 1.6 \(\pm\) 0.173 | 3.8 \(\pm\) 0.203 | 0.027 \(\pm\) 0.173 \(\times\) 10\(^{-2}\) | 0.040 \(\pm\) 0.176 \(\times\) 10\(^{-2}\) |
| Pb    | 25          | 4.5 \(\pm\) 0.176 | 6.6 \(\pm\) 0.173 | 0.075 \(\pm\) 0.173 \(\times\) 10\(^{-2}\) | 0.068 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) |
|       | 50          | 3.0 \(\pm\) 0.120 | 5.3 \(\pm\) 0.133 | 0.048 \(\pm\) 0.173 \(\times\) 10\(^{-2}\) | 0.067 \(\pm\) 0.115 \(\times\) 10\(^{-2}\) |
|       | 75          | 2.1 \(\pm\) 0.289 | 3.2 \(\pm\) 0.203 | 0.047 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) | 0.063 \(\pm\) 0.173 \(\times\) 10\(^{-2}\) |
| Mn    | 25          | 3.6 \(\pm\) 0.203 | 6.8 \(\pm\) 0.233 | 0.036 \(\pm\) 0.203 \(\times\) 10\(^{-2}\) | 0.075 \(\pm\) 0.115 \(\times\) 10\(^{-2}\) |
|       | 50          | 8.2 \(\pm\) 0.173 | 11.0 \(\pm\) 0.41 | 0.131 \(\pm\) 0.203 \(\times\) 10\(^{-2}\) | 0.161 \(\pm\) 0.176 \(\times\) 10\(^{-2}\) |
|       | 75          | 5.2 \(\pm\) 0.203 | 5.9 \(\pm\) 0.233 | 0.042 \(\pm\) 0.173 \(\times\) 10\(^{-2}\) | 0.091 \(\pm\) 0.115 \(\times\) 10\(^{-2}\) |
| Control |            | 7.8 \(\pm\) 0.145 | 9.5 \(\pm\) 0.145 | 0.098 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) | 0.144 \(\pm\) 0.145 \(\times\) 10\(^{-2}\) |

Note: Different letters (a, b, c) indicate significant differences at \(p < 0.01\) according to the Tukey-HSD.
tomato plants (Beyersmann & Hartwig, 2008). This change is due to oxidative stress (Azad, Shiva, & Malekpour, 2011) and the inhibition of chlorophyll biosynthesis as a result of lead accumulation in the plant tissue because lead also prevents photosynthetic activity of enzymes like δ-aminolevulinic acid dehydratase (Prasad & Prasad, 1987) or decrease essential elements absorption such as Mg²⁺ and Fe²⁺ by replacing these minerals with lead in the chlorophyll (Beyersmann & Hartwig, 2008; Haider, Kanwal, Uddin, & Azmat, 2006).

Results also demonstrated reduction in chlorophyll content at higher doses of Mn than in lower doses. This seems to be the interference of Mn on iron deficiency. Excess Mn has been reported to inhibit the chlorophyll biosynthesis process through a Fe-concerning process (Fecht-Christoffers, Braun, Lemaitre-Guillier, VanDorsselaer, & Horst, 2003; Sarkar, Pandey, Sud, & Chanemougasoundharam, 2004). The same observation was reported by Lei et al. (2007). In 50 ppm Mn treatment, chlorophyll content is higher than other treatments, i.e. 0.402 mg/g of total chlorophyll because a chloroplast-localized protein binds with Mn and makes it inactive (Führs et al., 2008), but at higher concentration the pigment content decreased to 0.271 mg/g because replacement of Mg and Fe occurred in their respective porphyrin (Sideris & Young, 1949). Almost similar trend of chlorophyll reduction by mercury was reported by Mondal, Das, and Datta (2015).

### Table 2. Pigment concentration and proline content in different treatments

| Metal | Conc. (ppm) | Chl “a” (mg/g) | Chl “b” (mg/g) | Chl “a”/chl “b” | Total chl (mg/g) | Carotenoid (mg/g) | Proline (mg/g) |
|-------|-------------|----------------|----------------|----------------|-----------------|------------------|----------------|
| Cr    | 25          | 0.113 ± 0.88 × 10⁻¹ | 0.090 ± 0.88 × 10⁻¹ | 1.256 | 0.211 ± 0.88 × 10⁻¹ | 0.0035 ± 0.18 × 10⁻¹ | 0.106 ± 0.26 × 10⁻² |
|       | 50          | 0.208 ± 0.88 × 10⁻¹ | 0.108 ± 0.203 × 10⁻² | 1.926 | 0.318 ± 0.145 × 10⁻² | 0.061 ± 0.12 × 10⁻³ | 0.109 ± 0.145 × 10⁻² |
|       | 75          | 0.074 ± 0.173 × 10⁻¹ | 0.055 ± 0.58 × 10⁻¹ | 1.345 | 0.135 ± 0.115 × 10⁻² | 0.00005 ± 0.11 × 10⁻³ | 0.112 ± 0.548 × 10⁻² |
| Pb    | 25          | 0.258 ± 0.145 × 10⁻¹ | 0.111 ± 0.145 × 10⁻² | 2.324 | 0.373 ± 0.88 × 10⁻³ | 0.177 ± 0.18 × 10⁻³ | 0.109 ± 0.145 × 10⁻² |
|       | 50          | 0.245 ± 0.58 × 10⁻¹ | 0.119 ± 0.307 | 2.059 | 0.366 ± 0.115 × 10⁻² | 0.0011 ± 0.12 × 10⁻³ | 0.116 ± 0.145 × 10⁻² |
|       | 75          | 0.067 ± 0.145 × 10⁻¹ | 0.050 ± 0.145 × 10⁻¹ | 1.34 | 0.117 ± 0.028 | 0.0055 ± 0.9 × 10⁻³ | 0.128 ± 0.145 × 10⁻² |
| Mn    | 25          | 0.119 ± 0.12 × 10⁻² | 0.066 ± 0.115 × 10⁻² | 1.803 | 0.194 ± 0.88 × 10⁻³ | 0.0035 ± 0.9 × 10⁻³ | 0.135 ± 0.548 × 10⁻² |
|       | 50          | 0.269 ± 0.88 × 10⁻¹ | 0.137 ± 0.115 × 10⁻² | 1.964 | 0.402 ± 0.233 × 10⁻² | 0.0073 ± 0.18 × 10⁻³ | 0.114 ± 0.173 × 10⁻² |
|       | 75          | 0.171 ± 0.145 × 10⁻¹ | 0.098 ± 0.347 | 1.745 | 0.271 ± 0.145 × 10⁻² | 0.0059 ± 0.12 × 10⁻³ | 0.131 ± 0.203 × 10⁻² |
| Control | 0.261 ± 0.115 × 10⁻² | 0.122 ± 0.173 × 10⁻² | 1.385 ± 0.115 × 10⁻² | 0.0866 ± 0.15 × 10⁻³ | 0.088 ± 0.115 × 10⁻² |

Note: Different letters (a, b, c) indicate significant differences at p < 0.01 according to the Tukey-HSD.

3.3. Proline accumulation

Proline content, which is a significant stress indicator, increases with increasing treatment concentration, but here also, in the 50 ppm Mn treatment proline content is little lower than other treatment but higher than control (Table 2).

Proline content in the Cr-treated plants showed increment pattern with higher Cr concentration. In lower Cr concentration, proline concentration was 0.106 mg/g, but when 75 mg/g Cr was applied, proline content increased to 0.112 mg/g, whereas in control plants proline content was much lower (0.088 mg/g). Proline is known to occur in many plant species and normally accumulates in large quantities in responses to metal toxicity (Tripathi & Gaur, 2004), and also for Cr (Rai, Vajpayee, Singh, & Mehrotra, 2004). It is noted that proline has an important role in osmotic adjustment, as well as, proline contributes to the stability of the sub cellular structures to scavenge free radicals and to buffer cellular redox potential under stress conditions (Ashraf & Foolad, 2007; Molinari et al., 2007). That is why proline accumulation increased with increasing chromium stress.

Increase in proline secretion was observed along with increasing lead concentration. Under heavy metal stress, high level of proline can eliminate hydroxyl radicals, maintain osmoregulation, prevent enzyme destruction (Kuznetsov & Shevyakova, 1997), and decrease the toxic effects of lead (Alia & Saradhi, 1991).
Proline accumulation in the treated plants was also higher in 75 ppm treatment than 25 ppm treatment. An increase of up to 20-fold content of proline in the leaves of metal non-tolerant *Silene vulgaris* has been reported earlier (Schat, Sharma, & Vooijs, 1997), which suggests its beneficial functions under heavy metal stress. In general, proline has three major functions under stress condition, namely osmoregulation, metal chelation, and antioxidant defense (Hall, 2002; Wu, Chen, Wei, & Zhang, 2004). As an osmolyte, proline might replace the water deficit developed due to an exposure to heavy metals, and it has also been reported that it takes part in stomatal closure to restrict metal uptake and translocation (Rajagopal, 1981).

### 3.4. Metal accumulation

The uptake and accumulation of lead, manganese, and chromium in different parts of the Gram seedlings are shown in Figures 1–3. From the result, it can be found that maximum accumulation of
chromium occurs in roots followed by shoots, and least in leaves which means that maximum amount of Cr is accumulated in the roots and residual amount is transported to the shoots and the leaves. Similar results were also reported in the case of wheat (Sharma & Sharma, 1996), corn (Sharma, Sharma, & Tripathi, 2003), cabbage (Lahouti & Peterson, 1979) and black bean (Karuppanapandian & Manoharan, 2008). Gupta et al. (2011) also showed higher amount of Cr was accumulated in the roots of aquatic macrophyte than shoots and leaves. Roots contain some low molecular weight proteins like phytochelatins which bind with the Cr ions and inhibit its translocation to shoots and leaves (Gupta et al., 2011).

The compartmentalization of heavy metals solely depends on variability of heavy metals and plant types. In the present experiment, it has been shown that most of lead accumulates in the roots than in shoots and leaves (Figure 2). However, very recently Mondal et al. (2015) demonstrated that maximum amount of mercury can accumulate in the shoot than root. On the other hand, maximum lead accumulation occurs in root, not in shoot and leaves. This means that the amount of lead absorbed by roots, bioaccumulated in the roots and cannot be transported to the other parts of the

| Concentration | Root | Shoot | Leaf |
|---------------|------|-------|------|
| 25 ppm        | ![a](image1.png) | ![a](image2.png) | ![b](image3.png) |
| 50 ppm        | ![a](image4.png) | ![a](image5.png) | ![c](image6.png) |
| 75 ppm        | ![a](image7.png) | ![a](image8.png) | ![c](image9.png) |
plants. Dahmani-Muller, van Oort, Gélie, and Balabane (2000) and Akinci et al. (2010) found similar results in different plants. This is due to different root tissues like endodermis (Ghani, 2010) act as barriers to apoplastic and symplastic lead transport and hence Pb transportation to shoot and leaves gets restricted (Trivedi & Erdel, 1992). Roots are very good storage for lead (Azad et al., 2011), lead adheres to the cell wall of the roots in pyrophosphate form (Marschner, 1995). In lower concentration (25 ppm) of Mn treatment, most of the metal accumulates in the root, but in higher concentrations, Mn distributed almost equally in root, shoot, and leaves. Führs et al. (2008) showed that chloroplast is a main target of Mn accumulation. In this experiment, a huge amount of Mn accumulates in the leaves where high concentration of metal was applied. There was a measurable amount of Mn in the shoots also. The main part of Mn accumulation in plant is the shoot (Millaleo, Reyes-Díaz, Ivanov, Mora, & Alberdi, 2010) where transportation of this metal from roots occurs through xylem, but it is immobilized in the phloem (Page & Feller, 2005).

There was an interesting observation that all the parameters showed higher result for 50 ppm Mn treatment than other treatment even than that of control. To some extent, manganese is essential for plant growth, by taking part in several metabolic processes, mainly in photosynthesis and as an enzyme antioxidant-cofactor (Millaleo et al., 2010). But excess of manganese can cause toxicity to plants. There are two phases in the process of manganese uptake by roots. The initial rapid phase is reversible and non-metabolic, where free exchange of manganese occurs with rhizosphere (Millaleo et al., 2010). In this phase, Mn$^{2+}$ is adsorbed by the negatively charged cell wall constituents of the root cell apoplastic spaces (Humphries, Stangoulis, & Graham, 2007). The second phase is a slow process, where Mn$^{2+}$ being less readily exchanged and its uptake into the symplast is dependent on plant metabolism (Maas & Moore, 1968), although the exact mechanisms are not clear (Humphries et al., 2007).
3.5. Micrograph

From the micrograph pictures (Figures 5–7) deformities are found in root, shoot, and leaf in comparison with control. In all the treatments, the hexagonal structure of the root and shoot cells became ruptured, where as clear hexagonal structure can be observed in case of control. In 25 ppm Cr concentration, the root and shoot cells were little ruptured but in higher concentration, the cell structure was completely damaged. No clear hexagonal structure was observed in root and shoot cells in 75 ppm Pb and Mn treatment also. In case of leaf too, there was clear structural changes in the stomata. Clear stomatal structures were observed in control plants but the stomata were ruptured in structure and were closed when exposed to high concentration of all the three heavy metals. Such damages are well documented in *C. arietinum* L. under cadmium stress (Mondal et al., 2013) and nodule ultra structural deformation by mercury in *Vigna radiata wilczek* L. (Mondal et al., 2015).

4. Conclusion

From the entire experiment, it was noted that with increasing metals (Cr and Pb) concentrations from 25 ppm to 75 mg/L in treatment pot decreased both root and shoot length along with root and shoot biomass. However, Mn showed little improvement in all growth physiological parameters at 50 ppm. Biochemical parameters also revealed that both Cr and Pb reduced 64.94 and 69.61% total chlorophyll, respectively, with respect to control. Chlorophyll “a” to “b” ratio was highest in Mn followed by Cr and Pb at higher concentration (75 ppm). Metal accumulation pattern indicate that Cr is less accumulated in root shoot and leaf compare to Mn and Pb in all the studied concentrations. However, the accumulation of Mn in shoot always higher compared to Pb in all studied concentrations. Ultrastructural damage was recorded highest for Cr in root, shoot and leaf and both the 25 and 50 ppm concentration. However, at 75 ppm Pb showed highest deformation for root and leaf.
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