

A Study on the Uses of Hyperaccumulator Plants to Remove Heavy Metals from the Polluted Soil

Yee Yee Nwe¹, Khin Myo Thwe², Thet Phoo Wai³, Myat Myat Moe⁴

Abstract

Heavy metals in the plants resources we used everyday are becoming increased toxicity and diseases in Myanmar. In this study, the seedlings of maize were treated with equal amount of cadmium (Cd), Lead (Pb) and Zinc (Zn) in the same concentration of 2 g/ 3 kg soil in separate plastic bags. After treating the maize seedling for 14 days and 28 days, they were subjected in the estimation of morphological as well as anatomical investigation. These experiments were conducted in triplicate and mean values were shown in Table and Figure. The effects of heavy metals on the plant parts and anatomical changes were done by using hand section cutting methods and research microscope. The results were compared to those of control plants and noted the changes in morphology and anatomy such as increase in size of cortex cells, decrease in size of central cylinder, smaller size of protoxylem and metaxylem. Similarly, changes in shape and size of epidermis, ground tissues and vascular tissues were also detected in the roots, stems and leaves of test plant seedlings.

Keywords: Anatomical changes of heavy metals (Cd, Pb and Zn) in *Zea mays* L.

Introduction

Contamination of the environment by heavy metals has been increased sharply at the beginning of the 20th century, as a result of industrial revolution and excessive population growth, posing major environmental and human health problems worldwide (Abdelhafez and Li, 2014). Several contamination sources contaminated large areas over the world, i.e., emissions from waste incinerators, car exhaust, residues from mining and military activities, smelting industry and the use of agricultural amendments (sludge or urban composts, pesticides, and mineral fertilizers (Abou-Shanabet *et al.*, 2011; Abdelhafezet *et al.*, 2012). Unlike organic contaminants, heavy metals are not biodegradable, and pose a critical concern to living organisms and the environment through their action as carcinogenic and mutagenic compounds (Wu *et al.*, 2018). Phytoextraction relies on plants with a high capacity to absorb heavy metals and remove them from the soil. The objective of this study was to analyze the potential of *Zea mays* L. for phytoextraction of Cadmium, Lead and Zinc contaminated soil. Heavy metals use of sewage sludge, compost, mining waste, chemical fertilizers and industrial development without control outputs, resulting accumulation of heavy metals in agricultural lands that has remained in the soil for many years. (Alloway *et al.*, 1991).

The increasing use of wide variety of heavy metals in industries and agriculture has caused a serious concern of environmental pollution (Sinha *et al.*, 2010). Phytoremediation is a promising new method that uses green plants to assimilate or detoxify metals and organic chemicals. The phytoremediation of metal-contaminated soils offers a low cost method for soil remediation and some extracted metals may be recycled for value (Chaney *et al.*, 1997). A hyperaccumulator is a plant capable of growing in soils with very high concentrations of metals, absorbing these metals through their roots, and concentrating extremely high levels of metals in their tissues (Rascio *et al.* 2011). Over 500 species of flowering plants have been identified as having the ability to hyperaccumulate metals in their tissues (Sarma *et al.*, 2011).

¹Lecturer, Department of Botany, Dagon University

²Assistant Lecturer, Department of Botany, Dagon University

³Demonstrator, Department of Botany, University of Medicinal “1”

⁴Professor and Head, Department of Botany, Dagon University

Hyperaccumulating plants hold interest for their ability to extract metals from the soils of contaminated sites (phytoremediation) to return the ecosystem to a less toxic state. The plants also hold potential to be used to mine metals from soils with very high concentrations (phytomining) by growing the plants then harvesting them for the metals in their tissues. These hyperaccumulation genes (HA genes) are found in over 450 plant species, including Brassicaceae, Poaceae and Asteraceae (Rascio *et al.*, 2011).

Contaminated soils pose a major environmental and human health problem, which may be partially solved by the emerging phytoremediation technology. Phytoremediation involves raising of plants hydroponically and transplanting them into metal-polluted soil and water where plants absorb and concentrate the metals in their roots and shoots. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal. Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Salt *et al.*, 1997). Phytoremediation technology is using plants to clean up contaminated sites and that is a promising loom to restore the environment and ecosystem. Pollution of the environment with toxic organic and heavy metal pollutants is one of the major problems that developed and developing nations are facing today. Large areas of land can be contaminated with Cd by anthropogenic activities such as mining and mineral processing of metallic ores, waste disposal, phosphate fertilizer application and wastewater irrigation. Soil Cd contamination is a great threat to human health since Cd is easily extracted by plants from the environment compared with other non-essential elements, and transferred to human food chain from the soils (Xiao *et al.* 2008). Cadmium is a ubiquitous non-essential element that possesses high toxicity and is easily accumulated from the environment by organisms (Rahimi and Nejatkhani 2010). Peer *et al* (2005) state that plants used for phytoremediation must have extraordinary ability to accumulate the contaminant known as hyper accumulators and also the use of tolerant plant. According to the above facts, the study is aimed to survey of preliminary hyperaccumulation plant, to study histological analysis of *Zea mays* L. to absorb and accumulate heavy metals, to evaluate morphological responses to varying soil metal contents and to deliver an important tool for bioremediation of heavy metal contamination in soil.

Materials and Methods

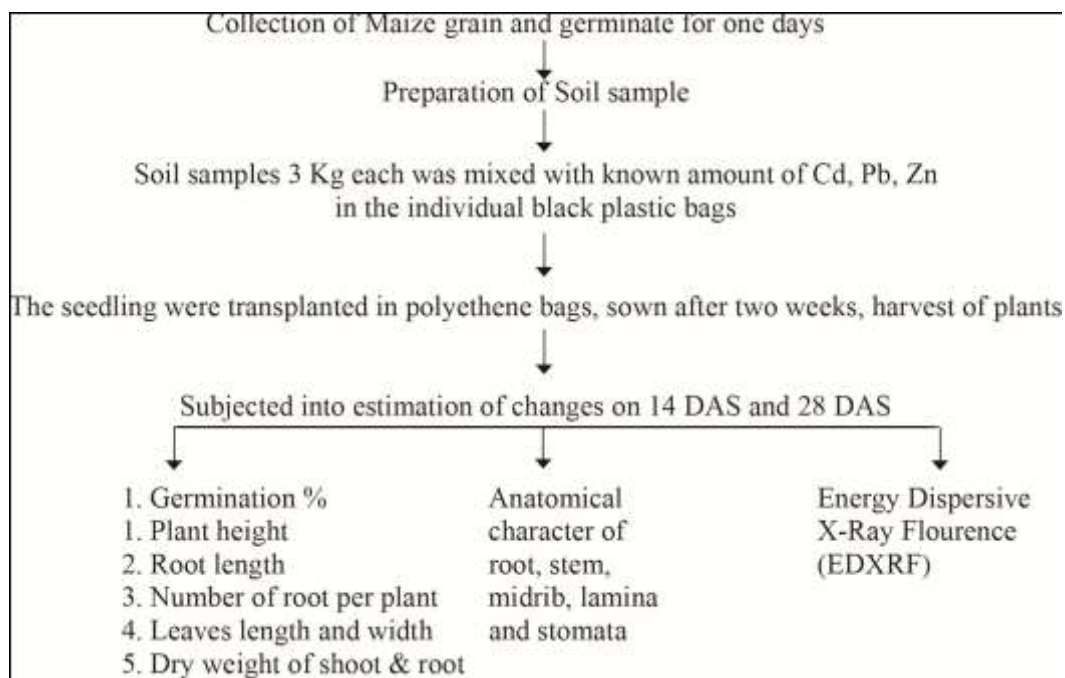


Figure 1 Flow Sheet of Scope Works

Results

Effect of heavy metal Cd, Pb and Zn on the structure *Zea mays L.*

After the experiments, the root and shoot weights of heavy metals treated plants were decreased than control. Plant heights of maize plants were found to be measured by Pb and Zn. But, the impact of Cd made shorter than control. In the case of No. of root per plants, it was observed, except pbdecrease root per plant in the tested plants. More or less equal plants height was estimated in the test plant. In the study of leaves length and width, all test plants were recorded to be smaller than control. The anatomical of root and the thickness of cortex in the test plant were recorded to be thicker than the control but diameter of central cylinder became decreased. In the case of pericycle and vascular bundle, the significant changes were confirmed under the microscope (Figure 5). Similarly, the size of epidermis, hypodermis, parenchyma ground tissue and no. of vascular bundles were significantly recorded to be increased in the stem of Pb treated plants. If compared to control, in the leaves tissue, the epidermis effect of heavy metals was prominently found to be smaller than control in upper epidermis and vascular bundle. But, all the xylem cell became increased due to the impact of heavy metal. In ground tissue, larger parenchyma was not to be distinct except Pb treated plant. But smaller parenchyma ground tissues were definitely larger than those of control. According to the (Figure 9), it can be concluded that all the tissues were increased in sizes because of the effect of heavy metals. Similar condition as above, the conditions in stomata were detected to be smaller than that of control. Finally, the concentrations of elements in the treated plant parts were detected by applying EDXRF. As shown in the (Figure 16, 17 and 18), heavy metals can be detected in root and shoot of Maize plant. But, the higher presence of Zn was confirmed in the EDXRF results. According to Qadiret *al.*, (2004) and Anjumet *al.*, (2007) the toxicity of heavy metals could change the physiological, morphological and anatomical properties. Mildvan (1970) was reported that these heavy metals in minute amount may support that metabolism of plant cells. But, too much concentration may intoxicate and interferes with cell biochemical reactions and become toxic to plants. In the conclusion, all the above results clearly pointed out that the effect of three heavy metals (Zn, Cd, and Pb) clearly changed the morphological as well as anatomical characteristic of Maize plant.

Expected Outcome

- To know that heavy metals were absorbed from contaminated soil through roots and stem and deposited in aerial parts.
- To study on the uses of Hyperaccumulator Plants to remove heavy metals from the polluted soil.
- Local farmer informed awareness of some edible plants that can cure as bioremediation for problem of soil contamination.



Figure2 Preparation of soil sample and seeds sowing

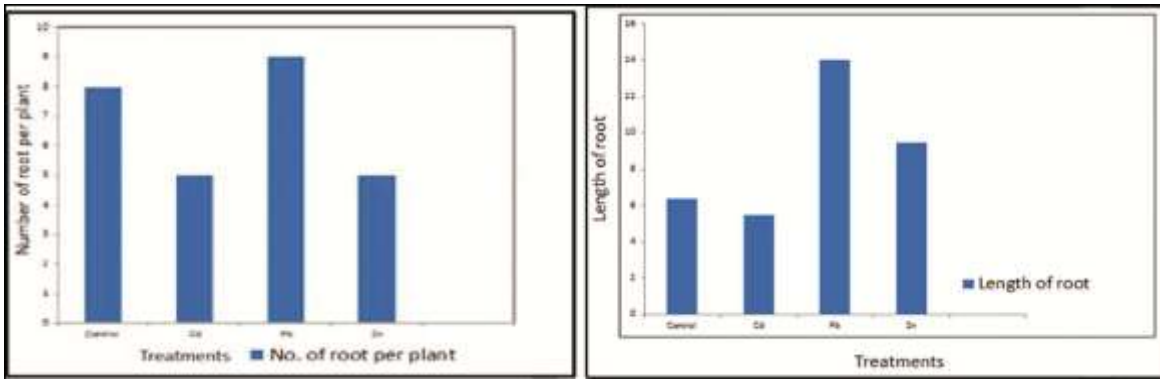


Figure3 Effect of Heavy Metals (Cd, Pb and Zn) on length of root and Number of Root per Plant (*Zea mays L.*)

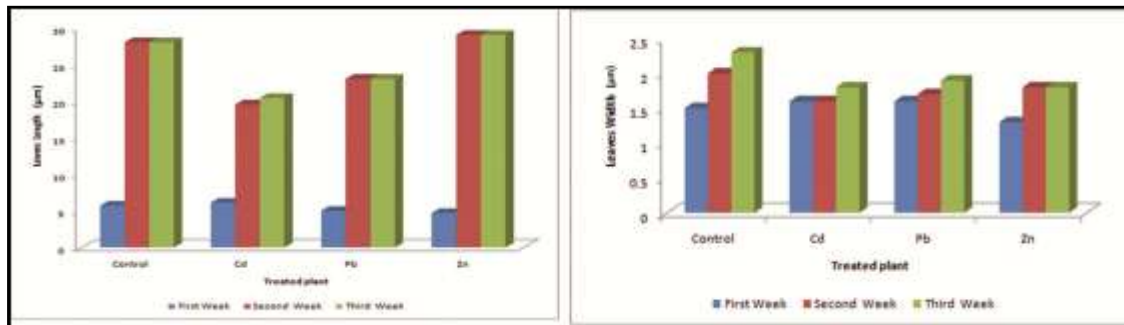


Figure 4 Effect of Heavy Metal (Cd, Pb and Zn) on Leaves length and width of Maize (*Zea mays L.*)

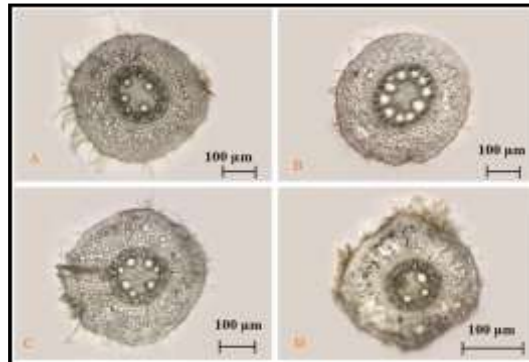


Figure5 T.S of root of *Zea mays L.*
A – Control B–Cd C – Pb D – Zn

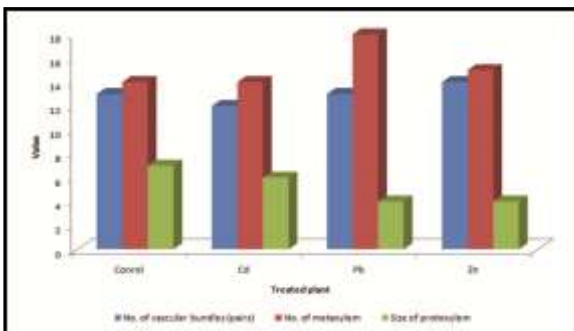


Figure 6 Effect of Heavy Metals (Cd, Pb and Zn) on the number of vascular bundle, metaxylem and protoxylem on Root

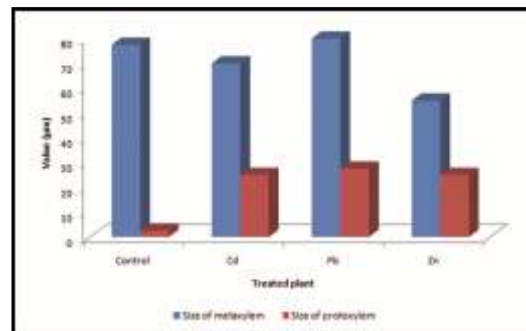


Figure 7 Effect of Heavy Metals (Cd, Pb and Zn) on diameter of metaxylem and protoxylem diameter Root

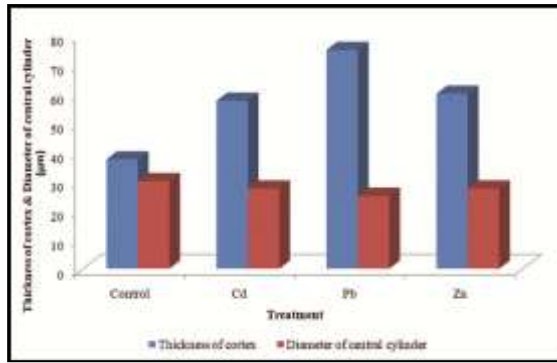


Figure 8 Effect of Heavy Metals (Cd, Pb and Zn) on cells layer of cortex and Diameter of central cylinder Root

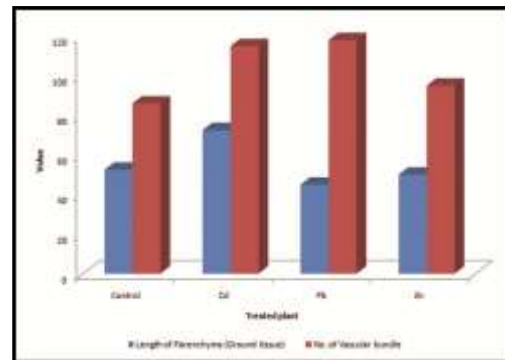


Figure 9 Effect of Heavy Metals (Cd, Pb and Zn) on diameter of parenchyma (Ground tissue) and No. of vascular bundle on Stem

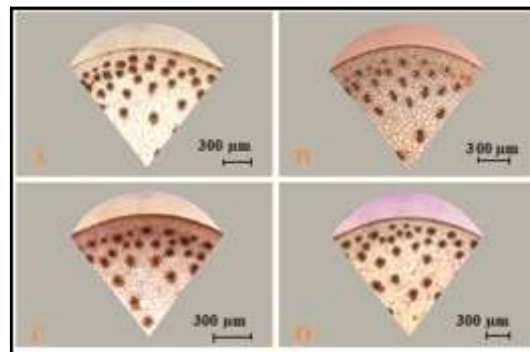


Figure 10 T.S of stem of Zea mays L.
A – Control B–Cd C – Pb D – Zn

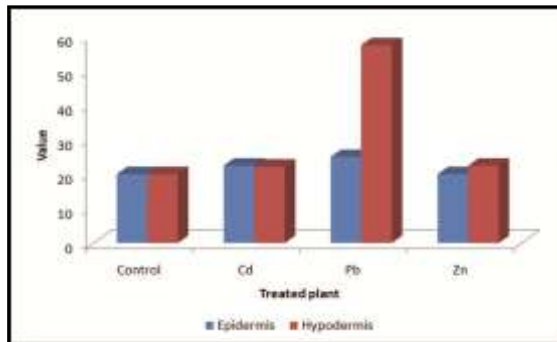


Figure 11 Effect of Heavy Metals (Cd, Pb and Zn) on diameter of cells of epidermis and hypodermis on Stem

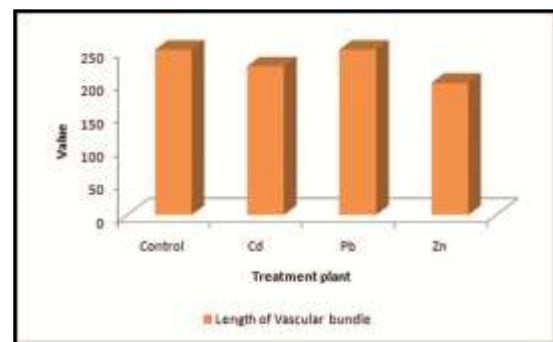


Figure 12 Effect of Heavy Metals (Cd, Pb and Zn) on diameter of vascular bundle on midrib of Maize



Figure 13 Transverse section of midrib and lamina

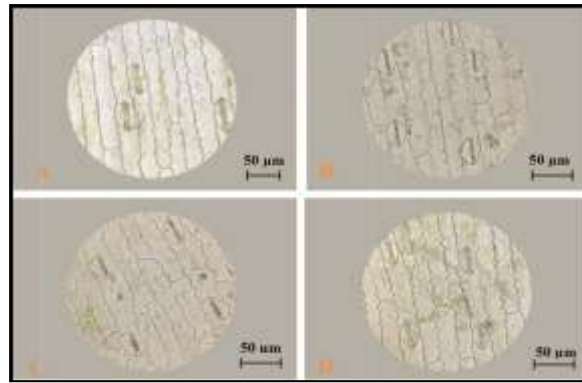


Figure 14 T.S of stomata of Zea mays L.
A – Control B–Cd C – Pb D – Zn

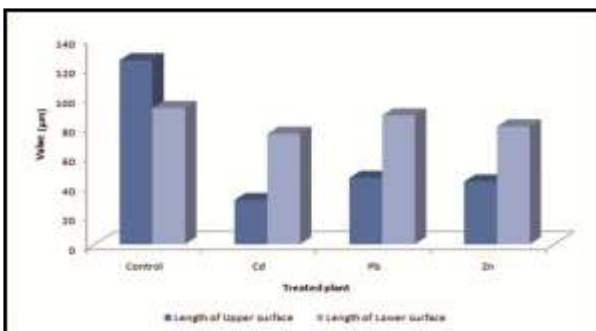


Figure 15 Effect of Heavy Metals (Cd,Pb and Zn) size of stomata of Upper and Lower surface on Maize

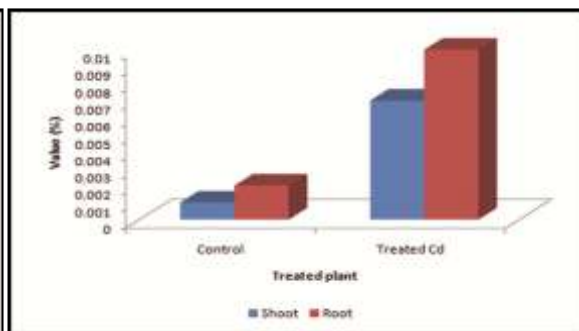


Figure16 Concentration of heavy metals contents (Cd) in the shoot and root treated plants after elemental analysis of EDXRF

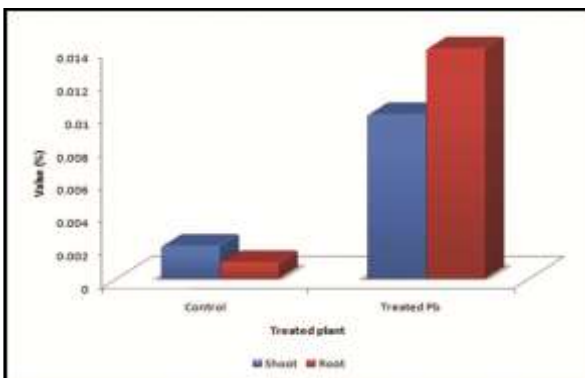


Figure17 Concentration of heavy metals contents (Pb) in the shoot and root treated plants after elemental analysis of EDXRF

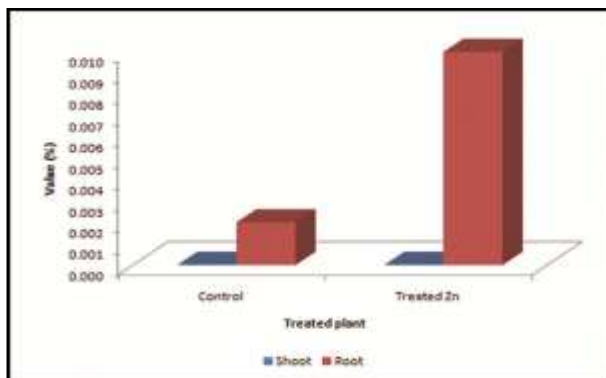


Figure18 Concentration of heavy metals contents (Zn) in the shoot and Root treated plants after elemental analysis of EDXRF

Discussion and Conclusion

Due to the industrial development and increased number of factories and automobile. the contamination of soil with heavy metals becomes widespread cadmium. (Cd), Lead (Pb) and Zinc(Zn), Mercury (Hg) are most common and very metals in the populated areas where

there were crowded with new industrial zone (Su *et al.* 2005). Although above heavy metals were considered to be non-essential for metabolic processes, these ions were easily absorbed by root of graminaceous plants. Small amount of toxicity systems was shown in anatomical structures of roots, stems and leaves of treated plants. In this investigation, the effects of Cd, Pb and Zn at the concentration of 0.667 $\mu\text{g}/\text{mg}$ soil were treated into soil and ten seeds of maize grains were sown in each plastic bag. Anatomical structures given by the stress of heavy metals were studied under the Olympus Microscope at 40 x10 magnification and results were shown in respective figures. When compared to the number of adventitious roots among the control with treated plants were appear in Pb treated plants (9 roots). But, fewer number was observed in the maize seedling, where there were 8 roots in control plant.

But, the longer root lengths were provided by Pb and Zn treated plant with the value of 7.0cm and 2.5cm where only 6.4cm long roots were recorded in control. Shorter root length of 5.5cm produced in Cd treatments is the persistent pollutant in own environment. It is a kind of heavy metal of anthropogenic origin and always accumulated in soils, sediments and water. Pb has no biological function and very toxic plants and animals. Although Pb is not an essential element, some plant species proliferate in Pb- contaminated soil and accumulate it in different plant parts. According to Arey and Sarkan (2012), roots are first organs to be in contact with the ions of heavy metal ions in the rhizosphere. These toxic ions can inhibit the elongation of roots due to the interference of heavy metals in the cell division processes. In 2009, Kozhevrikove *et al.*, reported that Pb ion may shorten the length of roots in *Zea mays*L. Similarly, Eunet *al* (2000) discovered that Pb treatment to maize plants resulted the accumulation of Pb ions in meristem and associated with changes in microtubule organization. In the present work, it was observed that the heavy metal ions made the cortex cell thicker than that of control as shown in (Figure 5). But, smaller central cylinder or vascular bundle was detected in heavy metal treated plants. The cell shape of pericycle and number of vascular bundle were not seriously changed.

The pronounce changes of size of metaxylem was recorded as the smaller vessels in Pb and Zn treated roots (70 x 77.5 μm) and (55 x 57.5 μm) in the control plant metaxylem is 77.5 x 82.5 μm . So, it can be regarded as the heavy metals shrink the size of metaxylem. Similar trend of decrease in size was also recorded in protoxylem. In the pith, although the Cd treated root was smaller control, the larger parenchyma pith cells were observed in Pb and Zn treated plants than control. In the case of stems, the shape and size of epidermal cells in control were circular and 20 x 25 μm . Smaller epidermal cells were observed in Zn treated plants and larger cells of rectangular shaped epidermis were found in Cd and Pb treated stems. In the control, there were 3 layers of hypodermis/cortex in the control as well as in Pb treated stems. Only 2 layers were seen in the stems of Cd and Zn treated plants. When compared to the parenchymatous ground tissues, large cells were recorded in Cd treated stems with 72.5 x 67.5 μm compared to 52.5 x 50 μm seen. When the number of vascular bundles was counted in control, 86 vascular bundles were recorded in control, 115 vascular bundles in Cd treated plant stem, 118 vascular bundles in Pb treated stem and 95 vascular bundles in Zn treated stem.

Moreover, all the sizes of vascular bundles were larger than that of control except Zn treated plant stems as revealed in (Figure 10). When the T.S of midribs were comparatively studied (Figure 13), the epidermal cell of treated distinctly reduced in size more than control plant. Parenchymatous mesophyll increased in size. The size of vascular bundle in the midrib became larger in Pb treated leaves, but smaller in Cd and Zn treated leaves. The size of metaxylem was significantly large in heavy metal treated leaves. The same results of increased sizes were also recorded Cd and Pb treated leaves except the same size as control in Zn treated maize leaves. It was concluded that the stress of heavy metals affected on maize plants depended on the parts of plants.

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