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Effect Of Copper And Cadmium On Germination And Anatomical Structure Of Leaf And Root Seedling In Maize (*Zea mays* L)

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Abstract: The aim of this work was to study the effect of copper (Cu) and/or cadmium (Cd) on grain germination and anatomical structure of Maize (*Zea mays* cv. Giza 2) seedling. The results indicated that Cu and/or Cd caused reduced the grain germination indices (FGP final germination percentage, MDG mean daily germination, GI germination index and CVG coefficient of velocity of germination), root anatomical traits as root diameter, central cylinder diameter, cortex thickness, cross section area of root and cross section area of central cylinder and leaf structures traits as thickness of midrib, upper epidermis and parenchyma and vascular bundle, in contrast they increased mean germination time MGT. The most inhibitory effect on grain germination indices and root anatomical traits was seen in Cd (150 µmol/L), but the highest increase in MGT was seen in a combination between Cu and Cd. The combination between Cu and Cd leads to a reduction in Cd toxicity when compared with the same concentration in Cd alone. It can concluded that both Cu and Cd either each alone or combined are toxic on maize, Cd is more toxic than Cu and the combination of Cu with Cd decreased its toxicity.

Key words: Maize; cadmium; copper; toxicity; germination; root anatomy; leaf anatomy.

INTRODUCTION

Indiscriminate in human activities such industrial products and using synthetic materials leads to drastically increase in concentration of different heavy metals in the environment (Moosavi *et al.,* 2012). Heavy metal toxicity is one of the major abiotic stresses leading to hazardous effects in plants (Hossain *et al.,* 2012).

Cadmium (Cd) pollution is regarded as one of the most harmful or toxic environmental issues that mainly resulted from mining, use of phosphatic fertilizers, sewage sludge and untreated waste water (Kováčik *et al.,* 2006), concentrations of Cd in agricultural soils have posed a significant threat to safe crop production and have therefore become a global concern (Nedjoud *et al*., 2012 and Soudeh and Zarinkamar, 2012). While copper (Cu) is widely prevalent and was considered as an essential element for all living organisms including plants but is toxic when present in excess. Cu occurs in the environment as hydrated ionic species, forming complex compounds with inorganic and organic legends (Singh *et al.,* 2007).

The limited information is available on the effects of heavy metal mixture on plant species (Houshmandfar and Moraghebi, 2011). The association of Cu and Cd in environment and their chemical similarity can lead to interaction between these two ions, resulting in the lowering of Cd toxicity (Gussarson *et al.,* 1995). Cd was somewhat more toxic than Cu; moreover, the combined effects of Cu and Cd provided a negative synergistic effect on grain germination (Michael Hogan, 2010).

Heavy metal toxic effect on the plants may cause alteration in their metabolic pathways, such as photosynthesis, respiration, growth and modifying plant anatomy (Gomes *et al.,* 2011). Grain is a developmental stage that is highly protective against external stresses in the plant life cycle (Sharma *et al.,* 2011). However, during germination and subsequent seedling stage they become stress sensitive. Responses of plants to adverse environmental conditions during grain germination and early seedling growth are considered critical for raising a successful agriculture crop under stress conditions (Aery and Sarkar, 2012). The inhibitory effect of heavy metals on grains germination could be the result of heavy metals toxicity or it could be the result of osmotic effect produced by the heavy metals (Burhan *et al.,* 2004). While Cu solutions significantly inhibit corn grain germination compared to the untreated control (Radoviciu *et al.,* 2009), the toxicity of Cu is higher if concentration increases. Generally, studies of Cu and Cd exposure indicated no adverse effects for cation concentrations of up to 0.01 mg/liter, however with increasing concentrations in the root zone, there was a steady decline in germination rates (Agarwal, 2009).

A few studies are carried out to date to investigate the impact of heavy metals on one or a few anatomical parameters of the plant (Khudsar *et al.,* 2001, Panou-Filotheou and Bosabalidis, 2004 and Kasim, 2006). Moreover, the understanding of the influence of toxic elements on root anatomy and element distribution is still limited (Vaculik *et al.,* 2012).

The aim of present work was to study effect of Cu and Cd either single or combined on germination and anatomical structure of Maize (*Zea mays* cv. Giza 2).

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MATERIAL AND METHODS

The present research work was conducted at the Biotechnology Lab, Biological Science Department, Faculty of Science, North Jeddah, KAU. Grains of Maize (*Zea mays* L.) cultivar Giza 2 were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. To study the different toxicity levels of Cu, Cd either single or in its combinations on germination and anatomical structure of Maize. The grains were kept at room temperature and each plastic pot (14 X 17 cm) was filled up with 0.5 kg of sand and Petmous at 1:1 ratio. Different concentrations of Cu (75 or 150 µmol/L) as $CuSo_4.5H_2O$, Cd (75 or 150 µmol/L) as $3CdSo_4.8H_2O$ and combinations 37.5 or 75 µmol/L were applied with sowing. The pots trials were conducted in growth chamber with using a completely randomized design with three replications and 10 grains per pots were used in this study. The pots were maintained in growth chamber under 16/8 hrs light/dark, photoperiod with 100 µmol $m^{-2}s^{-1}$ illumination at 25 ± 1^0C. After germination the seedling were irrigated with tap water every 2-3 days as control treatment, different concentrations of Cu, Cd and its combinations.

Number of germinated grains was recorded 8 days after planting as Final Germination Percentage (**FGP**) according to ISTA (1993 and 1999) where **FGP= Ng / Nt x 100,** Ng=total number of germinated grains, Nt=total number of grains evaluated. Mean Germination Time (MGT) was calculated according to Sadeghi *et al*., (2011). The Germination Index (**GI**) was calculated as described in the Association of Official Grains Analysts (**AOSA, 1983**) by following formula: **GI=no. of germinated grains/days of first count+…..+ no. of germinated grains / days of final count.** Coefficient of Velocity of Germination (CVG) determined by a mathematical manipulation **CV=ΣNi / ΣNiTi x 100** according to Scott *et al.,* (1984). Mean Daily Germination (MDG) which is index of daily germination was calculated from the following equation **MDG=FGP / d, FGP** is final germination percentage and **d** is days to the maximum of final germination.

For studying the various tissues in transverse sections of the primary root at middle part and third leaf at middle of leaf, samples were taken after 4 weeks from the sown. They were killed and fixed in FAA solution, (50 % alcoholic base), dehydrated and cleaned with ethyl alcohol and xylene respectively then infiltrated and embedded in pure paraffin wax (M.P. 56-58^o C) as described by Willey (1971). Sections of twenty microns thick were cut by Rotary microtome, stained with safranin and light green, mounted in D.P.X. then microscopically examined. The examination, measurements and photographing were done by Microscope Leica DMLS and digital camera DC300F with image recording and processing system IM50.

Statistical Analysis:

Results were expressed as mean ± SD (standard deviation). All data were subjected one way completely randomize ANOVA analysis to calculate the least significant difference (LSD) at $p<0.05$ with Costat computer program.

RESULTS AND DISCUSSION

a- Effect Of Heavy Metals On Germination Parameters:

The result indicated that Cu and/or Cd treatment was accompanied with a significant reduction in all grain germination indices (FGP, MDG, GI and CVG) in *Z. mays* cv. Giza 2, in contrast they increased MGT. The most inhibitory effect on grain germination indices was seen in Cd (150 µmol/L), but the highest increase in MGT was seen in a combination between Cu and Cd (Table 1). The decrease in grain germination of plant can be attributed to the accelerated breakdown of stored food materials in grain, by the application of heavy metal. Reduction in grain germination can also be attributed to alterations of selection permeability properties of cell membrane (Houshmandfar and Moraghebi, 2011). The percentage of grain germination was decreased as a result of Cd concentration increased in Canola, Wheat and Safflower (Moosavi *et al.* 2012), *Leucaena leucocephal* (Muhammad *et al.,* 2008), *Salicornia brachiata* (Sharma *et al.,* 2011), Safflower (Houshmandfar and Moraghebi, 2011). Moreover, Radoviciu *et al.,* (2009) indicated the same trend for Cu on corn grain germination.

In this study Cd was more toxic than Cu, moreover in combination Cu reduced Cd toxicity. This may be attributed to Cu inhibited Cd uptake and accumulation by plant (Lux *et al.,* 2011). The additional supply of Cu with Cd causes a certain degree of recovery in plant growth and the toxicity (Ali *et al.,* 1998). Also, Gussarson *et al.,* (1995) found that the concentration of Cd in root was decreased by Cu when its concentration was equal to or exceeded the concentration of Cd.

b- Effect Of Heavy Metals On Structure Of Root:

The results indicated that Cu and /or Cd lead to a significant decrease in root diameter, central cylinder diameter, cortex thickness, cross section area of root and cross section area of central cylinder. The highest decrease was seen in Cd (150 µmol/L). The combination between Cu and Cd leads to a reduction in Cd toxicity when compared with the same concentration in Cd alone (Table 2 and Figure 1). All treatments lead to a significant decrease in diameter of metaxylem elements except Cu and Cd combination (75 µmol/L) and in number of row cortex except Cu in both concentrations. The concentrations of Cu (75 µmol/L) and Cd (75 µmol/L) lead to a significant decrease in root/cylinder diameter and Cd (150 µmol/L) in diameter of protoxylem elements. On the other hand, Cu (150 µmol/L) alone or with Cd showed a significant increase in number of metaxylem elements. As well as Cd (150 µmol/L) alone or with Cu increased number of protoxylem element. At the same time, Cu at 75 µmol/L lead to a significant increase in root diameter/cortex thickness.

Table 1: Effects of different concentrations of Cu and/or Cd on Maize (*Zea mays* cv. Giza 2) germination.

Parameters / Treatments (µmol/ L)		Final Germination Percentage (FGP)	Mean Daily Germination (MDG)	Germination Index (GI)	Mean Germination Time (MGT)	Coefficient Velocity Germination (CVG)
	Control	96.67 ± 5.77^{a}	17.22 ± 2.55^{a}	2.11 ± 0.21^{a}	1.70 ± 0.26^{c}	0.60 ± 0.10^{a}
Cu	75	73.33 ± 5.77^{b}	11.03 ± 0.90^{b}	1.42 ± 0.12^{b}	2.36 ± 0.21^{b}	0.43 ± 0.04^{bc}
Cu	150	66.67 ± 5.77^{bc}	9.11 ± 0.78^{bc}	1.31 ± 0.14^{c}	2.39 ± 0.54^{b}	0.43 ± 0.09^{bc}
Cd	75	53.33 ± 5.77^{de}	8.10 ± 1.65^{c}	1.03 ± 0.17^{c}	2.33 ± 0.31^{b}	0.43 ± 0.06^{bc}
Cd	150	46.67 ± 11.55^{e}	7.43 ± 1.51^{c}	0.97 ± 0.19^{c}	1.94 ± 0.42^{bc}	0.53 ± 0.12^{ab}
Cu + Cd	75	63.33 ± 5.77^{bcd}	8.27 ± 0.68^{c}	1.05 ± 0.05^{c}	3.25 ± 0.29^{a}	0.31 ± 0.03^{c}
Cu + Cd	150	60.00 ± 0^{cd}	8.21 ± 0.62^{c}	0.99 ± 0.04^{c}	3.22 ± 0.25^{a}	0.31 ± 0.02^{c}
LSD at 0.05		11.46	2.45	0.26	0.60	0.13

The values are means ± SD
Deferent letters indicated significant difference from the control and between treatments at $p \leq 0.05$.

Roots are the first organs to be in contact with the toxic metal ions and the inhibition of root elongation under the influence of heavy metals may be due to metal interference with cell division which may include inducement of chromosomal aberrations and abnormal mitosis and cell elongation (Radha *et al.*, 2010 and Aery and Sarkar, 2012). A number of studies reported that reduction in root growth may be due to a decrease in cell division that led to increase the thickness of cell wall, and or a disorder in the activity and contents of phytohormones like auxin in the roots exposed to heavy metals (Schilcher *et al.,* 2005, Sharma and Dietz, 2006 and Soudeh and Zarinkamar 2012). In addition to the accelerated development of the endodermis and exodermis, many other aspects of root anatomy are altered when roots are exposed to elevated Cd and Cu concentrations in the rhizosphere (Seregin and Kozhevnikova, 2008).

Table 2: Effects of different concentrations of Cu and/or Cd on primary root structure of Maize (*Zea mays* cv. Giza 2).

Parameters / Treatments (µmol/ L)		Root diameter µm	Central cylinder diameter µm	Cortex thickness µm	number of rows cortex	Root/cylinder diameter	Root diameter/ Cortex thickness
	Control	1160 ± 20^{a}	535 ± 3^{a}	312.5 ± 8.5^{a}	9.67 ± 0.58^{a}	2.17 ± 0.03^{abc}	3.71 ± 0.04^{bcd}
Cu	75	$1065.\pm 13.23^{b}$	511 ± 11.14^{b}	277 ± 1.5^{b}	8.67 ± 1^{ab}	2.08 ± 0.02^{d}	3.85 ± 0.04^{a}
Cu	150	945 ± 16^{d}	444 ± 13.75^{d}	250.5 ± 1.8^{d}	9 ± 1^{ab}	2.13 ± 0.03^{cd}	3.77 ± 0.05^{b}
Cd	75	996 ± 19.08^{c}	459 ± 5.29^{d}	268.5 ± 7^{c}	8 ± 0.58^{b}	2.08 ± 0.02^{d}	3.71 ± 0.03^{bcd}
Cd	150	870 ± 11.36^{e}	398 ± 8^{f}	236 ± 2.5^{e}	8 ± 1^{b}	2.19 ± 0.02^{ab}	3.69 ± 0.03^{cd}
Cu + Cd	75	1042 ± 16.52^{b}	485 ± 10.58^{c}	278.5 ± 3.5^{b}	8 ± 1^{b}	2.15 ± 0.02^{bc}	3.74 ± 0.03^{bc}
Cu + Cd	150	921 ± 14^{d}	417 ± 13.53^{e}	252 ± 0.5^{d}	8 ± 1^{b}	2.21 ± 0.02^{a}	3.65 ± 0.05^{d}
LSD at 0.05		28.03	17.62	7.98	1.58	0.05	0.07
		Cross section area of root $(mm)^2$	Cross section area of central cylinder $(mm)^2$	Number of metaxylem elements	Number of protoxylem elements	Diameter of metaxylem elements µm	Diameter of protoxylem elements µm
	Control	1.057 ± 0.036^{a}	0.225 ± 0.003^{a}	5.33 ± 0.58^{cd}	15 ± 1^{cd}	96.33 ± 4.16^{a}	36 ± 6.08^{a}
Cu	75	0.891 ± 0.020^{b}	0.205 ± 0.008^{b}	6.33 ± 1^{bc}	15 ± 1^{cd}	77.33 ± 8.08^{bc}	34 ± 7^{ab}
Cu	150	0.702 ± 0.025^{d}	0.155 ± 0.010^{d}	$7 \pm 0,58^{ab}$	16 ± 1^{bc}	77.33 ± 8^{bc}	25 ± 5^{ab}
Cd	75	0.780 ± 0.032^{c}	0.166 ± 0.004^{d}	4.33 ± 0.58^{d}	14 ± 1^{d}	73 ± 7.09^{bc}	25 ± 2.65^{ab}
Cd	150	0.595 ± 0.016^{e}	0.124 ± 0.005^{e}	4.33 ± 0.58^{d}	17 ± 1^{ab}	79 ± 6.56^{b}	23 ± 4.36^{b}
Cu + Cd	75	0.853 ± 0.027^{b}	0.185 ± 0.008^{c}	6.33 ± 0.58^{bc}	16 ± 1^{bc}	85 ± 7.55^{ab}	31 ± 10.15^{ab}
Cu + Cd	150	0.667 ± 0.020^{d}	0.137 ± 0.009^{e}	7.67 ± 0.58^{a}	18 ± 1^{a}	66 ± 7^{c}	28 ± 9.54^{ab}
LSD at 0.05		45597.82	12419.15	1.14	1.75	12.31	12.04

The values are means ± SD
Deferent letters indicated significant difference from the control and between treatments at $p \leq 0.05$.

Elevated rhizosphere Cd concentrations often result in changes in the relative proportion and size of root tissues and cell types. Although many of these changes depend upon the rhizosphere Cd concentration, and appear to be species and tissue specific, they can be found both in plants permanently exposed to elevated Cd concentrations in the natural environment as well as in plants growing in artificial media in the laboratory (Lux *et al.,* 2011). Cu and Cd induced reduction in the cell size include all root cells whether they will remain parenchymatous in the cortex and pith or will develop into xylem vessels. The net outcome of this reduction in the cell size is the root diameter shrinkage (kasim, 2006). The reduction in the diameter of metaxylem vessels was the most remarkable result as it is one of the factors affecting their capacity as translocation conduits (Poschenrieder and Barceló, 1999). Pasternak *et al*., (2005) found that Cu impinges primarily on cell elongation and not so much on cell division. Reduction in the width of the root cortical parenchyma was mainly the result of a decrease in the mean cell size and not in the number of cell layers (Kasim 2006).

Several studies showed that the heavy metal stress by Cu or Cd an adverse effect on anatomical structure of *Sorghum bicolor*, especially when the plant was subjected to both metals simultaneously (kasim, 2006). Similar alterations in anatomical parameters of different crop plants have been previously reported (Kovačević *et al.,* 1999, Shalini *et al.,* 1999, Khudsar *et al.,* 2001 and Papadakis *et al.,* 2004). The up-take of heavy metals by the root seems to trigger a series of structural alterations with potential functional consequences in the plant. A marked decrease in cell size might be the result of a decrease in the elasticity of cell walls of the root as was previously demonstrated (Sieghardt, 1984 and Barceló *et al.,* 1986).

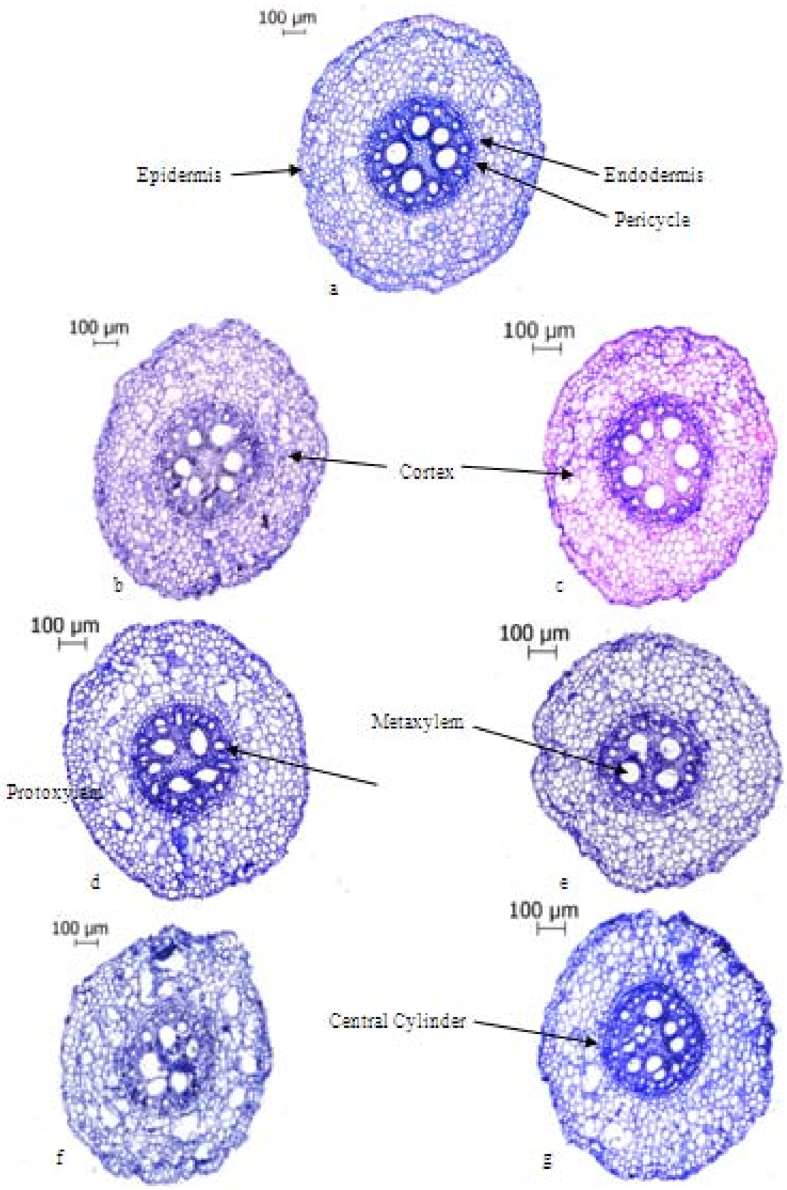

Fig. 1: Cross sections of primary root in Maize (*Zea mays* cv. Giza 2) treated with different concentrations of Cu and/or Cd. a; control, b; Cu 75, c; Cu 150, d; Cd 75, e; Cd 150, f; Cu & Cd (75) and g; Cu & Cd (150)

c- Effect Of Heavy Metals On Structure Of Leaf:

The results indicated that Cu and /or Cd lead to a significant decrease in thickness of midrib, upper epidermis and parenchyma and thickness of vascular bundle. The highest decrease was seen in Cd at concentration 150 µmol/L. The combination between Cu and Cd leads to a reduction in Cd toxicity when compared with the same concentration in Cd alone in thickness of midrib (Table 3 and Figure 2). All treatments lead to a significant decrease in thickness of lower epidermis and sclerenchyma, width of vascular bundle and diameter of xylem vessels except Cu (150 µmol/L) and in number of rows sclerenchyma lower vascular bundle except Cu and Cd (75 µmol/L). All a Cd treated groups except Cd with Cu at (75 µmol/ L) showed a significant decrease in thickness of phloem tissue and all groups except Cd with Cu at (150 µmol/L) showed a significant decrease in distance between large vascular bundle and nearest vascular bundle. Cd (150 µmol/L) and Cu with Cd at concentration 75 µmol/L lead to a significant decrease in number of rows parenchyma upper vascular bundle.

The changes in leaf cell enlargement caused by Cd may actually be due to specific morphogenetic effects rather than to impaired water balance (Djebli 2010). A number of studies reported that reduction of shoot elongation in the presence of Cd and Cu is due to reduction of photosynthesis. It has been reported that, heavy metals may cause anatomical changes in leaves and stems (Wan *et al.,* 2011 and Soudeh and Zarinkamar 2012). Cu or Cd alone or in combination caused significant reduction in width and thickness of leaf midrib, diameter of xylem vessels of all seedling organs of *Sorghum bicolor* L (Kasim, 2006).

Cd that reached the leaves resulted in further physiological and structural damages. Growth and anatomy of Cd treated *Solanum lycopersicum* became thinner and had less enlarged mesophyll cells following Cd treatment (Kovačević *et al.,* 1999). Despite numerous studies, the basic mechanisms through which Cd phytotoxicity takes place are still not completely understood, and remain an open question. This may be due to the fact that the apparent Cd effects may result from complex metal interferences with a great number of interacting physiological and metabolic processes, and also that in different species as well as in different organs a given activity may be differently and even oppositely affected by the metal. These differences in Cd impact between internode and leaf types are likely related to the different stages of organ development at which they were first exposed to Cd (Djebli 2010).

Table 3: Effects of different concentrations of Cu and/or Cd on third leaf structure of Maize (*Zea mays* cv. Giza 2).

Parameters / Treatments (µmol/ L)		Thickness of midrib µm	Thickness of lower epidermis and sclerenchyma µm	Thickness of upper epidermis and parenchyma µm	Thickness of vascular bundle µm	Width of vascular bundle µm
	Control	725.33 ± 13.65 a	62.67 ± 5.51 a	516.00 ± 16.52 a	146.67 ± 8.08 a	142.00 ± 6.56 a
Cu	75	568.33 ± 14.64 b	37.00 ± 4.58 e	398.00 ± 9.17 b	133.33 ± 1.15 b	122.00 ± 3.46 b
Cu	150	529.67 ± 7.02 c	61.00 ± 4.58 ab	335.33 ± 5.13 c	133.33 ± 2.89 b	145.67 ± 2.52 a
Cd	75	459.00 ± 5.57e	51.00 ± 4.36 cd	296.33 ± 3.06 d	111.67 ± 1.53 c	119.00 ± 3.61 b
Cd	150	381.00 ± 5.29 g	44.00 ± 3.00 de	234.67 ± 2.52 e	110.33 ± 2.08 d	95.33 ± 3.06 c
Cu + Cd	75	497.67 ± 7.37 d	50.00 ± 3.00 cd	340.00 ± 5.57 c	107.67 ± 2.08 c	121.00 ± 3.46 b
Cu + Cd	150	398.00 ± 6.24 f	55.67 ± 3.21 bc	236.00 ± 5.00 e	109.33 ± 3.51 c	122.33 ± 4.73 b
LSD at 0.05		16.24	7.24	14.12	6.56	7.19

		N. of rows sclerenchyma lower vascular bundle	N. of rows parenchyma upper vascular bundle	N. of xylem vessels	Diameter of xylem vessels µm	Thickness of phloem tissue µm	Distance between large vascular bundle and nearest vascular bundle µm
	Control	5.67 ± 0.58 a	6.33 ± 0.58 a	3.67 ± 0.58	36.00 ± 4.00 a	50.00 ± 3.61 ab	64.00 ± 12.49 ab
Cu	75	4.33 ± 0.58 bc	5.67 ± 0.58 ab	3.67 ± 0.58	27.67 ± 4.04 bc	46.67 ± 2.08 bc	52.00 ± 4.36 bc
Cu	150	5.33 ± 0.58 ab	5.67 ± 0.58 ab	3.67 ± 0.58	33.00 ± 2.65 ab	56.00 ± 3.61 a	70.00 ± 5.00 a
Cd	75	4.33 ± 0.58 bc	5.33 ± 0.58 ab	3.67 ± 0.58	23.33 ± 1.53 cd	37.67 ± 2.52 d	45.67 ± 6.43 c
Cd	150	4.33 ± 0.58 bc	4.67 ± 0.58 b	3.67 ± 0.58	20.33 ± 3.79 d	40.00 ± 5.00 cd	43.00 ± 4.36 c
Cu + Cd	75	4.67 ± 0.58 abc	5.00 ± 1.00 b	3.67 ± 0.58	21.00 ± 6.08 cd	38.67 ± 4.51 d	51.67 ± 14.47 bc
Cu + Cd	150	4.00 ± 1.00 c	5.33 ± 0.58 ab	3.67 ± 0.58	22.00 ± 4.58 cd	48.00 ±6.00 b	43.00 ± 2.00 c
LSD at 0.05		1.15	1.15	1.01	7.07	7.19	14.41

The values are means ± SD

Deferent letters indicated significant difference from the control and between treatments at p ≤0.05.

Owing to the dual role of Cu, as an essential micro-element on one hand and as a toxic environmental pollution on the other, complete exclusion during root uptake is not possible. Since Cu is more readily taken up and translocated to different plant organs than Cd (brune *et al.,* 1994 and All *et al.,* 1998), this may be the reason for the higher shoot regeneration in *Bacopa monniera* in the presence of Cu. Thus, by adding non toxic level of Cu to the Cd containing medium, the process of obtaining the Cd tolerant plant can be enhanced. Most of the Cd in these regenerates accumulates in the cytoplasm of the root, whereas Cu is readily translocated to shoot and accumulates in cell wall as well as cytoplasm (Gussason *et al*., 1995 and Chakravaty and Srivastava 1997). All *et al.,* (1998) attempts to transfer Cd tolerant plants to the soil are in progress. Whether the Cd tolerant character is inheritable is yet to be ascertained.

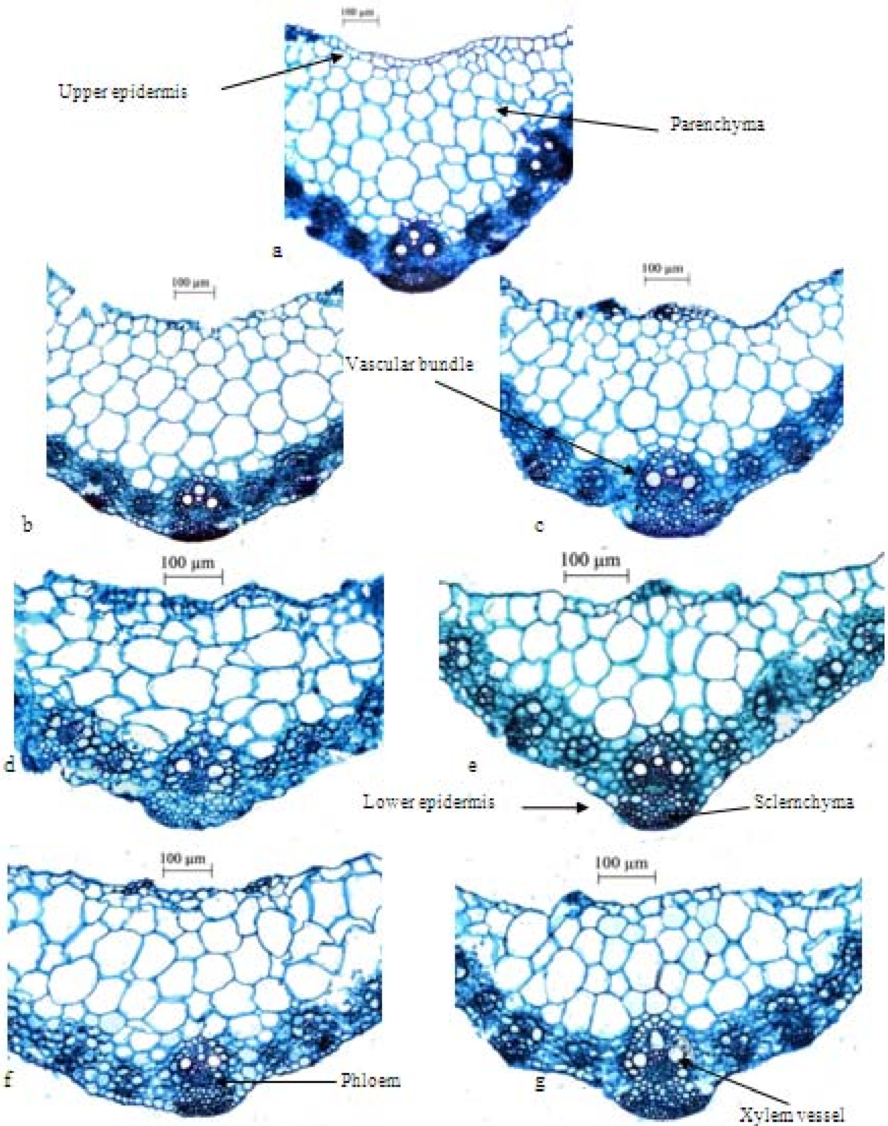

Fig. 2: Cross sections of third leaf at midrib in Maize (*Zea mays* cv. Giza 2) treated with different concentrations of Cu and/or Cd. a; control, b; Cu 75, c; Cu 150, d; Cd 75, e; Cd 150, f; Cu & Cd (75) and g; Cu & Cd (150)

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