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ISSN: 2348-1900
Plant Science Today
http://www.plantsciencetoday.online

OPEN ACCESS

Research Article

Early seedling growth affected by $CuSO_4$ and its combination with PEG 6000 in maize

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Article history
Received: 13 February 2019
Accepted: 25 March 2019
Published: 27 April 2019

Editor
Dr. Ana Isabel Carvalho
University of Trás-os-Montes and Alto Douro
Vila Real, Portugal

Publisher
Horizon e-Publishing Group

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Abstract

Several abiotic stress factors are faced by the plants in nature, including metal exposure and water deficit condition. The present study was undertaken to investigate the effects of copper and its combination with water deficit, on growth and anatomical characteristics of *Zea mays* L. (maize) cv. Ganga safed-2 seedlings. Seeds were treated with $CuSO_4$ (0-1000μM) for inducing Cu stress, PEG 6000 (0-10%) for inducing water deficit stress and their combination for combined stress for 5 days. Germination %, growth parameters, % phytotoxicity, and root anatomical characteristics were analyzed. Treatment of maize seeds with 0-1000μM $CuSO_4$ significantly reduced almost all the growth parameters, except germination %. Root growth was inhibited significantly at 100μM and higher concentrations of $CuSO_4$, however, for shoot growth, ≥300μM are inhibitory. Germination percentage was not affected by the supplementation of Cu, indicating the tolerant nature of Ganga safed-2 maize genotype at germination stage. Treatment with Cu (≥300μM) and PEG 6000 (10%), decreased the growth of maize seedlings with prominent effect on root by Cu and on the shoot by 10% PEG. Anatomical modifications in root were noticed with both the stresses, individually and in combination.

Keywords: heavy metals; water deficit; combined stress; anatomical modifications

Citation: Hirve M, Jain M. Early seedling growth affected by $CuSO_4$ and its combination with PEG 6000 in maize. Plant Science Today 2019;6(2):160-169. https://doi.org/10.14719/pst.2019.6.2.508

Indexing: Plant Science Today is covered by Scopus, CAS, AGRIS, CABI, Google Scholar, etc. Full list at http://www.plantsciencetoday.online

Introduction

Heavy metal contamination is the pervasive threat that makes a significant contribution to environmental pollution. Copper (Cu) is a naturally occurring element of earth crust, a transitional metal and usually present in soils within the range of 0-250 μg/g (1). However, its concentration in soils often varies due to its release into the environment by anthropogenic activities, such as, by the use of bactericides, fungicides and industrial wastes from manufacturing companies dealing with the production of metal, electric appliances, etc. (2,3). It is an essential trace element for plants required for normal growth and development. It may act as a structural element in regulatory proteins, like, plastocyanin and a constituent of many enzymes, such as, cytochrome C oxidase, Cu/zinc superoxide dismutase, ascorbate peroxidase, diamine oxidase, polyphenol oxidase etc. Being a component/cofactor for enzymes, it participates in various physiological processes, such as, photosynthesis, respiration, antioxidant mechanism and hormone metabolism (4-7). Apart from its essential role in plants, above threshold concentrations Cu becomes extremely toxic and causes inhibition of seed germination rate, root length and shoot elongation, root/shoot ratio and dry biomass as well as chlorosis and necrosis, that leads to inhibition of plant growth or even cell death (8,9). Excessive Cu alters the pigment and protein components (e.g., chlorophyll content and PSII), thylakoid membrane structure and CO_2-fixation reactions of photosynthesis. Further, it induces oxidative stress that leads to overproduction of reactive oxygen species that can severely damage membrane lipids, proteins and DNA (10,11). Furthermore, Cu toxicity can induce anatomical changes in plant roots, such as, decrease in thickening of endodermis, increase in thickness of exodermis and reduction of the diameter of vascular bundles (12). Structural modifications in the form of thickening of epiblema, endodermis, cell wall of xylem and cortical parenchyma, increase in the number of protoxylem and metaxylem elements, increased proportion of pith have been observed in several reports due to heavy metal elements, such as, copper, cadmium, chromium and lead (13-16).

Water deficit stress is an important abiotic factor that causes dehydration and osmotic imbalance in the cell, consequently water potential of the plant tissues is decreased that affects crop yields (17). Polyethylene glycol (PEG) compounds have been frequently used to stimulate water deficit effects in plants since, PEG of high molecular weight range (6000 or above) cannot enter the pores of plant cells (18,19). It reduces seed germination percentage, root length, seedling water content, decreases chlorophyll content and inhibits enzymes of chlorophyll biosynthesis, damages the thylakoid membrane structure thereby decreases photosynthesis and increases the activity of antioxidative enzymes (20-23). Water deficit and heavy metal toxicity are often co-occurring abiotic stresses in the natural environment. Recent studies showed that concurrent exposure of heavy metals Ni, Cu, Co, and Cr with drought alters xylem structure and reduces conduit density, hydraulic conductance and root and shoots growth that resulted in smaller plants and increases early mortality of *Acer rubrum* L. (Red maple) (24). Lead treatment in combination with drought has been found to significantly affect the dry mass production, photosynthetic activity and quantum yield of PSII and cellular ultrastructure of *Populus cathayana* L. (25). Combined application of excess Cu and water deficit on tobacco leaves showed increased proline and abscisic acid content with decreased leaf relative water content (26).

Zea mays L. is a cereal crop used as human food, animal feed and raw material for manufacture of many industrial products, so, it is crucial to understand its performance under various stresses and their combinations. Responses of plants under combination of stresses are limited to a small number of reports. Thus, present study was designed to understand the changes in growth characteristics and anatomical behavior in maize seedlings in relation to Cu stress and its combination with water deficit stress.

Materials and Methods

Uniform and healthy seeds of *Zea mays* L. cv. Ganga safed-2 (obtained from Ganga Kaveri seeds Pvt. Ltd., Hyderabad) were selected and surface-sterilized by soaking in 0.1% $HgCl_2$ for 1-2 min and then washed thoroughly with tap water followed by distilled water. Seeds were placed in 20 cm diameter Petri plates (10 seeds per plate) lined with filter paper discs and treated with different concentrations of $CuSO_4.5H_2O$ *viz*. 0, 10, 50, 100, 300, 500 and 1000 µM and allowed to grow in a light growth chamber in continuous light of intensity about 40W/m^2 at 25±3°C for five days. Distilled water was used as control. On the basis of observations of pilot experiments with 0-1000µM concentrations of Cu on growth parameters, 10 and 100µM concentrations were selected for combination studies with water deficit. Water deficit was induced by polyethylene glycol (PEG) 6000 (5% and 10% solutions). These concentrations of PEG were decided after performing germination and growth studies with 0-30% PEG solutions (data not shown). The combinations of PEG and Cu used were 5% PEG + 10µM $CuSO_4$, 5% PEG + 100µM $CuSO_4$, 10% PEG + 10µM $CuSO_4$ and 10% PEG + 100µM $CuSO_4$. After five days seedlings were examined for various growth parameters. Copper sulphate ($CuSO_4.5H_2O$) of SQ grade-Qualigens Fine Chemicals and PEG 6000 from HiMedia procured from a local dealer were used in the study.

Germination percentage was noted on 3rd day after emergence of 2 mm radicle by formula of Close and Wilson (27):

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds grown}} \times 100$$

Growth parameters, such as, root length, shoot length, root shoot ratio, seed vigor, root and shoot fresh and dry weights, tolerance index and percentage phytotoxicity in root and shoot as well as root anatomical features of maize seedling were determined on completion of 5th day of growth. Root and shoot length was measured with the help of thread and centimeter scale. Root shoot ratio was obtained by dividing lengths of root and shoot tissues. Seed vigor was calculated using formula of Baki and Anderson (28):

$$\text{Seed vigor} = \text{Seedling length} \times \text{Germination percentage}$$

Table 1: Effect of $CuSO_4$ on germination percentage and growth parameters in maize seedlings

$CuSO_4$ Concentration (µM)	Germination Percentage (%)	Root Length (cm)	Shoot Length (cm)	Root Shoot Ratio (R/S Ratio)	Seed Vigor
0	98±1.8 (100)	10±0.3 (100)	4.6±0.2 (100)	2.2±0.1 (100)	1464±46 (100)
10	98±1.8^{ns} (100)	9.1±0.4^{ns} (91)	4.6±0.2^{ns} (100)	2.0±0.1* (90)	1350±50^{ns} (92)
50	96±2.2^{ns} (98)	9.0±0.3^{ns} (89)	5.1±0.2^{ns} (109)	1.8±0.05*** (82)	1401±40^{ns} (96)
100	98±1.8^{ns} (100)	8.0±0.3* (80)	5.2±0.2* (112)	1.6±0.1*** (72)	1317±30^{ns} (90)
300	96±2.2^{ns} (98)	4.5±0.2*** (44)	3.3±0.2** (71)	1.8±0.2* (79)	773±34*** (53)
500	96±2.2^{ns} (98)	3.7±0.2*** (37)	2.9±0.2*** (62)	1.6±0.1*** (72)	641±31*** (44)
1000	94±2.2^{ns} (96)	2.1±0.1*** (21)	2.2±0.1*** (47)	1.3±0.1*** (58)	430±18*** (29)

ns = not significant, * , ** and *** = significant at $p<0.05$, $p<0.01$ and $p<0.001$ respectively. Values are mean ± SE and in parentheses relative values are given.

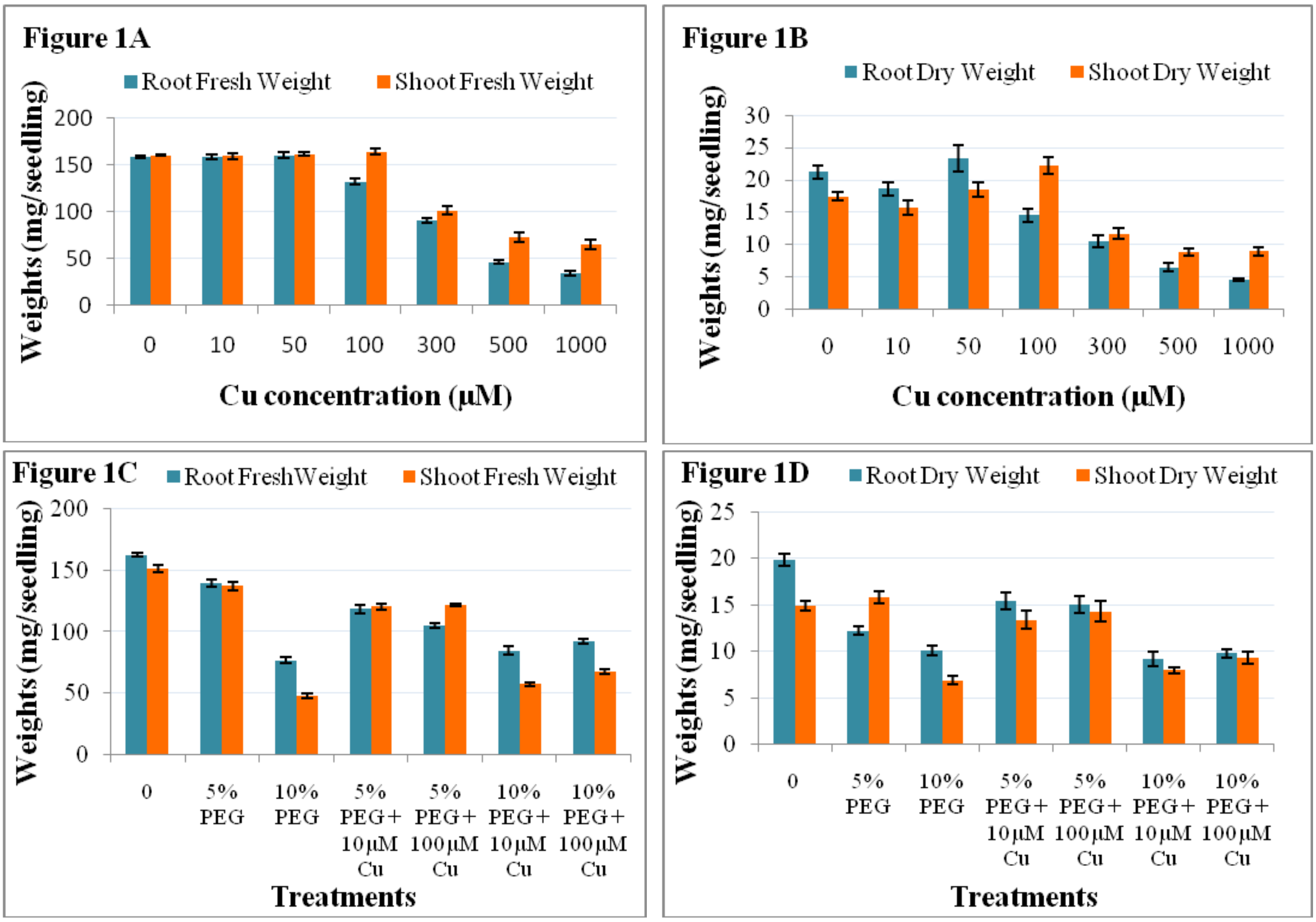


Fig. 1A: Effect of $CuSO_4$ on root and shoot fresh weights. **Fig. 1B**: Effect of $CuSO_4$ on root and shoot dry weights. **Fig. 1C**: Effect of combination of PEG 6000 and Cu on root and shoot fresh weights. **Fig. 1D**: Effect of combination of PEG 6000 and Cu on root and shoot dry weights.

Electrical weighing balance was used to determine fresh and dry weights of roots and shoots. Dry weight was obtained after keeping the tissues in a hot air oven at 80°C for a period of 4 days. Tolerance index was calculated by formula given by Iqbal and Rahmati (29):

$$\text{Tolerance index} = \frac{\text{Mean root length in treatment applied}}{\text{Mean root length in distilled water (control)}} \times 100$$

For calculation of percentage phytotoxicity in root and shoot, following equation was used (30):

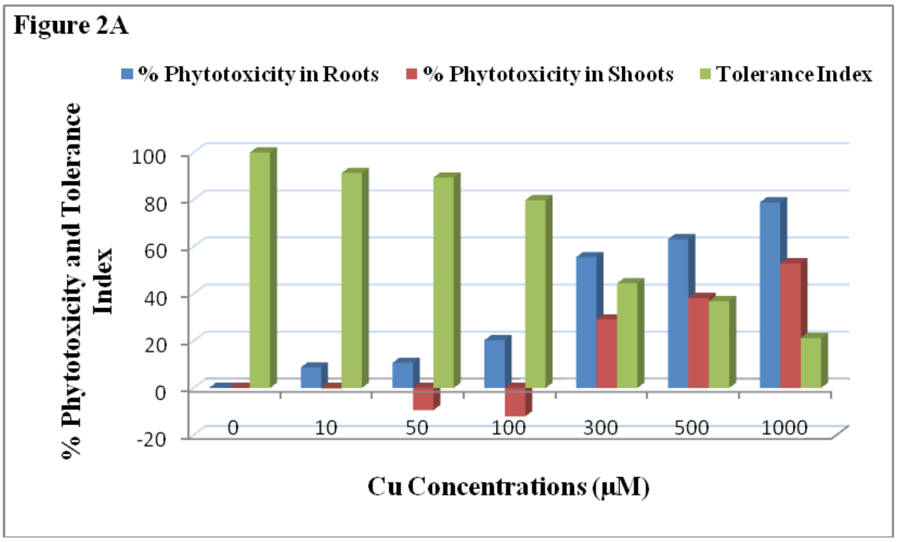
$$\%\ \text{Phytotoxicity} = \frac{\text{Root/Shoot length of control} - \text{Root/Shoot length of treatment}}{\text{Root/Shoot length of control}} \times 100$$

Histological sections of maize roots were prepared by free hand sectioning. Thin sections were stained with 0.05% toluidine blue solution,

Table 2: Effect of combination of PEG 6000 and $CuSO_4$ on germination percentage and growth parameters in maize seedlings.

PEG 6000 (%) and $CuSO_4$ (µM) Treatments	Germination Percentage (%)	Root Length (cm)	Shoot Length (cm)	Root Shoot Ratio (R/S Ratio)	Seed Vigor
0	98±1.3 (100)	10.5±0.2 (100)	4.7±0.1 (100)	2.3±0.1 (100)	1521±32 (100)
5% PEG	80±2.4** (82)	6.6±0.2*** (61)	3.6±0.1*** (64)	1.7±0.1^{ns} (87)	751±50*** (48)
10% PEG	73±2.0*** (74)	6.3±0.3*** (60)	1.9±0.1*** (41)	4.2±0.5*** (182)	598±24*** (39)
5% PEG + 10 µM $CuSO_4$	96±1.5^{ns} (98)	6.6±0.2*** (63)	3.6±0.1*** (77)	1.7±0.1*** (75)	774±20*** (51)
5% PEG + 100 µM $CuSO_4$	96±1.5^{ns} (98)	5.9±0.2*** (56)	3.4±0.1*** (73)	1.7±0.1*** (75)	691±21*** (45)
10% PEG + 10 µM $CuSO_4$	94±1.5^{ns} (96)	6.1±0.4*** (58)	1.6±0.1*** (35)	5.4±0.8*** (233)	730±39*** (48)
10% PEG + 100 µM $CuSO_4$	94±1.5^{ns} (96)	4.2±0.2*** (40)	1.6±0.1*** (35)	3.4±0.4** (146)	545±25*** (36)

ns = not significant, * , ** and *** = significant at p<0.05, p<0.01 and p<0.001 respectively. Values are mean ± SE and in parentheses relative values are given.



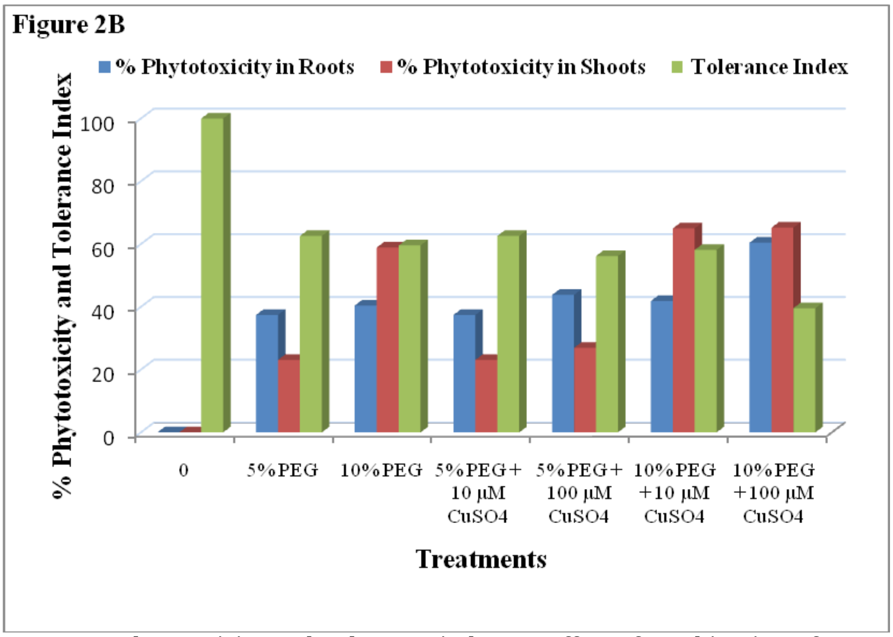


Fig. 2. A: Effect of $CuSO_4$ on % Phytotoxicity and Tolerance index. **B**: Effect of combination of PEG 6000 and $CuSO_4$ on % Phytotoxicity and Tolerance index.

prepared in 0.1M sodium acetate buffer (pH 4.4). Anatomical changes were then observed under Olympus microscope mounted with digital camera.

Each observation is a mean of at least 5 replicate experiments and data are presented as mean ± standard error. All data were analyzed by Student's *'t'* test for mean comparison and to test the significance of mean difference obtained for various treatments.

Results

Germination and growth parameters of Cu treated maize seeds are presented in Table 1. When seeds were treated with 0-1000µM concentrations of $CuSO_4$, there was no significant change in germination percentage. However, a concentration dependent decrease in root length was observed with increasing $CuSO_4$ concentrations, that was non-significant up to 50µM and significant at 100µM ($p<0.05$) and higher concentrations, ≥300 µM ($p<0.001$). Shoot length was marginally increased at 100µM Cu concentration. Further, at higher concentrations of Cu (300-1000µM), a significant reduction in shoot length was noted. Root-shoot ratio was decreased with increasing concentration of Cu. Seed vigor was reduced gradually and significantly at 300-1000µM concentrations with only 29% value of seed vigor at 1000µM Cu, while at lower Cu levels (10-100µM), the change in seed vigor was non-significant (Table 1).

Table 2 presents the data for germination percentage and seedling growth parameters of maize treated with PEG 6000 (5% and 10%) treatment alone and its combination with 10µM and 100µM $CuSO_4$. Germination percentage was significantly reduced with the treatment of both 5% and 10% PEG, however, when supplied along with 10µM and 100µM Cu, no significant change was observed (Table 2). Seedling root length, shoot length and seed vigor were significantly ($p<0.001$) decreased in both individual PEG and its combined treatments with Cu (Table 2). Root-shoot ratio was significantly reduced at 5% PEG treatment individually and in combination with Cu. However, the ratio was found to be significantly higher at 10% PEG treatment alone and in combination with metal (Table 2).

Effects of supplementation of Cu on fresh and dry weights of maize tissues are shown in Fig. 1A and 1B. At 300-1000µM concentrations, both root and shoot fresh and dry weights were decreased gradually and significantly ($p<0.001$), however, at low Cu levels *i.e.* 0-50µM, no significant change was noticed (Fig. 1A and 1B). At 100µM Cu concentration, fresh weight of root was reduced significantly, while, shoot fresh weight was increased (Fig. 1A). Similar pattern of change was observed with dry weights of root and shoot tissues (Fig. 1B). Treatment with only PEG and PEG+Cu, reduced the root and shoot fresh weights significantly (Fig. 1C). Root dry weight was decreased with individual PEG and combined treatments (Fig. 1D). However, shoot dry weight was slightly increased at individual 5% PEG treatment and decreased in combination; nevertheless, both the changes were non-significant. With the supply of 10% PEG both individually and in presence of Cu, shoot dry weight was reduced significantly (Fig. 1D).

When maize seeds were supplied with 300-1000µM concentrations of $CuSO_4$, percentage phytotoxicity increased with maximum toxicity of 79% in roots and 53% in shoots at highest concentration, however, at low levels (0-100µM), it was increased in roots and decreased in shoots (Fig. 2A). Percentage phytotoxicity due to PEG and combined treatments (Cu+PEG) increased in both root and shoot tissues (Fig. 2B). At 5% PEG and its combined treatment with Cu, % phytotoxicity was higher in roots as compared to shoots, while at 10% PEG and its combination with Cu, it was more in shoots than in roots (Fig. 2B). Due to increase in % phytotoxicity, tolerance index was consequently decreased in all treatments (Fig. 2A and 2B).

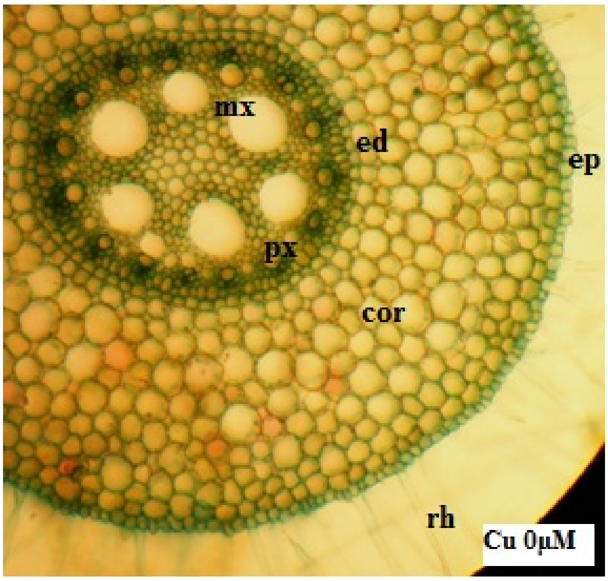


Fig. 3A: Anatomical section of untreated maize root: mx = metaxylem, px = protoxylem, ed = endodermis, cor = cortex, ep = epidermis and rh = root hairs. Bars= 600µm.

In root anatomical sections of maize seedlings, appropriate number of unicellular root hairs was seen in well defined epiblema cell lining when treated with 0-100µM Cu. Large sized metaxylem vessels and smaller pith in stellar region was identified (Fig. 3A and 3B). With increasing Cu concentration (≥300µM), a gradual increase in parenchymatous cell layers in cortex region, increased pith size with overall increase in area of stellar region and comparative decrease in size of metaxylem vessels was noticed (Fig. 3B). Due to increased cortex and pith size in stellar region, an overall increase in the width and

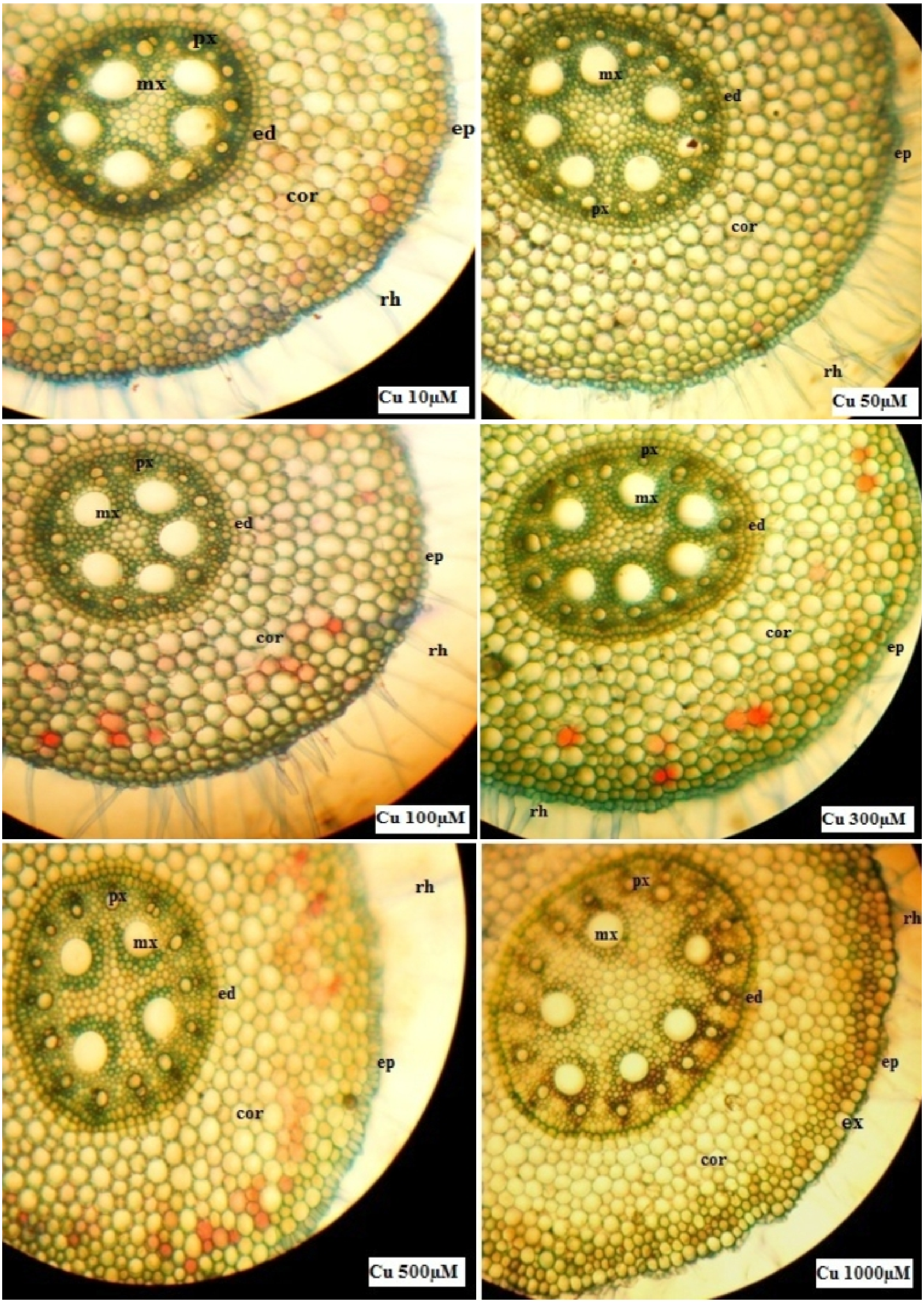


Fig. 3B: Anatomical sections of Cu treated maize roots: mx = metaxylem, px = protoxylem, ed = endodermis, ex = exodermis, cor = cortex, ep = epidermis and rh = root hairs. Bars= 600μm.

thickening of root was noted, especially at highest dose of metal (1000μM). Further, irregularity in distribution and arrangement of cells of epidermis and damage of root hairs was resulted, with the effect being more prominent at 1000μM $CuSO_4$. Due to damage of epidermal cells, an additional

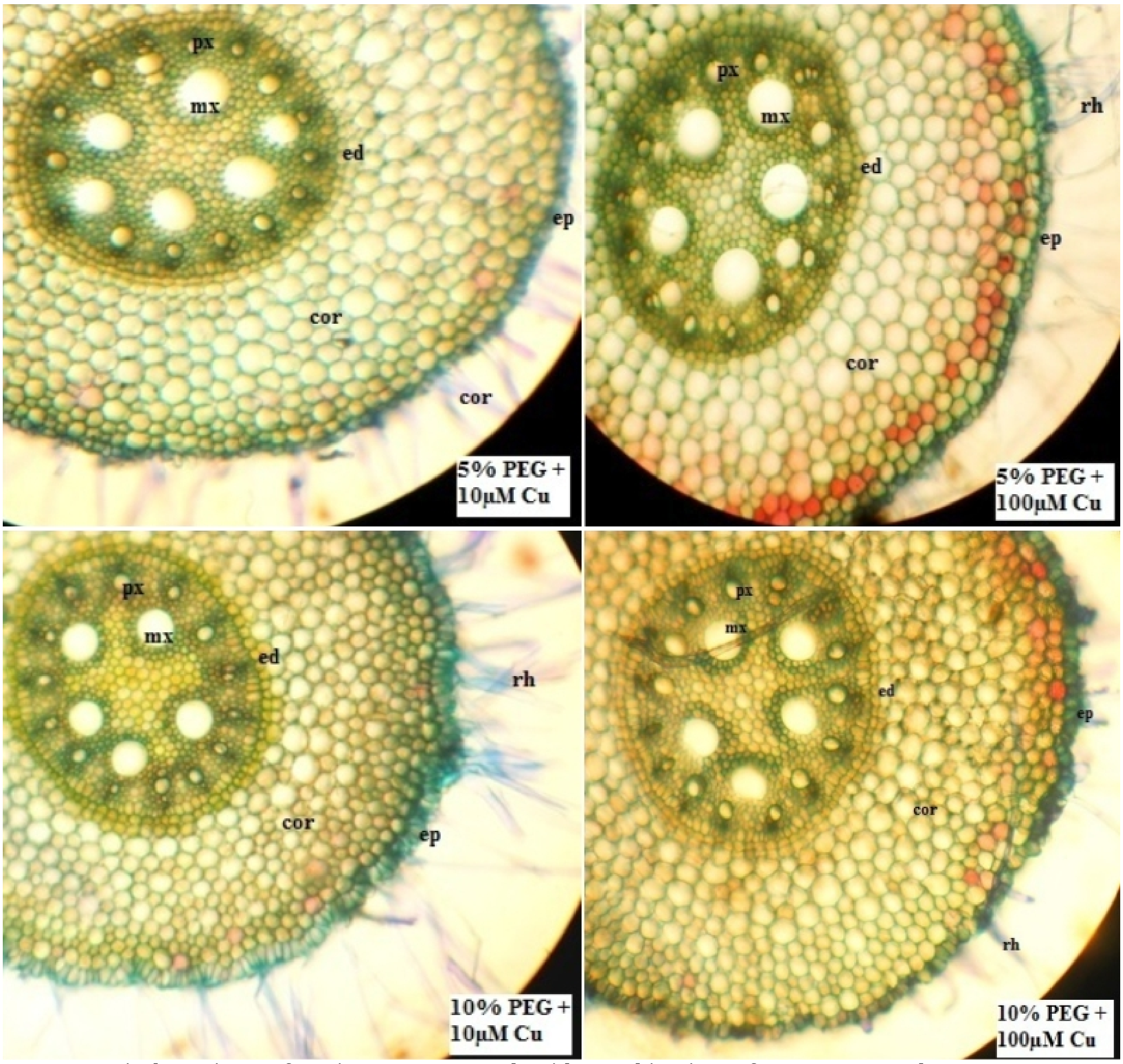


Fig. 3C: Anatomical sections of maize roots treated with combination of PEG 6000 and Cu: mx = metaxylem, px = protoxylem, ed = endodermis, cor = cortex, ep = epidermis and rh = root hairs. Bars= 600µm.

modification *i.e.* enlargement of cells in outermost lining of cortex was seen that causes thickening of overall exodermis of cortex (Fig. 3B). Appropriate number of root hairs was visualized when 10 and 100µM Cu combined with 5% PEG 6000 were supplied to the maize seedlings (Fig. 3C). However, in combination of 10µM Cu with 10% PEG increased number of root hairs with irregularities in shape and arrangement of epiblema cell lining was noticed. Further, thinning of roots and smaller cortex region was another structural change (Fig. 3C). No distinctive changes in stellar region were noticed in any of the combinations studied, however, treatment with combination of 100µM Cu and 10% PEG caused damage of epiblema with less number of root hairs (Fig. 3C).

Discussion

Copper being an essential heavy metal is required for normal plant growth and development. However, at elevated concentrations in soil it becomes toxic. Non-significant difference in % germination of maize seeds treated with varying concentrations of $CuSO_4$ in the present study indicates that probably Ganga safed-2 maize variety is resistant towards Cu toxicity even up to 1000 concentration at germination stage. It might be due to utilization of its own reserve by the seeds for germination and hence the process is not affected by presence of metal ions in the medium (31). Mahmood et al. (32) has reported a similar non-significant change in germination in maize at 3-12ppm Cu treatment, however, Radoviciu and coworkers (33) noticed slight improvement in germination % at 3ppm Cu in maize, that decreased on higher Cu doses (30 and 300ppm). Significant reduction in germination percentage obtained by treatment with PEG 6000, could be due to inhibition of seed imbibition process caused by reduced water potential gradient between seed and medium. This process delayed seed metabolism activation and hence germination (34). Supply of 100µM Cu significantly reduced root growth of maize seedling, while, shoot length was increased with the same treatment. This might be due to some protective mechanism for reduced translocation of Cu from root to shoot (35) or the

ISSN: 2348-1900

amount of Cu that transfers from root to shoot fulfills shoot Cu requirement in trace amounts to enhance its growth (36). An increased shoot length over control at 10ppm Cu has been reported in wheat (37). At concentration >100μM Cu, both root and shoot length were significantly reduced with the effect being more pronounced for the earlier one (Table 1). Similar to the observation of the present study, inhibitory effects of Cu on root and shoot has also been reported in wheat (37) and maize seedling (38). Heavy metal induced root inhibition may involve metal interference with cell division and hence abnormal mitosis (39). Water deficit induced by PEG treatment reduces the rate of cell division and elongation which results into decreased root and shoot length (40). Further, in water deficit condition, cell wall elasticity also decreases because of hormonal and hydraulic signals which in turn may reduce the final size of the cells and the growth (41). Significant reduction in root length by combined treatment of 10% PEG and 100μM $CuSO_4$ indicates synergistic effect of both the stresses as PEG induced water deficit condition becomes more severe in presence of Cu (Fig. 1C and 1D). For shoot length, significant reduction during combined treatment of 10% PEG and $CuSO_4$ seems to be due to prominent effect of water deficit stress only (Table 2).

Decreased fresh weight of root and shoot tissues might be related with reduced growth of these tissues under stress condition. Significant reduction in weights among combination of 10% PEG and $CuSO_4$ is probably due to more pronounced effect of water deficit condition only (Table 2). In maize, reduced root fresh weight and dry weight with PEG 6000 has also been observed by Li et al. (42). Lower activities of photosynthetic enzymes under water stress condition might be a possible cause of reduced dry matter (43). In the present study, 79% phytotoxicity was noticed in the roots at 1000μM Cu level, while in shoots the toxicity was 53% (Fig. 2A). Gang et al. (36) reported a percentage phytotoxicity of 95 and 91% at 500ppm Cu concentration in wheat root and shoot tissues, respectively. However, 100% phytotoxicity at Cu concentrations ≥300ppm has been reported in both root and shoots of tomato seedlings (44). Increase in percentage phytotoxicity in both root and shoot by the supplementation of PEG and its combination with Cu could be due to lesser water availability in 10% PEG containing medium which might be inadequate for growth. Anatomical changes in the cortical region due to Cu supplementation indicate root adaptation for survival under stress conditions. Increased thickness of the root as a result of greater number of cells in cortex and pith region is one of the morphological traits that contribute to heavy metal tolerance of maize (45). Thickening of exodermis under Cu stress in wheat has been suggested as a means of protection against penetration of excess toxic agents from the medium (12,14). Greater root system mainly of fine roots and large number of root hairs could be an adaptive strategy towards water deficit stress when PEG (10%) was supplemented along with 10μM Cu.

Conclusion

It seems that Cu concentration higher than 100μM is toxic for root environment, and doses of ≤100μM fulfils Cu requirement in shoot tissue of *Zea mays* L. cv. Ganga safed-2 cultivar. Unaffected germination percentage due to Cu supply demonstrates that probably this variety of maize is tolerant towards Cu toxicity at germination stage. Higher concentrations of Cu (≥300μM) and PEG 6000 (10%), negatively influence growth of maize seedlings with effects being substantial for root growth in Cu treated seedlings and for shoot growth in 10% PEG treated seedlings. Further, increase in the severity of stresses, individual and in combination causes more reduction in growth attributes. Furthermore, combination of both the water deficit and metal stress affects the root characteristics in a synergistic manner. Anatomical modifications in root architecture due to both the stresses represent an adaptive trait towards tolerance of maize.

Acknowledgement

Rajiv Gandhi National SRF to Mamta Hirve from UGC, New Delhi, is gratefully acknowledged.

Authors' contribution

MJ conceived and designed the experiments. MH carried out the experiments, analyzed data and wrote the manuscript with inputs from MJ.

Competing interests

The authors declared that they have no competing interest.

References

1. Alloway BJ. Heavy Metals in Soils. Chapman & Hall, London. 1995.

2. Panagos P, Ballabio C, Lugato E, Jones A, Borrelli P, Scarpa S, Orgiazzi A, Montanarella L. Potential sources of anthropogenic copper inputs to European agricultural soils. Sustainability. 2018;10:2380. https://doi.org/10.3390/su10072380

3. Al-Saydeh SA, El-Naas MH, Zaidi SJ. Copper removal from industrial wastewater: A comprehensive review. J Industrial and Engineering Chem. 2017;56:35-44. https://doi.org/10.1016/j.jiec.2017.07.026

4. Rodriguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science. 1999;283:996-8. https://doi.org/10.1126/science.283.5404.996

5. Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, Ye H. Copper cofactor delivery in plant cells. Curr Opin Plant Biol. 2006;9:256-63. https://doi.org/10.1016/j.pbi.2006.03.007

6. Burkhead JL, Gogolin Reynolds KA, Abdel-Ghany SE, Cohu CM, Pilon M. Copper homeostasis. New Phytologist. 2009;182:799-816. https://doi.org/10.1111/j.1469-8137.2009.02846.x

7. Martinez-Penalver A, Grana E, Reigosa MJ, Sanchez-Moreiras AM. (2012). The early response of *Arabidopsis thaliana* to cadmium- and copper-induced stress. Environ Exp Bot. 2012;78:1-9. https://doi.org/10.1016/j.envexpbot.2011.12.017

8. Maksymiec W, Krupa Z. Effects of methyl jasmonate and excess copper on root and leaf growth. Biol Plant. 2007;51:322-6. https://doi.org/10.1007/s10535-007-0062-4

9. Diwaker G, Abdullah. Toxicity of copper and cadmium on germination and seedling growth of maize (*Zea mays* L.) seeds. Indian J Sci Res. 2011;2:67-70.

10. Patsikka E, Kairavuo M, Sersen F, Aro EM, Tyystjarvi E. Excess copper predisposes photosysten II to photoinhibition in Vivo by outcompeting iron and causing decrease in leaf chlorophyll. Plant Physiol. 2002;129:1359-67. https://doi.org/10.1104/pp.004788

11. Lequeux H, Hermans C, Lutts S, Verbruggen N. Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. Plant Physiol Biochem. 2010;48:673-82. https://doi.org/10.1016/j.plaphy.2010.05.005

12. Atabayeva S, Nurmahanova A, Akhmetova A, Narmuratova M, Asrandina S, Beisenova A, Alybayeva R and Lee T. Anatomical peculiarities in wheat (*Triticum aestivum* L.) varieties under copper stress. Pak J Bot. 2016;484:1399-1405.

13. Singh S, Srivastava PK, Kumar D, Tripathi DK, Chauhan DK, Prasad SM. Morpho-anatomical and biochemical adapting strategies of maize *Zea mays* L. seedling against lead and chromium stresses. Biocatal Agric Biotechnol. 2015;4:286-95. https://doi.org/10.1016/j.bcab.2015.03.004

14. Gomes MP, de Sae Melo Marques TC, Oliveira M, Nogueira G, Castro EM, De Soares AM. Ecophysiological and anatomical changes due to uptake and accumulation of heavy metal in *Brachiaria decumbens*. Scientia Agricola. 2011;68:566-73. https://doi.org/10.1590/s0103-90162011000500009

15. Gowayed SMH, Almaghrabi OA. Effect of copper and cadmium on germination and anatomical structure of leaf and root seedling in maize (*Zea mays* L). Austr. J. Basic Appl Sci. 2013;7:548-55.

16. Suseela MR, Sinha S, Singh S, Saxena R. Accumulation of chromium and scanning electron microscopic studies in *Scirpus lacustris* L. treated with metal and tannery effluent. Bull Environ Contam Toxicol. 2002;68:540–48. https://doi.org/10.1007/s001280288

17. Boyer JS. Plant productivity and environment. Science. 1982; 218:443–8. https://doi.org/10.1126/science.218.4571.443

18. Oertli JJ. The response of plant cells to different forms of moisture stress. J plant physiol. 1985;121:295-300. https://doi.org/10.1016/s0176-1617(85)80022-5

19. Pane RF, Damanik RI, Khardinata EH. Germination performance of selected local soybean (*Glycine max* (L.) Merrills) cultivars during drought stress induced by Polyethylene Glycol (PEG). Earth Environ. Sci. 2018;122:012054. https://doi.org/10.1088/1755-1315/122/1/012054

20. Meher, Shivakrishna P, Reddy KA, Rao DM. Effect of PEG- 6000 imposed drought stress on RNA content, relative water content (RWC) and chlorophyll content in peanut leaves and roots. Saudi J Bio.Sci. 2018;25:285-289. https://doi.org/10.1016/j.sjbs.2017.04.008

21. Huseynova IM, Suleymanov SY, Aliyev JA. Structural functional state of thylakoid membranes of wheat genotypes under water stress. Biochimica et Biophysica Acta. 2007;1767:869-75. https://doi.org/10.1016/j.bbabio.2007.01.014

22. Jain M, Mittal M, Gadre R. Effect of PEG-6000 imposed water deficit on chlorophyll metabolism in maize leaves. J Stress Physiol and Biochem. 2013;9:262-71.

23. Sarmadi M, Karimi N, Palazon J, Ghassempour A, Miralili MH. Improved effects of polyethylene glycol on the growth, antioxidative enzymes activity and taxanes production in a Taxus baccata L. callus culture. Plant Cell Tissue and Organ Culture. 2019; https://doi.org/10.1007/s11240-019-01573-y

24. de Silva ND, Cholewa E, Ryser P. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple (*Acer rubrum* L.). J Exp Bot. 2012;63:5957-66. https://doi.org/10.1093/jxb/ers241

25. Han Y, Wang L, Zhang X, Korpelainen H, Li C. Sexual differences in photosynthetic activity, ultrastructure and phytoremediation potential of *Populus cathayana* exposed to lead and drought. Tree Physiol. 2013;33:1043-60. https://doi.org/10.1093/treephys/tpt086

26. Ku HM, Tan CW, Su YS, Chiu CY, Chen CT, Jan FJ. The effect of water deficit and excess copper on proline metabolism in *Nicotiana benthamiana*. Biol Plant. 2012;56:337-43. https://doi.org/10.1007/s10535-012-0095-1

27. Close DC, Wilson SJ. Provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensis* seed. For Ecol Manage. 2002;170:299-305. https://doi.org/10.1016/s0378-1127(01)00768-x

28. Baki AA, Anderson JD. Relationship between decarboxylation of glutamic acid and vigour in soybean seed. Crop Science. 1973;13:222-6. https://doi.org/10.2135/cropsci1973.0011183x001300020023x

29. Iqbal MZ, Rahmati K. Tolerance of *Albizia lebbeck* to Cu and Fe application. Ekologia CSFR. 1992;11:427-30.

30. Chou CH, Lin HJ. Autointoxication mechanism of *Oriza sativa* L. Phytotoxic effects of decomposing rice residues in soil. J Chem Ecol. 1976;2:353-67. https://doi.org/10.1007/bf00988282

31. Stefani A, Arduini I, Onnis A. *Juncus acutus*: Germination and initial growth in presence of heavy metals. Ann Bot Fenn. 1991;28:37-43.

32. Mahmood S, Hussain A, Saeed Z, Athar M. Germination and seedling growth of corn (*Zea mays L*.) under varying levels of copper and zinc. Int. J. Environ. Sci. Tech. 2005;2:269-74. https://doi.org/10.1007/bf03325886

33. Radoviciu EM, Tomulescu IM, Merca VV. Effects Induced following the treatments with copper, manganese and zinc on corn seeds germination (Carrera, Turda 200 And HD-160). Analele Universitatii din Oradea, Fascicula Biologie. 2009;XVI (1):105-7.

34. Singh N, Rajendran A, Niraj P. Response of maize hybrids to Peg- induced osmotic stress at pre germination and germination phase. Int J Tropical Agri. 2016;34:2367-73.
35. Nishizono H, Kubta K, Suzuki S. Accumulation of heavy metals in cell walls of Polygonum cuspidatum roots from metalliferous habitats. Plant. Cell Physiol. 1989;30:595-8. https://doi.org/10.1093/oxfordjournals.pcp.a077780
36. Reichman SM. The Responses of Plants to Metal Toxicity: A review focusing on Copper, Manganese and Zinc. Australian Minerals & Energy Environment Foundation, Melbourne, Victoria, 3000, Melbourne, Australia. 2002.
37. Gang A, Vyas A, Vyas H. Toxic effect of heavy metals on germination and seedling growth of wheat. J Environ Res Develop. 2013;8:206-13.
38. Gupta D, Abdullah. Toxicity of copper and cadmium on germination and seedling growth of maize (*Zea mays L.*) Seeds. Indian J Sci Res. 2011;2:67-70.
39. Munzuroglu O, Geckil H. Effect of metals on seed germination, root elongation and coleoptile and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus*. J Arch Environ Contam Toxicol. 2002;43:203-13. https://doi.org/10.1007/s00244-002-1116-4
40. Choi WY, Kang SY, Park HK, Kim SS, Lee KS, Shin HT, Chai SY. Effects of water stress by PEG on growth and physiological traits in rice seedlings. Korean J. Crop Science. 2000;45:112-7.
41. Nilson ET, Orcut DM. The physiology of plants under stress. JohnWiley and Sons, New York, USA. 1996.
42. Li X, Mu C, Lin J. The germination and seedlings growth response of wheat and corn to drought and low temperature in spring of Northeast China. J Animal and Plant Sci. 2014;21:3212-22.
43. Abdalla M, El-Khoshiban NH. The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* cultivars. J Appl Sci Res. 2007;3:2062-74.
44. Ashagre H, Almaw D, Feyisa T. Effect of copper and zinc on seed germination, phytotoxicity, tolerance and seedling vigor of tomato (*Lycopersicon esculentum* L. cultivar Roma VF). Int J Agri Sci Res. 2013;2:312-7.
45. Stanova A, Durisova E, Banasova V, Gurinova E, Nadubinska M, Kenderesova L, Miroslav Ovecka M, Ciamporova M. Root system morphology and primary root anatomy in natural non-metallicolous and metallicolous populations of three *Arabidopsis* species differing in heavy metal tolerance. Biologia. 2012;67:505-16. https://doi.org/10.2478/s11756-012-0040-y