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Effects of Heavy Metal: Copper on the Ultrastructure of Wheat

Zhongliang Su[a,*], Chengwei Liang[a], Yongchao Bao[a], Xueyan Liu[a], Yonghong Sun[b], Chaoyu Song[b], Jianmei Chen[b], Qingzeng Guo[a], Jinheng Zhang[a]

[a]College of Chemical engineering, Qingdao University of Science &Technology, Qingdao, China, 266042.
[b]Qingdao Academy of Agriculture Science, Qingdao, China, 266100.
albertszhl@126.com

Copper was one of common soil contaminants in China. In order to research the stress mechanism of copper to wheat, the structure of subcellular in mesophyll cell were studied at different copper concentration and growth stage in natural conditions. The results showed that 1) at the tillering stage, under the level of L4 (900 mg/kg), comparing to the control, the cell structures were not intact. The number of chloroplast increased. The volume of chloroplast was enlarged. The arrangement of the thylakoids was disorder. 2) At the jointing stage, the membrane of chloroplast under concentrations of copper (L4) ruptured, and thylakoid spilled from chloroplast. 3) at the booting stage, the membrane of chloroplast under concentrations of L4 (900 mg/kg) broke and thylakoid spilled. The number of mitochondrion increased. 4) At the mature stage, some chloroplast disappeared under the concentration of L1 (100 mg/kg). The volume of chloroplast of became lager than it in control. The number of mitochondrion increased. Most chloroplasts under the level of L3 (600 mg/kg) disappeared, the number of mitochondrion increased and the cell wall was damaged. At the level of L4 (900 mg/kg), most organelles disappeared. In a word, the chloroplast in mesophyll cells of wheat was the most sensitive organelle under the condition of copper stress. The copper concentration and the developmental stage of wheat affected the damaged degree of subcellular structure of mesophyll cells. The organelle rupture in mesophyll cells caused by heavy metal was a key factor that led to the death of wheat.

1. Introduction

With the rapid increase of pollution, the food security had become increasingly serious. More and more attention was paid to pollution study, such as toxicity mechanism to plant. Heavy metal was the common contamination (Mikulasek and Cuhorka, 2016), especially in soil. Heavy metal in soil could be absorbed by plant and harm plant and the health of human being. The common heavy metal in soil was copper, cadmium and zinc. The source of copper in soil included its natural background value, pesticide spraying, chemical fertilizer application, special area of sewage irrigation, copper and zinc mining smelting, electroplating industrial wastewater and metal processing.

Copper was an essential nutrient for plant growth, the decrease of copper in plant could affect the synthesis of the pigment and cytochrome oxidase, which led to the inhibition of growth and photosynthesis and respiration. However, when the copper content in plant was higher than a certain level, it would cause a high degree of plant toxicity, inhabiting its growth and yield. The excess copper could come into human body by food chain and was harmful to human being. The excess of copper in the soil could inhibit the development of plant root and promote the growth of fibrous roots, resulting in hardening of root tip (Bi, 2013). During the process above, a common signal that involved oxidative posttranslational modifications of specific cell cycle proteins might be necessary (Pena et al., 2015). Excessive copper could destroy the structure of cell and affect the function of organelles (Li et al., 2006). The excess copper in plant could cause electrolyte leakage from cells as well as increased lipid peroxidation and protein carbonylation (Gajewska and Skłodowska, 2010).

In this study, plot experiments were conducted to explore the stress mechanism of excess copper to winter wheat in natural environment. The organelles structure of wheat leaf cell at different growth stages and copper levels were studied using transmission electron microscope. This study laid a foundation for exploring the stress mechanism of heavy metal to plant for further work.

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2. Materials and methods

2.1 Material source and culture

Wheat seed was Jimai 22, planting density was 187.5 kg/hm^2. The planting time was October 2014. As shown in Table 1, there were four levels of copper. The wheat leaves were sampled at tillering stage, jointing stage, booting stage and mature stage.

Table1: The copper concentration gradient in the study. CK blank; CuL1 treated by copper at 100 mg/kg; CuL2 treated by copper at 300 mg/kg; CuL3 treated by copper at 600 mg/kg; CuL4 treated by copper at 900 mg/kg.

	CK	CuL1	CuL2	CuL3	CuL4
concentration (mgkg^{-1})	0.00	100	300	600	900

2.2 Preparation of transmission electron microscope sample

The central parts of wheat leaves were sampled and then quickly placed into vials containing 2.5 % glutaraldehyde solution. They were sunk to the bottom of the vials using the method of vacuum-pumping and stored in 4 ^oC for a day. The leaves were soaked in 0.1 mol/L phosphate buffer for15 min and changed the buffer for 6 times. After fixed using 0.1 mol/L osmic acid for 2h, 0.1 mol/L phosphate buffer were used for washing the samples for 15 min and repeated the process 3 times. Subsequently, the samples were gradually dehydrated for 15min with 30 %, 50 %, 70 %, 85 %, 90 %, 95 % and 100 % ethanol. The samples were washed for 30min with epoxy propane after they were soaked into propylene oxide and ethanol for 12 h. The samples were sequentially soaked into propylene oxide and embedding medium with the ratio of 2:1, 1:1 and 1:2. The embedding medium was used to immerse the samples for 3 - 4 h. After that, the samples were placed on embedding plate containing embedding medium, and baked at 27 ^oC, 45 ^oC and 60 ^oC. Before observed using transmission electron microscope (TEM), the samples were stained with uranyl acetate and lead citrate after slicing.

3. Results Analysis

3.1The ultrastructure of Wheat mesophyll cell at tillering stage

In tillering stage, the shape of chloroplast in blank samples was fusiform, cytoplasm was transparent, thylakoid lamella was clear and the arrangement of grana was along the longitudinal axis of chloroplasts (Figure 1a). However, compared to the blank, in the samples (L4), thylakoids in chloroplasts disintegrated. There was almost no grana stacking, and the number of osmiophilic granules in chloroplasts increased (Figure 1b, 1c, 1d). Moreover, chloroplasts in some mesophyll cells disappeared, and cell nucleus was destroyed. The number of mitochondria in these cells decreased or disappeared, and cell wall thickness was not uniform any more (Figure 1b, 1c, 1d).

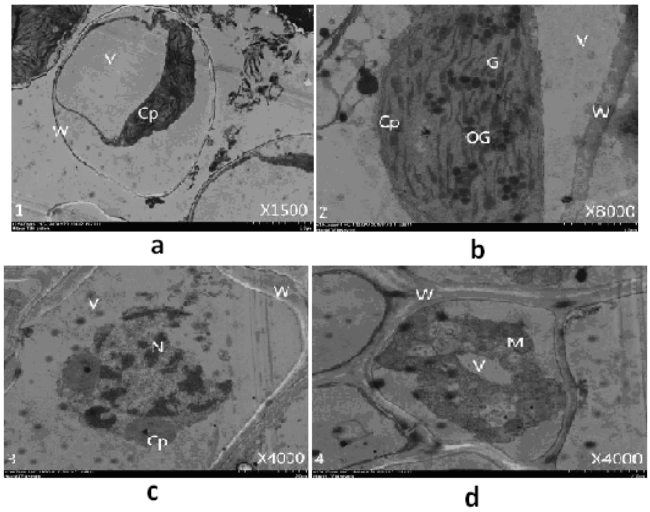

Figure 1: The ultrastructure of wheat mesophyll cell at tillering stage. a) blank; b), c) and d) treated by copper at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.2The ultrastructure of wheat mesophyll cell at jointing stage

In jointing stage, in blank samples, the shape of chloroplast is spherical and the site of chloroplast is close to the cell wall, the arrangement of grana was along the long axis of chloroplasts (Figure 2a). In samples (L4), chloroplast membrane ruptured, thylakoid escaped from chloroplasts and the number of osmiophilic granules increased significantly (Figure 2b).

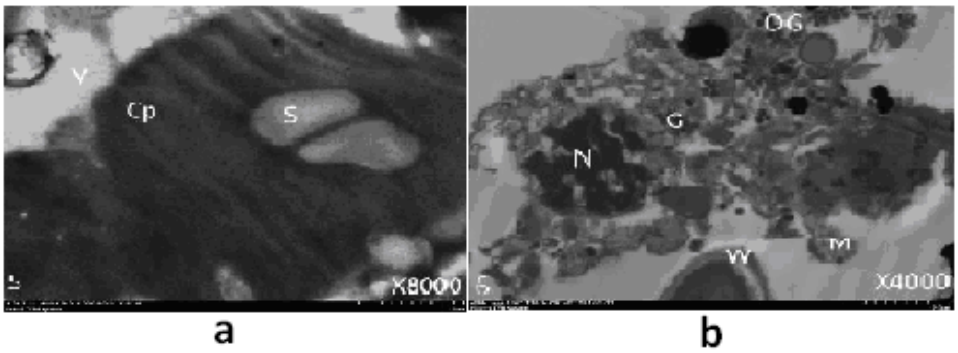

Figure 2: The ultrastructure of wheat mesophyll cell at jointing stage. a) blank; b) treated by copper at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.3 The ultrastructure of wheat mesophyll cells at booting stage

In booting stage, in L1 samples, the chloroplast membrane ruptured, thylakoid escaped from chloroplast; in some cells, chloroplasts became distortion (Figure 3a). However, in L2 samples, the density of thylakoid increased, the shape of chloroplast deformed and the number of mitochondria increased (Figure 3b). In L3 samples, the chloroplast was deformed, the number of osmiophilic granules increased, the distribution of mitochondria was along the chloroplast (Figure 3c, 3d). In some mesophyll cells of L4 samples, the cells were deformed, chloroplast membrane ruptured, thylakoid escaped and chloroplast swelled seriously (Figure 4a, 4b).

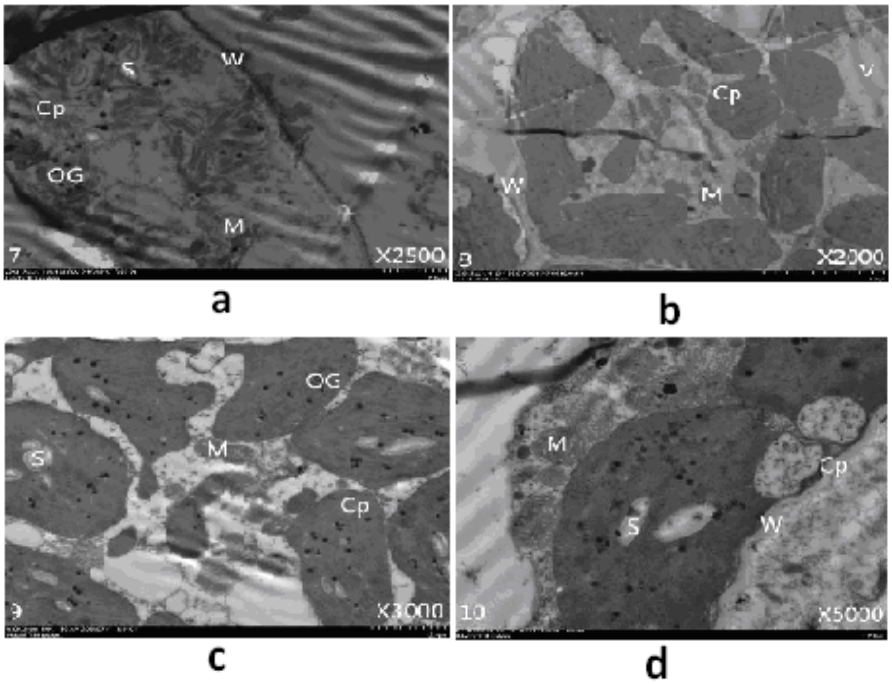

Figure 3: The ultrastructure of wheat mesophyll cell at booting stage. a) blank; b) treated by copper at level of L2; c) and d) treated by copper at level of L3. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

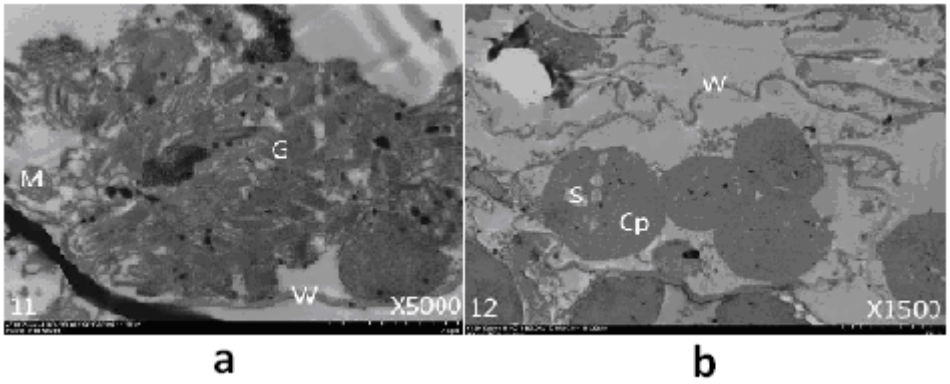

Figure 4: The ultrastructure of wheat mesophyll cell at booting stage. a) and b) treated by copper at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

3.4 The ultrastructure of wheat mesophyll cell at mature stage

At this stage, the shape of chloroplast in mesophyll cells was spindle with normal size and close to the cell wall; the grana arranged along the long axis; there were some starch grains and osmiophilic granules in the cells (Figure 5a). In L1 samples, some chloroplasts swelled seriously, disappeared or completely broken; the number of mitochondria increased (Figure 5b, 5c). In L2 samples, the chloroplast disappeared or completely broken and the degree of stacking in some thylakoids decreased significantly (Figure 5d, 5e). In L3 samples, chloroplasts disappeared or completely ruptured, the number of mitochondria increased and the cell wall was damaged (Figure 6a, 6b). In L4 samples, the organelles were severely deformed or completely destroyed, and the chloroplast swelled and occupied most of the cell space (Figure 6c, 6d).

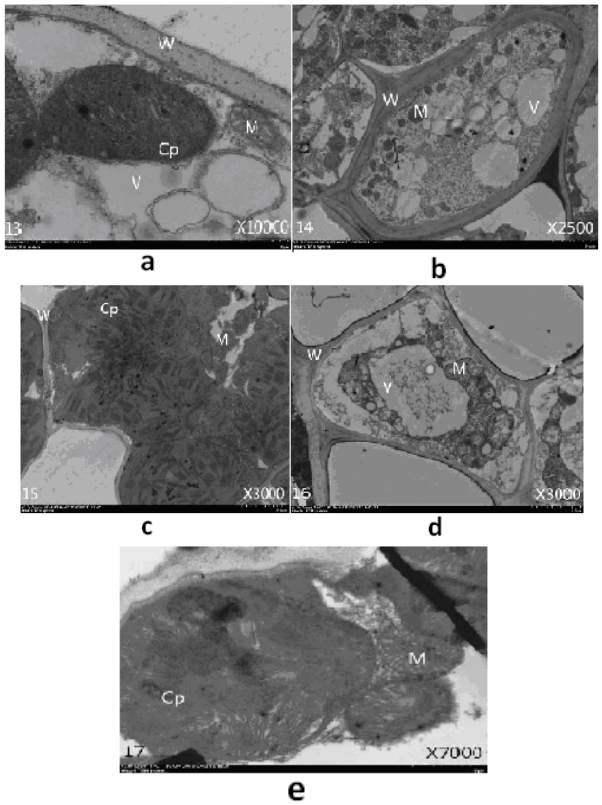

Figure 5: The ultrastructure of wheat mesophyll cell at mature stage. a) blank; b) and c) treated by copper at level of L1; d) and e) treated by copper at level of L2. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

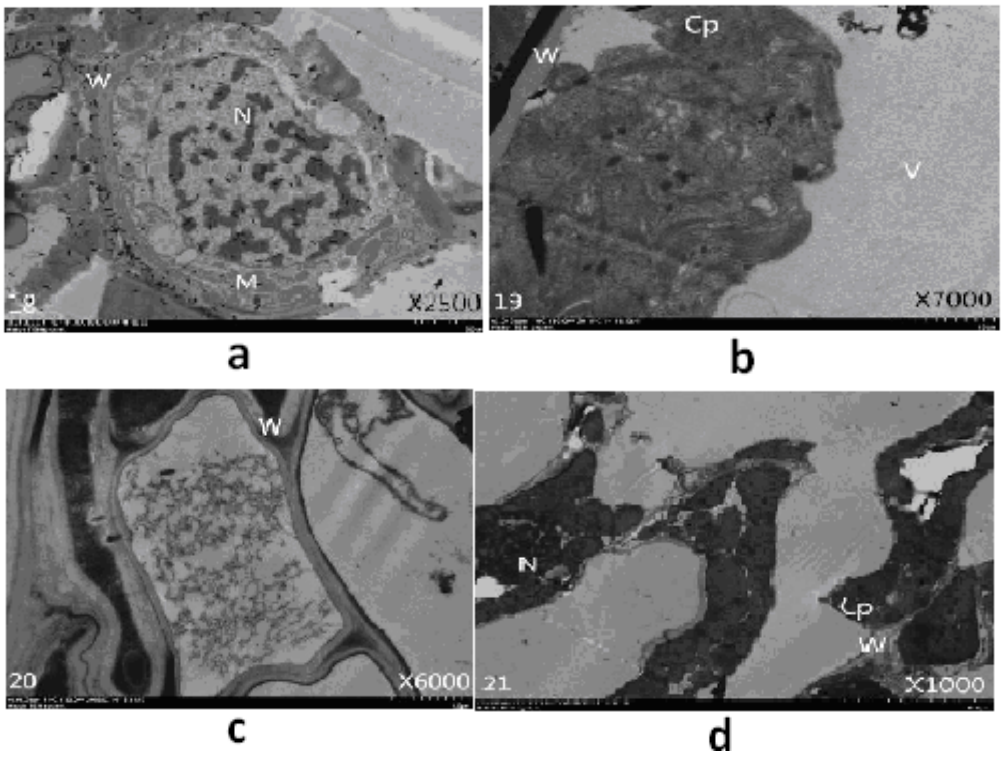

Figure 6: The ultrastructure of wheat mesophyll cell at mature stage. a) and b) treated by copper at level of L3; c) and d) treated by copper at level of L4. Notes: V---vacuole; Cp---chloroplasts; M---mitochnodrion; W---wall; S---starch granule; G---grana layer; SL---stroma layer; OG---osmiophilic granule.

Discussion

The stress of copper on plants firstly poisoned roots of plants. Due to the accumulation of large amount of copper in plant roots, the taproots became short and more fibrous roots were sent forth, which resulted in bad absorption of water and nutrients, led to stunted plant (Lukatkin et al., 2014). A part of the copper was transferred to the overground part of the plant, and it destroyed the internal structure of the leaves. Leaf was one of the most sensitive organs to stress (Ge et al., 2010). It could be seen by transmission electron microscope that at tillering stage, chloroplast was the most seriously damaged in all the ultrastructure in mesophyll cells under the stress of excess copper, proving that the chloroplast was the most sensitive organelle in wheat mesophyll cells. Liang et al. (2011) had reported the same results. In addition to chloroplast, cell wall, nucleus and mitochondria were also damaged by excess copper (Jiang et al., 2013). At wheat jointing stage, the most obvious characteristics of copper stress in mesophyll cell was the chloroplasts rapture. At the booting stage, in addition to the obviously chloroplast rapture, mitochondria was also serious stressed. With the increase of cooper concentration, the number of mitochondria increased firstly and then decreased. In the mature stage, the chloroplast was destroyed completely and the number of mitochondria increased firstly and then decreased until disappeared. These results were caused by the interaction of copper stress and wheat resistance, i.e. at low cooper concentration, the wheat was stressed and the respiration of single mitochondria was weakened, so the wheat had to increase the number of mitochondria to resist the stress of copper. However, with the increase of the concentration of copper in wheat, the osmotic pressure in mitochondria improved subsequently. And this would lead to the rapture of mitochondria. A common characteristic of wheat under copper stress was that the volume and number of chloroplasts was larger than the blank.

The stress effect on all organelles in mesophyll cells by the same level of copper was not completely consistent, which was called the growth asynchronism of plant (Li et al., 2007). When wheat was stressed by copper, part of cells generated defensive capacity, (That is, the number and volume of chloroplast increased, the distribution of chloroplast was along cell wall, and the number of mitochondria also increased), and other cells maintain normal activity. Overall, the physiological activity in some cells was lost to protect other cells avoiding damage (Li et al., 2007). This asynchronism could be observed not only by microscope but also by the phenotype of wheat (etiolated leaf edge and leaf exfoliation).

There were three stress effects by copper on mesophyll cells of wheat. The root length shortened, which resulted in the decrease of water in plant, the enhanced activity of lipid peroxidation in mesophyll cells, and the damage of the structure of cell membrane (Meng et al., 2011). The toxicity caused by excess copper resulted in the decrease of chlorophyll and further weakened photosynthesis of wheat. The excess copper in wheat could directly damage the structure of membrane or changed the osmotic pressure of cells, which led to the rapture of thylakoid, chloroplast and mitochondrial until disappearance (Fan et al., 2011). In order to resist the toxicity by excess copper, three measures were taken by wheat: the number of chloroplasts and mitochondria in cells were increased; normal physiological activities were maintained in some of the cells by the mechanism of physiological non-synchronization; the level of copper in the cell was reduced by thickening cell wall and increasing the residual copper on the cell wall (Strange and Macnair, 1991).

Conclusions

Chloroplasts in wheat mesophyll cells were damaged by excess copper. With the increase of copper level, the chloroplasts gradually became deformed and broken. The number of mitochondria increased at low contration of copper. And the mitochondria began to rupture and disappear at high concentration of copper. High concentration of copper in wheat could damage the rest of the cell organelles and cell wall of mesophyll cells. The results in this paper could be used to explain the abnormal growth and death of wheat at subcellular level under the stress of copper. This study laid a foundation for the further research, i.e., exploring the stress mechanism of heavy metal on wheat at molecular level.

Acknowledgements

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References

Bi R.C. (Ed), 2013, Introduction to Soil Pollution, Science Press, Beijing, China, ISBN: 9787030376787, 107-114.

Fan H., Dong K.H., Hou Y.P., Fang Z.H, 2011, The Effects of NaCl Stress on Ultrastructure of Salt-born Plant Artemisia Alkaloids, Journal of Grassland, 19(3), 482-486.

Gajewska E., Skłodowska M., 2010, Differential Effect of Equal Copper, Cadmium and Nickel Concentration on Biochemical Reactions in Wheat Seedlings, Ecotoxicology & Environmental Safety, 73(5), 996-1003.

Ge X.X., Ke F.F., Hao Q., Yu J.J., Guan X.L., 2010, The Adaptive Changes of North Four Euonymus Broad-leaved Evergreen Plants Ultrastructure of Mesophyll Cell in the Einter, Journal of Microscopy, 29 (2), 167-172.

Jiang Z.Y., Wang J.Y., Yang X.M., 2013, Effect of Copper, Lead and Zinc on Growth of Astragalus Membranaceus (Fisch.) Bge. Varmongholicus (Bge.) Hsiao and Its DNA Damage, Journal of Southern Agriculture, 44(2), 205-209.

Li Q., Deng Y., Yu L.J., 2007, The Observation with Scanning Electron Microscope and Ecological Adaptability on Two Lonicera Plant Leaf Epidermis, Guang Xi Plant, 27(3), 146-151.

Li Y., Wang L.Z., Yang G.X., He G.Y., 2006, Effects of Aluminum Stress on Wheat Embryo Induction and Ultrastructure, Biotechnology, 16(5), 25-29.

Liang W.B., Xue S. G., Shen J.H., 2011, The Effects of Mn Stress on Phytolacca Americana Leaf Morphological Structure and Ultrastructure of Chloroplast. Journal of Ecology, 31 (13), 3677-3683.

Lukatkin A., Egorova I., Michailova I., Malec P., Strzałka K., 2014, Effect of Copper on Pro- and Antioxidative Reactions in Radish (Raphanus sativus L.) in Vitro and in Vivo, Journal of Trace Elements in Medicine & Biology, 28(1), 80-6.

Meng F.J., Pang H. Y., Wang J.Z., Li S.Y., Wang Y.J., 2011, Mesophyll Chloroplasts Ultrastructure of Two Kinds of Robinia Pseudoacacia under NaCl and Na_2SO_4 Stress, Journal of Ecology, 31 (3), 734-741.

Mikulasek P., Cuhorka J., 2016, Removal of Heavy Metals Ions from Aqueous Solutions by Nanofiltration, Chemical Engineering Transactions, 47, 379-384.

Pena L.B., Méndez A.E., Matayoshi C.L., Gallego S.M., 2014, Early Response of Wheat Seminal Roots Growing under Copper Excess, Plant Physiology & Biochemistry, 87,115-123.

Strange J., Macnair R.M., 1991, Evidence for A Role for the Cell Membrane in Copper Tolerance of Mimulus Guttatus Fischer ex DC, New Phytologist, 119(3), 383-388.