http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (4): 649-655, 2005
ISSN 1028-8880

Comparative Effects of Copper and Cadmium on Growth and Lipid Content in Maize Seedlings (*Zea mays* L.)

[1]Radhouane Chaffai, [2]Ali Tekitek and [1]Ezzedine El Ferjani
[1]Laboratoire de Biologie et Physiologie Cellulaires Végétales,
Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie
[2]Laboratoire de Biochimie et de Biologie Moléculaire,
Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie

Abstract: Maize seedlings were grown in hydroponic nutrient solutions and treated for four days with 100 μM $CuSO_4$ or $Cd(NO_3)_2$. The effects of copper and cadmium on growth parameters and lipid composition of maize organs were studied. The results showed that copper reduced more significantly than cadmium the fresh and dry matter production at the aerial part of the plant. The two metals were found to be localized in major part in roots. We focus on the soluble and cell wall fractions of these metals. Hence, the soluble fraction of Cd was greater than that of copper. By contrast, the cell wall fraction of Cu was more significant than that of Cd. Results showed that glycolipids were more affected than phospholipids and steryl lipids. Thus, copper reduced more significantly the glycolipids content in roots and shoots. Moreover, we have suggested that the increase in MDA content in roots by copper could be the result of membrane lipoperoxidation.

Key words: Copper, lipid peroxidation, lipids, *Zea mays* L.

INTRODUCTION

Phospholipids, glycolipids and free sterols are major cell components that are primarily localized in plant membranes[1]. Damage of membrane lipids under heavy metal stress has been documented in several plant species[2]. Oxidative damage disrupts cellular membranes resulting in the conversion of unsaturated fatty acids into small hydrocarbon fragments such as MDA[3,4]. The lipid peroxidation processes may severely affect the functioning of biological membranes and may finally cause cell death[3,4]. Copper is an essential redox component required for a wide variety of processes including the detoxification of free radicals, lignification of plant cell walls, photosynthesis and electron transfer reactions of respiration[5]. However, excess of copper causes oxidative damage. The free copper ions can react with water to produce free radical hydroxyls, which in turn react to cause membrane lipid peroxidation[6].

Cadmium, a non-essential element, is strongly phytotoxic[7,8] and causes an increase in lipid peroxidation and lipoxygenase activity[9]. Excess cadmium has been also shown to enhance leaf senescence[9]. This metal is spilled extensively in the nature, present in the environment by the powerhouses, the heating systems, the metallurgic industries, the incinerators of waste, the urban traffic, the cement industrial unit and present as impurity in the phosphate fertilizer[8].

In this study, we analyzed the effect of copper and cadmium excess on growth parameters and lipid content in maize seedlings.

MATERIALS AND METHODS

Plant material and growth conditions: The corn (*Zea mays* L.; Var. LG 23/01) seeds were disinfected with 10% (v/v) H_2O_2 for 20 min then rinsed many times with distilled water and germinated in darkness at 25°C. After 4 days, the seedlings were transferred to plastic beakers filled with continuously aerated basal nutrient solution. For treatment purposes, eight-day-old seedlings were transferred to $CuSO_4$ or $Cd(NO_3)_2$ containing solutions that are renewed only once. The composition of nutrient solution (pH 5.7) was as follows: (mM) 2 KNO_3, 2.5 $Ca(NO_3)_2$, 1 KH_2PO_4, 1 $MgSO_4$; (in μM) 50 Fe as Fe-K-EDTA complex, 30 B as H_3BO_3, 10 Mn as $MnSO_4.H_2O$, 1 Cu as $CuSO_4.5H_2O$, 1 Zn as $ZnSO_4.7H_2O$ and 0.2 Mo as $(NH_4)_6Mo_7O_{24}.4H_2O$. Cultures were performed in conditioned room with 16 h photoperiod, using mercury lamps providing a light intensity of

Corresponding Author: Radhouane Chaffai, Laboratoire de Biologie et Physiologie Cellulaires Végétales, Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie
Tel: +216 22 822 523 Fax: +216 72 591 906 E-mail: radhouanec2002@yahoo.fr

150 μmol m^{-2} s^{-2}, a day/night temperatures of 25/22±1°C and 65±5%, relative humidity.

Cu and Cd analysis: At harvest, plants were divided into roots and shoots. The roots were washed three times with distilled water. The dry weight was determined after desiccation at 70°C for 7 days. For total metal determination, desiccated plant material was mineralized with nitroperchloric acid mixture HNO_3/$HCLO_4$ (4:1, v/v). The soluble Cu or Cd was determined in protein extracts auditioned with 3.7% HCl (1:2, v/v).

For analysis of Cu and Cd cell wall fractions, the cell wall is isolated according to Cathala *et al.*[10]. Roots were rinsed several times with distilled water and treated with non-ionic detergent 1% (v/v) Triton X-100 for 30 days. The solution was renewed everyday for the first two weeks and less frequently afterwards until it became clear. The cell wall fragments were rinsed for many times with distilled water oven-dried (70°C) and thereafter, converted to ash.

Cu and Cd content were analyzed by atomic absorption flame spectrophotometery (Perkin Elmer-model 2380).

MDA determination: The extent of lipid peroxidation was estimated according to Heath and Packer[11]. The plant materials were ground in 0.25% (w/v) in 10%TCA. The mixture was then heated at 95°C for 30 min and immediately cooled inside ice beaker and centrifuged at 1000 g for 10 min.

The absorbance of the supernatant was measured at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration was calculated using the extinction coefficient (155 mM^{-1} cm^{-1}).

Lipid extraction: The lipids were extracted according to the method of Folch *et al.*[12] modified by Bligh and Dyer[13]. The plant tissues were fixed in boiling water for 5 min to denature phospholipases[14] and then homogenized in chloroform : methanol mixture (2:1, v/v). The homogenate was centrifuged at 3000 rpm for 20 min. The lower chloroformic phase containing lipids was aspirated and evaporated at 40°C using rotary evaporator (Büchi). The residue was immediately redissolved in toluene/ethanol (4:1, v/v).

Total lipid determination: The total lipids were quantified by the absorbance at 215 nm. An aliquot of lipid extract (200 μL) was evaporated. The dry residue was dissolved in 3 mL ethanol. Total lipids were quantified using standard calibration curve of corn oil.

Steryl lipid determination: Steryl lipids in total lipids were determined according to Huang *et al.*[15]. An aliquot of lipid extract (200 μL) was evaporated in glass tubes. After addition of 1 mL of acetic acid, the tubes were vortexed and 2 mL of Liebermann-burchard reagent (1 mL of concentrated H_2SO_4 was added to 20 mL of acetic anhydride) were added. The tubes were incubated at room temperature in darkness for 1 h and the absorbance was measured at 525 nm. Cholesterol (Sigma) was used as a standard.

Glycolipids determination: The glycolipids were quantified in total lipid extracts by measuring sugar content according to Roughan and Batt[16]. An aliquot of lipid extracts (200 μL) was placed in glass tubes to evaporate. After addition of 0.5 mL 2% (v/v) phenol and 2 mL of concentrated H_2SO_4, the tubes were vortexed and incubated for 10 min. After centrifugation at 2-000 g for 5 min, the supernatants were used for the determination of the sugars. Absorbance was measured at 480 nm. Galactose (Sigma) was used as standard.

Phospholipids determination: The phospholipids were quantified by measuring the inorganic phosphorous content in lipid extracts according to Bartlett[17].

A lipid sample (200 μL) was evaporated in glass tubes, heated on flame and 0.5 mL of concentrated H_2SO_4 was added. After the appearance of a white smoke, few drops of H_2O_2 were added. The tubes were cooled and the volume was adjusted to 2 mL with distilled water. Six milliliter of acetate buffer containing copper sulfate 0.01 M, sodium acetate 0.33 M and acetic acid 2 M (pH 4.0), 1 mL 5% (w/v) ammonium molybdate and 1 mL reducing agent (2 g of paramethylaminophenol sulfate in 100 mL 10% (w/v) sodium sulfite) were added consecutively. The absorbance of the resulted colored solution was measured at 880 nm. A standard calibration curve was prepared using KH_2PO_4.

RESULTS AND DISCUSSION

Effect on growth: The exposure of maize seedlings to 100 μM $CuSO_4$ resulted in the inhibition of growth. Coppered reduces the production of fresh and dry matter of roots to the extent of 45.4 and 16.1%, respectively (Fig. 1A and C). While cadmium affected similarly the fresh and dry weight of root, the reduction was of a lesser extent by 37 and 14.1%, respectively (Fig. 1A and C). In the same way, copper affected more negatively the shoot growth than cadmium. The reduction of shoot fresh weight by copper is 49% while it was only 15% by cadmium. Moreover, copper decreases the

Table 1: Total amount of copper and cadmium in roots and shoots of maize seedlings treated with Cu or Cd for 4 days

		Metal content (μg g^{-1} DW)	
		Control	100 μM
Cu	Root	537±41.85	1321.1±174.93
	Shoot	34.27±2.07	49.31±1.52
Cd	Root	nd	2323.43±110.86
	Shoot	nd	214.03±4.44

The results presented were the mean values±standard error obtained from at least five independent experiments. nd, not detected

Table 2: Copper and cadmium in soluble fractions of roots and shoots of maize seedlings treated with 100 μM $CuSO_4$ or $Cd(NO_3)_2$ for 4 days

	Soluble fraction			
	μg g^{-1} DW		% of total content	
	Root	Shoot	Root	Shoot
0	82.49±13.38	nd	15.36±2.49	nd
100 μM Cu	269.08±28.18	6.48±0.85	20.37±2.13	13.15±1.73
0	Nd	Nd	Nd	Nd
100 μM Cd	904.82±25.14	38.1±7.92	38.94±1.08	17.8±3.70

The results presented were the mean values±standard error obtained from at least five independent experiments. Nd, not determined

shoot dry weight by 37% while cadmium did not register any effect.

Also, the copper had a more significant decreasing effect on water content than cadmium. The decrease was of 39 and 22% in roots and shoots respectively, while no significant changes were observed for cadmium treatment (Fig. 1 E and F).

Metal analysis

Total Cu and Cd : The Cd contents of maize organs were more significant than copper's and the accumulation of both metals was more important in roots than in shoots (Table 1). It has been shown elsewhere that the roots had a tendency to accumulate higher quantities of Cu than the aerial parts of plants[18]. There is also evidence that copper should be excluded from the leaves because of its inhibitory function against photosynthesis[19]. Restricted translocation of Cu from roots to shoots was also reported in *Agrostis stolonifera*[20].

Present results indicated that cadmium had higher mobility compared to copper. Translocation of Cd from roots to shoots has been studied in several species, including ryegrass (*Secale cereale*)[21], tomato (*Lycopersicon esculentum*)[22] and bean (*Phaseolus vulgaris*)[23]. Though there is evidence that Cd^{2+} binding to phytochelatins has little effect on the xylem translocation of Cd to shoots[24,25], the vacuolar compartmentalization of Cd may be a more effective mechanism for inhibiting long-distance transport within the plant. Movement of Cd from roots to shoots is likely to occur via xylem and to be driven by transpiration from

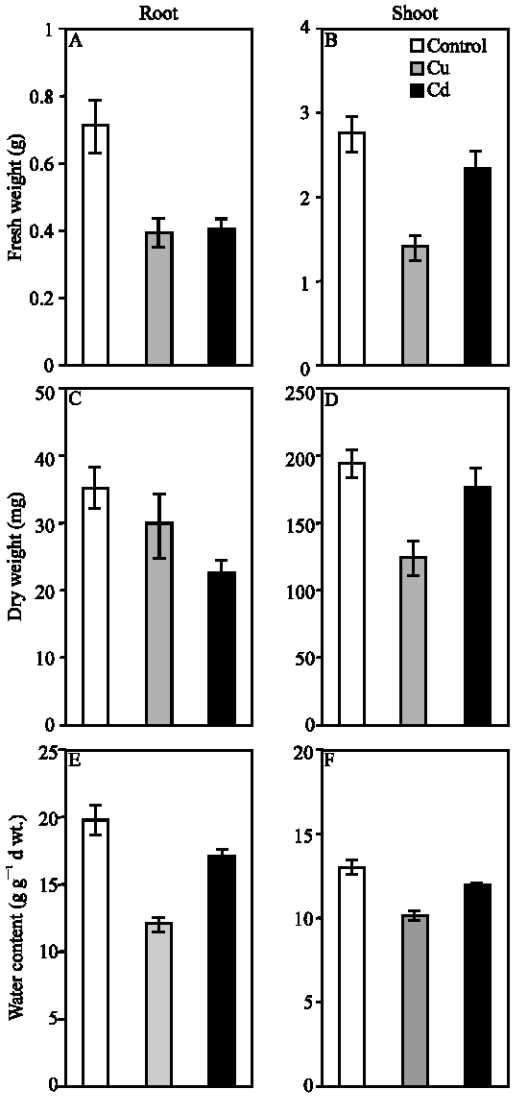

Fig. 1: Effects of copper and cadmium (100 μM) on fresh weight (A, B), dry weight (C, D) and water content (E, F) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results were given as the mean±standard error of at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

the leaves. Evidence for this was provided by Salt *et al.*[25], who showed that ABA-induced stomatal closure, dramatically reduced Cd accumulation in shoots of Indian mustard. Cellular sequestration of Cd can have significant effects on the levels of free Cd in the symplast and potentially influence the movement of Cd across the plant.

Table 3: Root cell-wall fractions of copper and cadmium of maize seedlings treated with 100 μM $CuSO_4$ or $Cd(NO_3)_2$ for 4 days

	Cell wall fraction	
	Cu	Cd
μg g^{-1} DW	97.34±11.80	67.45±8.82
% total root	9.18±1.55	2.9±0.38

The results presented were the mean values±standard error obtained from at least five independent experiments

Soluble fraction of Cu and Cd: Table 2 showed that the soluble fraction of Cd was larger than Cu in both shoots and roots. Indeed, the soluble fraction of Cd and Cu were 38.5 and 20.5%, respectively of the total root. Ionic Cd^{2+} concentration in the cytosol can be regulated by at least two processes: Cd^{2+} binding to phytochelatins[26] and cellular compartmentalization, particularly in the vacuole[27]. It has been shown that Cd movement across the plasma membrane of root cells occurs via concentration-dependent processes that exhibit saturated kinetics in maize[28].

Judging by the portion of heavy metals that is transported to the cytoplasm, it is suggested that there are some metal-binding substances present in the cytoplasm, which are involved in metal tolerance[29].

Cell wall fractions: With regard to cell wall fractions, the results were opposite to those obtained for soluble fractions, as the rate of Cu is greater than of Cd's one. In fact, the root cell-wall fractions represented 9.2% for Cu and 3% for Cd contents (Table 3). Hence, compared with Cu, cell wall contributed less effectively to the prevention of Cd from entering the cytoplasm.

In contrast with the present results, evidence on *Polygonum cuspidatum* grown in Cu-contaminated habitat indicated that only 10 to 25% of the total Cu was present in the soluble forming roots. This means that most of the Cu in the roots or in the cortex was associated with the cell wall fraction[30].

MDA content: Cadmium did not induce significant changes in MDA content in roots, but there was an increase when copper is introduced. The MDA content in shoots was increased by copper treatment, but decreased by cadmium (Fig. 2). The observed change was in accord with the results of Gora and Clijsters[31] which indicate that copper stimulates the formation of lipid peroxidation products, MDA and ethane in the leaves of *Phaseolus vulgaris* seedlings. The alteration of lipid membrane composition is the cell-response towards the environment stresses allowing the restoration of optimum physical properties[32]. Likewise, the lipid peroxidation in the roots of copper-sensitive *Silene cucubalus* induced by excess of copper occurs within a single day[2], whereas much higher copper concentrations are needed to induce lipid

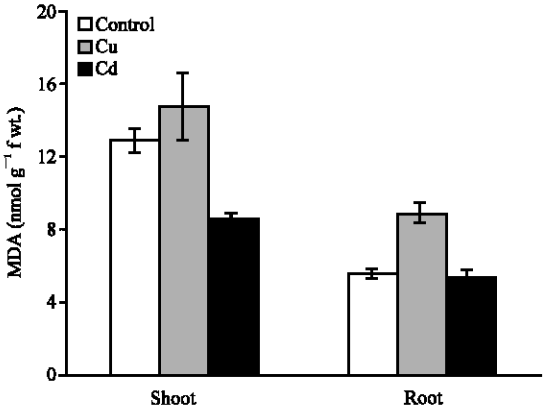

Fig. 2: Effects of copper and cadmium (100 μM) on MDA content in maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

peroxidation in copper tolerant genotypes of plants[6]. Copper is thought to play an important role in the onset of lipid peroxidation. It is capable of converting hydroperoxyds into reactive alloys and proxy radicals[33,34]. Copper catalyzes the formation of hydroxyl free radicals, which trigger the peroxidative process[35]. Because of its ability to catalyze the formation of harmful free radicals, copper at high concentrations can thus cause oxidative stress[36]. It can also catalyze the peroxidative destruction of thylacoïd membranes in isolated chloroplasts[37].

Lipid content: The total lipids increase in roots similarly for both Cu and Cd. In shoots, there was a significant increase in the total lipids only for the Cd treatment (Fig. 3A). It has been shown elsewhere that exposure of spinach plants to excess copper resulted in a significant decrease in the acyl lipid contents and induced changes in the lipid and fatty acid composition in the chloroplasts membranes[38].

The glycolipids concentrations decreased under copper treatment in roots but no significant change was noticed in Cd-treated ones. In shoots, a decrease of the concentrations of glycolipids was observed for both metals (Fig. 3B). Phospholipids are not influenced by Cu treatment neither in roots nor in shoots, while cadmium treatment, increased the concentrations of phospholipids in roots with no change in shoots (Fig. 3C).

There was no significant effect on steryl lipids in roots and shoots by both elements (Fig. 4A and B). The high level content of sterols is a major characteristic of the

Table 4: Effect of $CuSO_4$ and $Cd(NO_3)_2$ on SL/PL ratio in roots and shoots of maize seedlings treated for 4 days

	SL/PL		
	Control	Cu	Cd
Root	0.57±0.09	0.35±0.09	0.25±0.03
Shoot	7.17±1.61	6.11±0.96	3.42±0.65

The results presented were the mean values±standard error obtained from at least five independent experiments

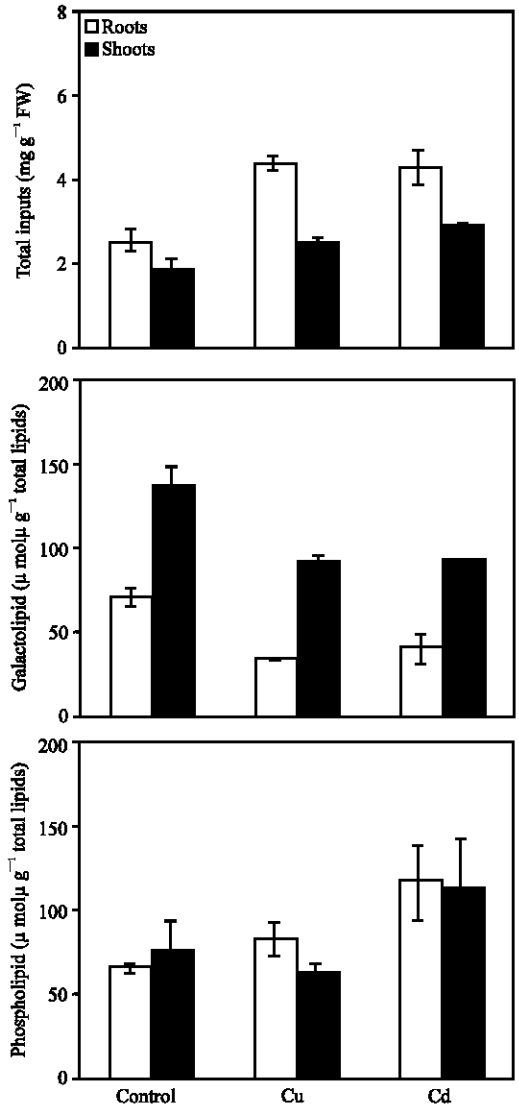

Fig. 3: Effects of copper and cadmium (100 µM) on total lipids (A), glycolipids (B) and phospholipids © of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

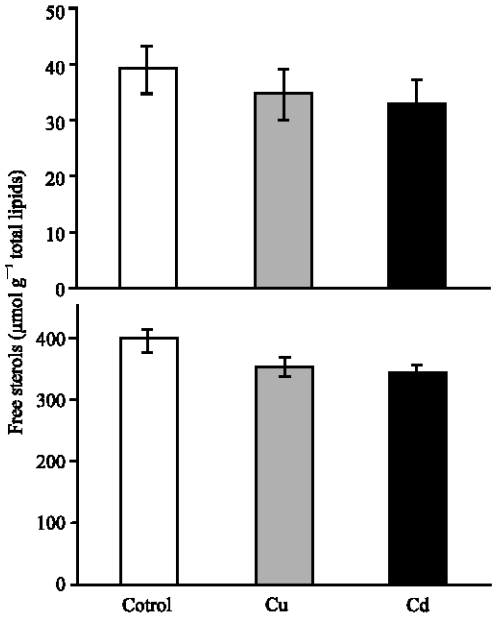

Fig. 4: Effects of copper and cadmium (100 µM) on steryl lipids in root (A) and shoot (B) of maize seedlings grown in hydroponics for 14 days then treated for 4 days. The results presented were the mean values±standard error obtained from at least five independent experiments. Significant differences between treated and control plants were determined using Student's t test ($p<0.05$)

cell membrane[38]. As evidenced by Zhang *et al.* [39]high sterol rates can be detected in microsomal membranes isolated in wheat. Similar results were reported with respect to other species[40]. A decrease in free sterols in leaf membranes of *Arabidopsis thaliana* was detected after 14 days of cold acclimatization[41].

The SL/PL ratio was decreased by Cu and Cd in roots compared to control plants (Table 4). The same result was noted for the shoots.

Present results showed that Cu and Cd treatment inhibited the biomass production in maize organs. It seems that copper affected more noticeably the aerial part of plants than cadmium did. In addition, these metals were accumulated preferentially in the root part. While cadmium was accumulated in greater level than copper in the shoots, its effect was of to a lesser degree on this part of plant.

The cell-wall fraction did not represent the major part of the total content of roots. This led to suggest that this fraction did not contribute efficiently in the metal exclusion mechanism. On the other hand, the non-soluble cytosolic fraction of roots represented a large proportion of the total content.

We noted that copper affected more negatively the growth of the root and especially of the aerial part of the plant. The two metals induced similarly a decrease of the glycolipid content in shoots. This could be due to the fact that copper confers the potential to cause oxidative damage and cause membrane lipid peroxidation.

ACKNOWLEDGMENTS

We are very grateful to Dr. Ben Jamaa Sami for his helpful advices and to Ms. Najeh Galei for her excellent and skillful help in preparing the manuscript.

REFERENCES

1. Harwood, J.L, 1980. Plant Acyl Lipids: Structure, Distribution and Analysis. In: The Biochemistry of Plants (Eds. Stumpf, P.K. and E.E. Conn) Academic Press, New York, USA., pp: 91-116.
2. De Vos, C.H.R., H. Schat, R. Vooijs and W.H.O. Ernst, 1989. Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. J. Plant Physiol., 135: 164-169.
3. Kappus, H., 1985. Lipid Peroxidation; Mechanisms, Analysis, Enzymology and Biological Relevance. In: Oxidative Stress (Ed. Sies, H.). Academic Press, London, pp: 273-310.
4. Tappel, A.L., 1973. Lipid peroxidation damage to cell components. Fed. Proc., 32: 1870-1874.
5. Fox, T.C. and M.L. Guerinot, 1998. Molecular biology of cation transport in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., 49: 669-696.
6. De Vos, C.H.R., H. Schat, M.A.M. De Waal, R. Vooijs and W.H.O. Ernst, 1991. Increased resistance to copper-induced damage of the root cell plasmalemma in copper tolerant *Silene cucubalus*. Physiol. Plant, 82: 523-528.
7. Clijsters, H. and F. Van Assche, 1985. Inhibition of photosynthetic by heavy metals. Photosynthesis Res., 7: 31-40.
8. Di Toppi, L.S. and R. Gabbrieli, 1999. Response to cadmium in higher plants. J. Exp. Bot., 41: 105-130.
9. Gallego, S.M., M.P. Benavides and M.L. Tomaro, 1996. Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. Plant Sci., 121: 151-159.
10. Cathala, N., M.H. Ghorbal, A. Lamant and L. Salsac, 1978. Obtention des parois cellulosiques à l'aide d'un détergent: Etude préliminaire de leur composition minérale. C.R. Académie des Sciences Paris, 286: 1025-1027.
11. Heath, R.L. and L. Packer, 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125: 189-198.
12. Folch, J., M. Lee and G.H. Sloane-Stanley, 1957. A simple method for the isolation of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.
13. Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.
14. Douce, R., 1964. Identification et dosage de quelques glycérophosphatides dans des souches normales et tumorales de scosonères cultivés *in vitro*. C.R. Académie des Sciences Paris, 259: 3066-3068.
15. Huang, T.C., C.P. Chen, V. Wefler and A. Raftery, 1961. A stable reagent for the Liebermann-Burchard Reaction. Ann. Chem., 33: 1405.
16. Roughan, P.G. and R.D. Batt, 1968. Quantitative analysis of sulfolipids and glycolipids in plant tissues. Ann. Biochem., 22: 74-88.
17. Bartlett, G.R., 1959. Phosphorus assay in column chromatography, J. Biol. Chem., 234: 466-468.
18. Cathala, N. and L. Salsac, 1975. Absorption du cuivre par les racines de maïs (*Zea mays* L.) et de tournesol (*Helianthus annuus* L.). Plant and Soil, 42: 65-83.
19. Graham, R.D., 1981. Absorpion of Copper by Plant Roots. In: Copper in Soils and Plants (Eds. Loneragan, J.F., A.D. Robson and R.D. Graham). Academic Press, New York, USA., pp: 141-163.
20. Wu, L., D.A. Thurman and A.D. Bradshaw, 1975. The uptake of copper and its effect upon respiratory processes of roots of copper-tolerant and non-tolerant clones of *Agrostis stolonifera*. New Phytol., 75: 225-229.
21. Jarvis, S.C., L.H.P. Jones and M.J. Hopper, 1976. Cadmium uptake from solution by plants and its transport from root to shoots. Plant and Soil, 44: 179-191.
22. Petit, C.M. and S.C. Van De Geijen, 1978. *In vivo* measurement of cadmium (115 m Cd) transport and accumulation in the stems of intact tomato plants (*Lycopersicon esculentum*, Mill.). I. Long distance transport and local accumulation. Planta, 138: 137-143.
23. Hardiman, R.T. and B. Jacoby, 1984. Absorption and translocation of Cd in bush beans (*Phaseolus vulgaris* L.). Physiol. Plant, 61: 670-674.
24. Florijn, P.J., J.A. Nelemans and M.L. Van Beusichem, 1993. Evaluation of structural and physiological plant characteristics in relation to the distribution of cadmium in maize inbred lines. Plant and Soil, 154: 103-109.

25. Salt, D.E., C.R. Prince, I.J. Pickering and I. Raskin, 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol., 109: 1427-1433.
26. Grill, E., E.L. Winnacker and M.H. Zenk, 1985. Phytochelatins: The principal heavy-metal complexing peptides of higher plants. Science, 230: 674-676.
27. Rauser, W.E., 1995. Phytochelatins and related peptides: structure, biosynthesis and function. Plant Physiol., 109: 1141-1149.
28. Mullins, G.L. and L.E. Sommers, 1986. Cadmium and zinc influx characteristics by intact corn (*Zea mays* L.) seedlings. Plant and Soil, 96: 153-164.
29. Rauser, W.E., 1987. Compartmental efflux analysis and removal of extracellular cadmium from roots. Plant Physiol., 85: 62-65.
30. Kubota, K., H. Nishizono, S. Suzuki and F. Ishii, 1988. A copper-binding protein in root cytoplasm of *Polygonum cuspidatum* growing in a metalliferous habitat. Plant Cell Physiol., 29: 1029-1033.
31. Gora, L. and H. Clijsters, 1989. Effects of Copper and Zinc on the Ethylene Metabolism. In: *Phaseolus vulgaris* L. In: Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants (Eds. Clijsters, H., M. De Proft, R. Marcel and M. Van Poucke). Kluwer Academic Publishers Dordrecht, The Netherlands, pp: 219-228.
32. Thompson, G.A.Jr., 1992. The Regulation of Membrane Lipid. CRC Press, Boca Raton, FL.
33. Chan, P.C., O.G. Peller and L. Kesner, 1982. Copper (II)-catalysed lipid peroxidation in liposomes and erythrocyte membranes. Lipids, 17: 331-337.
34. Girotti, A.W., 1985. Mechanisms of lipid peroxidation. J. Free Rad. Biol. Med., 1: 87-95.
35. Halliwell, B. and J.M.C. Gutteridge, 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J., 219: 1-14.
36. Fernandes, A., M.L. Mira, MS. Azevedo and C. Manso, 1988. Mechanisms of hemolysis induced by copper. Free Rad. Res. Comm., 4: 291-298.
37. Sandmann, G. and P. Böger, 1980. Copper-mediated lipid peroxidation processes in photosynthetic membranes. Plant Physiol., 66: 797-800.
38. Zhang, G., J.J. Slaski, D.J. Archambault and G.J. Taylor, 1996. Aluminum-induced alteration in lipid composition of microsomal membrane from an aluminum-resistant and aluminum sensitive cultivar of *Triticum aestivum*. Physiol. Plant, 96: 683-691.
39. Zhang, G., J.J. Slaski, D.J. Archambault and G.J. Taylor, 1997. Alteration of plasma membrane lipids in aluminum-resistant and aluminum-sensitive wheat genotypes in response to aluminum stress. Physiol. Plant, 99: 302-308.
40. Palta, J.P. B.D. Whitaker and L.S. Weiss, 1993. Plasma membrane lipids associated with genetic variability in freezing tolerance and cold acclimation of *Solanum* species. Plant Physiol., 103: 793-803.
41. Uemura, M., R.A. Joseph and P.L. Steponkus, 1995. Cold acclimation of *Arabidopsis thaliana*, effect on plasma membrane lipid composition and freeze-induced lesions. Plant Physiol., 109: 15-30.