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BRIEF COMMUNICATION

Calcium-mediated enhancement of copper tolerance in *Elodea canadensis*

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Abstract

The alleviative effects of exogenous calcium on copper phytotoxicity were investigated in *Elodea canadensis* plants. There was a significant accumulation of Cu in the plants after their exposure to 0.01 mM Cu accompanied by many symptoms of toxicity. Increased uptake of Cu severely reduced content of photosynthetic pigments, soluble proteins, and free proline. The total antioxidant capacity (T-AOC), reduced glutathione (GSH), and non-protein thiol (NP-SH) were severely suppressed in Cu-stressed plants resulting in a rapid increase in content of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide, lipid peroxidation, and cell death. Simultaneous application of Ca markedly increased the content of photosynthetic pigments, soluble proteins, free proline, T-AOC, GSH, and NP-SH, and reduced oxidative damage as indicated by lowered content of MDA, $O_2^{\bullet-}$, and H_2O_2; and decreased cell death. Furthermore, application of Ca reduced Cu uptake and effectively reversed the Cu-induced nutrient imbalance.

Additional key words: alleviation, antioxidants, carotenoids, chlorophyll, glutathione, H_2O_2, mineral nutrition, $O_2^{\bullet-}$, proline, proteins.

The contamination of aquatic ecosystems by heavy metals has attracted considerable concern around the world due to their toxic effects on plant metabolism and public health (Nagajyoti *et al.* 2010). Copper is an essential trace element needed for growth and development of plants. It acts as a co-factor in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative reactions, cell wall metabolism, and hormone signaling (Flemming and Trevors 1989, Maksymiec 1997). However, previous studies have suggested that Cu could be highly toxic to plants at the supraoptimal amount. For example, Cu can inhibit growth, cause physiological and biochemical disturbances, and induce symptoms of plant senescence (Flemming and Trevors 1989, Maksymiec 1997). Moreover, Cu catalyzes the Fenton/Haber-Weiss reaction and is directly involved in the generation of oxidative stress due to overproduction of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), *etc*. (Stohs and Bagchi 1995).

As an essential macronutrient, calcium is a crucial regulator of growth and development in plants (Hepler 2005) and has been shown to stabilize cell membranes, change the pH of cells, and prevent solute leakage from the cytoplasm (Hirschi 2004). Furthermore, there is a considerable body of research that shows that Ca is involved in the signal transduction of environmental stimuli and related gene expression (McAinsh *et al.* 1996, He *et al.* 2005). Recent findings have revealed that Ca could increase plant tolerance to abiotic stresses such as salinity (Bonilla *et al.* 2004), water stress (Nayyar and Kaushal 2002), and heat stress (Larkindale and Knight 2002). Increased Ca concentration in growth media have

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Abbreviations: Car - carotenoid; Chl - chlorophyll; GSH - reduced glutathione; MDA - malondialdehyde; NP-SH - non-protein thiols; $O_2^{\bullet-}$ - superoxide anion; PCs - phytochelatins; ROS - reactive oxygen species; T-AOC - total antioxidant capacity; TBA - thiobarbituric acid.

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also been reported to protect algae and terrestrial plants against heavy metal toxicity by precipitation, complex formation, reduced metal accumulation, enhancement of antioxidants, and increased metabolism (Ouzounidou *et al.* 2006, Drażkiewicz and Baszyński 2008, Wang and Song 2009, Fan *et al.* 2012, Le *et al.* 2012). A commonly proposed mechanism is the restoration of Ca content (Rengel 1992). There have also been reports on the protective role of Ca against the inhibition of photosystem II by Cu and Cd (Maksymiec and Baszyński 1999, Drażkiewicz and Baszyński 2008).

Elodea canadensis, a common submerged macrophyte, is widely distributed around the world and has a rapid growth rate. It is also a potential accumulator of heavy metals and these accumulated metals have been shown to induce several biochemical, physiological and cellular changes within the plant (Mal *et al.* 2002, Fritioff and Greger 2007, Dogan *et al.* 2009). However, as far as can be ascertained, there have been few reports on the role of Ca in response to heavy metal stress in aquatic plants. In this study, *E. canadensis* was chosen as the experimental material to investigate whether exogenous Ca confers protection against Cu toxicity. This study will be helpful in understanding the physiological and biochemical detoxification strategies that aquatic plants adopt against heavy metal stress when Ca is added.

Elodea canadensis Michx. plants were obtained from unpolluted pond in Nanjing, China and were grown for 3 months in large hydroponic tubes filled to 1/4 with soil. Before treatment, the growing shoots (top 10 cm of the plant) were separated from the mother plant, washed three times with running tap water and acclimatized in 1/10 Hoagland solution under laboratory conditions (114 μmol m^{-2} s^{-1} irradiance, a 14-h photoperiod and day/night temperature of 25/20°C). There were three treatments: control (1/10 Hoagland solution), 0.01 mM Cu (the sublethal concentration of Cu for *E. canadensis*) and 0.01 mM Cu + 20 mM Ca. The concentrations were chosen on the basis of preliminary experiments (data not shown). The plants were grown in aquaria under the above-mentioned laboratory conditions for 4 d. All the solutions were refreshed every 2 d. All experiments were carried out in triplicate and each replicate contained 20 plants. After harvesting, the plants were washed with double distilled water, blotted, frozen in liquid nitrogen, and stored at -80 °C until analysis.

Cu and other nutrients were analyzed by inductively coupled plasma - atomic emission spectrometry (*ICP-AES*, *Leeman Labs*, Prodigy, USA). Chlorophyll (Chl) and carotenoid (Car) content was determined according to the method used by Lichtenthaler (1987). The rate of $O_2^{\bullet-}$ generation was determined following the method used by Elstner and Heupel (1976). H_2O_2 content was measured according to Pick (1986) with some modifications. Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content using the thiobarbituric acid (TBA) method as described by Heath and Packer (1968). Cell death was measured using the Evans blue staining method as described by Baker and Mock (1994). The protein content was estimated following the method of Bradford (1976). The level of T-AOC was determined using a T-AOC testing kit (Nanjing Jiancheng Bioengineering Institute). GSH content was determined according to the method used by Anderson (1985). NP-SH compounds were extracted and assayed according to Ellman (1959) and the proline content was estimated using ninhydrine acid reagent according to Bates *et al.* (1973).

All values presented are expressed as means ± standard deviations (SD; $n = 3$). Significant differences between treatments were analyzed statistically using one-way *ANOVA* followed by multiple comparison tests (LSD). Different letters indicate significantly different values at $P < 0.05$.

A substantial accumulation of Cu was observed in *E. canadensis* plants after 0.01 mM Cu treatment over 4 d (Table 1). This confirmed the results reported by Giampaoli *et al.* (2012). Cu has the ability to bind thiol groups and disrupt the redox status of a cell, thereby enhancing the production of ROS which can induce lipid peroxidation (Stohs and Bagchi 1995). In this study, Cu accumulation stimulated ROS generation and lipid peroxidation which suggested that Cu can cause oxidative stress in *E. canadensis* plants. A sharp increase in content of $O_2^{\bullet-}$, H_2O_2, and MDA (Table 2) may have caused membrane damage, leading to accelerated cell death (Table 2). In previous studies, the accumulation of Cu and other toxic metals in plants resulted in significant reduction in the uptake of a number of nutrients (Ouzounidou *et al.* 2006, Xu *et al.* 2010). A similar result was also observed in relation to the contents of P, K, Mg, Na, and B in Cu-treated *E. canadensis* (Table 1). These nutrient deficiencies might be also the result of reduced energy availability (which the membrane transport systems require in order to function) caused by possible damage to chloroplasts and mitochondria. In addition, a slight increase in Ca was seen in the Cu-treated plants (Table 1) which could be attributed to the fact that Cu causes an influx of Ca through the plasma membrane Ca^{2+} channels (Inoue *et al.* 2005). These results suggested that Cu disrupted selective mineral uptake in the cells of *E. canadensis* plants and disturbed the internal nutrient balance. However, application of Ca had a negative effect on Cu accumulation (Table 1). The results of this study are consistent with previous studies that the addition of Ca decreased Cu content (Zaki and Fathi 2004, Fan *et al.* 2012). The decline in the accumulation of Cu after Ca treatment suggested an antagonistic interaction between Cu and Ca due to their similar ionic form (Maksymiec and Baszyński 1998). Ca^{2+} stabilizes lipid bilayers by binding to phospholipids and thus provides structural integrity to cellular membranes (Hepler 2005). The decrease in ROS, lipid peroxidation, and Evans blue uptake could also explain the alleviative effects of Ca on

Table 1. Effects of exogenous Ca on the content of Cu and other elements [μg g^{-1}(f.m.)] in *E. canadensis* plants exposed to 0.01 mM Cu for 4 d. Means ± SD (n = 3). Effects of treatments not sharing common letters are significantly different at $P < 0.05$ as determined using one-way *ANOVA* followed by LSD.

Content	Control	Cu	Cu + Ca
Copper	5.2 ± 1.8 c	89.8 ± 5.7 a	47.6 ± 5.7 b
Calcium	1116.7 ± 64.3 b	1246.7 ± 94.5 b	1673.3 ± 47.3 a
Phosphorus	1417.7 ± 158.3 a	565.3 ± 69.0 c	877.7 ± 81.1 b
Potassium	4113.3 ± 328.1 a	2190.0 ± 278.4 c	2950.0 ± 216.6 b
Magnesium	374.0 ± 26.5 a	234.0 ± 65.0 b	324.7 ± 16.9 a
Iron	32.3 ± 2.6 a	41.1 ± 5.7 a	35.3 ± 5.3 a
Boron	2.7 ± 0.4 a	1.5 ± 0.4 b	2.4 ± 0.5 a
Sodium	955.3 ± 59.4 a	481.3 ± 23.4 c	693.7 ± 17.0 b

Table 2. Effects of exogenous Ca on the levels of Chl *a*, Chl *b*, Car, $O_2^{\bullet-}$, H_2O_2, MDA, cell death, soluble protein, T-AOC, GSH, NP-SH, and proline in *E. canadensis* plants exposed to 0.01 mM Cu for 4 d. Means ± SD (n = 3). Effects of treatments not sharing common letters are significantly different at $P < 0.05$ as determined using one-way *ANOVA* followed by LSD.

Parameters	Control	Cu	Cu + Ca
Chl *a* [mg g^{-1}(f.m.)]	0.50 ± 0.05 a	0.25 ± 0.02 b	0.45 ± 0.04 a
Chl *b* [μg g^{-1}(f.m.)]	0.19 ± 0.02 a	0.14 ± 0.01 b	0.20 ± 0.01 a
Car [μmg g^{-1}(f.m.)]	0.13 ± 0.02 a	0.05 ± 0.003 c	0.10 ± 0.002 b
$O_2^{\bullet-}$ [nmol g^{-1}(f.m.) min^{-1}]	2.68 ± 0.16 c	9.38 ± 0.48 a	4.71 ± 0.21 b
H_2O_2 [nmol g^{-1}(f.m.)]	3.24 ± 0.28 c	6.29 ± 0.13 a	4.75 ± 0.42 b
MDA [nmol g^{-1}(f.m.)]	13.03 ± 0.91 c	23.03 ± 1.92 a	14.16 ± 0.41 b
Cell death [A_{600}]	0.31 ± 0.02 c	0.74 ± 0.01 a	0.47 ± 0.04 b
Soluble protein [mg g^{-1}(f.m.)]	2.92 ± 0.23 a	1.72 ± 0.04 c	2.35 ± 0.20 b
T-AOC [U g^{-1}(f.m.)]	27.44 ± 3.28 a	8.06 ± 2.43 c	19.61 ± 2.52 b
GSH [mg g^{-1}(f.m.)]	10.16 ± 1.17 a	2.47 ± 0.44 c	6.48 ± 0.73 b
NP-SH (μmol g^{-1}(f.m.))	46.87 ± 1.99 a	28.75 ± 1.76 c	35.28 ± 3.54 b
Proline [μg g^{-1}(f.m.)]	50.29 ± 4.81 a	32.48 ± 1.53 b	45.54 ± 1.71 a

Cu-induced membrane damage. Hence, the imbalance of nutrient elements in *E. canadensis* plants under Cu stress could be greatly alleviated by the application of Ca (Table 1) due to decreased membrane damage.

Redox cycling between Cu^+ and Cu^{2+} can catalyze the production of highly toxic free radicals through the Haber-Weiss reaction which causes damage to the photosynthetic apparatus and catalyzes the degradation of proteins through oxidative modification and increased proteolytic activity (Srivastava *et al.* 2006). Content of Chl *a*, Chl *b*, Car and soluble protein decreased significantly in *E. canadensis* plants treated with Cu alone (Table 2). The addition of Ca significantly counteracted the inhibition of pigment and protein biosynthesis caused by Cu. It is suggested that this is due to the efficient control of Cu-induced oxidative damage and a major increase in antioxidant capacity due to the Cu + Ca treatment which effectively mitigated Cu toxicity.

Cu led to a significant inhibition of T-AOC (Table 2). This was possibly due to the overproduction of ROS, which would induce oxidative damage. It is evident from these results that the equilibrium between the production of ROS and protective antioxidants became unbalanced. The addition of Ca caused a significant increase in the content of T-AOC and thus mitigated oxidative damage induced by Cu stress. A significant increase in the activity of antioxidative enzymes has also been reported in Ca + Cd treated *Trifolium repens* by Wang and Song (2009). Ca could regulate the activities of target proteins directly or *via* calmodulin which after binding to Ca^{2+} activates a number of protein kinases (Wang and Song 2009). This may explain one of the roles of Ca in improving the antioxidant capacity of plants under Cu stress.

Cu stress generated a sharp reduction in GSH and NP-SH concent (Table 2). Sulfate assimilation and GSH biosynthesis take place in chloroplasts, so damage to chloroplast ultrastructure and membrane integrity by Cu may affect the sulfur metabolism in plants (Srivastava *et al.* 2006). The results of this study were in agreement with the earlier findings of Singh *et al.* (2010) in

chickpea. The decreased content of NP-SH could be due to its consumption during the synthesis of phytochelatins (PCs) which require GSH as precursor (De Vos *et al.* 1992). The fact that GSH became depleted during PCs synthesis suggested the possibility of PCs synthesis in *E. canadensis* under Cu stress but no evidence for the induction of PCs was found in this study. However, Ca application significantly enhanced the content of NP-SH and GSH (Table 2). It has been shown that NP-SH compounds, in particular GSH, play a prominent role in the detoxification of free radicals in plants (De Vos *et al.* 1992). Extensive investigation is required to elucidate the roles of PCs in Cu and Ca + Cu-treated *E. canadensis*.

Dhir *et al.* (2004) demonstrated that hydrophytes (*Ceratophyllum*, *Wolffia* and *Hydrilla*), unlike mesophytes, exhibited a decline in proline content in response to Cd pollution. These results are in accordance with this study which showed a Cu concentration-dependent decline in proline content (Table 2) in the opposite to the findings of Ku *et al.* (2012). However, the plants subjected to the Cu + Ca treatment showed a higher proline content compared to the plants subjected to the Cu alone. Proline has been shown to be involved in the protection of enzyme activity, the maintenance of cellular osmoticum, the NADPH/$NADP^+$ ratio, cytosolic pH, as well as helping to scavenge ROS (Mehta and Gaur 1999). The increase in proline content induced by exogenous Ca indicated that proline was involved in the adaptation of *E. canadensis* to Cu stress when additional Ca was supplied.

In conclusion, these results have shown that application of 20 mM Ca together with 0.01 mM Cu significantly decreased the excessive accumulation of Cu, maintained cellular nutrient balance, reduced the degradation of pigments and soluble proteins, promoted the synthesis of proline, alleviated oxidative damage, maintained high antioxidant capacity (T-AOC, GSH, and NP-SH), and effectively ameliorated the phytotoxicity of Cu in *E. canadensis*.

References

Anderson, M.E.: Determination of glutathione and glutathione disulfide in biological samples. - Methods Enzymol. **113**: 548-555, 1985.

Baker, C.J., Mock, N.M.: An improved method for monitoring cell death in cell suspension and leaf disc assays using Evans blue. - Plant Cell Tissue Organ Cult. **39**: 7-12, 1994.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. - Plant Soil **39**: 205-207, 1973.

Bonilla, I., El-Hamdaoui, A., Bolaños, L.: Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress. - Plant Soil **267**: 97-107, 2004.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

De Vos, C.H.R., Vonk, M.J., Vooijs, R., Schat, H.: Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in *Silene cucubalus*. - Plant Physiol. **98**: 853-858, 1992.

Dhir, B., Sharmila, P., Saradhi, P.P.: Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. - Aquat. Toxicol. **66**: 141-147, 2004.

Dogan, M., Saygideger, S.D., Colak, U.: Effect of lead toxicity on aquatic macrophyte *Elodea canadensis* Michx. - Bull. Environ. Contam. Toxicol. **83**: 249-254, 2009.

Drażkiewicz, M., Baszyński, T.: Calcium protection of PS2 complex of *Phaseolus coccineus* from cadmium toxicity: *in vitro* study. - Environ. exp. Bot. **64**: 8-14, 2008.

Ellman, G.L.: Tissue sulfhydryl groups. - Arch. Biochem. Biophys. **82**: 70-77, 1959.

Elstner, E.F., Heupel, A.: Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. - Anal. Biochem. **70**: 616-620, 1976.

Fan, J., He, Z., Ma, L.Q., Nogueira, T.A.R., Wang, Y., Liang, Z., Stoffella, P.J.: Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils. - J. Hazard. Materials **199-200**: 375-382, 2012.

Flemming, C.A., Trevors, J.T.: Copper toxicity and chemistry in the environment: a review. - Water Air Soil Pollut. **44**: 143-158, 1989.

Fritioff, A., Greger, M.: Fate of cadmium in *Elodea canadensis*. - Chemosphere **67**: 365-375, 2007.

Giampaoli, P., Tresmondi, F., Lima, G.P.P., Kanashiro, S., Alves, E.S., Domingos, M., Tavares, A.R.: Analysis of tolerance to copper and zinc in *Aechmea blanchetiana* grown *in vitro*. - Biol. Plant. **56**: 83-88, 2012.

He, Z., Li, J., Zhang, H., Ma, M.: Different effects of calcium and lanthanum on the expression of phytochelatin synthase gene and cadmium absorption in *Lactuca sativa*. - Plant Sci. **168**: 309-318, 2005.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Hepler, P.K.: Calcium: a central regulator of plant growth and development. - Plant Cell **17**: 2142-2155, 2005.

Hirschi, K.D.: The calcium conundrum. Both versatile nutrient and specific signal. - Plant Physiol. **136**: 2438-2442, 2004.

Inoue, H., Kudo, T., Kamada, H., Kimura, M., Yamaguchi, I., Hamamoto, H.: Copper elicits an increase in cytosolic free calcium in cultured tobacco cells. - Plant Physiol. Biochem. **43**: 1089-1094, 2005.

Ku, H.M., Tan, C.W., Su, Y.S., Chiu, C.Y., Chen, C.T., Jan, F.J.: The effect of water deficit and excess copper on proline metabolism in *Nicotiana benthamiana*. - Biol. Plant. **56**: 337-343, 2012.

Larkindale, J., Knight, M.R.: Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. - Plant Physiol. **128**: 682-695, 2002.

Le, T.T.Y., Peijnenburg, W.J.G.M., Hendriks, A.J., Vijver,

M.G.: Predicting effects of cations on copper toxicity to lettuce (*Lactuca sativa*) by the biotic ligand model. - Environ. Toxicol. Chem. **3**: 355-359, 2012.

Lichtenthaler, H.K.: Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. - Methods Enzymol. **148**: 350-382, 1987.

Maksymiec, W.: Effect of copper on cellular processes in higher plants. - Photosynthetica **34**: 321-342, 1997.

Maksymiec, W., Baszyński, T.: The role of Ca ions in changes induced by excess Cu^{2+} in bean plants. Growth parameters. - Acta Physiol. Plant. **20**: 411-417, 1998.

Maksymiec, W., Baszyński, T.: The role of Ca^{2+} ions in modulating changes induced in bean plants by an excess of Cu^{2+} ions. Chlorophyll fluorescence measurements. - Physiol. Plant. **105**: 562-568, 1999.

Mal, T.K., Adorjan, P., Corbett, A.L.: Effect of copper on growth of an aquatic macrophyte, *Elodea canadensis*. - Environ. Pollut. **120**: 307-311, 2002.

McAinsh, M.R., Clayton, H., Mansfield, T.A., Hetherington, A.M.: Changes in stomatal behavior and guard cell cytosolic free calcium in response to oxidative stress. - Plant Physiol. **111**: 1031-1042, 1996.

Mehta, S.K., Gaur, J.P.: Heavy-metal-induced proline accumulation and its role in ameliorating metal toxicity in *Chlorella vulgaris*. - New Phytol. **143**: 253-259, 1999.

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M.: Heavy metals, occurrence and toxicity for plants: a review. - Environ. Chem. Lett. **8**: 199-216, 2010.

Nayyar, H., Kaushal, S.K.: Alleviation of negative effects of water stress in two contrasting wheat genotypes by calcium and abscisic acid. - Biol. Plant. **45**: 65-70, 2002.

Ouzounidou, G., Moustakas, M., Symeonidis, L., Karataglis, S.: Response of wheat seedlings to Ni stress: effects of supplemental calcium. - Arch. Environ. Contam. Toxicol. **50**: 346-352, 2006.

Pick, E.: Microassays for superoxide and hydrogen peroxide production and nitroblue tetrazolium reduction using an enzyme immunoassay microplate reader. - Methods Enzymol. **132**: 407-421, 1986.

Rengel, Z.: Role of calcium in aluminum toxicity. - New Phytol. **121**: 499-513, 1992.

Singh, V., Bhatt, I., Aggarwal, A., Tripathi, B.N., Munjal, A.K., Sharma, V.: Proline improves copper tolerance in chickpea (*Cicer arietinum*). - Protoplasma **245**: 173-181, 2010.

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Gupta, D.K.: Copper-induced oxidative stress and responses of antioxidants and phytochelatins in *Hydrilla verticillata* (L.f.) Royle. - Aquat. Toxicol. **80**: 405-415, 2006.

Stohs, S.J., Bagchi, D.: Oxidative mechanisms in the toxicity of metal ions. - Free Radical Biol. Med. **18**: 321-336, 1995.

Wang, C.Q., Song, H.: Calcium protects *Trifolium repens* L. seedlings against cadmium stress. - Plant Cell Rep. **28**: 1341-1349, 2009.

Xu, Q.S., Hu, J.Z., Xie, K.B., Yang, H.Y., Du, K.H., Shi, G.X.: Accumulation and acute toxicity of silver in *Potamogeton crispus* L. - J. Hazard. Mater. **173**: 186-193, 2010.

Zaki, F.T., Fathi, A.A.: Impact of copper on some physiological aspects of *Nostoc muscorum* with special references to the detoxifying role of calcium. - Acta bot. hung. **46**: 423-433, 2004.