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THE INHIBITION OF POLYMERIZATION OF GLUCOSE IN CARBOHYDRATE UNDER CU STRESS IN *VIGNA RADIATA*

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Abstract

This study was planned to explore the effect of Cu on polymerization of glucose into carbohydrate under Cu stress in seedlings of 15 day old *Vigna radiata* in relation with photosynthetic pigments. Copper is an important trace metal in carbohydrate metabolism, it effects on the sugar contents when in excess. The interaction of Cu with photo-inhibitory and sugar metabolism showed a direct relation in between carbohydrate metabolism and photosynthesis. A decrease in glucose contents may be attributed with Cu toxicity which inhibits the fixation of CO_2 that in turns related with formation of first organic complex. A drastic decrease in carbohydrate contents may be related with Cu interference in polymerization of glucose into carbohydrates. Relative growth rate (RGR) and leaf mass ratio (LMR) of stressed plants were significantly lower than that of control plants. Copper was shown to restrain the growth of the seedlings as it is most important immobile micronutrient that inhibits new root growth. A radical disorder in soluble reducing and non reducing sugars like, glucose, sucrose, total soluble sugars and carbohydrate with decrease in chloroplast pigments can be associated with reduction in leaf photosynthesis and also growth reduction is more likely due to a reduction in whole plant leaf area. Reduced leaf expansion with reduced photosynthetic activity may be related with fewer K contents in seedlings which play a crucial role in leaf expansion.

Introduction

Plants in general are very sensitive to Cu toxicity, displaying metabolic disturbances and growth inhibition at Cu contents in the tissues only slightly higher than the normal levels (Cook *et al*., 1997). Copper should not be applied to soils without a demonstrated need through soil and plant analysis. Toxic effects from over-application can last many years. The reduced mobility of Cu in soil and sediments, due to its strong binding to organic and inorganic colloids, constitutes, in a way, a barrier to Cu toxicity in land plants. Excess Cu inhibits a large number of enzymes and interferes with several aspects of plant biochemistry, including photosynthesis, pigment synthesis, and membrane integrity (Florence *et al.*, 2002). Perhaps its most important effect is associated with the blocking of photosynthetic electron transport, leading to the production of radicals which start peroxidative chain reactions involving membrane lipids (Fernandes & Henriques, 1999). The chlorophyll (Chl) concentration increased with increasing leaf Cu concentration, however, the Chl a/b ratio decreased. Since with an increasing leaf Cu concentration, the leaf area decreased more markedly than the leaf dry mass, the net photosynthetic rate (PN) per leaf area increased and per dry mass decreased (Sheldon & Menzies, 2005). Fargašová (2001) reported highest growth inhibition and reduced chlorophyll contents caused by copper metal as compared to other heavy metal like Cd, Zn and Pb in the roots and shoots of *Sinapis alba*. Sugars are considered as an important metabolism in plant metabolism not only because it is the first organic compound formed in the plants as a results of photosynthesis but also provide the major source of respiratory energy. Also sugars play a number of ecological roles in plant protection against wound, infection and detoxification of external stress (Pistocchi, *et al.*, 1997). Alaoui-Sossé *et al*., (2004) reported that leaf expansion declined, leaves became a weak sink and this might account for the observed accumulation of carbohydrates in leaves due to Cu toxicity. This accumulation could induce a feedback inhibition of photosynthesis. They also reported that the significant accumulation of starch and sucrose did not occur in roots and seemed to be confined to leaves.

The phytotoxic effects of Cu are strongly reliant on many factors with different origin. Thus, the sensitivity of the proposed bio-indicators should be tested for the actual species in an experimental design well suited for screening studies. The aim of this work was to study the effects of excess Cu on seedlings of *Vigna radiata* growth in relation with sugar metabolism, mineral metabolism and photosynthetic apparatus as sensitive bio indicators

Material and Method

The bean seeds were surface-sterilized in 10-3 M $HgCl_2$ for 2 min (Azmat *&* Hasa*n*, 2008), washed in distilled water, and germinated between wet filter paper towels at room temperature in the dark for 2 days. Then the plants were cultivated hydroponically in natural environment. After an initial growth period of 15 days, the seedlings were taken from their pots and separated into leaves, shoot and roots, then washed with distilled water. Weight and length of root, shoot and leaves were measured. Chlorophylls *a* and *b* were extracted in 80% acetone, measured spectrtrophotometrically and determined as follows:
Chlorophyll *a* (mg/L) = $12.7abs_{663}$ $-2.60abs_{645}$
Chlorophylls *b* (mg/L) = $22.9abs_{645}$ $-4.68abs_{663}$
Chlorophyll total= Chlorophylls *a*+ Chlorophylls *b*

The carbohydrate, glucose, sucrose and total soluble sugars were determined by the method described by Riazi *et al*., (1985) and Buysse & Merckx (1993 respectively) in 80% ethanol through spectrophotometer. Sodium and potassium were determined by flame photometry after digestion in HNO_3 and HCl (Azmat & Haider 2007). Data were subjected to statistical analysis.

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Results and Discussion

Physico-morphological indicators: The results observed in this study, specified that a number of different physical estimates of 15 days old seedlings of *Vigna radiata* like length, weight, LMR (leaf mass ratio), SMR (shoot mass ratio), RMR(root mass ratio), and LWR (length weight ratio) (Azmat & Hasan 2008) are significantly affected by excess Cu in nutrient medium and might be used as a bio-indicators in plant test system (Table 1). The measured "effective concentrations" leading to 10% decrease in the value of the used estimates (length and weight) varied around 10 ppm to 25ppm Cu mg kg^{-1} for plant dry weight (DW) accumulation, leaf area (LA) formation as well as for net photosynthetic rate. Dry mass and leaf area of seedlings exposed for 15 days to 10 and 25 mg kg^{-1} significantly differed from control plants values (Figs. 1&2). But these plant parameters at the highest Cu treatment (25 mg kg^{-1}) were significantly lowered (0.005), from control plants (37%) (Figs. 1-2). Values of measured parameters showed decline and the visual symptoms of Cu toxicity were accompanied by a reduction in plant growth, expressed as a relative growth rate.The results were significant in comparisons with the control plants ($p<0.001$).

Table 1. Physical visual morphological parameters of 15 d old seedlings of *Vigna radiata*.

Cu[ppm]	LMR	SMR	RMR	LWR
0	-	-	-	99.10
10	0.828 ± 0.02	0.442 ± 0.01	0.842 ± 0.02	70.11
15	0.741 ± 0.03	0.393 ± 0.01	0.650 ± 0.02	61.84
20	0.618 ± 0.02	0.357 ± 0.12	0.619 ± 0.02	57.49
25	0.455 ± 0.01	0.201 ± 0.023	0.091 ± 0.02	16.73

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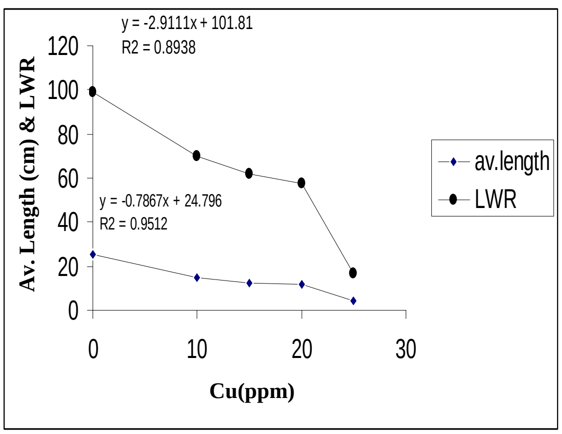

Fig. 1. Effect of Cu on length and length weight ratio of seedlings of *Vigna radiata*.

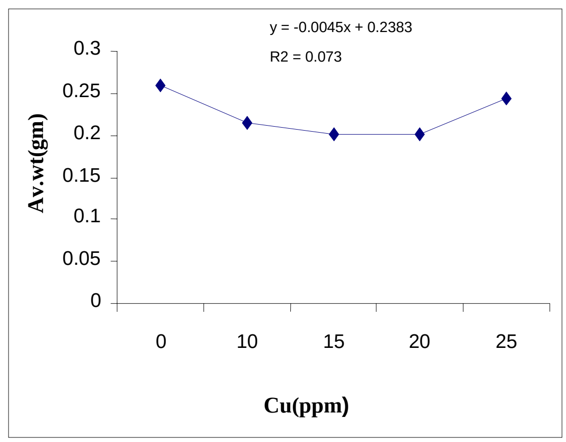

Fig. 2. Effect of Cu on fresh mass of seedlings of *Vigna radiata*.

These results showed that V*igna rdiata* is Cu sensitive species. Growth inhibition; LMR, SMR, RMR, and (LWR) were physical visual symptoms of accumulation of Cu (Table 1). Growth inhibition and reduction of biomass production are general response of plants to metal toxicity, which results due to inhibition in elongation and division of cells by metal addition. It has been observed that growth of root was more sensitive because root could play an important role in retention of metal by preventing an excess of toxic accumulation in the shoot. Seedling exposed to higher concentration and for long time shows drastic reduction in morphological and sugar metabolism which directly related with the processes of photosynthesis in seedlings. These results suggested that there was an interaction between seedling growth stages and applied Cu concentrations on roots. At 25ppm of Cu level total inhibition in root length per plant and average length of root system were observed ($p<0.05$), and the inhibiting effects increased with the time of Cu stress.

Root growth was most likely reduced due to a direct Cu^{2+} toxicity; it is believed that the shoot growth reduction was characteristic to a decrease in Na and K contents in seedling tissue and the formation of interveinal chlorosis as reported earlier (Kopittke *et al*., 2008, 2006). At high Cu^{2+} concentration, roots were brown in color, short and thick, had bent root tips due to cracking of the epidermis and outer cortex and had local swellings behind the roots tips with a reduction in cell elongation. Inhibition of root and root hair formation at high concentration caused significant reduction in cell elongation with lateral root formation. These investigations revealed that Cu, bind to the walls of cells, causing increased cell wall rigidity and eventual cell rupturing of the rhizodermis and outer cortex in the elongating zone due to which reduced shoot growth observed. The reduction of shoot growth can be correlated with the toxic effect of Cu^{2+} on photosynthesis, root respiration and protein synthesis in roots (Kopittke *et al*., 2008, 2006) (Figs. 1&2).

Photosynthesis and sugar metabolism: The total chlorophyll contents were significantly affected by Cu application in nutrient medium in root, shoot and leaves of seedlings. Changes in concentrations of copper and of soluble carbohydrates were in parallel but not opposing which results decrease in rate of photosynthates (Table 2), The contents of chlorophyll *a* and *b* were severely reduced by Cu treatments relative to untreated plants specially with the 25 mg kg^{-1} treatment, when the contents of Chl *a* and Chl *b* fall 41.4% and 44.5%, respectively in leaves of seedlings ($p<.001$). This might be due to a reduction in CO_2 fixation through distorted nitrogen metabolism (Azmat & Khan 2011) and sugar metabolism. Lack of sugar utilization or formation for growth due to excessive heavy metal concentration may be stronger than inhibition of CO_2 fixation (Haider *et al.,* 2006).

Table 2. Sugars and chloroplast contents of 15 d old seedlings of *Vigna radiata*.

Cu[ppm]	0	10	15	20	25
Root					
Chlorophyll a(mg/gm)	0.392 ± 0.02	0.342 ± 0.05	0.299 ± 0.01	0.274 ± 0.01	0.257 ± 0.01
Chlorophyll b(mg/gm)	0.370 ± 0.01	0.237 ± 0.02	0.233 ± 0.05	0.223 ± 0.03	0.181 ± 0.05
Total Chlorophyll (mg/gm)	0.762	0.579	0.532	0.497	0.438
Glucose (µg/ml)	67 ± 12	65 ± 12	64 ± 15	63 ± 9	62 ± 9
Sucrose (µg/ml)	200 ± 15	68 ± 16	40 ± 13	36 ± 10	34 ± 11
Total soluble sugars (µg/ml)	40 ± 04	33 ± 04	31 ± 10	29 ± 08	28 ± 0.06
Carbohydrate (µg/ml) 10^{-4}	0.637 ± 0.06	0.588 ± 0.04	0.575 ± 0.06	0.614 ± 0.11	0.497 ± 0.02
Sodium (ppm)	14 ± 0.9	13 ± 0.8	12 ± 0.8	08 ± 0.44	05 ± 0.6
Potassium (ppm)	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.07	0.08 ± 0.05	0.06 ± 0.05
Leaf					
Chlorophyll a(mg/gm)	0.898 ± 0.30	0.788 ± 0.23	0.641 ± 0.25	0.591 ± 0.14	0.484 ± 0.26
Chlorophyll b(mg/gm)	0.866 ± 0.54	0.744 ± 0.26	0.544 ± 0..41	0.405 ± 0.14	0.321 ± 0.32
Total Chlorophyll (mg/gm)	1.764	1.532	1.165	0.996	0.805
Glucose(µg/ml)	137 ± 14.2	72 ± 11.0	63 ± 11.6	61 ± 14.0	60 ± 11.0
Sucrose (µg/ml)	204 ± 16.3	202 ± 18.0	132 ± 16.0	116 ± 11.9	96 ± 11.3
Total soluble sugars(µg/ml)	54 ± 05.0	34 ± 04.0	33 ± 02	31 ± 03	30 ± 04
Carbohydrate (µg/ml) 10^{-4}	2.81 ± 0.02	1.96 ± 0.03	1.612 ± 0.034	1.21 ± 0.01	1.16 ± 0.01
Sodium (ppm)	18 ± 0.9	17 ± 0.8	16 ± 0.56	15 ± 0.7	13 ± 0.6
Potassium (ppm)	0.39 ± 0.02	0.25 ± 0.01	0.2 ± 0.01	0.16 ± 0.02	0.09 ± 0.03
Shoot					
Chlorophyll a(mg/gm)	0.756 ± 0.03	0.537 ± 0.02	0.467 ± 0.01	0.348 ± 0.01	0.334 ± 0.02
Chlorophyll b(mg/gm)	0.695 ± 0.03	0.492 ± 0.04	0.356 ± 0.04	0.343 ± 0.04	0.320 ± 0.4
Total Chlorophyll (mg/gm)	1.451 ±	1.029 ±	0.823 ±	0.691 ±	0.654 ±
Glucose (µg/ml)	116 ± 15	63 ± 14	62 ± 12	61 ± 12	56 ± 12
Sucrose (µg/ml)	208 ± 19	196 ± 17	50 ± 19	48 ± 18	38 ± 17
Total soluble sugars(µg/ml)	33 ± 09	31 ± 08	26 ± 19	22 ± 18	21 ± 09
Carbohydrate (µg/ml) 10^{-4}	1.48 ± 0.08	1.05 ± 0.2	1.04 ± 0.2	1.08 ±	0.95 ± 0.3
Sodium (ppm)	13 ± 01	12 ± 03	11 ± 01	09 ± 01	08 ± 02
Potassium (ppm)	0.03 ± 0.01	0.06 ± .01	0.08 ± .01	0.11 ± 0.01	0.12 ± 0.01

Investigation also reveals that high dose of Cu concentration in nutrient medium reduced the uptake of minerals ions like Na and K which also indicated that Cu exposure induced changes in mineral metabolism specially on K due to which reduced leaf expansion was observed ($p<.001$) (Table 2). Results reported in Table 2 showed that reduced leaf area was also responsible for reduced photosynthesis as well as fixation of CO_2. Decrease photosynthetic pigments mirror to one of the main site of heavy metal injury in plants and also inhibitory effect on pigment biosynthesis as well as pigments degradation. This was attributed to chlorophyll molecules which failed to integrate normally. A lower ratio of chlorophyll *a* to *b* and sugars contents, showing disturbed photo system II both indicated that copper treatment resulted in a larger light-harvesting antenna (Hanan 2007; Caspi *et al.,* 1999; Bernal *et al.,* 2004).

Sugars considered as a main source of energy provider to operate respiratory system. Results of analyzes sugars showed that distorted chloroplast pigment results in the decrease in glucose “first organic complex compounds” (Graham, 1980.) which when dimerise gives sucrose and then polymerization results in the formation of carbohydrate. These results indicate that Cu act as negative biocatalyst and inhibit or slow down the processes of polymerization. It can also be related with failure of absorption of light due to degraded structure of chlorophyll. This rapid destruction of chlorophyll or chlorophyll-protein complexes both in light and in the dark is due to accumulation of Cu in seedlings which increases in the plant body with increased in metal concentrations. This indicated a more destructive effect of Cu metals on the chlorophyll contents of species (Cook *et al.,* 1997). High concentrations of Cu are known to activate oxidative damage and alter cell-membrane properties by lipid peroxidation, thereby demonstrating the inhibitory effect on the enzymes involved in chlorophyll production (Shakya, 2008). The underlying mechanism for the Cu (II) inhibition of photosystem I and the destruction of the chlorophyll-protein complexes, may be caused by the formation of H_2O_2 in a Cu(II) catalyzed autooxidation of ascorbate. Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigments content are linked to visual symptoms of plant illness and photosynthetic productivity. And also inhibit the sugar conversion into carbohydrate which in turns related with biosynthesis of chlorophyll.

Mechanism of interaction of Cu in seedlings: The interaction of Cu toxicity with photo inhibitory and sugar metabolism was investigated and results proposed that Cu interferes with the biosynthesis of the photosynthetic machinery modifying the pigment and protein composition of photosynthetic membranes as heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient like Na and K. The depletion in photosynthetic pigments and sugar contents demonstrated that Cu enhances the adverse effects of light. (Yruela *et al*., 1996, Pätsikkä *et al*., 1998, 2001) and interaction is on the site where glucose converted into sugar and sugar polymerize into carbohydrate. The photosynthetic pigments are some of the most important internal factors, which in certain cases are able to limit the photosynthesis rate. It is believed that they are some of the receptor points of the toxic effects of Cu as free radical generation is one the initial responses of plants to stress which is most common in presence of metals and seriously disrupt normal metabolism through oxidative damage to cellular components. The decrease photosynthetic activity, due to decrease in CO_2 absorption and formation of reactive oxygen and hydroxyl free radical which involve in reduced biosynthesis of chlorophyll. This process, which includes the functional impairment of PSII electron transport and the structural damage of the PSII reaction center, is known as photo-inhibition. These results showed that that Cu is an efficient catalyst in the formation of reactive oxygen species (ROS), it was suggested that the Cu act as a photo inhibitor due to production of hydroxyl radicals which interfere both on photosynthesis and carbohydrate metabolism (Yruela *et al*., 1996). Because It is well known, fact that transition metals like Cu catalyze the formation of hydroxyl radicals ($OH^.$) from the non-enzymatic chemical reaction between superoxide ($O_2^{.-}$) and H_2O_2 (Haber-Weiss reaction) (Halliwell & Gutteridge, 1984). The reduced chlorophyll contents (Table 2) in seedlings of leaves grown in the presence of high Cu concentrations made leaves more susceptible to photo-inhibition as a consequence of a Cu-induced K deficiency.

Conclusion

It was concluded that Cu induced oxidative stress through decline in ratio of essential mineral elements with decrease in biomass as well as leaf area which effects on absorption of CO_2 and reduced water uptake results in the decrease in total carbohydrates and chlorophyll contents or in other words it may act synergistically to reduce energy transfer of photo system 11 retarding the reaction center to receive the light energy.

References

Alaoui-Sossé, B., P. Genet, F. Vinit-Dunand, M. Toussaint, D. Epron and P. Badot. 2004. Effect of copper on growth in cucumber plants (*Cucumis sativus*) and its relationships with carbohydrate accumulation and changes in ion contents. *Plant Science*, 166(5): 1213-1218.

Azmat, R. and S. Haider. 2007. Effect of Pb stress on Phytochemistry of *Phaseolus mungo* and *Lens culinaris. Asian Journal of Plant Sciences*, 6(2): 332-337.

Azmat, R. and S. Hasan. 2008. Photochemistry of light harvesting pigments of *Lens culinaris* under Al stress. *Pak. J. Bot*., 40(2): 779-784.

Azmat, R. and N. Khan. 2011 Nitrogen metabolism as a Bio Indicator of Cu Stress in *Vigna radiata Pak. J. Bot*., 43(1): 515-520.

Bernal, M., M. Roncel., J.M. Ortega., R. Picorel and I. Yruela. 2004. Copper effect on cytochrome b_{559} of photosystem II under photoinhibitory conditions. *Physiol. Plant*., 120: 686-694.

Buysse, J. and R. Merckx. 1993. An improved colorimetric method to quantity sugar content of plant tissue. *J. Expt. Bot*., 44: 1627-9

Cook, C.M., A. Kostidou, E. Vardaka and T. Lanaras. 1997. Effects of copper on the growth, photosynthesis and nutrient concentrations of *Phaseolus* plants. *Photosynthetica*, 34(2): 179-193

Caspi, V., M. Droppa, G. Horváth, S. l Malkin, J.B. Marder and V. I. Raskin. 1999. The effect of copper on chlorophyll organization during greening of barley leaves *Photosynthesis Research,* 62(2): 165-174

Fernandes, J.C. and F.S. Henriques.1999. Biochemical, physiological and structural effects of excess copper in plants. *The Botanical Review*, 57(3): 246-273.

Fargašová, A. 2001. Phytotoxic Effects of Cd, Zn, Pb, Cu and Fe on *Sinapis Alba* L., seedlings and their accumulation in roots and shoots. *Biologia Plantarum*., 44(3): 471-473.

Florence, V.D., E. Daniel, A.S. Badr and B. Pierre-Marie. 2002. Effects of copper on growth and on photosynthesis of mature and expanding leaves in cucumber plants. *Plant Science*, 163(1): 53-58.

Graham, R.D. 1980. The distribution of copper and soluble carbohydrates in wheat plants grown at high and low levels of copper supply. *Zeitschriff für Pflanzenernāhrung und Bodenkunde*, 143(2): 161-169.

Haider, S., S. Kanwal., F. Uddin and R. Azmat. 2006. Phytotoxicity of Pb: II. Changes in Chlorophyll Absorption Spectrum due to Toxic Metal Pb Stress on *Phaseolus mungo* and *Lens culinaris Pakistan Journal Biological. Sciences*, 9(11): 2062-2068.

Halliwell, B. and J.M.C. Gutteridge. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J*., 219: 1-14.

Hanan, El-Said Deef. 2007. Copper treatment and their effects on growth, carbohydrate, minerals and essential oils contents of *Rosmarinus officinalis*. *World Journal of Agriculturel Science*, 3(3): 322-328.

Kopittke, P.M., F.P.C. Blamey and N.W. Menzies. 2008 Toxicities of soluble Al, Cu and La include ruptures to rhizodermal and root cortical cells of cowpea. *Plant and Soil*, 303(1-2): 217-227.

Kopittke, P.M. and N.W. Menzies. 2006. Effect of Cu toxicity on growth of cowpea (*Vigna unguiculata*). *Plant and Soil,* 279(1-2): 287-296.

Pistocchi, R., F. Guerrini, V. Balboni and L. Boni. 1997. Copper toxicity and carbohydrate production in the microalgae *Cylindrotheca fusiformis* and *Gymnodinium* sp. *European Journal of Phycology*, 32(2): 125-132.

Pätsikkä, E., E-M. Aro and E. Tyystjärvi. 1998. Increase in the quantum yield of photo inhibition contributes to copper toxicity *In vivo*. *Plant Physiol*., 117: 619-627.

Pätsikkä, E., E-M. Aro and E. Tyystjärvi. 2001. Mechanism of copper-enhanced photo inhibition in thylakoid membranes. *Physiol. Plant*, 113: 142-150.

Riazi, A., K. Matruda and A. Arslam. 1985. Water stress induces Changes in concentration of proloine and other solutes in growing regions. *J. Exp. Bot.*, 36(172): 1716-1725.

Sheldon, R. and N.W. Menzies. 2005. The Effect of Copper toxicity on the growth and root morphology of Rhodes Grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil,* 278(1-2): 341-349.

Shakya, K., M.K. Chettri and T. Sawidis. 2008. Impact of heavy metals (Copper, Zinc and Lead) on the Chlorophyll Content of Some Mosses. *Archives of Environmental Contamination and Toxicology*, 54(3): 412-421.

Yruela, I., J.J. Pueyo, P.J. Alonso and R. Picorel. 1996. Photo inhibition of photo system II from higher plants: effect of copper inhibition. *J. Biol. Chem*., 271: 27408-27415.

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