JOURNAL OF ADVANCED
BOTANY AND ZOOLOGY

Journal homepage: http://scienceq.org/Journals/JABZ.php

Research Article Open Access

Assessment of Copper Caused Stress in Terms of Morphometric, Pigmental, Biochemical and Enzymatic Characteristics of *Vigna Unguiculata (L)*

M.Duraipandian, R.Sevugaperumal, and V.Ramasubramanian*

Department of Botany, Ayya Nadar Janaki Ammal College (Autonomous) Sivakasi 626124, India.

*Corresponding author: V.Ramasubramanian, E-mail: drvrams@gmail.com

Received: June 1, 2015, Accepted: July 9 2015, Published: July 9, 2015.

ABSTRACT

Metal contamination issues are common in india and elsewhere, in the cases of metal toxicity in mining industries, coal-burning power plants, foundries and smelters. Copper is an essential nutrient but in excess concentration responsible for environmental pollution. The excess of copper is released from many industries to cause soil and water pollution. The copper is a heavy metal highly accumulated in soil by anthropogenic activities. In the present study, the seedlings of (cow pea) *Vigna unguiculata* (L) walp were treated with various concentrations of copper chloride and its impact on the morphometric, pigmental, biochemical and enzymatic characters were studied. After ten days of treatment with different concentrations of copper chloride (2mM, 4mM, 6mM, 8mM, & 10 mM), the growth parameters such as leaf area, fresh weight, dry weight, shoot length, root length were found decreased than in the control. Pigmental characters such as chlorophyll a, chlorophyll b, total chlorophyll, carotenoides content were decreased with the increase in the concentrations of copper chloride, but the content of anthocyanin was increased and the biochemical parameters such as soluble sugar and protein content were found decreased with the increase in the concentrations of copper chloride. But the contents of free amino acid, proline and leaf nitrate were increased with increase in the concentrations of copper chloride. The activities of enzyme such as catalase and peroxidase were found increased with the increase in the concentration of copper chloride. But the NRA enzyme activity was decreased. The Atomic Absorption of Spectroscopy (AAS) technique was employed to confirm the presence of copper chloride in treated plants and control. Comparison of the values of treated plants with control reveals that copper chloride has seriously affected the cow pea plants.

Key words: Vigna Unguiculata (L), Copper Caused Stress, Metal contamination, metal toxicity

INTRODUCTION

With the beginning of the industrial revolution, pollution of the biosphere with toxic metals has accelerated dramatically. The Natural processes such as continental dusts, volcanic eruptions and anthropogenic activities like combustion of fossil fuel, mining, phosphate fertilizers, metal working industries and military activities, lead to accumulation and emission of heavy metals in environment. Elements such as Cu, Zn, Ni, Co, Fe, Mn and Mo are essential mineral nutrients that play an important function in the biosynthesis of proteins, nucleic acids, secondary metabolites, growth substances, gene expression. Chlorophyll, lipid and carbohydrate metabolism require in trace amounts. Heavy metal toxicity is finally attributed to altered plant metabolism, inhibition of photosynthesis, respiration, and altered activities of several key enzymes [1]. Heavy metals are natural constituents of soils and occur naturally in the environment. Nowadays, contamination of soils by toxic metals and metalloids is of major concern worldwide [2]. The problem of Heavy metal pollution is continuously worsening due to a series of human activities, leading to an intensification of research dealing with the phytotoxicity of these contaminants and with the mechanisms used by plants to counter their detrimental effects [3]. Heavy metals are not degradable and intervention stay in soil for centuries. Heavy metals contamination has has reached toxic levels in the air, land and water of many parts of the world [4]. Copper element is found naturally in water bodies [5]. The copper is most frequently discharged elements into the environment [6]. Excess of copper causes an elevated susceptibility to photo inhibition of Ps-II *in vitro* and *in vivo*. It has long been known that high concentration of copper when added to the incubation medium of isolated thylakoids inhibits PS-II electron transfer activity on the acceptor sites. In addition, copper toxicity is related to disturbances in the uptake of essential elements [7]. Copper participates in the reduction of electron transfer in photosynthesis in the form of plastocyanin. However, copper at high level becomes strongly phototoxic to cell and causes inhibition of plant or even death [8].

MATERIAL AND METHODS

Both control and experimental seeds were allowed to grow in soil. After seven days the seedling of *Vigna unguiculata* (L)

Walp were treated with various concentrations of copper chloride (2mM, 2mM, 6mM, 8mM & 10mM v/v). After ten days of copper treatment various morphometric, pigmental, biochemical and enzymatic characters were analysed.

Ten days of copper treated plants of *Vigna unguiculata* (L) Walp were used for measuring the morphometric characters such as shoot length, root length, leaf area, fresh weight and dry weight, the pigmental characters such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids [9]. were analysed.

The biochemical and enzymatic characters such as total soluble sugar & amino acid content [10]. protein content [11]. An increase in the aminoacid and proline content after match, sugar industrial effluent treated has already been reported. [12] & [13]. Leaf nitrate [14]. *In vivo* nitrate reductase activity [15], peroxidase and catalase activity [16] were analysed as per the methods proposed by those given within the parenthesis.

RESULT AND DISCUSSION

The results obtained on the effect of different concentration of copper chloride were summarized and discussed as follows. The results showed that the morphometric characters such as root length, shoot length, leaf area, fresh weight and dry weight decreased (Table1) with increase in the concentration of copper chloride the levels of chlorophyll and carotenoids decreased but the level of anthocyanin increased (Table 2). Similarly the total soluble sugar and protein (Table 3) also showed a declining trend. In contrary the leaf nitrate, free amino acid and proline (Table 3) and antioxidant enzyme such as peroxidase and catalase increased with the increasing in the metal concentration but nitrate reductase activity decreased (Table 4). The metal content of the treated and control seedlings of *vigna unguiculata* (L) Walp was finally estimated using AAS study in Table (5).

Copper can induce degradation of biological membranes, chlorophyll breakdown and protein denaturation and DNA mutation [17]. The reduction in sugar contents may be attributed to reduction in chlorophyll contents of the leaf and also a decline in protein. This change might have already affected the photosynthetic activity of the plant and hence the reduction in contents [18].

Accumulation of proline has been frequently used as biochemical marker for water stress in plants [19]. In stress condition the inhibition of growth of cells, leaves and the whole plant were accompanied by an accumulation of nitrate in plant tissue particularly in leaves [20].

The leaf nitrate content was found to be more in copper treated plants paralleling with the reduction in nitrate reductase activity. The peroxidase activity was reported to be increased with the increase in the concentration of the copper chloride, and cause a major impact on the chlorophyll degradation. Catalase is an antioxidant and scavenging enzyme, found to be increased with the increasing concentration of copper chloride. Both the catalase and peroxidase catalyze the degradation of H_2O_2, which is natural metabolite and also toxic to plants [21].

Table 1.Impact of various concentration of Copper chloride on the Morphometric characteristics of Vigna unguiculata (L) Walp.

Concentration	Shoot Length	Root Length	Leaf area	Fresh weight	Dry weight
Control	49.73 ± 0.851 (100)	15.26 ± 0.800 (100)	12.83 ± 0.316 (100)	2.35 ± 0.236 (100)	0.74 ± 0.076 (100)
2Mm	46.63 ± 0.664 (93.77)	13.76 ± 0.676 (90.21)	11.57 ± 0.346 (90.21)	1.68 ± 0.153 (71.67)	0.65 ± 0.061 (87.50)
4mM	41.03 ± 0.633 (82.51)	12.33 ± 0.578 (95.93)	10.40 ± 0.279 (81.06)	1.18 ± 0.073 (50.42)	0.58 ± 0.048 (75.57)
6mM	39.43 ± 0.417 (79.29)	12.13 ± 0.548 (80.82)	9.85 ± 0.263 (76.78)	1.05 ± 0.050 (44.76)	0.52 ± 0.024 (70.54)
8mM	28.63 ± 0.260 (57.57)	11.16 ± 0.375 (73.18)	9.52 ± 0.216 (74.21)	0.86 ± 0.021 (36.69)	0.44 ± 0.012 (59.37)
10mM	21.86 ± 0.176 (43.97)	9.63 ± 0.176 (63.13)	8.02 ± 0.174 (62.52)	0.70 ± 0.016 (30.03)	0.24 ± 0.008 (32.59)

Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error (˙)

Table 2. Impact of various concentration of Copper chloride on the photosynthetic pigments of Vigna unguiculata (L) Walp.

Concentration	Chlorophyll a (mg/gLFW)	Chlorophyll b (mg/gLFW)	Total chlorophyll (mg/gLFW)	Carotenoids (mg/gLFW)	Anthocyanin (mg/gLFW)
Control	18.98 ± 0.692 (100)	8.72 ± 0.137 (100)	27.71 ± 0.638 (100)	0.86 ± 0.036 (100)	10.63 ± 0.235 (100)
2mM	15.95 ± 0.422 (83.88)	8.50 ± 0.241 (97.48)	24.45 ± 0.277 (88.26)	0.56 ± 0.217 (64.80)	11.31 ± 0.311 (106.3)

4mM	14.21 ± 0.233 (77.90)	7.79 ± 0.240 (89.32)	22.45 ± 0.438 (81.02)	0.52 ± 0.199 (60.58)	13.28 ± 0.399 (124.9)
6mM	12.61 ± 0.208 (66.33)	6.70 ± 0.139 (76.82)	19.31 ± 0.178 (69.71)	0.44 ± 0.272 (51.81)	16.84 ± 0.446 (158.4)
8mM	10.39 ± 0.129 (54.65)	4.63 ± 0.221 (53.10)	15.02 ± 0.193 (54.22)	0.36 ± 0.104 (42.16)	22.78 ± 0.486 (214.2)
10mM	9.19 ± 0.067 (48.34)	1.57 ± 0.119 (18.02)	10.76 ± 0.090 (38.85)	0.230 ± 0.054 (26.69)	26.00 ± 0.815 (244.4)

Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error (・)

Table 3. Impact of various concentration of Copper chloride on the Biochemical characteristics of *Vigna unguiculata* (L.) Walp.

Concentration	Total soluble sugar (mg/g LFW)	Total soluble protein (mg/g LFW)	Amino acid (µMole/g LFW)	Proline (mg/g LFW)	Leaf nitrate (mg/g LFW)
Control	1.56 ± 0.633 (100)	7.60 ± 0.403 (100)	2.93 ± 0.315 (100)	0.49 ± 0.029 (100)	0.007 ± 0.001 (100)
2mM	1.15 ± 0.053 (73.62)	6.30 ± 0.220 (82.96)	4.24 ± 0.487 (144.8)	0.54 ± 0.034 (109.9)	0.011 ± 0.001 (152.9)
4mM	1.10 ± 0.042 (70.64)	5.73 ± 0.129 (75.48)	5.46 ± 0.573 (186.6)	0.62 ± 0.052 (126.1)	0.015 ± 0.002 (194.3)
6mM	0.98 ± 0.023 (62.77)	4.77 ± 0.108 (62.85)	5.99 ± 0.683 (204.5)	0.66 ± 0.057 (134.7)	0.019 ± 0.002 (254.3)
8mM	0.74 ± 0.017 (47.66)	3.70 ± 0.032 (48.71)	6.16 ± 0.151 (210.4)	0.77 ± 0.081 (157.2)	0.022 ± 0.003 (288.6)
10mM	0.46 ± 0.008 (29.57)	2.41 ± 0.013 (31.81)	7.13 ± 0.175 (243.3)	0.84 ± 0.086 (171.2)	0..028 ± 0.003 (362.8)

Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error (・)

Table 4. Impact of various concentration of Copper chloride on the Enzymes activity of *Vigna unguiculata* (L.) Walp.

Concentration	Catalase (mg/g LFW)	Peroxidase (mg/g LFW)	NR Activity (µMole/g LFW)
Control	4.75 ± 0.145 (100)	8.55 ± 0.199 (100)	4.48 ± 0.370 (100)
2mM	5.08 ± 0.283 (107.0)	9.35 ± 0.215 (109.4)	4.20 ± 0.266 (93.8)
4mM	5.64 ± 0.387 (118.7)	10.20 ± 0.224 (119.3)	3.83 ± 0.239 (85.54)
6mM	6.09 ± 0.414 (128.2)	11.04 ± 0.229 (129.1)	3.20 ± 0.177 (71.49)
8mM	6.51 ± 0.490 (136.9)	11.84 ± 0.259 (138.5)	2.90 ± 0.160 (64.88)
10mM	7.022 ± 0.547 (147.7)	12.71 ± 0.322 (148.6)	2.63 ± 0.115 (58.88)

Values in parenthesis indicate percent activity; value represents mean of 10 samples with their standard error (・)

Table 5. AAS result of Copper chloride in Vigna unguiculata (L.) Walp.

SAMPLE	AAS in ppm
SAMPLE 1 (Control)	0.8945ppm
SAMPLE 2 (2 mM)	1.2721ppm
SAMPLE 3 (4 mM)	2.7951ppm
SAMPLE 4 (6 mM)	3.9080ppm
SAMPLE 5 (8 mM)	5.7756ppm
SAMPLE 6 (10 mM)	9.9820ppm

The result of present investigation clearly showed that the comparison of the values given in parenthesis of treated plants with control reveals that copper chloride has seriously affected the cowpea plants.

REFERENCES

1. Hossain, H. A. Hossain, M. Z. and Fujita, M. 2009, "Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA

cloning of glyoxalase I gene,"Australian Journal of Crop Science, vol. 3, no. 2, pp. 53–64.
2. Rascio, N and Navari-Izzo, F. 2011, "Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?" Plant Science, vol. 180, no. 2, pp. 169–181.
3. Villiers. F. Ducruix, C. Hugouvieux.V. et al., 2011, "Investigating the plant response to cadmium exposure by proteomic and metabolomic approaches," Proteomics, vol. 11, no. 9, pp.1650–1663.
4. Nriagu, J. O., "A silent epidemic of environmental metal poisoning?" Environ Pollut, 50 (1988) 139.
5. Delmail, D., Dabrousse, P., Hourdin, P. Larcher, L., Moesch, C. and Botineau, M. 2011. Differential responses of Myriophyllum alterniflorum DC (Haloragaceae) organs to copper: physiological and developmental approaches. Hydrobiologia, vol. 664, no. 1, p. 95-105.
6. Moore, JW. 2011. Inorganic contaminants in surface water: research and monitoring priorities. London: Springer. 348 p.
7. Van Assche, F. and Clijsters, H. 1986, "Inhibition of photosynthesis in Phaseolus vulgaris by treatment with toxic concentration of zinc: effect on ribulose-1,5-bisphosphate carboxylase/oxygenase," Journal of Plant Physiology, vol. 125, no. 3-4, pp. 355–360.
8. Mocquot B, Vangernsveld J, Clijsers H, Mench. 1996. Copper toxicity in young maize (Zea mays L.) plants effects on growth, mineral and chlorophyll contents and enzymes activities. Plants soil, 182:287-300.
9. Wellburn, A.R. and Lichtenthaler, H. 1984,"In Advances in photosynthesis Research",(ed.Sybesma) Martinus Nijhoff, Co., The Hague, Vol.II, and pp: 9-12.
10. Jayaraman, J. 1981. Laboratory manual in Biochemistry, Willey-Eastern Company Limited, Madras, pp. 1-65.
11. Lowry, O.H. Rosenbury, N.J. Farr, A.L. and Randall, J. 1951, Bio.Chem.193:262-275.
12. Jeyarathi, K,P. and Ramasubramanian, V. 2002. Analysis of sugar mill Effluents and its impact on the growth and biochemical characteristics of Abelmascus esculentus (L) Mediakus. In: Recent Trends in Biotechnology (Ed.Harikumar,V.S) Agrobios Publication, Jodhpur, pp. 245-253.
13. Ramasubramanian, V., Ravichandran,V. and Kannan, N. 1993. Analysis of industrial effluents and their impact on the growth and metabolism of Phaseolus mungo,L.Commun. Soil. Sci. Plant. Anal., 24(17&18): 2241-2249.
14. Cataldo, D.A. Garland, T.R. and Wildung, R.E. 1978. Nickel in plants. I. Uptake kinetics using intact soybean seedlings. Plant Physiology and Biochemistry 62: 563-565.
15. Jaworski, E.G., 1971. Biochem. Biophy. Res. Commun. 43: 1274-1279.
16. Kar,M. and Mishra, D., 1976. Plant Physiol. 57: 315- 319.
17. Apel, K. and Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology, vol. 55, p. 373- 399. PMid:15377225.
18. Swaminathan,K., Arjunan,J. and Gurusamy,R. 1998. Effect of glucose factory effluents on the seed germination and seedling development of groundnut (Arachis hypogea,L.). Envirion. Biol., 2: 187-189.
19. Alia, P. and Saradhi, P.P. 1991. Proline accumulation under heavy metal stress. J.Plant Physiol. 138: 534-538.
20. Sinha and Nicholas,D.J.D. 1981. In : the physiology and biochemistry of drought resistance in plants (ed. Paleg,L.G. and Aspinall,D.) Academic Press, London. Pp:145- 169.
21. Balasimha, D. 1982. Regulation of peroxidase in higher plants a review. Plant. Physiol.Biochem.,9 (2): 130-143.

Citation: V.Ramasubramanian et al. (2015). Assessment of Copper Caused Stress in Terms of Morphometric, Pigmental, Biochemical and Enzymatic Characteristics of Vigna Unguiculata (L). J. of Advanced Botany and Zoology, V3I2. DOI: 10.15297/JABZ.V3I2.05.