EFFECTS OF COPPER STRESS ON BIOCHEMICAL AND ANTIOXIDANT RESPONSES OF *Enydra fluctuans* Lour. (Asteraceae)

Sultana Parven*, Aparajita De*, Abhik Gupta*, Aasif Abdullah Baba*

* Assam University, Department of Ecology and Environmental Science, Silchar, Assam, India

corresponding author: Aasif Abdullah Baba, e-mail: aasifenv@gmail.com

Original scientific paper
Received: December 12th, 2024
Accepted: February 18th, 2025
HAE-24139
https://doi.org/10.33765/thate.15.4.2

ABSTRACT

Enydra fluctuans, an edible aquatic macrophyte with medicinal properties, was exposed to different concentrations of copper (0.11 - 3.03 mg L^{-1}) to determine its effects on chlorophyll *a* (Chl 'a') and chlorophyll *b* (Chl 'b'), protein and carbohydrate content, catalase activity and proline content. The copper exposure at 0.11 mg L^{-1} initially increased the chlorophyll *a* concentration, while concentrations of 0.51 mg L^{-1} and higher caused a decrease. One-way ANOVA showed significant differences ($p < 0.05$) between Cu-exposed plants and control plants for chlorophyll *a*, but not for chlorophyll *b*. At cooper concentrations of 1.09 - 3.03 mg L^{-1}, protein content was significantly reduced in the leaf, upper and lower parts of the stem. Cu at 0.11 - 0.51 mg L^{-1} caused insignificant increase in carbohydrate content in the leaf (except on day 15) and the upper and lower parts of the stem, while a significant decrease occurred in the leaf at 1.09 - 3.03 mg L^{-1} Cu on day 15, and only at 3.03 mg L^{-1} in the upper and lower parts of the stem. Catalase activity and proline content were significantly elevated in all plant tissues at Cu exposure of 0.11 mg L^{-1} and above. Therefore, the study showed that although Cu can significantly affect *Enydra fluctuans* at relatively low concentrations, the plant has the ability to cope with Cu stress by increasing the activities of the antioxidant enzyme catalase and the accumulation of the non-enzymatic antioxidant proline.

Keywords: *chlorophyll, protein, carbohydrate, Enydra fluctuans, catalase, proline*

INTRODUCTION

The rapid increase in industrialization and urbanization has caused contamination of natural ecosystems, including water systems, with a multitude of contaminants such as sewage, heavy metals, pesticides and others, resulting in the deterioration of water quality worldwide. Heavy metals are well-known environmental pollutants that contaminate freshwater ecosystems such as rivers and lakes, thereby affecting their biological communities through processes of bioaccumulation and concentration in the food chain. Heavy metal pollution of freshwater systems has also assumed serious proportions in India [1, 2].

Many metabolic biomolecules, such as photosynthetic pigments, carbohydrates, and others, are severely affected by heavy metal toxicity. Proteins play significant roles in ion transport, mitochondrial enzyme activities, cellular trafficking, gene transcription, etc [3]. Higher concentrations of toxic heavy metals can alter or restrain the activities of proteins in plants. It was found that increased concentrations of Cu cause a decrease in the content of proteins in young leaves of *Helianthus annuus* [4]. Toxicity of heavy metals affects the functioning of amylolytic enzymes that finally interfere with the accumulation of carbohydrate in plants [5].

Fluctuations in the activity of catalase were observed among different plant species when exposed to different concentrations of Cu. *Astragalus neo-mobayenii* showed an increase in catalase activity with increasing Cu concentration [6], while there was a decrease in catalase activity in the stem of *Helianthus annuus* seedlings at a concentration of 7.98 mg L^{-1} $CuSO_4$ [7].

The aim of this research was to examine the toxic effects of Cu (0.1 - 3.03 mg L^{-1}) on chlorophyll *a* and *b*, protein, carbohydrate and proline contents and catalase activity in the aquatic macrophyte *Enydra fluctuans*. *Enydra fluctuans* (watercress) belongs to the Asteraceae family and is found in South and Southeast Asia, and parts of East Asia. In addition to being an edible leafy green vegetable known for its nutritional values, it is used in traditional medicine to treat several diseases such as bronchitis, smallpox, and skin diseases. It is also used for its anticancer, antimicrobial, analgesic and hepatoprotective properties. The plant occurs abundantly and is widely consumed in the north-eastern region of India [8].

MATERIALS AND METHODS

Plant material and stock culture

Enydra fluctuans with prominent roots and clear nodes were collected from water bodies of Irongmara area in Cachar district, Assam, India (24.689 ºN; 92.742 ºE). Stock cultures were grown according to standard methods [9, 10]. The plants were grown hydroponically to develop new branches. New branches from a single, healthy plant were cut and transplanted into pots filled with soil containing 50 % Hoagland nutrient solution. The pH of the nutrient medium was adjusted between 5.8 and 6.2. Tap water was added whenever necessary to compensate for water loss due to evapotranspiration. Healthy growing shoots of similar length from one plant were cut and washed properly with tap water. Finally, all the shoots were acclimatized for a week in 50 % Hoagland medium at 22 - 25 °C, with 12 h of light (intensity of 100 - 120 μ mol^{-2} s^{-1}) and 12 h of darkness.

Chemicals

The experiment was performed in hydroponic microcosms consisting of 1000 ml conical flasks [11]. Acclimatized plants were treated with graded concentrations of Cu as $CuCl_2 \cdot 2H_2O$ (0.11, 0.51, 1.09, 2.54, and 3.03 mg Cu L^{-1}). The aqueous medium consisted of 1 liter of 50 % Hoagland nutrient [12]. A control was maintained with plants grown in 50 % Hoagland nutrient media without added Cu. Samples were collected at the end of 5, 10 and 15 days.

Biochemical analysis

The following methods were used for biochemical analysis: for chlorophyll, protein, carbohydrates, catalase activity and proline the methods of Arnon [13], Lowry et al. [14], Dubois et al. [15], Hakiman and Maziah [16] and Bates et al. [17] were used, respectively.

Metal analysis

Actual concentrations of Cu in the culture medium were determined by flame atomic absorption spectrophotometer at Tripura State Pollution Control Board, Agartala, Tripura, India.

Statistical analysis

Data were subjected to analysis of variance (one-way ANOVA) with multiple comparisons made by Tukey test at $p \leq 0.05$ using Statistical Package for the Social Sciences (SPSS) 20 for Windows.

RESULTS AND DISCUSSION

Exposure to Cu resulted in an insignificant increase in Chl ‘a’ on day 5, with a significant decrease occurring at 1.09 mg L^{-1} and above. On day 10, reductions became significant at 0.11 mg L^{-1}, and on day 15 at 0.51 mg L^{-1}. Reductions in Chl ‘b’ were not significant. The highest reduction in Chl ‘a’ was 70.85 %, while for Chl ‘b’ it was 69.41 % of that in control on day 10 at 3.03 mg L^{-1} Cu. The study showed that Chl ‘a’ was highly sensitive to Cu stress, as a significant decrease occurred at 0.11 mg L^{-1} Cu on day 10 and 0.51 mg L^{-1} on day 15 (Figure 1). On the other hand, Chl ‘b’ was more resistant. These values were comparable to the results obtained by Rama Devi and Prasad [18], who noted a significant decrease in Chl ‘a’ at Cu concentrations of 2 and 4 µM (equivalent to 0.13 and 0.25 mg L^{-1} Cu, respectively), although the decreases in Chl ‘b’ were not significant, as shown in Figure 1. Shakya et al. [19] observed significant changes in the content of chlorophyll in a leafy *Marchantiophyta* when exposed to a wide range of $CuCl_2$, including extremely low and high concentrations (10^{-10} to 10^{-2} M). On the other hand, several studies have shown that Cu can cause a decrease in chlorophyll in bean (*Phaseolus vulgaris* L.) seedlings at relatively higher concentrations of 6.35, 12.71 and 19.06 mg L^{-1} [20]. Although Cu inhibited chlorophyll accumulation, slowed its integration into photosystems, caused a delay in the formation of photosystem I (PS I), and decreased the photosynthetic capacity of *Hordeum vulgare* plants, the effects were significant only at 1.0 mM, but not at 0.1 and 0.01 mM Cu (~ 63.55, 6.36, and 0.64 mg L^{-1}, respectively) [21].

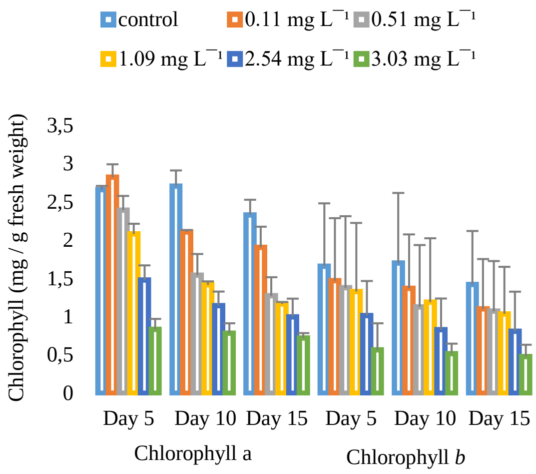

Figure 1. Effect of Cu on chlorophyll *a* and *b* concentrations in leaves of *Enydra fluctuans* exposed to different concentrations of Cu (0.11 - 3.03 mg L^{-1}) and control after 5, 10 and 15 days

Proteins were relatively less sensitive to Cu than Chl ‘a’, with a significant decrease occurring at exposure concentrations of 1.09 - 3.03 mg L^{-1} (Figure 2). A decrease in protein content was observed in *Brassica oleracea var. botrytis* L. plants exposed to 0.5 mM (~ 31.77 mg L^{-1}) [22] and in *Helianthus annuus* L. at 0.4 - 0.6 mM (~ 25.42 - 38.13 mg L^{-1}) [23]. These concentrations were several times higher than those used in this study, suggesting that Cu is able to suppress protein synthesis at much lower concentrations. It is known that free radicals generated under oxidative stress, such as those caused by heavy metals, result in degradation of proteins that are known to serve as indicators of oxidative damage [24, 25], suggesting that the reduction of protein content in plants due to metal stress could be due to changes in the synthesis as well as oxidation of proteins. All of this could likely be the result of increased carbonylation of proteins.

The decrease in carbohydrate content showed a pattern similar to that of proteins, with significant decreases occurring in leaves at 1.09 - 3.03 mg L^{-1} on day 15, and only at 3.03 mg L^{-1} in the upper (days 5, 10 and 15) and lower parts of the stem (days 5 and 15) (Figure 3). On the other hand, Cu at a concentration of 0.11 - 0.51 mg L^{-1} caused insignificant increase in the carbohydrate content in the leaves (except on day 15) and the upper and lower parts of the stem. This reflects the essential nature of Cu at trace concentrations, which nevertheless becomes toxic at higher doses. Exposure to Cu concentrations of 1, 10 and 100 mg L^{-1} inhibited the synthesis of carbohydrates in *Spirodela polyrrhiza* [26]. Contrary to these findings, Cu at 50, 200 and 800 ppm in the growth medium increased carbohydrate concentrations in *Rosmarinus officinalis* L. [27]. Cr (VI) concentrations of 0.01, 0.1, 0.9 and 2 mg L^{-1} caused a decrease in carbohydrate content in the leaves, upper and lower parts of the stem of *Ipomoea aquatica* [11]. Thus, effects on carbohydrates could show metal- and plant-specific variations.

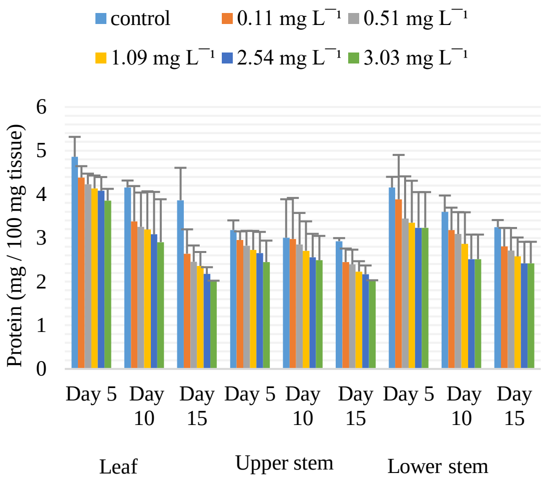

Figure 2. Effect of Cu on protein concentrations in leaves, upper and lower stem parts of *Enydra fluctuans* exposed to different concentrations of Cu (0.11 - 3.03 mg L^{-1}) and control after 5, 10 and 15 days

The toxic effects of various heavy metals are known to alter plant metabolic functions including respiration, photosynthesis and activities of enzymes [28]. An increase in catalase activity was induced at Cu concentrations ranging from 0.11 - 1.09 mg L^{-1} in leaves and upper and lower parts of the stem, while proline production was induced at 0.51 mg L^{-1} and higher concentrations. It can be observed that protein and carbohydrate contents were significantly affected only at or above 1.09 mg L^{-1} Cu, while Chl ‘a’ was degraded at Cu concentrations of only 0.11 - 0.51 mg L^{-1}. Therefore, it can be concluded that decreases in Chl ‘a’ caused improvements in catalase activity and proline synthesis in *E. fluctuans*, and therefore Chl ‘a’ can be identified as a sensitive biomarker of Cu stress in this plant. Teisseire and Guy [29] reported higher catalase activity in *Lemna minor* exposed to $CuSO_4$ (0.8 - 1.6 mg L^{-1}), and suggested that the plant uses such mechanisms to defend itself against the formation of reactive oxygen species (ROS) stimulated by heavy metals.

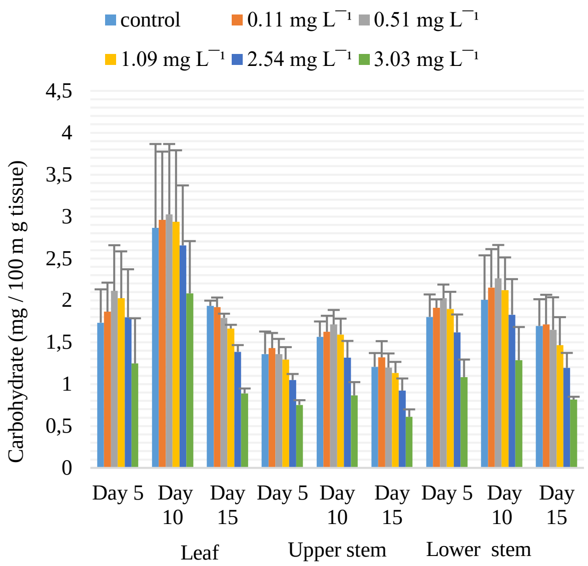

Figure 3. Effect of Cu on carbohydrate concentrations in leaves, upper and lower stem parts of *Enydra fluctuans* exposed to different concentrations of Cu (0.11 - 3.03 mg L^{-1}) and control after 5, 10 and 15 days

Cu stress increased proline concentrations in the leaves of *Helianthus annuus* [4]. A likely mechanism is altered water balance in plants due to heavy metal stress, which in turn may stimulate proline accumulation [30]. This research highlights the fact that although low and environmentally relevant concentrations of Cu could cause toxic effects on *E. fluctuans*, the plant attempted to mitigate these effects by increasing the activities of the antioxidant enzyme catalase and accumulating the non-enzymatic antioxidant proline.

CONCLUSION

Among the various biochemical and enzymatic biomarkers used in this study, Chl 'a' was shown to be a sensitive biomarker, and proteins and carbohydrates were relatively less vulnerable. Increased catalase activity and proline synthesis are also induced in the early phase of Cu stress, and may be valuable in monitoring the effects of Cu. *E. fluctuans* can survive in water bodies contaminated with Cu due to its biochemical defense mechanisms. However, this also adds a note of caution for the consumers of this plant, either as a vegetable or as a source of traditional medicine, due to the risk of Cu toxicity, especially in areas known to have Cu-contaminated waters and sediments.

REFERENCES

[1] S. Das, A. Mitra, S. Zaman, P. Pramanick, T.R. Chaudhuri, A.T. Raha, Zinc, copper, lead and cadmium levels in edible finfishes from lower gangetic delta, American Journal of Bio-pharmacology Biochemistry and Life Sciences 3(2014) 1, 8-19.

[2] S.K. Gupta, M. Chabukdhara, P. Kumar, J. Sinha, F. Bux, Evaluation of ecological risk of metal contamination in river Gomti, India: A biomonitoring approach, Ecotoxicology and Environmental Safety 110(2014), 49-55. https://doi.org/10.1016/j.ecoenv.2014.08.008

[3] P. Aducci, L. Camoni, M. Marra, S. Visconti, From Cytosol to Organelles: 14–3-3 Proteins as Multifunctional Regulators of Plant Cell, IUBMB Life 53(2002) 1, 49–55. https://doi.org/10.1080/15216540210813

[4] R. Kastori, M. Petrović, N. Petrović, Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower, Journal of Plant Nutrition 15(1992) 11, 2427-2439. https://doi.org/10.1080/01904169209364485

[5] R. Kaur, R. Bhardwaj, A.K. Thukral, Growth and heavy metal uptake in *B. juncea* L. seedlings as affected by binary interactions between nickel and other heavy metals, Canadian Journal of Pure and Applied Sciences 4(2010) 1, 1011-1019.

[6] P. Karimi, R.A. Khavari-Nejad, V. Niknam, F. Ghahremaninejad, F. Najafi, The effects of excess copper on antioxidative enzymes, lipid peroxidation, proline, chlorophyll and concentration of Mn, Fe and Cu in *Astragalus neo-mobayenii*, The Scientific World Journal 2012(2012) 1, Article ID: 615670. https://doi.org/10.1100/2012/615670

[7] H. Jouili, E. El Ferjani, Effect of copper excess on superoxide dismutase, catalase, and peroxidase activities in sunflower seedlings (*Helianthus annuus* L.), Acta Physiologiae Plantarum 26(2004) 1, 29-35. https://doi.org/10.1007/s11738-004-0041-8

[8] R. Chakraborty, B. De, N. Devanna, S. Sen, North-East India an ethnic storehouse of unexplored medicinal plants, Journal of Natural Product and Plant Resources 2(2012) 1, 143-152.

[9] A. Göthberg, M. Greger, K. Holm, B.-E. Bengtsson, Influence of nutrient levels on uptake and effects of mercury, cadmium, lead in water spinach, Journal of Environmental Quality 33(2004) 4, 1247-1255. https://doi.org/10.2134/jeq2004.1247

[10] L. Bedabati Chanu, A. Gupta, Phytoremediation of lead using *Ipomoea aquatica* Frosk. in hydroponic solution, Chemosphere 156(2016), 407-411. https://doi.org/10.1016/j.chemosphere.2016.05.001

[11] N. Haokip, A. Gupta, Phytoremediation of chromium and manganese by *Ipomoea aquatica* Forssk. from aqueous medium containing chromium-manganese mixtures in microcosms and mesocosms, Water and Environment Journal 35(2021) 3, 884-891. https://doi.org/10.1111/wej.12676

[12] D.R. Hoagland, D.I. Arnon, The water-culture method for growing plants without soil, Circular 347(1950) 2, 1-32.

[13] D.I. Arnon, Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*, Plant Physiology 24(1949) 1, 1-15. https://doi.org/10.1104/pp.24.1.1

[14] O.H. Lowry, N. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, Journal of Biological Chemistry 193(1951) 1, 265-275. https://doi.org/10.1016/s0021-9258(19)52451-6

[15] M. DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric Method for Determination of Sugars and Related Substances, Analytical Chemistry 28(1956) 3, 350-356. https://doi.org/10.1021/ac60111a017

[16] M. Hakiman, M. Maziah, Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions, Journal of medicinal plant research 3(2009) 3, 120-131.

[17] L.S. Bates, R.P. Waldren, I.D. Teare, Rapid determination of free proline for water-stress studies, Plant and Soil 39(1973) 1, 205-207. https://doi.org/10.1007/bf00018060

[18] S. Rama Devi, M.N.V. Prasad, Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a floating macrophyte: response of antioxidant enzymes and antioxidants, Plant Science 138(1998) 2, 157-165. https://doi.org/10.1016/S0168-9452(98)00161-7

[19] K. Shakya, M.K. Chettri, T. Sawidis Impact of heavy metals (copper, zinc and lead) on the chlorophyll content of some mosses, Archives of Environmental Contamination and Toxicology 54(2008), 412-421. https://doi.org/10.1007/s00244-007-9060-y

[20] F.K. Zengin, O. Munzuroglu, Effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgris* L.) seedlings, Acta Biologica Cracoviencia Series Botanica 47(2005) 2, 157-164.

[21] V. Caspi, M. Droppa, G. Horváth, S. Malkin, J.B. Marder, V.I. Raskin, The effect of copper on chlorophyll organization during greening of barley leaves, Photosynthesis Research 62(1999), 165-174. https://doi.org/10.1023/A:1006397714430

[22] J. Chatterjee, C. Chatterjee, Phytotoxicity of cobalt, chromium and copper in cauliflower, Environmental Pollution 109(2000) 1, 69-74. https://doi.org/10.1016/s0269-7491(99)00238-9

[23] F.K. Zengin, S. Kirbag, Effects of copper on chlorophyll, proline, protein and abscisic acid level of sunflower (*Helianthus annuus* L.) seedlings, Journal of Environmental Biology 28(2007) 3, 561-566.

[24] S. Sinha, R. Saxena, S. Singh Chromium induced lipid peroxidation in the plants of *Pistia stratiotes* L.: role of antioxidants and antioxidant enzymes, Chemosphere 58(2005) 5, 595-604. https://doi.org/10.1016/j.chemosphere.2004.08.071

[25] P. Kumar, R. Kumar, N.S. Nagpure, P. Nautiyal, B. Kushwaha, A. Dabas, Genotoxicity and antioxidant enzyme activity induced by hexavalent chromium in *Cyprinus carpio* after in vivo exposure, Drug and Chemical Toxicology 36(2013) 4, 451-460. https://doi.org/10.3109/01480545.2013.776581

[26] W. Xing, W. Huang, G. Liu, Effect of excess iron and copper on physiology of aquatic plant *Spirodela polyrrhiza*(L.) Schleid, Environmental Toxicology 25(2010) 2, 103-112. https://doi.org/10.1002/tox.20480

[27] H. El-Said Deef, Copper treatments and their effects on growth, carbohydrates, minerals and essential oils contents of *Rosmarinus officinalis* L, World Journal of Agricultural Sciences 3(2007) 3, 322-328.

[28] M.A. Hossain, P. Piyatida, J.A.T. da Silva, M. Fujita, Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in

detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation, Journal of Botany 2012(2012) 1, Article ID: 872875. https://doi.org/10.1155/2012/872875

[29] H. Teisseire, V. Guy, Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*), Plant Science 153(2000) 1, 65-72. https://doi.org/10.1016/S0168-9452(99)00257-5

[30] S.S. Sharma, K.-J. Dietz, The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress, The Journal of Experimental Botany 57(2006) 4, 711-726. https://doi.org/10.1093/jxb/erj073

Acknowledgements

Sultana Parven is grateful to the University Grants Commission, India, for providing non-NET fellowship. The authors thank the Sophisticated Analytical Instrument Facility (SAIF), North Eastern Hill University, Shillong-793022, India, and Tripura State Pollution Control Board, Agartala, Tripura-799006, for copper determination. The authors are also grateful to the e-library facility by DELCON located at Bioinformatics Centre, Assam University, Silchar, India.