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Effect of Co and Cu (Heavy Metals) on the Anatomical and Histochemical Aspects of *Eichhornia Crassipes* (Mart.) Solms

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ABSTRACT

An attempt has made to study the accumulation of heavy metals such as copper and cobalt in *Eichhornia crassipes* (Mart.) Solms, as bioaccumulation of this compounds leads to various health risks. The study was conducted by treating the plant *Eichhornia crassipes* (Mart.) Solms with 800 μ M of Co and Cu separately and compared with the one kept as control. At the end of 60th day it was observed that these heavy metals were present in plants roots, stolon, petiole and leaves and the degradation of vascular tissues was noticed. The histochemical analysis showed the reduced contents of starch and protein metabolites in the metal treated conditions.

Keywords - Heavy Metals, Copper, Cobalt, *Eichhornia crassipes* (Mart.) Solms.

1. INTRODUCTION

Ecosystems are defined by the network of interactions among organisms, and between organisms and their environment [1]. An organism is always in the state of perfect balance with the environment. The favorable unpolluted environment has a specific composition. When this composition gets changed by addition of harmful substances, the environment is called polluted environment and the substances that pollutes it are called pollutants. Pollutants may be of several types depending upon where each pollutants acts. Pesticides, fertilizers, detergents, acids, salts etc are water pollutants whereas metal and their compounds, plastics, fertilizers, pesticides etc. are soil pollutants. A small quantity of a toxic chemical may have little impact if it is spilled into the ocean from a ship. But the same amount of the same chemical can have a much bigger impact pumped into a lake or river, where there is less clean water to disperse it. Water pollution occurs when pollutants are discharged directly or indirectly into water bodies. Among various water pollutants, heavy metals are of major concern because of their persistent and bio-accumulative nature. They are highly toxic and cause ill effects at very low concentrations [5].

Eichhornia crassipes (Mart.) Solms is a free floating aquatic plant well known for its removal of pollutants from water [9]. Water hyacinth is listed as one of the most productive and active plants on earth. It has been used to treat a variety of waste waters and to produce high protein cattle food, pulp, paper, fiber and more importantly, biogas as energy source [7]. The present study aims at determining the effect of Co and Cu (heavy metals) on anatomical and histochemical character of *Eichhornia crassipes* (Mart.) Solms.

2. MATERIALS AND METHODS

Before starting the experiment, young plants of *E. crassipes* was collected from Chittoor river of Ernakulam District and Kerala state. The plants were acclimated to room conditions for a short duration prior to experimentation.

Tanks of similar size were taken. Eight liters of distilled water had been added to each tank. *Eichhornia* plants were introduced. Stock solution of heavy metal Copper and Cobalt are prepared. For this 800 μ M of Cu and Co weighed and separately treated with eight liters of distilled water. Metallic solution kept in the tanks considered as treated condition and

the other tank to which no metallic solution was added considered as controlled condition. The tanks were then transferred to an open place. For each treatment 3 replicates were maintained.

For anatomical studies, transverse sections of stem, leaf, root and petiole of *Eichhornia crassipes* (Mart) Solms. was taken with the help of sharp blade. The sections were stained in saffranin mounted in glycerin on a slide. Such sections were studied under the microscope. The anatomical features were observed and recorded. The microphotographs of the sections were also taken. For histochemical localization, fresh materials alone were used. For uniform results, variables like time, temperature and concentration of the stains were kept constant. Starch was localized in *E. crassipes* according to Iodine – Potassium Iodide reaction (I2KI) method. The peelings were mounted in Iodine – Potassium Iodide solution made by dissolving 2gm of potassium iodide in 100 ml of water and then dissolving 2 gm of iodine in the potassium iodide solution. The blue black colour indicated the presence of starch. The total proteins was localized by Mercuric Bromophenol blue Method. The peels were immersed in the Mercuric Bromophenol blue dye solution for 15 minutes. Washed for 20 minutes in 0.5% acetic acid to remove the excess dye. Washed in water for 15 min. These parameters are noted down after 60 days.

3. RESULTS AND DISCUSSION

In the present study, it is evident that the Co and Cu treated plants showed a decrease in growth, number of complex tissues and development of plant. For anatomical studies, the different plant parts like petiole, root, leaves and stolon are taken and sections were analyzed under the microscope. Degradation of vascular tissues occurred due to the effects of Cobalt and Copper. In normal condition (control) plants shows good health and vascular tissues are abundant with water. Studies on effect of heavy metals Cadmium, Cobalt, Mercury and Lead in some members of Malvaceae [2], corn and soya beans [4], [8], supports these findings. The study on anatomical features like transverse section of stem, root, leaves and petiole revealed increased numbers of complex tissues layers like xylem and phloem for controlled plants and decreased in plant treated with heavy metals Co and Cu (Fig 1, 2, 3 & 4).

In leaf, the epidermis layers were more in control than in treated conditions. Raphid crystals were present in treated conditions. In petiole, the vascular bundles were lesser in treated conditions, especially in Cu treated conditions. The air spaces were prominent in treated conditions. In stolon, the vascular bundles and air spaces showed the same as in petiole. In roots, the stellar region of control showed more xylem bundles alternating with phloem than treated conditions. The number of cortex layers was greater in control. The presence of heavy metals happens to be injurious to plant growth, affecting water absorption and other metabolic process in plants. It was proven by [6].

Histochemical localization of metabolites in foliar epidermis was observed. For the localization of Starch, Iodine – Potassium Iodide reaction, Johansen's method was adopted. In all the three conditions, the blue black color developed and this indicated the presence of starch. (Fig. 5). In control the starch grains were seen as well defined, deeply stained black granules in the palisade cells of the leaves. In treated conditions, the granules were not well defined. The intensity of the staining was lessened. When the protein was localized, in all the three conditions, blue colour developed and thus indicated the presence of proteins. (Fig 6). In control the proteins were stained as blue granules in the palisade cells of the mesophyll cells of the leaves. They were deeply stained. In treated conditions, a very low activity of the proteins were noted. The protein granules were very few and the blue staining was very poor. They were not compactly packed as in control. Scientists are of opinion that, heavy metal can cause specific inhibition of photosynthesis [3]. When the photosynthesis process is inhibited, the overall growth performance of the plant will be affected as in the present study. It is found that heavy metals are capable of decreasing the chlorophyll content, carotenoid, DNA, RNA, protein and dry weight [11], which in turn will lead to a decrease in the overall growth performance of the plant. It was found that heavy metals inhibit the total carbohydrate, protein and nucleic acid content. It also inhibits the cell division of the plant. As these heavy metals affect the overall growth performance of the plant, its growth reduction leads to the decrease in the phytomass of the plant. Thus the phytomass of the plants showed considerable decrease over the control. As reported by the scientist, the Cu may be interfering with the proteins,

which are the structural components of the chloroplast and it may be blocking either the synthesis or the activity of enzyme proteins responsible for chlorophyll biogenesis [10].

Fig. 1. Effect of Co and Cu on T.S. of leaf anatomy of *Eichhornia crassipes* (Mart.) Solms.

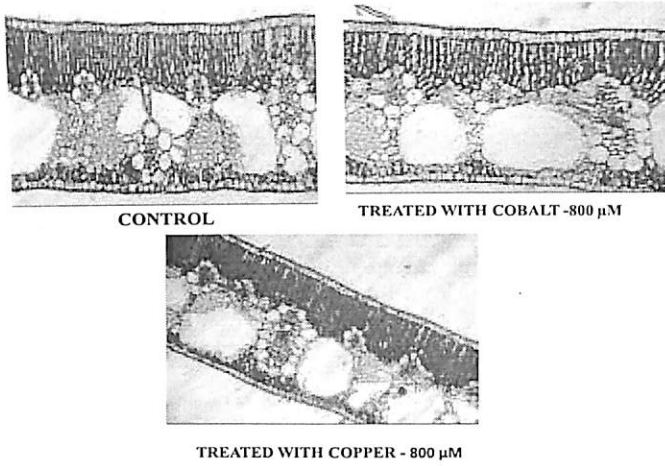


Fig. 2. Effect of Co and Cu on T.S. of petiole anatomy of *Eichhornia crassipes* (Mart.) Solms.

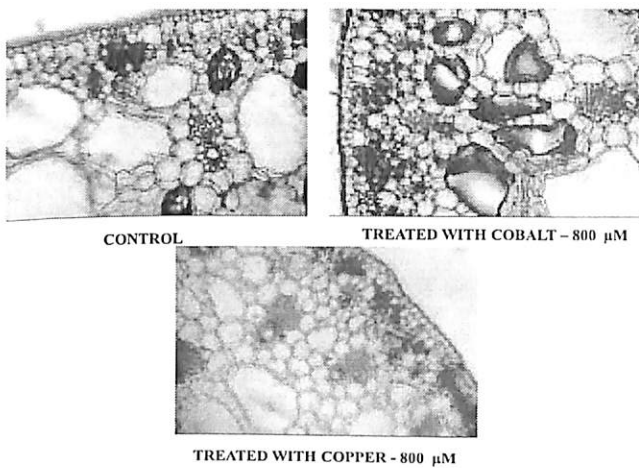


Fig. 3. Effect of Co and Cu on T.S. of stolon anatomy of *Eichhornia crassipes* (Mart.) Solms.

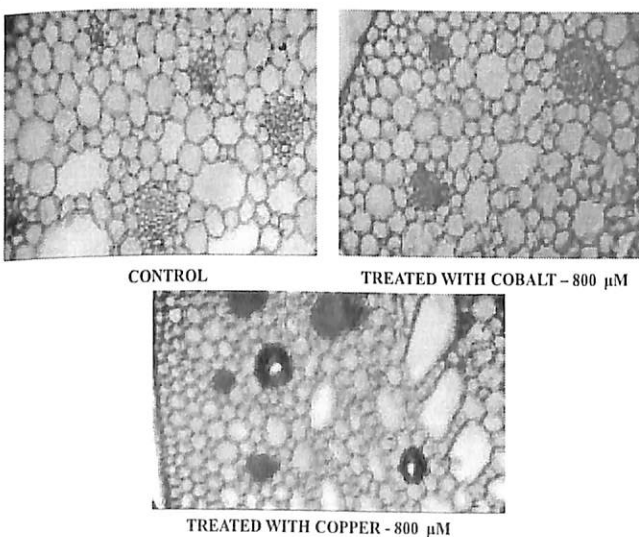


Fig. 4. Effect of Co and Cu on T.S. of root anatomy of *Eichhornia crassipes* (Mart.) Solms.

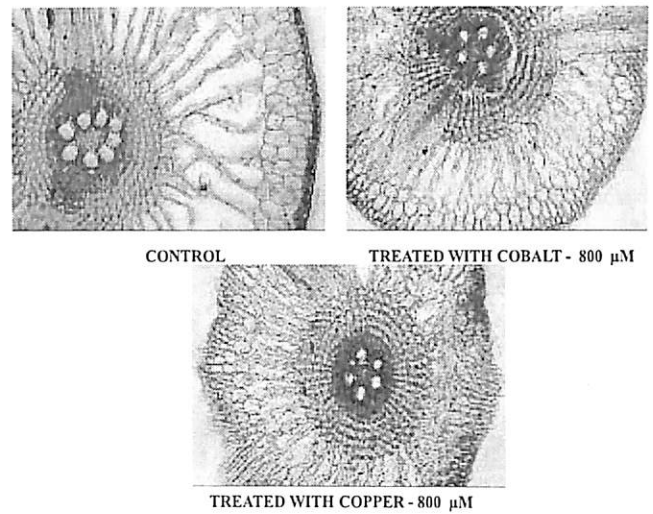


Fig 5. Histochemical localization of Starch in the leaf of *Eichhornia crassipes* (Mart.) Solms. showing the effect of Co and Cu.

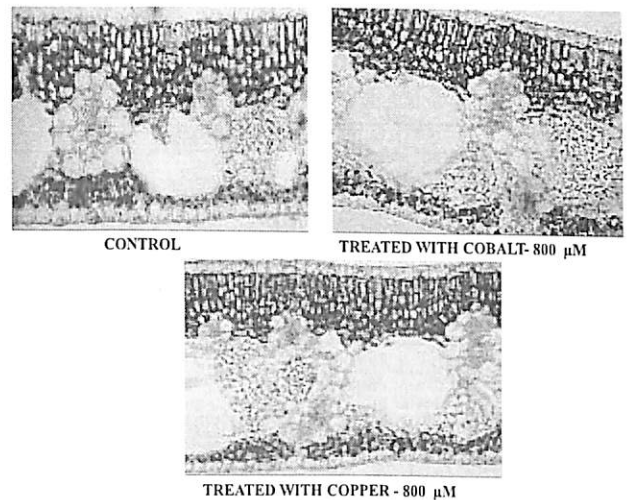
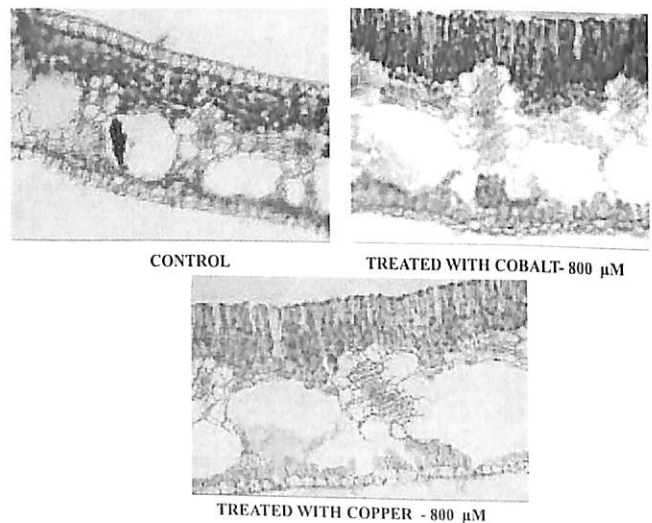


Fig 6. Histochemical localization of Protein in the leaf of *Eichhornia crassipes* (Mart.) Solms. showing the effect of Co and Cu.



4. CONCLUSION

The result of the present study clearly indicated that even at lower concentrations of heavy metals the plant showed reduced growth rate and it is also harmful to humans as well as animals. From the results obtained, it can be stated that a small amount of heavy metals has a negative impact on the growth of the plant and it has shown to accumulate large amount of metals in its parts which is said to be major cause of heavy metal poisoning, pollution and bioaccumulation. Smaller concentrations of the applications of heavy metals naturally stunt the growth, development and reproduction of the plants. It is imperative to have proper understanding of plant response and pollutant concentration relationship with environmental conditions to preserve our nature and natural resources. Disintegrated the xylem forms, disturb transports water, and dissolved mineral nutrients throughout the plant from the roots. The functions of phloem also partially prevent (transports sugars, proteins and minerals around the plant). Then the plants stunted in growth. In the present study, the heavy metals such as Co and Cu accumulations in leaves were found to be at toxic level, which is harmful to humans.

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