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EFFECT OF MANGANESE AND COPPER STRESS ON A LEAF BIOCHEMICAL PARAMETER OF A MANGROVE *AVICENNIA MARINA* (FORSK.) VIERH

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ABSTRACT
Increasing industrialization and release of industrial effluent including heavy metals is a major environmental problem worldwide. The effluent includes numerous heavy metals, of which manganese and copper, has been selected for experiments. Changes in chlorophyll contents (Total chlorophyll and ratio chl a/b were examined by the application of different concentrations of copper and manganese in seedlings of a mangrove, *Avicennia marina*(Forsk.)Vierh. The seedlings were grown in pots in green house conditions in the soil amended with various levels of manganese and copper (100,200,300 and 400, 500 ppm). The estimation of chlorophyll was done on 10th, 20th and 30th day after the metals were spiked in the soil. The results indicated that the content of total chlorophyll increased at low levels of copper and manganese but started declining at higher concentrations while ratio of chl a/b keeps on increasing throughout the experimental period. The increase in total chlorophyll at lower concentrations indicate that these metals, being vital micronutrient for the growth of plants, helped in increasing chlorophyll content but decline at high concentrations indicates the adverse effect of their toxicity.

KEYWORDS: Pigment, Chlorophyll, *Avicennia Marina*, Industrialization, Effluent, Toxicity.

INTRODUCTION
Highly toxic metals like cadmium and lead have no known functions in plants but heavy metals like manganese and copper, though essential for normal plant growth and development, can be toxic when present in excess in the environment. Heavy metals are not biodegradable and remain persistent in the environment. (MacFarlane and Burchett, 2002).[1]

Manganese is an essential micronutrient for all vital functions. It acts as a cofactor of various enzymes, plays a critical role in a reaction catalysed in photosystem II. (Marschner, 1995)[2] It also aids in synthesis of chlorophyll. Its toxicity can be an important factor limiting plant growth, particularly in acidic, poorly drained soils (Horst,1998).[3]

Copper is an essential element for plant growth and plays a significant role in many physiological processes including photosynthesis, respiration, cell wall metabolism, nitrogen fixation etc. Plants usually find an ample supply of copper in soils and copper at higher concentrations may act as a stress factor triggering physiological responses (Yruela 2005).[4]

Excess of copper can result in production of ROS and free radicals, which binds to the sulphydryl groups of membrane proteins or increase rates of lipid peroxidation and ultimately damages cell membranes (Liu et al. 2004).[5] Studies have shown that toxic action is substitution of copper for magnesium in chlorophyll molecules and reduces photosynthesis (Kupper et al. 1996).[6] Due to inhibition of photosynthesis by excess copper concentration, production of free radicals increase and hence leaf senescence.(Luna et.al, 1994).[7] In fact mangrove plants are growing in a complicated environment including multiple heavy metals. Hence it is necessary to study the correlation between mangroves and heavy metals. *Avicennia marina* is dominant mangrove species. This study investigated the effect of manganese and copper stress on chlorophyll content in seedlings of *Avicennia marina*.

MATERIALS AND METHODS
Collection and treatment
The seeds of *Avicennia marina* were collected manually from the field. Seeds were soaked in water till the pericarp was removed. Soil in different pots were treated with different concentrations of manganese and copper (100,200,300,400,500ppm).

Experiment duration
Experiment was conducted upto one month after treatment of soil with different concentrations of Mn and Cu.

Experimental design: Four seeds were sown in a pot. Three pots were kept for one concentration. All the pots treated with different concentrations of manganese and copper as well as untreated as control were provided adequate environment for their optimum growth. Pots without any treatment of heavy metals were kept as control for comparison purposes.

Analysis procedure: The experiments were triplicated. Chlorophyll content was estimated on 10th, 20th and 30th day after the treatment of soil. Estimation of chlorophyll was done by Machlachlan and Zalik (1963)[8] method.

RESULTS

The results of the effect of different concentration of manganese and copper on chlorophyll content of a mangrove *Avicennia marina* are as follows.

Chlorophyll a is found to be greater than chlorophyll b in control as well as in treated seedlings.

In case of manganese total chlorophyll increased in seedlings treated upto 200 ppm and started decreasing after that but still decline was not very sharp till 400 ppm and maximum decline at maximum concentration level was 17.2% decrease on 30th day as compared to control.(Fig.1).

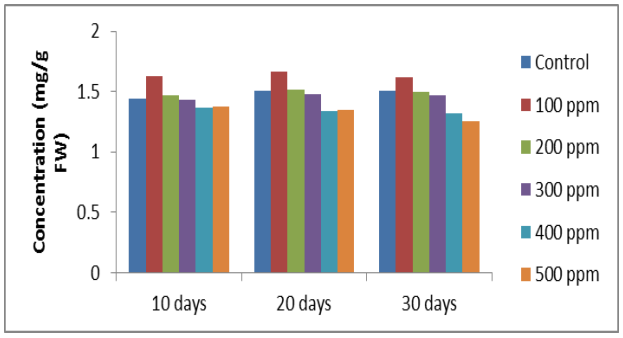

Fig. 1. Total chlorophyll content in *Avicennia marina* treated with different concentrations of manganese.

Ratio of chlorophyll a and b kept on increasing with increase in concentration indicating that chlorophyll b is being more severely affected than chlorophyll a. There was 39.8% increase on 30th day of maximum concentration applied as compared to control.(Fig .2)

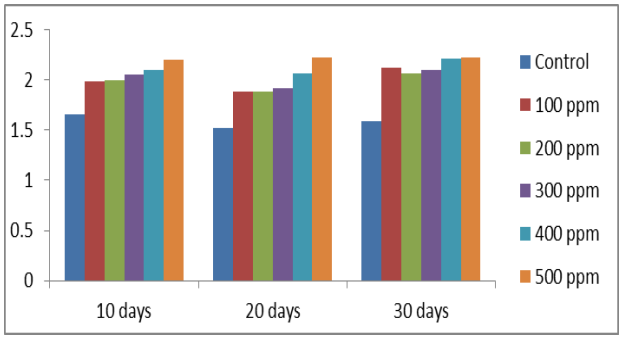

Fig. 2. Ratio of chl a/b in *Avicennia marina* treated with different concentrations of manganese.

In case of copper, total chlorophyll increased upto 200 ppm as in case of manganese and after that it started declining but the decline was much more as compared to manganese and decreased upto 33.3% as compared to control while in case of manganese it was 17.2% decrease.

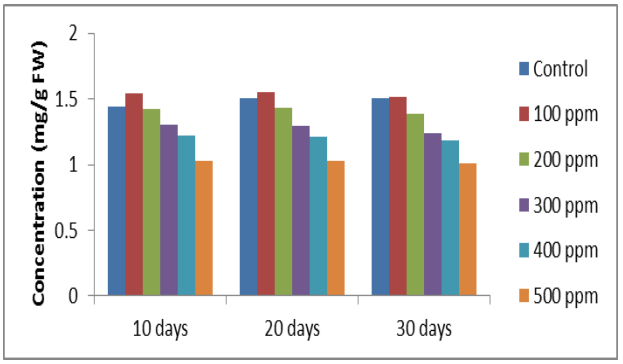

Fig. 3. Total chlorophyll content in *Avicennia marina* treated with different concentrations of copper.

Ratio of chlorophyll a and b in case of copper increased as compared to control but the increase was tremendous and it increased upto 64.26% as compared to control on 30th day of the seedlings treated with maximum concentration applied.

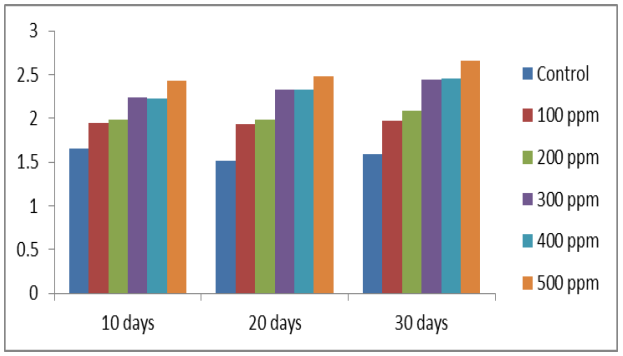

Fig. 4. Ratio of chlorophyll a and b in *Avicennia marina* treated with different concentrations of copper.

DISCUSSION

The present study demonstrates that manganese and copper being essential micronutrient, their application upto low concentration level increased chlorophyll content. The reason of increase in chlorophyll might be due to manganese which aids in synthesis of chlorophyll Also according to Marschner 1995, manganese plays a critical role as an accumulator of positive charge equivalents in a reaction catalysed in photosystem II.(Marchner, 1995).[2] In case of copper, according to Romeu –Moreno and Mas, 1999, an increase in photosynthetic pigments occur because of copper induced reduction in carbondioxide fixation and as such photosynthesis doesnot decrease atleast initially. (Romeu-Moreno and Mas, 1999)[9] Increased chlorophyll content due to copper at low level may be due to the copper acting as structural and catalytic components of

protein, enzymes and as a cofactor for normal development of chlorophyll biosynthesis. This is showed by the studies of (Ouzounidou(1994 a)[10], Mocquot et al.(1996)[11] Umabese and Motajo(2008)[12], Mysliwa-Kurdzeil and Strazalka (2002)[13] under copper treatment.

At higher concentrations, manganese and copper behave as toxic elements and decline in chlorophyll content was reported. Decline in chlorophyll content in the plants exposed to heavy metals stress is believed to be probably due to inhibition of important enzymes, such as 6-aminolevulinic acid dehydratase (ALA-dehydratase) (Padmaja et al. 1990)[14] and protochlorophyllide reductase (Van assche and Clijsters,1990)[15] associated with chlorophyll biosynthesis; and/or (b) impairment of supply of Mg^{2+} and Fe^{2+}. At higher concentration level of manganese, chlorophyll content decreased. Other studies have also shown same results, plants reacts with excess manganese with a drop in photosynthetic rate. (Macfie and Taylor, 1992; Macfie et al., 1994).[16,17] Manganese toxicity has also been associated with swollen chloroplasts in G.max(Wu,1994).[18] Copper also acts as toxic element at higher concentrations and cause decrease in chlorophyll content. Same results were shown in other studies also. Presence of excess copper significantly decrease chlorophyll content and inhibtis growth. (Ralph and Burchett, 1998; Fargasova, 2001)[19,20] According to Fernandes and Henrique excessive copper can be accumulated in plant tissue and become toxic to plants affecting several physiological and biochemical processes and growth. (Fernandes and Henrique, 1991).[21] The results showing toxicity of copper at higher levels are comparable with the reports of Lidon and Henriques (1992)[22], Romeu-moreno aand mas (1999)[9], Mysliwa-Kurdzeil and Strazalka(2002).[13]

CONCLUSION

Increasing industrialization and effect of heavy metals present in the effluent, released by industries, on the plants in the vicinity of industries, has become a major problem worldwide. Mangrove ecosystem being ecologically and economically very important, the mangrove *Avicennia marina* was used as experimental plant for estimating the effect of two metals manganese and copper on chlorophyll content of seedling of *Avicennia marina*. The results reported in this study showed that both manganese and copper at low concentrations behave as a micronutrient, vital for the appropriate growth of the seedlings of *Avicennia marina* as well as chlorophyll content increased at low levels, but at higher levels of application, these metals became toxic to the plant and affected chlorophyll content especially by decreasing chlorophyll b.

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