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PHYTOACCUMULATION OF HEAVY METALS AND FLUCTUATION IN PHOTOSYNTHETIC PIGMENTS IN SELECTED AQUATIC MACROPHYTES

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Abstract: Bioassay was conducted by raising three macrophytes such as *Hydrilla verticillata*, *Pistia stratiotes* and *Salvinia molesta* in water containing varying concentration of the heavy metal solution of copper, lead and cadmium (1, 5, 10 and 20 mg L^{-1}) for ten days. The concentration of photosynthetic pigments like chlorophyll a, b, total chlorophyll and carotenoids present in the plant samples were estimated and chlorophyll a/ b ratio, total chlorophyll/carotenoid ratio were computed. The reduction of the photosynthetic pigments was negligible (< 15%) with varying concentration of copper treatment while significant (>80%) with lead and cadmium irrespective of the genera. Chlorophyll a to b ratio showed reduction as the concentration of treatment increased which may be attributed to the alteration in the rate of pigment synthesis or its degradation. The carotenoid content and chlorophyll/ carotenoid ratio often changed in metal treated plants. *S. molesta* exhibited comparatively higher reduction in the pigments (>75%) on exposure to cadmium and lead. The accumulation pattern of the heavy metals in *H. verticillata*, *P. stratiotes* and *S. molesta* was Cu>Pb> Cd. The biosorption of heavy metals exceeded the toxic limit in all macrophytes.

Key words: Chlorophyll, Carotenoids, Chlorophyll a/ b ratio, Total chlorophyll/carotenoid ratio

INTRODUCTION

The pollution of aquatic systems by organic and inorganic contaminants as well as metals is a global issue. Industrialization and rapid advancements in agriculture practices, mining activities as well as smelting of metalliferous ores aggravate the problem of heavy metal pollution. Macrophytes are aquatic macroscopic plants, both cryptogams and phanerogams growing luxuriantly in the shallow euphotic zone of the water bodies. Many aquatic macrophytes viz., submerged, emergent and floating plants are capable of cleaning organic and inorganic contaminants from polluted water and are potent tools in the abatement of heavy metal pollution in aquatic ecosystems which receive industrial effluents and municipal wastewater. They are employed in pollution abatement due to low cost, easy handling and abundance in aquatic ecosystems. Ecophysiological studies under heavy metal stress provide an insight in to the efficiency of macrophytes in phytoremediation.

Objectives

The present study helps assess the metal accumulation efficiency of selected macrophytes viz., *Hydrilla verticillata*, *Pistia stratiotes* and *Salvinia molesta* and evaluate the photosynthetic pigments in the macrophytes under heavy metal stress.

MATERIALS AND METHODS

Three free floating fresh water macrophytes were employed for the bioassay viz., *Hydrilla verticillata, Pistia stratiotes* and *Salvinia molesta*. *Hydrilla verticillata* and *Pistia stratiotes* are monocotyledons belonging to Hydrocharitaceae and Araceae family respectively while *Salvinia molesta* is an aquatic fern growing luxuriantly as an invasive weed in polluted waters. Macrophytes were collected from a fresh water pond and transferred to the plastic containers and exposed to heavy metal stress for ten days in water containing varying concentration of the metal solution (1, 5, 10 and 20 mg L^{-1}) viz., copper sulphate, lead nitrate and cadmium sulphate with four treatment concentration (T1, T2,T3, T4). Estimation of chlorophyll a, b, total chlorophyll and carotenoids present in the plant samples was carried out by standard method (Arnon, 1949).

A known quantity of the tissue was homogenized in 80% acetone and homogenate was filtered and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and absorbance was measured at 490 nm, 645nm and 663nm and the amount of various photosynthetic pigments was calculated and expressed in mg g^{-1}. Experiments conducted in triplicate and mean and standard deviation were computed.

RESULTS AND DISCUSSION

The photosynthetic pigments such as chlorophyll a, b, total chlorophyll, carotenoids and chlorophyll a/b as well as total chlorophyll/carotenoid ratio exhibited fluctuations in macrophytes under exposure to varying concentration of copper, cadmium and lead as compared to control (Fig. 1 to 6). The reduction of the photosynthetic pigments was negligible

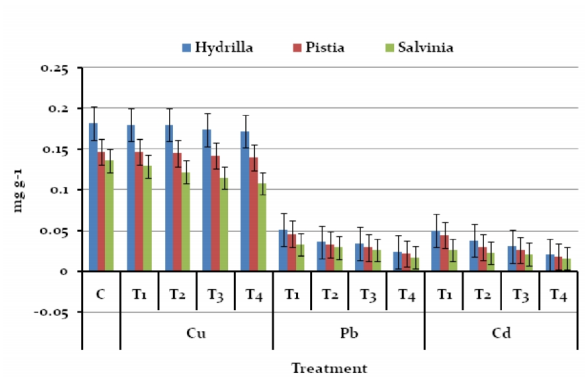

Fig. 1. Chlorophyll a in macrophytes

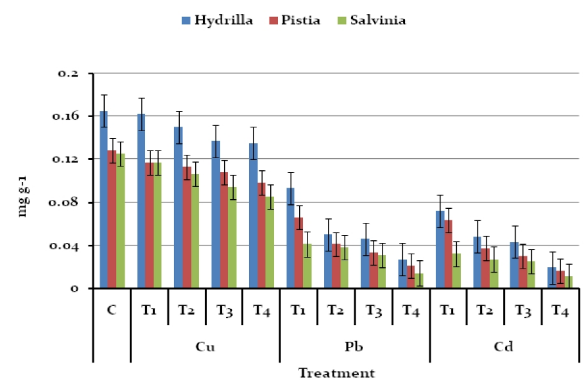

Fig. 2. Chlorophyll b in macrophytes

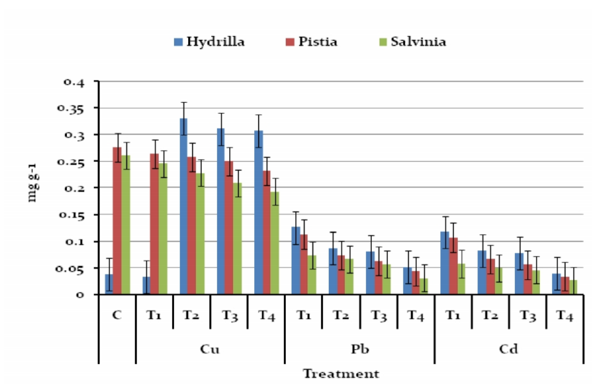

Fig. 3. Total Chlorophyll in macrophytes

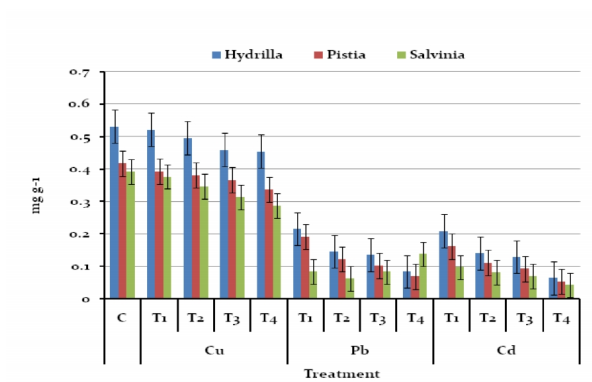

Fig. 4. Carotenoids b in macrophytes

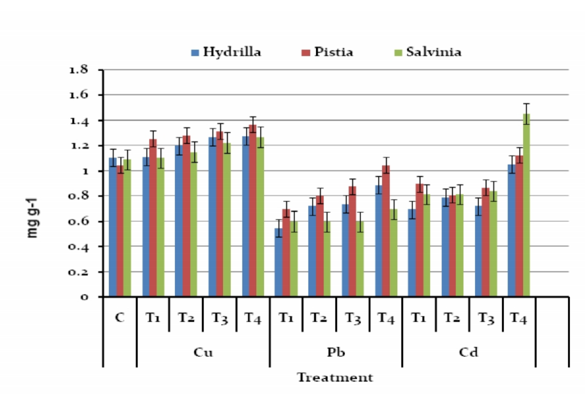

Fig. 5. Chlorophyll a/b ratio in macrophytes

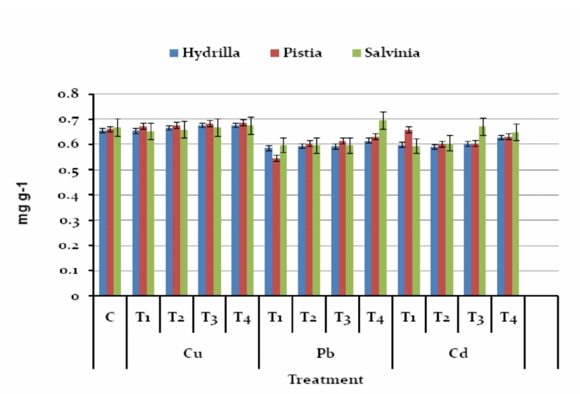

Fig. 6. Total chlorophyll/carotenoid ratio in macrophytes

with varying concentration of copper treatment while significant with lead and cadmium irrespective of the genera. The above observation may be attributed to the fact that copper is an essential element for the plant metabolism where as lead and cadmium were non essential and rather toxic.

Minimum reduction in pigments was encountered in macrophytes grown in varying concentration of copper (< 15% reduction). The estimation of pigments in *H. verticillata, P. stratiotes* revealed reduction of >80% while >90% was evident in *S. molesta* under higher treatment concentration of lead and cadmium. The results in the study remained in unison with the reports of Naguib *et al.* (1982). Heavy metals influenced and altered chl a/b ratio which may be attributed to the alteration in the rate of pigment synthesis or its degradation. Cd^{2+} is found to decrease chl a/b ratio (Stiborova *et al.*, 1986) and considered to be the most effective inhibitor of chlorophyll biosynthesis, lower pigment content and photosynthetic activity (Mysliwa and Strazatka, 2002). Cd^{2+} affected chlorophyll biosynthesis even at lower concentration remained in agreement with the results obtained herein. Low concentration of cadmium enhanced the level of chlorophyll and carotenoids irrespective of the genera remained in unison with the reports of Rai *et al.*(1998) and cadmium was found to be more toxic than copper (Hou *et al.*, 2007). Pigment degradation observed during senescence was found to be influenced by heavy metals by increasing chlorophyllase activity (Abdel - Basset *et al.*, 1995). Lower pigment content results from the inhibition of biosynthesis of photosynthetic pigments as well as differential proportion of various accessory pigments. Kupper *et al.* (1998) observed that heavy metals substitute the central magnesium in chlorophyll causing important type of damage in submerged aquatic plants grown in metal contaminated environments. The central magnesium atoms of the chlorophyll are accessible to heavy metals which in turn enter in to the production of stable heavy metal – chlorophyll complex creating damage to the pigment with low light intensity and irradiance.

Heavy metals influenced chl a/b ratio and alteration observed in the ratio may be attributed to the variation in the rate of pigment synthesis or its degradation. Cd^{2+} is found to decrease chl a/b ratio while an increase was observed treated with Cu^{2+} and Pb^{2+}in maize and barley (Stiborova *et al.*, 1986). Decrease in the a/b ratio was observed irrespective of the genera remained in unison with the reports of Baszyruski *et al.* (1980). The carotenoid content and chlorophyll/ carotenoid ratio often changed in metal treated plants.

The heavy metal content in plant tissue (Fig. 7 to 9) varied with the concentration of metal content in the medium. The accumulation of copper followed a trend *S. molesta>H. verticillata>P. stratiotes* at lower concentration of treatment while at higher concentration the

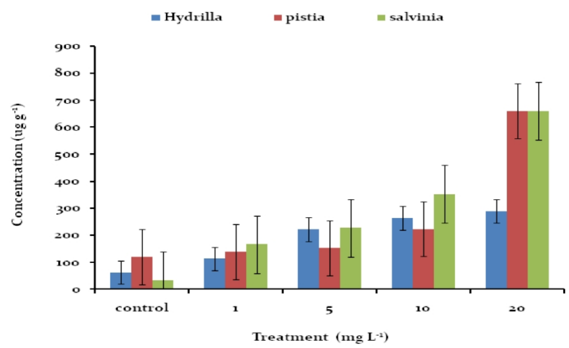

Fig. 7. Biosorption of Copper in macrophytes

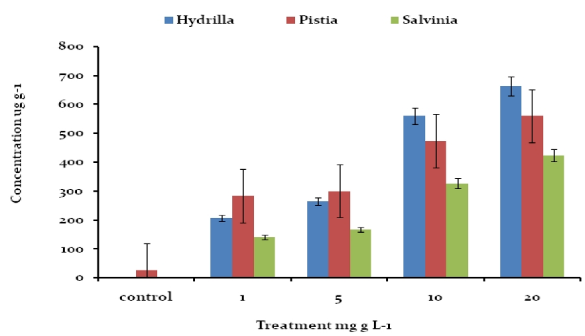

Fig. 8. Biosorption of Lead in macrophytes

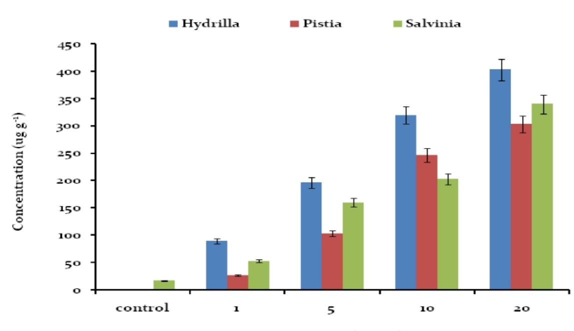

Fig. 9. Biosorption of Cadmium in macrophytes

sequence became *S. molesta* = *P. stratiotes*>*H. verticillata*. The accumulation efficiency of lead in the macrophytes under investigation exhibited a pattern with *H. verticillata*> *P. stratiotes* > *S. molesta*. The sequence of absorption of cadmium was *H. verticillata*>*S. molesta*> *P. stratiotes*. The accumulation pattern of the heavy metals in *H. verticillata*, *P. stratiotes* and *S. molesta* was Cu>Pb> Cd. The biosorption of heavy metals in bioassay exceeded the toxic limit in all macrophytes. Submerged aquatic plants transport heavy metals in acropetal and basipetal directions and the translocated metals normally get accumulated in the cell walls and mesophyll (Prasad, 2004). The immobilization and detoxification of the metal is accomplished by the specific molecules in the cell like phytochelatins of the category of organic acids and polypeptides thus forming metal – phytochelatin complex and transported and stored in to the vacuoles. Chaney *et al*. (1978) computed the foliar concentration of copper and concluded that the value above 25 $\mu g\ g^{-1}$ is indicative of phytotoxicity. Leeper (1978) opined that plants normally contain copper with a range of 4 to 15 $\mu g\ g^{-1}$ and the toxic limit is 30 $\mu g\ g^{-1}$. Critical toxic level of copper is 20-30 $\mu g\ g^{-1}$ dry wt. (Ouzounidou *et al*., 1992). . The normal range of concentration of lead in plants is 0.1 to 10 $\mu g\ g^{-1}$ (Leeper, 1978) and had very short retention time in water and major portion of lead is bound to the cellwalls of plants. Leeper (1978) reported that the normal range of cadmium in plants was 0.2 to 1.8 $\mu g\ g^{-1}$ with a toxic limit of 100 $\mu g\ g^{-1}$. In general, the biosorption of copper, lead and cadmium exceeded the toxic level in all macrophytes in the present investigation.

The present study revealed the metal accumulation efficiency of macrophytes such as *H. verticillata*, *P. stratiotes* and *S. molesta* and resultant variation of pigment profile

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