ABSTRACT
Heavy metals are some of the major environmental pollutants released into water as a result of industrial processing. These are toxic and non-biodegradable. The contaminated waters and soils pose major environmental, agriculture and human health problems worldwide. These problems may be partially mitigated through “Phytoremediation”. A biotechnological process over-ruling chemical technologies. Phytoremediation is also most applicable for aquatic pollution because of its cost effectiveness, aesthetic and long term applicability. In the present study bio-adsorption studies were carried out in both Hydrilla and Pistia sp. with three different initial Copper (II) concentrations (i.e. 5ppm, 10ppm, 15ppm). Triplicates were maintained in each concentration by using 3 L capacity plastic tubs. Water samples were collected every 24 hr interval over a period of 10 day exposure. Samples were analyzed using Atomic Absorption Spectrophotometer (AAS). Pistia showed the maximum removal percentage of copper on day 9 and Hydrilla on day 7 of exposure at 5 ppm concentration. Increase in the concentration decreased the removal percentage in both plants. Morphological observations and plant growth also varied at varying concentrations and exposure duration. Heavy metal removal efficiency was higher (P<0.05) with Hydrilla than with Pistia at lower concentrations, however, the removal efficiency was more with Pistia at higher concentrations.

Key words : Quantification, Cholesterol, Ascaridia galli, Domestic fowl

Environmental pollution is one of the most hazardous ecological crises to which humans are subjected today. Population explosion coupled with rapid industrialization has led to the contamination of natural resources with harmful pollutants like pesticides, heavy metals, toxic chemicals, etc. The heavy metals, which are toxic and non-biodegradable, are the major environmental pollutants released into water as a result of industrial processing. The polluted waters and soils cause major environmental, agricultural and human health problems. The toxicity due to metal ions is due to their ability to bind with protein molecules (Kar and Sahoo, 1992) and prevent replication of DNA and subsequent cell divisions. Rapid industrialization has further aggravated the problem through indiscriminate discharge of untreated and partially treated industrial effluents thereby increasing the concentration of Cd, Pb, Zn, Cu and Mn in the near by water bodies (Totawat, 1993). These heavy metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability (Knasmuller et al., 1998; Baudouin et al., 2002). Recently biotechnology has opened doors to unique uses of different eco-friendly technologies overruling chemical technologies. Most of the chemical and engineering technologies have almost failed to remove pollutants. These problems may be partially solved by a new and emerging biotechnological process called “Bioremediation”.

In “bioremediation” procedures “Phytoremediation” is the most applicable and advantageous method to clean up aquatic environment. Ilya Raskin (1994) defined phytoremediation as “a process in which plants are used for environmental remediation that involves removing organics and metals from soils and water”. Of late phytoremediation is attracting increasing attention from scientists and regulators because it appears to be cheaper than chemical and engineering oriented methods and may also offer immediate and long-term environmental benefits. Since last decade phytoremediation has emerged as a new, low-tech, cost effective technology that uses plants and their associated microbial flora for environmental clean up (Raskin et al., 1994; Salt et al., 1995a; Salt et al., 1998).

Plants are reported to have the capacity to withstand relatively high concentrations of metals or organic chemicals without being affected by toxicity. They can also take up and transform organic chemicals to less toxic metabolites in some cases. At sites contaminated with heavy metals, plants are used either to stabilize or remove the metals from the soils and ground water through three mechanisms: (1) Phytoextraction: The use of metal
accumulating plants to remove toxic metals. (2) Rhizofiltration: The use of plant roots to remove toxic metals from polluted waters. (3) Phytostabilization: The use of plants to eliminate the bioavailability of toxic metals in soils.

Bioremediation perhaps, is the best way to treat effluents as they operate under milder conditions with minimum generation of byproducts. Use of microorganisms and aquatic weeds for removal of toxic heavy metals from industrial effluents has been documented. Cynobacteria (Verma et al., 1995) have been used for removal of chromium from industrial effluents. Water hyacinth (Prasad et al., 1983), powdered leaves (Suseela et al., 1987) and water weeds (Singaram, 1994 and Tobin et al., 1998) have been used for the removal of chromium from spent liquor and tannery effluents. Hydrilla and Nymphaea (Sood et al., 1994) have been used for removal of lead, cadmium and mercury.

The present paper is a part of our study aimed at understanding the bio-adsorption efficiency of aquatic weeds exposed to heavy metals (copper II) at different concentrations.

**MATERIALS AND METHODS**

Aquatic weed plants *Pistia stratoletes* (free floating) and *Hydrilla vorticillata* (submerged macrophyte) were collected from near by natural ponds in Tirupati and surrounding areas. Both plants were acclimatized to laboratory conditions in cement tanks containing fresh water for one week. After acclimatization both plants were exposed to three different initial concentrations (5ppm, 10ppm, 15ppm) of metal solution of Cu(II) prepared by dissolving copper nitrate in order to give known concentration of metal required at the beginning of the experiment.

**Experimental design:**

 triplicates were maintained in each concentration using plastic tubs of 5lit capacity having a total volume of 3lit of sample solution in each tub. 1000ppm stock solution and 500ppm of secondary stock solution were prepared and kept ready for use. Desired concentrations of copper solution were added to each plastic tub, prepared from secondary stock solution. Young medium sized healthy plants were selected. *Pistia* and *Hydrilla* plants with equal biomass were kept in each tub. Water levels were marked and all the tubs were exposed to enough light during the 10 day exposure period. Tap water was added to maintain the same level in each tub every day. Samples were collected at every 24 hr interval and filtered through Whatman No.42 filter paper. Filtered samples were analyzed using Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer Analyst-100 with HGA 2380). In this study, a probability of $p<0.05$ was considered to be statistically significant.

**RESULTS AND DISCUSSION**

The results on bio-adsorption studies, using *Pistia* and *Hydrilla* plants, which were exposed for 10 days to copper (II) at three different initial concentrations (i.e. 5, 10,15ppm) are presented in Table 1 and 2, Fig. 1 and 2, respectively. These results clearly indicate that at lower initial concentrations (5ppm) Pistia plants showed higher ($p<0.05$) removal and greater adsorption efficiency although the physiological and morphological conditions remained normal (Table 1 and Fig. 1). Highest removal percentage (94.63) of copper (II) was noticed on day 9.
at initial concentration (5ppm).

In case of Hydrilla, removal efficiency was greater up to 7th day of exposure. Significantly (p<0.05) higher removal efficiency and removal percentage (96.02) were observed on day 7 at initial concentration of 5ppm (Fig. 2 and Table 2). For every 24hr interval the removal or adsorption efficiency varied drastically. This may be due to direct or indirect disturbances and metabolic imbalances caused by copper in the internal physiological processes of the plant.

Some morphological changes were also observed during the experimental period. At lower concentrations (5ppm) the plant growth and morphological characteristics were normal up to 3rd day in both the plants. At higher concentrations morphological characters of the plants changed very rapidly during early days. Discoloration of leaves started from day 6 onwards in both the plants. But, at lower initial concentrations (5ppm) the changes were very slow compared to those at higher initial concentrations with longer exposure period where the leaves are wrinkled and more than 50% of the leaves discolored. Changes in growth were dependent on the initial concentrations of metals and exposure duration. These changes may be due to metal toxicity which reduces chlorophyll content (Neelu et al., 2000) and the rate of photosynthesis in the plants (Vassilev et al., 1998). Removal efficiency was higher in Hydrilla compared to Pistia at lower initial concentration (5ppm). But Pistia showed higher removal efficiency at higher initial concentration (10ppm, 15ppm). It is obvious that phytoremediation is an effective technology for removing and detoxifying metals and metalloids such as Cd, Se and As from the environment for recultivation and reclamation of polluted sites. Phytoremediation works best when supplemented by non-biological remediation technologies for decontamination of most polluted sites. Because pollutant distribution and concentration are heterogeneous for sites, the most efficient and cost effective remediation solution may be a combination of different technologies such as excavation of the most contaminated sites followed by polishing the site with the use of plants (Vinita Hooda, 2007).

It has been observed that copper (II) can be removed effectively at lower concentrations. Hydrilla showed greater removal efficiency at lower concentrations (5ppm) while Pistia showed greater removal efficiency at higher initial concentration (10ppm, 15ppm). Thus phytoremediation appears to be a useful technique to cleanup polluted waters very effectively. Aquatic plants with extreme growth and rapid spreading character can be easily employed as a cost effective and eco-friendly management technique in metal pollution control programmes. These aquatic plants are easily harvestable and can easily be removed and dispersed. This study demonstrates that bioremediation technique is one of the most inexpensive method to remove metals effectively from aquatic environment. Apparently mechanisms of most of the biological processes underlying phytoremediation such as plant metal uptake, translocation, accumulation and / or degradation and plant microbe interaction, are poorly understood and need further research.

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**Table 2: Per cent removal of copper [Cu(II)] by Hydrilla plant at different concentrations**

<table>
<thead>
<tr>
<th>Exposure period (days)</th>
<th>5 ppm</th>
<th>10 ppm</th>
<th>15 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.58</td>
<td>19.49</td>
<td>49.91</td>
</tr>
<tr>
<td>2</td>
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<td>22.51</td>
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<td>54.14</td>
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<td>4</td>
<td>73.48</td>
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<td>35.9</td>
<td>57.57</td>
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<td>58.94</td>
</tr>
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<td>96.02</td>
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<td>9</td>
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<td>10</td>
<td>93.66</td>
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<td>59.88</td>
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</tbody>
</table>
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