The anchored hydrophyte, *Hydrocotyle umbellata* L., was employed for the removal of toxic metals from tannery sludge concentrations (w/v) from a tanneries wastewater treatment plant. Different concentrations of wet tannery sludge were prepared and plants of *H. umbellata* showed a good tolerance for all the prepared concentrations. Plants were retrieved after 30, 60 and 90 days and the sludge concentrations showed reduction in sodium chloride, chlorides and chemical oxygen demand (COD). The accumulation of toxic metals in the plants was significantly increased, with increasing exposure time of plants. A higher amount was accumulated in the roots than in the shoots. The bioconcentration factor of Cr was higher than that of Zn and Cu at the same exposure time, indicating a higher accumulation potential of Cr by *H. umbellata*. The order of uptake efficiency was Cr > Zn > Na > Cu and the maximum metal uptake was observed after 90 days of exposure of *H. umbellata*, being 18,200 mg kg\(^{-1}\) for chromium, 15,560 mg kg\(^{-1}\) for zinc 7,692 mg kg\(^{-1}\) for sodium and 6,660 mg kg\(^{-1}\) for copper in the roots. These plants not only tolerated up to 60% concentration of tannery sludge but also reduced chromium content of sludge to a considerable extent.

**Key words:** Phytoremediation, rhizofiltration, tannery sludge, heavy metals, hydrophytes.

**INTRODUCTION**

Recently, several studies have demonstrated the potential of wetland plants in aquatic phytoremediation, a process that includes rhizofiltration, phytofiltration, and constructed wetlands (Brooks and Robinson, 1998; Qian et al., 1999; Dushenkov and Kapulnik, 2000). Due to the relative novelty of the technology, much of the current research is still aimed at the selection of wetland plant species, based on the type of elements to be remediated, the local environmental and geographical conditions and the remediative capabilities of the plant.

A cursory review of the current literature (Raskin and Ensley, 2000; Terry and Banuelos, 2000) reveals that the desirable attributes of a plant for the removal of toxic metal from water include tolerance to high environmental concentration of the metal, its ability to accumulate very high levels of the metal, ability to bioconcentrate the metal in low-concentration environments, fast growth and high biomass production, limited root to shoot translocation (especially for plants used in rhizofiltration), and the ability to phytoaccumulate metals across a broad range of nutrient levels. Moreover, for constructed wetlands, site-specific requirements include the ability to sustain the desirable characters across a broad range of climatic conditions, as well as the native origin of the species.

Ahalya et al. (2004) have reported that discharge of heavy metals into aquatic ecosystems has become a matter of concern over the past few decades. These pollutants are introduced into the aquatic system significantly as a result of various industrial operations. The pollutants of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel. Plants have been employed in the decontamination of heavy metals from polluted water and have demonstrated high performances in treating mineral tailing water and industrial effluents. Several aquatic species have the ability to remove heavy metals from water, including Water Pennywort (*Hydrocotyle umbellata* L.) (Dierberg et al., 1987), Duckweed (*Lemna minor* L.) (Mo et al., 1989) and Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms) (Zhu et al., 1999).

Leather industry is one of the major sources of pollution in Pakistan and one of the most affected situations is that
of Kasur, Pakistan. A wastewater treatment plant has been set up in Kasur in order to minimize pollution before throwing the effluents of leather industry into Rohi Nullah, falling into the River Sutlej. In this treatment unit, called the Kasur Tannery Waste Management Agency (KTWMA), a large quantity of sludge is obtained which is of a very toxic nature due to a high amount of metals in it. There is no proper method of disposal than dumping it initially in a permanent sludge lagoon and later shifting it to a landfill site nearby. It cannot even be used to fertilize the soil because of its toxic nature.

The efficiency of removal of excess toxic and heavy metals from tannery sludge by growing a tolerant anchored hydrophyte, *Hydrocotyle umbellata* in different concentrations of tannery sludge was investigated. The study was designed for assessing the rhizofiltration capabilities of *H. umbellata*.

**MATERIALS AND METHODS**

Samples of sludge were collected from the Kasur Tannery Waste Management Agency (KTWMA) located at the Depalpur Road, Kasur, Pakistan. Sludge samples were collected in plastic drums and transported to the Department of Botany, University of the Punjab, Lahore, Pakistan.

For the preliminary experiment, different concentrations of fresh semi-solid (wet) sludge; 20, 40, 60, 80 and 100% were prepared in plastic beakers of 1 L capacity amounting to 800 ml in each beaker for experimental treatments by homogenously mixing the sludge with tap water. Tap water was taken in case of control. Plants of the aquatic hydrophyte *H. umbellata* were collected from the Botanical Garden, University of the Punjab, Lahore, Pakistan. Five plants of uniform size were grown in each treatment with five replicates of each. Based upon the tolerance of plants, the concentrations selected for the actual experiment were 20, 40 and 60% along with control (0%). Different concentrations of freshly transported semi-solid sludge were prepared in plastic beakers of 1 L capacity as described earlier. The experiment was set up in a wire house in the Dept. of Botany in a “Completely Randomized Design” with factorial arrangement (Steel and Torrie, 1981). Five replicate plants of uniform size were taken in each beaker. The experiment comprised of four treatments with five replicates each, to be analyzed at monthly intervals.

A detailed physico-chemical analysis of the wet sludge was carried out to determine pH, conductivity, total dissolved solids (TDS), NaCl, amount of chlorides, chemical oxygen demand (COD) and metals. The pH, TDS, NaCl percentage and conductivity were measured with the help of a Multiparameter Meter (Hanna, Model HI 98107) and a conductivity meter (Hanna Model HI 9835), respectively. COD was determined according to the procedure described by Greenberg et al. (1998). Chlorides were measured titrimetrically (Saeed, 1980). The estimation of Na was done on a Flame photometer (PPF7 & PPF7/C JENWAY) while estimation of heavy metals was done on an Atomic Absorption Spectrophotometer (AA-1275/ VARIAN, Australia).

After retrieval, the plants were acid digested (perchloric and nitric acid digestion method) as described by Greenberg et al. (1998) and subjected to spectrophotometry for metal analyses.

The statistical analyses of the data were carried out using Analyze-it-Free Software available on the web.

**RESULTS**

*H. umbellata* showed excellent tolerance for different concentrations of tannery sludge. During the initial adjustment phase, it showed some wilting but afterwards flourished well and developed new leaves and branches. The plants of *H. umbellata* tolerated all concentrations of tannery sludge until 90 days of exposure and were still very healthy and green at the time of retrieval after 90 days. In the preliminary experiment, plants of *H. umbellata* started to wither and decay in higher concentrations; also showing some necrosis. After two weeks, the plants of *H. umbellata* were unable to tolerate the 80 and 100% concentrations of sludge. Thus, these concentrations were excluded from the actual experiment and it was carried out with control 0, 20, 40, and 60% concentrations.

The results of pH and conductivity indicated minor fluctuations but significant reduction in pH of higher concentration was observed (Figure 1). The general trend was an increase in the TDS content of different concentrations with increasing concentration and with prolonged period of plant growth. There was a significant reduction in the amount of NaCl in all concentrations after the growth of *H. umbellata* and the reduction increased with the increase in period of growth. The reduction was more pronounced at elevated concentration levels of sludge. Chlorides showed similar reductions (Figure 2). The COD values also showed an increase with increasing concentration of sludge. After growing plants of *H. umbellate*, a significant reduction in COD was observed and reduction was more pronounced with increasing growth period of plants (Figure 2).

The metal contents of the different sludge concentrations before and after exposing plants of *H. umbellata* are shown in Figure 3. Among the toxic metals, amounts were in the order sodium > chromium > zinc > copper. The percentage reduction in the amount of metals was observed in different concentrations of tannery sludge after growing *H. umbellata* for 90 days (Figure 4). The percentage reduction was in the order of chromium > sodium > zinc > copper. In all cases, metal uptake was higher in the roots than shoots. The amount of metals increased with increasing concentration of sludge and with increasing exposure time of plants in the sludge.

Among the toxic metals, chromium uptake was the greatest corresponding to the amount of these metals in the sludge (Figure 6). The general trend in uptake of metals was in the order Cr > Na > Zn > Cu. In all cases, the amount of metals increased with increasing exposure time of plants within the sludge concentrations. The maximum uptake was observed after 90 days exposure of *H. umbellata*, amounting to 18,200 mg kg⁻¹ for chromium, 15,560 mg kg⁻¹ for zinc, 7,692 mg kg⁻¹ for sodium and 6,660 mg kg⁻¹ for copper in the roots (Figure 5). The amount was almost reduced to two thirds in shoots and the maximum uptake was observed after 90 days exposure of plants; 12,820 mg kg⁻¹ for chromium, 9,436 mg kg⁻¹ for zinc, 4,488 mg kg⁻¹ for copper and 3,260 mg kg⁻¹ for sodium (Figure 6). The values for coefficient of bioaccumulation of metals in both roots and shoots were quite high for Zn and Cr as compared to Cu (Table 1). The
least bioaccumulation was observed in case of Na.

DISCUSSION

Phytoremediation is a fast developing field and it will hopefully become an integral part of the environment management and risk reduction process. Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr and Zn (Kumar et al., 1995; Ensley, 2000).

The characteristics of the ideal plant for rhizofiltration have been described by Dushenkov and Kapulnik (2000).
Plants to be used should be able to bioaccumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal (Boushy, 1991). Moorhead and Reddy (1990) studied the effectiveness of *H. umbellata* in the use of oxygen to reduce biological oxygen demand, ammonia and nitrogen concentrations in sewage and found it to be very effective in the conversion of organic carbon into carbon dioxide. It can also withstand cooler temperatures of water and air, and thus can also be combined with more warm-seasoned plants to maximize the treatment process. The experiment with *H. umbellata* revealed a significant reduction in all of the pollution parameters like COD and amount of NaCl, chlorides and bicarbonates. Metal analyses revealed that *H. umbellata* was the best selected plant, which not only tolerated the highest concentration of the sludge but also exhibited a significant uptake of chromium, zinc and copper in the same order.

In Pakistan tanning is one of the most prominent and potentially polluting industry in terms of waste generation, containing rather high concentrations of metals, particularly important being Cr. Chromium merits a special reference for its extreme toxicity due to the interaction of its compounds with living cells (Costa, 1997). In chromium uptake and bioaccumulation studies, prepared concentrations of chromium have been used in experiments (Sinha et al., 2002; Maine et al., 2004; Shiny et al., 2004; Choo et al., 2006). In a few cases, hydrophytes have been used to remove metals from tannery effluent concentrations (Sinha et al., 2002). This is the first study of removal of metals from toxic tannery sludge concentrations using an anchored emergent hydrophyte, *H. umbellata* without any obvious symptoms of toxicity.
Significant metal uptake was observed in *H. umbellata* at higher concentrations of sludge and increased with increasing concentration of sludge. This ability to tolerate high amount of metal levels is apparently associated with the ability of the plant to limit translocation of metals to the shoot. Plants are used either to stabilize or to remove metals from the soil and contaminated water through various mechanisms among which rhizofiltration is a very important mechanism. Root exudates and changes in rhizosphere pH may also cause metals to precipitate onto root surfaces. As they become saturated with metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza, 1998; Zhu et al., 1999). Once absorbed by the plants, toxic or heavy metals can be stored in different plant parts converted into less harmful substances within plants, changed into gaseous form or released into the air through transpiration. Therefore, the proper disposal of such metal contaminated plants also requires a careful strategy so that the metals taken up from the environment must not spread in the
Table 1. Coefficient of bioaccumulation in roots and shoots of *H. umbellata* in different concentrations of sludge after 90 days exposure.

<table>
<thead>
<tr>
<th>Metals concentrations</th>
<th>Amount of metal in sludge concentrations (mg L⁻¹)</th>
<th>Amount of metal in roots after 90 days (mg kg⁻¹ dry weight)</th>
<th>Amount of metal in shoots after 90 days (mg kg⁻¹ dry weight)</th>
<th>Coefficient of bioaccumulation in roots</th>
<th>Coefficient of bioaccumulation in shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na 20%</td>
<td>285.2</td>
<td>4,012</td>
<td>1,572</td>
<td>14.06</td>
<td>5.51</td>
</tr>
<tr>
<td></td>
<td>40% 450.5</td>
<td>6,248</td>
<td>2,384</td>
<td>13.86</td>
<td>5.29</td>
</tr>
<tr>
<td></td>
<td>60% 625.5</td>
<td>7,692</td>
<td>3,260</td>
<td>12.29</td>
<td>5.21</td>
</tr>
<tr>
<td>Cr 20%</td>
<td>75.2</td>
<td>11,560</td>
<td>6,268</td>
<td>153.73</td>
<td>88.03</td>
</tr>
<tr>
<td></td>
<td>40% 120.5</td>
<td>15,600</td>
<td>6,268</td>
<td>129.46</td>
<td>52.01</td>
</tr>
<tr>
<td></td>
<td>60% 178.7</td>
<td>18,200</td>
<td>12,820</td>
<td>101.84</td>
<td>71.70</td>
</tr>
<tr>
<td>Cu 20%</td>
<td>49.5</td>
<td>3,248</td>
<td>1,888</td>
<td>65.61</td>
<td>38.14</td>
</tr>
<tr>
<td></td>
<td>40% 68.4</td>
<td>5,060</td>
<td>3,460</td>
<td>73.97</td>
<td>50.58</td>
</tr>
<tr>
<td></td>
<td>60% 82.5</td>
<td>6,660</td>
<td>4,488</td>
<td>80.72</td>
<td>54.4</td>
</tr>
<tr>
<td>Zn 20%</td>
<td>54.0</td>
<td>7,024</td>
<td>5,336</td>
<td>130.07</td>
<td>98.81</td>
</tr>
<tr>
<td></td>
<td>40% 76.5</td>
<td>8,760</td>
<td>5,990</td>
<td>114.50</td>
<td>78.30</td>
</tr>
<tr>
<td></td>
<td>60% 108.5</td>
<td>15,560</td>
<td>9,436</td>
<td>143.41</td>
<td>86.96</td>
</tr>
</tbody>
</table>

Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov et al., 1995; Salt et al., 1995; Flathman and Lanza, 1998). Dushenkov et al. (1995) were of the view that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. In contrast, Zhu et al. (1999) suggested that the efficiency of the process can be increased by using plants which have a better ability to absorb and translocate metals within the plant. Despite this difference of opinion, it is apparent that proper plant selection is the key to ensuring the success of rhizofiltration as a water cleanup strategy.

In the hyperaccumulator plant *Leersia hexandra*, chromium bioaccumulation in the leaves has been observed to be 5,005 mg kg⁻¹ dw while in roots it was as high as 18,656 mg kg⁻¹ from prepared culture solutions of Cr (Zhang et al., 2007). In this study a higher amount of metals was observed in the roots than in shoots. Even though the amount of metals was less in the leaves, still it was as high as 12,820 mg kg⁻¹ of Cr in case of *H. umbellata*. Fritioff and Gregor (2006) while studying distribution of heavy metals like Zn, Cu, Cd and Pb taken up by leaves, stems and roots of *Potamogeton natans*, found the highest accumulation in the roots in conformity with the present findings. According to Lasat (2000), hyperaccumulators are plants that have an innate capacity to absorb metal at levels 50 - 500 times greater than average plants. *H. umbellata* showed a good hyperaccumulation of all the metals, especially of importance, is the heavy metal chromium. Thus, this plant can qualify as a hyperaccumulator according to the criterion given by Baker and Brooks (1989).

The percentage reduction in the metal content of the sludge after a 90 day exposure of *H. umbellata*, typically in case of chromium, signifies the fact that regular growth and harvesting of these plants in specially prepared treatment ponds with a 60% sludge concentration for three such cycles will completely remove all the metals from tannery sludge whereby it can be applied as fertilizer in fields.

**Conclusion**

Some plants have the ability to accumulate metals from the substratum but the problem is the sensitivity of the hydrophytes towards the concentrations of the tannery sludge which represent a very toxic type of waste. *H. umbellata* proved to be a very important nutrient fixer and a valuable member of the aquatic communities as far as toxic sludge like that of tanneries is concerned. It can be employed as an on-site remediation strategy near the treatment plant, so that after the sludge becomes free of heavy metals, it can be used as a fertilizer in cultivated fields.

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