Evaluation of phytostabilization, a green technology to remove heavy metals from industrial sludge using *Typha latifolia* L.

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**ABSTRACT**

The effect of the glass industry on heavy metal characterization was assessed in the sludge/sediments of 5 discharge pits (sites) for effluents of functional glass factories and/or finishing workshops in the study area. A comprehensive profile of Zn, Mn, Co, Cd, Pb, Cr, Ni, Cu and As levels in industrial sludge was obtained. Correlation coefficients for metal pairs indicate varying levels (positive/negative) of correlation in the samples. A statistically significant difference (P = 0.001) was obtained for each metal on comparing mean metal content among sludge samples. Phytoremedial potential of *Typha latifolia*, a locally thriving plant species was also evaluated. Shoot and root samples from *T. latifolia* L. were taken for heavy metal analysis and leaf samples for pigment analysis to gauge its tolerance of heavy metals. Total chlorophyll and free proline content indicate that the species has adapted itself to multi metal stress in the rhizosphere. BCF > 1 (Bioconcentration factor) and TF < 1 (Translocation factor) were obtained in *T. latifolia* samples in the case of Mn, Cr and As at all 5 sites; Zn at 4 sites; Co, Cd and Ni at 2 sites each, and as such it can be advocated as a potential phytostabilising species for these metals. The species demonstrated limited capabilities in remediating Pb and Cu.

**Keywords:** phytostabilisation, bioavailability, *Typha latifolia*, phytoremediation

**INTRODUCTION**

Environmental pollution is a major concern because of the continuous urbanization and industrial development [1,2]. Heavy metals can accumulate in soil and sediments via several pathways, including the disposal of liquid effluent, terrestrial run-off and leachate-carrying chemicals originating from numerous urban, industrial and agricultural activities, and atmospheric deposition. Industrial effluents contain large number of pollutants in higher concentration [3]. High levels of heavy metals can be found in soils around industrial area due to discharge and dispersion of industrial effluent and wastes into nearby agricultural soils and streams [4,5]. Characterization of the chemical and physical properties of industrial effluents is important to assess the environmental risk caused by the mobility of toxic heavy metals, which are contained in this type of wastes [6].

One of the features that distinguish metals from other toxic pollutants is that they are not biodegradable, so they tend to accumulate and persist in the soil for a very long time [7]. Although they do not occur naturally in living organisms and have been reported not to have any known function in them [8], these toxic metals are absorbed by and tend to accumulate in humans and animals through food, air and water. Lead, cadmium, copper, zinc, nickel, chromium and arsenic are the metals most frequently reported to have the highest impact on organisms [9,10].

Phytoremediation may be a feasible and practical ‘natural’ remediation technique for contaminated soils, and may be defined as “the engineered use of green plants to remove, contain or render harmless environmental contaminants like heavy metals, trace elements, organic compounds and radioactive compounds in soil or water [11]. Phytostabilization is one of the strategies of phytoremediation that
aims to reduce the mobility and bioavailability of pollutants in environment and not removing them [12]. A significant fraction of metals can be stored within or adsorbed on the root surface [13] contributing to long-term stabilization of pollutants.

It has been demonstrated [14-16] that, wild native plants may be better phytoremediators for waste lands than the known metal hyperaccumulators like *Thlaspi caerulescens* and *Alyssum bertolonii* because these are slow growing with shallow root systems and low biomass. Even if the soil is naturally high in a particular metal, native plants often become adapted over time to the locally elevated levels [17,18] so in the case of native flora and soils, metal toxicity issues, mostly, do not arise. Species of *Sesbania, Avena, Crotalaria, Crinum asiaticum* and *Calotropis procera*, lemongrass, vetiver, and other wild grasses have been reported for heavy metal bioindicating and phytoremedial purposes [19-22]. Allowing native species to remediate soils is an attractive proposition since a plant community comparable to that existing in the vicinity can be established. The outcome is, thus, both site remediation and ecological restoration [23].

Phytoremedial potential of plants is influenced by the mobility and bioavailability of heavy metals in soil, and thus translocation patterns of contaminants can be used to assess the phytoremedial potential of plants. Plants with a high bioconcentration factor (ratio of metal content in root to that in soil) as well as a low TF (ratio of metal content in shoot to that in root) are potential phytostabilisers as they keep the translocation of metals from roots to shoots as low as possible [24]. *Gentiana pennelliana* having BCF values of 11, 22 and 2.6 for Pb, Cu and Zn has been reported as a suitable phytostabilizer [25].

Firozabad, known for its glass industry, is a rapidly expanding town in north central India. Chemicals like KNO$_3$, Na$_2$SO$_4$, AsO$_3$, ZnO, CuO, PbCr$_2$O$_7$, CdS, NiO$_2$ etc. are utilized as refining and colouring agents in this industry. Finishing and decorating procedures like clustering and metalizing in which various heavy metal (Fe, Zn, Cd, Cu etc.) salts are widely used. These processes involve spraying metal salt and acid mixtures or vacuum coating, all done by illiterate workers, often in the open, without any safeguards for themselves or the environment. The town and its residents are subjected to heavy pollution levels due to the rapid pace of industrialization, urbanization and development and efforts to remediate and reclaim soil and water bodies industrial areas are urgently needed. In this context, the present study was undertaken to assess the heavy metal content of effluents and associated soil and sediments of the glass industry. Accumulation of various heavy metals was also investigated in *Typha latifolia* growing at the sampling sites to evaluate its phytoremedial potential, as a native, tolerant plant species.

**MATERIALS AND METHODS**

**Experimental design**

A survey was carried out to identify discharge pits/reservoirs for effluents of functional glass factories and/or finishing workshops in the study area. Sludge/sediment samples were collected from these pits. The flora growing in and around the pits was identified. *Typha latifolia* was seen thriving in the contaminated matrix where neighboring flora showed signs of phytotoxicity and stunted growth patterns. Shoot and root samples from *T. latifolia* were taken for subsequent metal analysis to evaluate its phytoremedial potential. Chlorophyll and proline were also investigated to check overall health and tolerance of metal stress in the species. *T. latifolia* leaf samples were also collected from plants growing in the Keetham bird sanctuary near Agra to obtain control values for chlorophyll and proline content.

**Description of study area**

Firozabad is located at 164 MSL in north central India (27°09’N 78°24’E), in western Uttar Pradesh state, around 240 km away from New Delhi, the national capital. The river Yamuna flows close by.
The glass industry of Firozabad is centuries old and articles manufactured in the city include chandeliers, lanterns, souvenirs, statuettes, vases, tableware, stemware, mugs and tumblers, bottles, bulbs, laboratory apparatus, glass accessories for automobiles as well as glass bangles and beads. As per the District Industry Centre, there are about 185 registered functional large and medium sized production units. Approx. 4000 small and micro-scale processing and finishing units exist. Employing about 40,000 workers, the industry melts around 2000 metric ton glass every day and boasts an annual turnover of nearly 40 billion rupees (about $ 890 million).

**Description of *Typha latifolia***

*Typha latifolia* L. also known as Cat-tail belongs to family Typhaceae, is an aquatic or semiaquatic perennial herbaceous plant that grows in marshy areas and boggy places, along borders of lakes and water reservoirs. The plant is 1.5 to 3 metres high and it has 2-4cm broad leaves. Rhizomes grow horizontally just below the soil surface is tough, stout, coarse, and extensive. Shallow fibrous roots are attached to the rhizomes.

**Collection of samples**

Five random sludge samples were collected from the bottom of each effluent reservoir. The samples were air dried, passed through <80 mesh sieve and stored in clean zip-lock polythene bags until further use. Shoot and root samples were collected in triplicate from *T. latifolia* at spots from where sludge samples were collected and kept in separate zip-lock bags. In the lab, first the adhering sludge was removed with distilled water. Then the samples were further washed thoroughly 3-4 times with de-ionized water and allowed to drip dry completely in a dust free chamber. Fresh leaf samples for chlorophyll and proline analysis were stored immediately in ziplock bags and kept in an icebox. They were analyzed within an hour of sampling. Control samples were treated in the same manner.

**Analysis of samples**

Sludge samples were tested for various parameters to obtain a clear profile of soils at the sites selected. Important soil properties are shown in table 1. pH was measured by a pH meter (Systronics pH system 361) with a water soil ratio of 1:2.5. Organic carbon was measured by modified Walkley-Black rapid dichromate oxidation method [26]. Available potash by flame photometer (mediFlam), available Nitrogen by micro-kjeldahl method and available phosphate was estimated according to Jackson [27]. Cation exchange capacity was calculated according to Bower et al. [28]. Sludge and plant samples (< 80 mesh) from all sites were wet-digested using aqua regia (3ml HNO₃ + 9 ml HCl) for soil and 5 ml HNO₃ + 2 ml H₂O₂ for shoot and root samples. The filtrate was analyzed by flame atomic absorption spectrophotometry or by graphite furnace atomic absorption spectrophotometry using a Solaar M2 - Thermo Unicam instrument. Chlorophyll content in leaf samples was determined on fresh weight basis [29]. 40 mg fresh leaves were placed in 10 ml 80% acetone in a sealed, dark bottle in a refrigerator. After 5 days optical density of the solution was measured by a spectrophotometer at different wavelengths i.e. 480, 510, 630, 645, 652 and 665nm and chlorophyll content was calculated using relevant formulae.

Free proline was extracted and determined according to Bates et al. [30]. 1g fresh leaf sample was extracted in 2 ml 3% sulphasalicylic acid to which 2 ml each of ninhydrin and glacial acetic acid was added and the mixture boiled in a water bath for 1 hr. the reaction was terminated by plunging this in an ice bath. 4 ml toluene was added and absorbance measured at 520 nm in a spectrophotometer and calculated against standard proline.
Assessment of phytoremedial potential of plant

To evaluate the phytoremedial (phytostabilisation) potential of Typha latifolia, the following factors were calculated: (1) Bioconcentration Factor \([\text{BCF}] = \frac{\text{metal content in root}}{\text{metal content in soil}}\), (2) Translocation Factor \([\text{TF}] = \frac{\text{metal content in shoot}}{\text{metal content in root}}\).

Statistical analyses

Pearson’s coefficient for correlation was statistically analyzed at a significance level of \(P < 0.05\) and \(P < 0.01\). The statistical significance of differences among means was determined by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Concentrations of heavy metals in the sludge samples have been summarized in table 2. Mean concentrations of Zn, Mn, Co, Cu and As were highest in samples from Site 1. Cr and Ni levels were highest at Site 4 while Cd and Pb levels were highest at Site 5 and 4, respectively. Compared to the threshold value for industrial soils suggested by the Canadian Environmental Quality Guidelines (2003), levels of As were consistently higher at all sites (as much as 24 times). Cu levels were nearly thrice the limit at 2 sites. Ni content was higher than the threshold suggested at 1 site. Zn (1 sample) and Cd (2 samples) levels were near the thresholds while Pb and Cr levels were below them. Correlation coefficients (\(P = 0.01\)) in table 3 indicate significant positive correlation between concentrations of Zn and Mn versus nearly all the metals. Mostly negative correlation was obtained for Cd and Pb values with all other metals. Cr content did not exhibit any correlation except with Zn while Ni, Cu and As showed variable correlation with the other metals. One way analysis of variance (ANOVA) indicated a statistically significant difference (\(P = 0.001\)) in mean content of each metal among sludge samples. These were further compared to isolate the site(s) that differ from the others using Fisher’s LSD test as a multiple comparison procedure.

Heavy metal content above/near the suggested industrial thresholds indicates the extent of damage caused to the pedosphere. When compared with the thresholds suggested for residential soils, the picture is even more alarming with all metals crossing the values at least at 1 site. This becomes relevant in the light of the fact that some of the study sites fall within densely populated residential zones. Besides, a couple of these pits are in fact more like a swampy bits of land where cattle were seen grazing the grass. At three places the pits were surrounded by agricultural land sown with leafy vegetables. In this context the presence of such toxic effluents and sediments compromises the health of not only the local inhabitants but also the consumers of the agricultural produce of such areas.

Soil properties, environmental conditions, biological activity as well as the chemical properties of the pollutant, all influence its bioavailability. Soil pH seems to have the greatest effect on the availability of metals in soil [31]. Sludge samples from all five sampling zones (Table 1) had pH values in the range
Table 2. Heavy metal content in sludge samples (mg/kg)

<table>
<thead>
<tr>
<th>Sites</th>
<th>Zn</th>
<th>Mn</th>
<th>Co</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Ni</th>
<th>Cu</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Range</td>
<td>336.2-406.30</td>
<td>28.7-38.22</td>
<td>56.3-72.34</td>
<td>11.35-164.25</td>
<td>36.54-43.25</td>
<td>32.4-42.31</td>
<td>258.13-368.47</td>
<td>262.47-306.44</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>362.4</td>
<td>33.63</td>
<td>63.69</td>
<td>13.44</td>
<td>138.18</td>
<td>37.8</td>
<td>283.87</td>
<td>293.37</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>255.5</td>
<td>17.15</td>
<td>13.74</td>
<td>21.32</td>
<td>186.4</td>
<td>33.61</td>
<td>20.1</td>
<td>175.32</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>214.69</td>
<td>17.15</td>
<td>13.74</td>
<td>21.32</td>
<td>186.4</td>
<td>33.61</td>
<td>20.1</td>
<td>175.32</td>
</tr>
<tr>
<td>S4</td>
<td>Range</td>
<td>310.28-354.62</td>
<td>18.64-21.30</td>
<td>-0.578*-10.47</td>
<td>-0.895*-16.47</td>
<td>-0.578*-10.47</td>
<td>-0.895*-16.47</td>
<td>-0.578*-10.47</td>
<td>-0.895*-16.47</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>334.2</td>
<td>19.18</td>
<td>11.57</td>
<td>21.42</td>
<td>185.6</td>
<td>33.61</td>
<td>20.1</td>
<td>175.32</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>283.4</td>
<td>27.2</td>
<td>21.01</td>
<td>24.1</td>
<td>234.6</td>
<td>42.63</td>
<td>20.1</td>
<td>175.32</td>
</tr>
</tbody>
</table>

F value: '*' statistically significant difference at (P ≤ 0.001) in mean metal contents in sludge at the different sites; SD - Standard deviation; a - Canadian environmental quality guidelines 2003 proposed by The Canadian Council of Ministers of the Environment.

Table 3. Correlation coefficient.

<table>
<thead>
<tr>
<th>Mn</th>
<th>Co</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Ni</th>
<th>Cu</th>
<th>As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.786*</td>
<td>0.705*</td>
<td>-0.578*</td>
<td>-0.895*</td>
<td>0.524*</td>
<td>0.562*</td>
<td>0.914*</td>
</tr>
<tr>
<td>Mn</td>
<td>0.598*</td>
<td>-0.476*</td>
<td>-0.724*</td>
<td>0.376</td>
<td>0.510*</td>
<td>0.836*</td>
<td>0.759*</td>
</tr>
<tr>
<td>Co</td>
<td>-0.219</td>
<td>-0.710*</td>
<td>0.079</td>
<td>-0.001</td>
<td>0.597*</td>
<td>0.878*</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.710*</td>
<td>-0.324</td>
<td>-0.476*</td>
<td>-0.744*</td>
<td>-0.324</td>
<td>-0.744*</td>
<td>-0.324</td>
</tr>
<tr>
<td>Pb</td>
<td>-0.289</td>
<td>-0.379</td>
<td>-0.866*</td>
<td>-0.798*</td>
<td>-0.289</td>
<td>-0.379</td>
<td>-0.866*</td>
</tr>
<tr>
<td>Cr</td>
<td>0.919*</td>
<td>0.548*</td>
<td>-0.040</td>
<td>0.548*</td>
<td>0.667*</td>
<td>0.044</td>
<td>0.670*</td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.01 level (2-tailed) (n = 25).

Organic carbon content of soil samples was also low. These parameters indicate that metal ions were more available to plant roots. Metals like Pb are mostly immobile in the soil, which reduces their bioavailability [32] and subsequent uptake by the plant, Cd on the other hand is usually less adsorbed by soil and organic matter which makes it more available to plants [33], even though it is far more toxic.

Heavy metal concentrations in shoots and roots of *T. latifolia* are presented in table 4. The results indicate that all the metals displayed an identical trend of accumulation in shoot and root samples. Translocation patterns indicate that in *T. latifolia*, Zn, Mn, Co, Cd, Pb, Cr, Ni, Cu and As are all selectively partitioned in the roots. As and Pb are not easily transferred to above-ground plant biomass, being stored mainly in root cells [34], whereas Zn is easily accumulated in green tissues like leaves [35]. Although *T. latifolia* was not seen to hyperaccumulate any of the metals, the uptake ranges are encouraging when compared to reported toxic concentrations in plants (Table 5). In the case of roots, concentrations...
Figure 1. Chlorophyll pigment in *T. latifolia* (leaf).

Figure 2. Free Proline content in *T. latifolia*. 
of all metals studied except Mn and Co were above the reported phytotoxic levels [36-39], up to 80 times in the case of As, 35 times for Cr, 3.5 times for Cu and 4.5 times for Pb. The concentration of Pb, Cr, Cu and As was seen to surpass phytotoxic levels in aboveground (shoot) parts too.

The Bioconcentration Factor (BCF) and Translocation Factor (TF) values (Table 6) help to identify the suitability of plants for phytostabilisation by explaining the accumulation characteristics and translocation properties of metals in plants. TF values indicate that none of the metals tested was allocated in greater proportion to the aboveground biomass in T. latifolia. The root system is the primary target in heavy metal toxicity [40] hence this indicates the high metal tolerance of the species and supports the hypothesis that T. latifolia is naturalized to the contaminated matrix in which it had germinated and grown, and that its metabolism can handle the multi-metal contamination. In plants with low TF, there is less translocation of metals to the aboveground portions. The lower translocation of metals to the above-ground portions may be due to immobilization of metals in roots by vacuole sequestration or cell wall binding, thereby preventing interaction with high-molecular-weight compounds in the plant cell cytoplasm [41]. According to Mendez and Maier [24], a plant suitable for phytostabilisation should have BCF > 1 and TF < 1. Such values were obtained in T. latifolia in the case of Mn, Cr and As at all 5 sites; Zn at 4 sites; Co, Cd and Ni at 2 sites each. Significantly, T. latifolia demonstrated limited capability in containing Pb and Cu in the root zone.

Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient [42,43]. Reduction in total chlorophyll levels in plants due to Pb, Cu, Zn, Co, Cd, Cr and Hg has been observed [44-46]. As seen from figure 1, it is clearly evident that chlorophyll content in plants growing in contaminated pits was not very different from the control values. At some sites total chlorophyll content was actually more than the control values (sites 2, 3 and 4). However, analysis of variance indicates that the difference in mean total chlorophyll content was not statistically significant (P < 0.05). These findings are in accordance with studies showing small amounts of heavy metals like Pb having a stimulatory effect on plant growth [47,48].

Accumulation of free proline in response to heavy metal exposure seems widespread among plants. Available evidences suggest that proline increases the stress tolerance of plants through such mechanisms as osmoregulation, protection of enzymes against denaturation, and stabilization of protein synthesis [49,50]. Detailed studies have shown accumulation of free proline in algal as well as plant species due to metals like Pb, Cu, Co, Cd, Zn and Hg [21,44,45,51]. However, in the present study it was observed (Figure 2) free proline levels in leaf samples of T. latifolia were not very markedly different from those at the control site as confirmed by ANOVA where no significant difference in mean free proline content among samples was obtained (P < 0.05). According to Handique and Handique [52], toxic heavy metals like Pb, Hg and Cd induce higher proline accumulation in lemongrass following short term exposure (two months after transplantation) than long term exposure (nine months after transplantation). This can be a possible explanation for the lack of significant difference in proline levels. In all probability the plants at the sites studied, being perennials had adapted themselves to the higher levels of heavy metals in the soil.

CONCLUSION

The results of the present investigation indicate that all the metals investigated showed differences in the magnitude of accumulation and distribution patterns in the sediments/sludge. Specifically, arsenic content was many times the suggested threshold in all samples. In the light of toxic and carcinogenic nature of heavy metals, the presence of such high concentrations in such densely populated areas as well as close vicinity of agricultural fields is alarming. From the pigment analysis of T. latifolia its ability to withstand
high concentrations of heavy metals in the rhizosphere is confirmed. Translocation patterns of metals in the plant indicate major partitioning in the root itself, combined with the specially low TF values indicate its suitability as a phytostabiliser for Zn, Mn, Cr and As with some potential for Co, Cd and Ni also. The species demonstrated difficulties in mobilizing Pb and Cu in the root zone and as such has limited potential for phytoremediation of these two metals. Such plants with promising capabilities under the outlined categories, their efficiencies can be further enhanced by developing transgenic plants in this specific context.

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