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Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent

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ABSTRACT

In this study, the mercury (Hg) removal capacities of two aquatic macrophytes, *Pistia stratiotes* and *Azolla pinnata*, were investigated against the coal mining effluent. These plants reduced mercury from the effluent via rhizofiltration and subsequent accumulation in plant. The removal rate of *P. stratiotes* and *A. pinnata* was 80% and 68%, respectively, after 21 days of exposure to the effluent containing 10 $\mu g\,L^{-1}$ of Hg. As mercury from the effluent was accumulated in the root and shoot tissues of both aquatic macrophytes, they were proven to be a root accumulator with a translocation factor of less than one during the entire study. The decreasing Hg content in effluent (from 10 to 2.0 $\mu g\,L^{-1}$) was reflected by its accumulation in roots ($0.57 \pm 0.02\ mg\,g^{-1}$ in *P. stratiotes*) and leaves of the experimental plants ($0.42 \pm 0.01\ mg\,g^{-1}$, *P. stratiotes*). As a result, Hg concentrations in the coal mining effluent were tightly associated with those observed from macrophytes. Considering the high removal efficiencies of Hg by these aquatic macrophytes, these plants can be recommended for the actual treatment of Hg-containing waste waters.

1. Introduction

Presence of mercury (Hg) in aquatic ecosystem is detrimental, as it is one of the most harmful heavy metals from human health perspective [1,2]. Municipal waste, fossil fuel combustion, chlor alkali plants, non ferrous metallurgy, and effluents from gold and coal mines are designated as important sources of Hg into the aquatic ecosystem [3]. In aquatic ecosystems, Hg is found in both organic and inorganic forms with two ionic states, Hg (I) and Hg (II); being soluble in water, both of them are bioavailable [4,5]. The salt $HgCl_2$, one of the common forms of inorganic mercury, has the potential to damage the gastrointestinal tract, affect sperm formation, and cause kidney failure [6]. As an organic compound, methyl mercury (CH_3Hg) is identified as the most toxic. It affects the immune system, alters genetic and enzyme systems, damages the nervous system (including coordination and the senses of touch, taste, and sight), and disrupts the endocrine system [7].

The capacity of aquatic macrophytes to absorb and accumulate heavy metals including Hg from waste water has attracted a great deal of attention from many researchers [8–11]. Several species of aquatic macrophytes have been used to remove mercury from water and wastewater. They also concluded that mercury accumulation in the roots of *Pistia stratiotes* was about four times higher than the shoots at lower concentrations and about twice as much at higher concentrations (e.g. 20 $mg\,L^{-1}$). Kamal et al. [12] demonstrated that three aquatic plants (parrot feather, creeping primrose, and water mint) removed up to 99.8% of mercury contained in multi metal solutions prepared in the laboratory in 21 days. Some transgenic plants can accrue 2–3 times more metals than non-genetically engineered species [13].

The accumulation rate of heavy metals has been tested between the roots and leaves of many aquatic macrophyte species. A few of them have been identified as bioindicators of metal pollution such as *Eichhornia crassipes* [14], *Lemna minor* [15], and *Spirodela polyrrhiza* [16,17]. Although many species of aquatic macrophytes have been utilized for the removal of several heavy metals, relatively little is known about the capability of these plants for phytoremediation.

A survey on previous phytoremediation studies indicates that a few species of aquatic macrophytes were investigated under the simulated metal solution conditions [18]. As the removal of heavy metals in industrial effluents has been dealt rather scarcely, the removal capacities of different aquatic plants need to be critically assessed to harness their ability for heavy metal removal from industrial sources. Considering the higher tolerance level of the plants towards the metal uptake, future research on this subject can offer economically feasible and ecologically viable methodologies for heavy metal removal from waste water [11].

Azolla pinnata is a small aquatic fern (1–5 cm), maintaining a symbiotic association with cyanobacteria *Anabaena azollae* Strasb

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(Nostocaceae) (Fixer of atmospheric nitrogen). *A. pinnata* inhabits the surface of eutrophic, warm, and still waters (ponds, swamps) in temperate and tropical regions. Although it is known for excellent removal capacity of heavy metals, few studies to date have specifically dealt with its Hg removal capacity [19]. *P. stratiotes* is another aquatic plant which floats on the water surface with its submersible roots (beneath floating leaves). This plant can exhibit a high accumulation capacity of heavy metals while it grows on nutrient rich medium [20]. Nonetheless, the Hg removal capacity of these plants has not been investigated previously with respect to a coal mining effluent. To extend our knowledge in the field of Hg removal by plant species, this study was designed and conducted to assess and compare the Hg removal capacities of the two important aquatic macrophytes, small water fern (*A. pinnata)* and. water lettuce (*P. stratiotes*). This work was initially pursued to help resolve the pollution problem of Bina open cast coal mine, which is one of the 10 open cast coal mines in Northern Coalfields Limited (NCL), Singrauli, India. The coalfield in the Singrauli region (between 24°37′ to 24°12′N and 81°48′ to 82°52′E) belongs to one of the largest coal-power complexes in the world (13% of coal production and 14% of power generation in India). These coal mines serve as a potential source of Hg for the surrounding aquatic ecosystem. The washing process for coal quality improvement renders the waste water to be left as the major pollution route of Hg in the surrounding aquatic ecosystem [21].

2. Materials and methods

2.1. Study area and experimental setup

In this study, two types of experimental aquatic macrophytes were selected: water lettuce (*P. stratiotes*) and small water fern (*A. pinnata*). These plants were grown in coal mining effluents for diverse purposes, i.e., for the treatment of nutrients, biochemical oxygen demand, heavy metals, etc. These aquatic macrophyte samples selected were collected from a nearby unpolluted site in Govind Ballabh Pant Sagar reservoir where Hg concentration was below detection limit [22]. Both plants (*P. stretiotes* and *A. pinnata*) were placed in a 150 L glass aquaria filled with the 100 L of coal mining effluent (containing the mercury). The initial concentration of mercury was kept at $10 \pm 0.1\ \mu g\ L^{-1}$ in all the experimental sets. A control tank (without plants) was also prepared at the same concentration level with the mining effluent.

Before the experiments, these plants were washed and rinsed thoroughly with distilled water. Plants were placed in tanks with the maximum cover, and tanks were mildly aerated twice per day. All plants were left continuously to a photoperiod of 14 (light) and 10 h (dark) with the tanks covered to minimize evaporation. Coal mining effluents for the experiments were analyzed at weekly intervals (0, 7th, 14th, 21st, and 28th days). Plant tissues (root and leaves) were also analyzed at the same intervals. The water level was maintained constant in the aquaria with the addition of distilled water, if needed. This step was needed to maintain the constant dilution factor for the experimental solutions. For the analysis of mercury, 40 mL of effluent was pipetted out of each tank into glass jars and mixed thoroughly. These samples were refrigerated at 4 °C, until the content of mercury was quantified. Sampling and analysis of the coalmine effluent and biological samples were done using Standard Methods for Examination of Water and Wastewater [22].

2.2. Mercury analysis in roots and leaves

Before the mercury analysis, the plants were separated into roots and leaves. The plant parts were washed, dried, and stored at 4 °C for 1 week. Samples were then cut into small pieces and dried to a constant mass in a fan forced oven (80 °C for 24 h). This temperature was used to sufficiently remove the moisture without thermal decomposition. The oven dried material was chopped and grounded to facilitate organic matter digestion. The fine-sized grounded material was used for the digestion in HNO_3:$HClO_4$ (5:1 v/v) at a temperature of 60 to 70 °C.

The dried plant tissues were weighed and ground to powder for the analysis of total nitrogen-N (microKjeldahl method) [23] and total phosphorus-P (wet oxidation method) [24]. Potassium was analyzed by flame photometer [25]. Chlorophyll was analyzed after being extracted in 80% chilled acetone by Arnon's method [26]. The protein content of leaf material was estimated following Lowry et al. [27]. Bovine serum albumin was used as a standard for protein estimation.

The cold vapor technique for mercury analysis (total mercury) was conducted by employing PerkinElmer MSH-10 connected to a PerkinElmer 2380 spectrophotometer. A solution of 3% $NaBH_4$ in 1% NaOH as a reducing agent was used following the procedure described in the European Standard EN 1483 [28]. In this study, all the analysis of Hg was made as the total Hg by converting all the available forms of Hg (e.g., Hg^{+} and Hg^{2+}) into elemental form. The certified reference materials were analyzed according to EN 1483 for mercury. The accuracy of the Hg analysis was evaluated with both certified reference material and matrix spiked. The results of the reference material showed that the recovery values fell in an acceptable range of between 97% and 105%. One sample and one acid blank in every batch digestion were spiked with a known amount of Hg spike standard. The analysis of spike recoveries showed that the sample digestion was complete without matrix interference. The matrix spike recovery for Hg was in the range of 98–106%, which is also in the acceptable range. Precision was established by analyzing a replicate sample in every batch digestion and was within 10% of the relative standard error. The detection limits were evaluated using the procedure recommended by the United States Environmental Protection Agency, Method 1631 [29]. To this end, seven aliquot replicates of reference matrix or the sample matrix with the analytes of interest at a concentration within one to five times of the estimated detection limit were analyzed by the same analytical procedure. The standard deviation of the seven replicates was multiplied by 3.14 (the Student's *t*-value at six degrees of freedom) to yield the method detection limit (MDL). In this study, the MDL value of Hg was $0.1\ \mu g\ L^{-1}$ (or 4 ng in absolute mass terms).

Mercury concentrations in the coal mining effluent and in plant material are expressed in $\mu g\ L^{-1}$ and $\mu g\ g^{-1}$ (dry weight), respectively, by averaging the data of three replicates, unless specified otherwise. Correlation and regression analysis were performed by using the SPSS 12 package.

3. Results and discussion

3.1. Mercury concentrations of mining effluent and removal of Hg by macrophytes

The analysis of physico-chemical properties of the mining effluent showed that those taken from Bina open cast coal mine contained high levels of total suspended solids (TSS), total dissolved solids (TDS), total-phosphorus (TP), and total-nitrogen (TN), and heavy metals (Table 1). Initial concentration of mercury was maintained at $10 \pm 0.1\ \mu g\ L^{-1}$ in all the experimental sets. Mercury concentrations in the coal mining effluent were high enough to exceed the permissible limit or in the upper bounds of the permissible limits prescribed by the central pollution control board (CPCB) of India [30]. Significant correlations existed between the pH levels and the Hg concentrations. It is important to note that

Table 1
The physico-chemical properties of coal mining effluents used in this study (prior to phytoremediation treatment).

Parameters	Values[a]
Temperature (°C)	28.5 ± 0.7
pH	8.3 ± 0.20
DO[b] (mg L^{-1})	0.87 ± 0.13
BOD[c] (mg L^{-1})	70 ± 2.5
COD[d] (mg L^{-1})	145 ± 3.5
Total suspended solids (mg L^{-1})	1098 ± 10
Total dissolved solids (mg L^{-1})	746 ± 7
Total nitrogen (mg L^{-1})	48.2 ± 4.03
Total phosphorus (mg L^{-1})	6.03 ± 0.33
Hg (mg L^{-1})	0.011 ± 0.001

[a] Mean ± SD, $n = 3$.
[b] DO: dissolved oxygen.
[c] BOD: biochemical oxygen demand.
[d] COD: chemical oxygen demand.

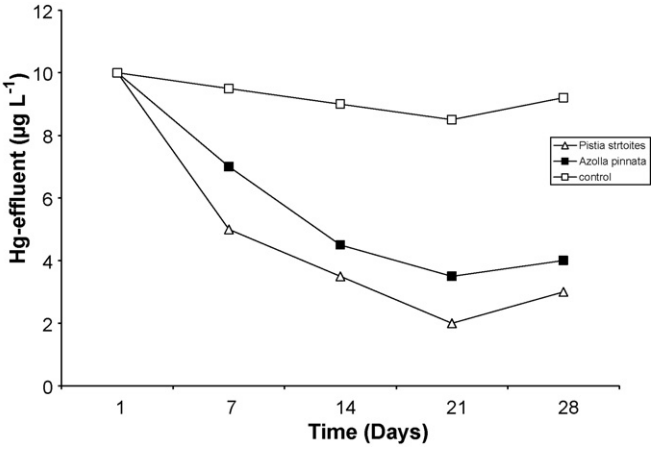

Fig. 1. Temporal changes in Hg concentrations (µg L^{-1}): (a) in the effluent, (b) in the effluent with *P. stratiotes*, and (c) in the effluent with *Azolla pinnata*.

metal concentration is normally low at high pH, although the metal precipitation increases in the form of their respective salts with increasing pH. Fig. 1 shows the results of the mining effluent experiments for the entire period (28 days). After the incubation of macrophytes in the coal mining effluent, the mercury concentration decreased gradually to a minimum value (2 ± 0.01 µg L^{-1} in *P. stretiotes*) by the 21st day of the experiment. *P. stratiotes* and *A. pinnata* removed 80% and 68% of Hg, respectively, from the coal mining effluent in 21 days period (Table 2). These removal rates can be compared with those reported from previous studies such as macrophyte, *A. pinnata* (75–83%) [31], *Vallisinaria spiralis* [32], three aquatic plants of *Myriophylhum aquaticum, Ludwigina palustris,*and *Mentha aquatic* (97–99.7%) [12], and the Indian mustard plant *Brassica juncea* (80–93%) [19]. The removal of Hg in our study was higher, if the absolute total quantities of Hg removed by the plants are compared. Because our study was performed with higher effluent volume and the experimental plant removed significant amount of Hg successfully, these factors contributed to a higher net removal rate of Hg. However, a slight increase in the mercury concentration on the 28th day of the experiment suggests a change in uptake capacity of the plants that restricted the metal bonding site to further incorporate Hg. In addition, such phenomenon might have caused the decaying of plant tissues from which metals are re-released into the effluent. Both plant species examined in this study removed much of the Hg from mining effluent within the 21 day period, regardless of differences in physiology. A greater reduction of Hg from both experimental plants (*P. stratiotes* and *A. pinnata*) may imply their enhanced metal accumulation capacity.

Table 2
Mercury removal efficiencies of *Azolla pinnata* and *P. stratiotes* against the mining effluent.

Plant types	Initial Hg content (µg L^{-1}) (Day = 0)	Final Hg content (µg L^{-1}) (Day = 21)	Removal efficiency (%)
A. pinnata	10.0	3.20	68.0
P. stratiotes	10.0	2.0	80.0
Control	10.0	6.50	7.1

3.2. Accumulation of Hg in roots and leaves of the macrophytes

An analysis of the Hg level in aquatic macrophytes at each selected experimental interval revealed that its accumulation in both plants was higher in roots than in the leaves (Table 3). In this study, the highest accumulation of Hg was recorded on the 21st day, as there were slight reductions in the last results (on the 28th day analysis) for the plant tissues. This may be due to the decay of plants and the re-release of metals into the effluent. The highest accumulation of Hg was observed on the 21st day of analysis (0.57 ± 0.02 mg g^{-1}) in the root of *P. stratiotes.* This result was slightly higher than that of *A. pinnata* (0.45 ± 0.01 mg g^{-1}) (Table 3). In this study, there was a strong and inverse correlation between the Hg concentration (plant parts) and in the mining effluent (Fig. 2, $p < 0.001$). The higher removal of Hg by experimental plants can be associated with a number of factors such as their fast growth, high biomass accumulation, high bioaccumulation capacity, and high affinity towards uptake. Variations in the metal uptake may be explained partly by the difference in the plant growth rate and in the efficiency towards metal absorption. Plants growing in the natural conditions might have developed some types of regulatory mechanism for an adaptation which allowed their growth at exceptionally higher concentrations. This factor may also play an important role in efficient removal and accumulation of Hg from coal mining effluents.

In this study, roots served as the only exchange surface for Hg, as roots alone were in contact with effluent. These root systems can oxidize the rhizosphere which increases availability of metal (and hence the removal of metals). The rhizosphere is a region approximately 1 mm around the root. Both a large root system and an increased number of fine roots can oxidize the rhizosphere to a great extent with the increasing metal uptake [33]. Rhizosphere remediation may be the result of active processes mediated by plants and/or microbes. Pollutants can be phytostabilized simply via erosion prevention and hydraulic control as described above. Hence, plant species growing on metal contaminated water should have restricted the translocation of metals into the aerial parts [14]. The higher accumulation of mercury in the roots may be due to the abundance of anionic sites in the cell wall [34]. This fact makes the roots the primary exposure site to toxic metals present in the surrounding medium. Note that there is also a passive adsorption of pollutants to the plant surface. Low metal accumulation in leaves (relative to root) can be explained partially as the protective mechanism to maintain photosynthesis and to provide tolerance to the plants even at excessively high concentrations of metals [35,36].

An inverse correlation was significant between the mercury concentration (in root and in leaf) and coalmine effluents (Fig. 2). The variation of Hg concentration was seen evident in both coal mining effluents and the plant parts (roots and leaves) as a function of time (Fig. 2). This model confirms a significant removal of mercury ($p < 0.001$) from the coalmine effluent, while accumulation increases in the root and leaves of macrophytes through time.

3.3. Effects of Hg accumulation on nutrients in macrophytes

In Table 3, TN content in roots of *P. stretiotes* decreased from 39.6 ± 1.90 (Day 0) to 25.4 ± 0.42 mg g^{-1} (Day 21). Accumulation

Table 3
Accumulation pattern of Hg (mg L^{-1}) and the related variation in N, P, and K (mg g^{-1} dry weight each) of *A. pinnata* and *P. stratiotes* grown in coal mining effluent through time[a].

Plant Time	Element	Plant parts[b]	Time period				
			Initial	7th day	14th day	21st day	28th day
Pistia stretiotes	Hg	R	BDL	0.41 ± 0.02	0.52 ± 0.02	0.57 ± 0.02	0.55 ± 0.02
		L	BDL	0.30 ± 0.01	0.36 ± 0.01	0.42 ± 0.01	0.40 ± 0.01
	N	R	39.5 ± 1.9	35.3 ± 12	30.6 ± 1.2	29.7 ± 0.7	25.4 ± 0.42
		L	38.2 ± 1.7	30.6 ± 1.5	27.2 ± 0.8	25.4 ± 1.8	22.3 ± 1.0
	P	R	4.64 ± 0.5	4.10 ± 0.4	3.9 ± 0.1	3.5 ± 0.4	2.8 ± 0.4
		L	4.8 ± 0.2	4.5 ± 0.4	3.9 ± 04	3.4 ± 0.2	2.4 ± 0.1
	K	R	48.4 ± 2.1	39.6 ± 0.8	34.6 ± 0.2	32.4 ± 0.2	26.2 ± 0.3
		L	41.4 ± 1.8	34.4 ± 1.1	32.4 ± 0.9	31.7 ± 0.9	29.6 ± 0.6
Azolla pinnata	Hg	R	BDL	0.34 ± 0.01	0.42 ± 0.01	0.45 ± 0.01	0.44 ± 0.01
		L	BDL	0.24 ± 0.01	0.27 ± 0.01	0.30 ± 0.01	0.33 ± 0.01
	N	R	36.3 ± 1.3	34.3 ± 0.9	30.6 ± 1.0	29.2 ± 1.2	25.2 ± 0.8
		L	34.4 ± 1.2	31.4 ± 1.3	27.6 ± 0.7	25.7 ± 0.8	23.4 ± 0.8
	P	R	4.8 ± 0.3	4.2 ± 0.3	3.9 ± 0.1	3.5 ± 0.1	3.1 ± 0.2
		L	4.1 ± 0.2	3.7 ± 0.3	3.4 ± 0.4	2.9 ± 0.3	2.4 ± 0.2
	K	R	45.5 ± 2.7	40.4 ± 2.0	35.4 ± 1.9	30.2 ± 1.1	26.5 ± 0.5
		L	43.4 ± 2.1	39.8 ± 2.4	35.8 ± 2.0	31.4 ± 0.9	28.4 ± 1.7

[a] All values in mean ± SD, $n = 3$.
[b] R = root, L = leaves.

of mercury in the plant tissues appeared to suppress the plant concentrations of N, P and K. Because the highest concentration of Hg was observed in the roots and leaves of the plant on the 21st day, such effects were reflected by the reduced concentrations of N, P, and K. The minimum values for N, P, and K were however observed on the 28th day, due to prolonged stress caused by Hg accumulation over an extended period. Root samples of *A. pinnata* also experienced a reduction in TN from 36.3 ± 1.3 (Day 0) to 25.2 ± 0.80 mg g^{-1} (Day 21), while its content in leaf samples decreased from 34.4 ± 1.2 (Day 0) to 23.4 ± 0.8 mg g^{-1}(Day 21). Leaves of *P. stretiotes* also showed a similar trend in TN data: 38.2 ± 1.7 (Day 0) to 22.3 ± 1.0 mg g^{-1} (Day 21). It is interesting to note that Hg concentrations in all samples resumed a slight increase at the end.

Analysis of chlorophyll and protein content in the leaves of the experimental plants exhibited a sharp decrease on the 21st day of analysis. Such a reduction was more significant in *A. pinnata* (0.76 ± 0.04 to 0.38 ± 0.04 mg g^{-1} fresh weight) than in *P. stratiotes* (0.69 ± 0.03 to 0.41 ± 0.05 mg g^{-1} fresh weight) (Table 4). A constant decrease in chlorophyll and protein content was probably due to the stress caused by a higher concentration of Hg. A high mercury concentration in plants has been reported to reduce chlorophyll by inhibiting biosynthesis [37]. Strong inverse correlations were apparent between chlorophyll (and protein content) in the foliage

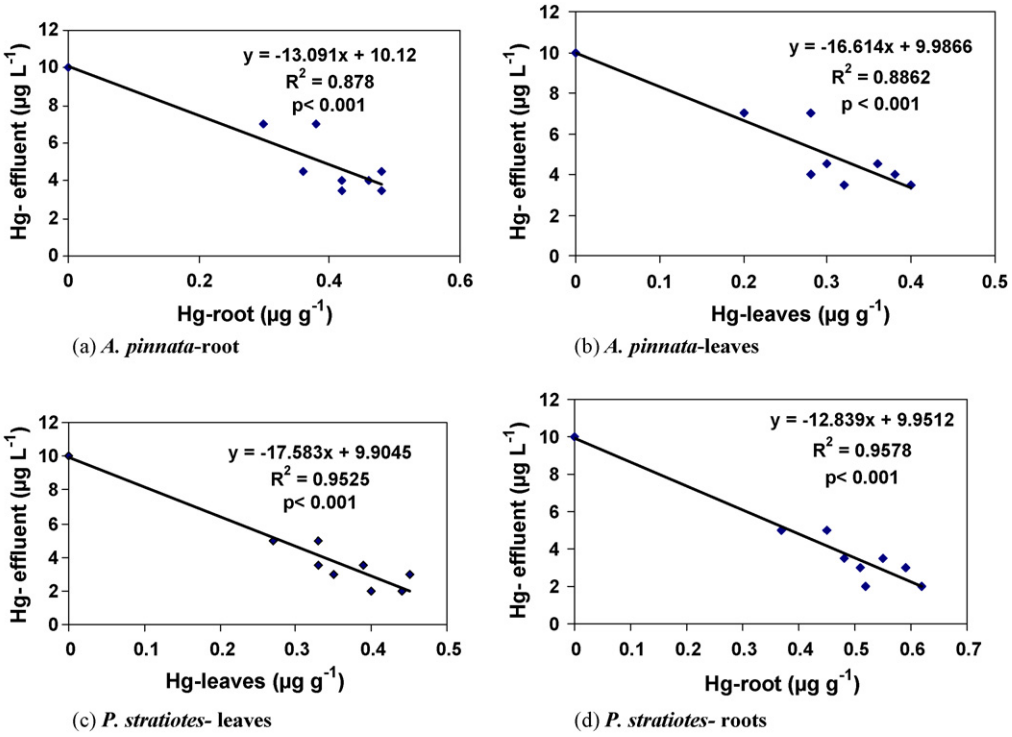

Fig. 2. Results of linear regression analyses between mercury in mining effluent (µg L^{-1}) and roots and leaves (µg g^{-1} dry weight) of macrophytes grown in a coal mine effluent. (a) Between mercury in mining effluent and *A. pinnata*-roots. (b) Between mercury in mining effluent *and A. pinnata*-leaves. (c) Between mercury in mining effluent and *P. stratiotes*-roots. (d) Between mercury in mining effluent *and P. stratiotes*-leaves.

Table 4
Changes in chlorophyll (mg g^{-1}), protein (mg g^{-1}) content, and translocation factors for *A. pinnat*a and *P. stratiotes* tested against a coal mining effluent[a].

Macrophytes		Initial	7th day	14th day	21st Day	28th day
A. pinnata	Chlorophyll	0.76 ± 0.04	0.65 ± 0.03	0.60 ± 0.02	0.49 ± 0.03	0.38 ± 0.04
	Protein	5.92 ± 0.2	4.94 ± 0.1	4.10 ± 0.4	3.52 ± 0.4	3.23 ± 0.3
P. stratiotes	Chlorophyll	0.69 ± 0.03	0.61 ± 0.02	0.54 ± 0.04	0.49 ± 0.05	0.41 ± 0.05
	Protein	6.0 ± 0.3	5.50 ± 0.2	4.93 ± 0.3	4.41 ± 0.2	3.70 ± 0.40

[a] Mean ± SD, $n = 3$.

Table 5
Translocation factor for *A. pinnat*a and *P. stratiotes* grown in a coal mining effluent during 28 days phytoremediation experiment.

Macrophytes	Initial	7th day	14th day	21st Day	28th day
A. pinnata	–	0.70 ± 0.03	0.64 ± 0.02	0.66 ± 0.02	0.63 ± 0.02
P. stratiotes	–	0.73 ± 0.03	0.69 ± 0.02	0.74 ± 0.03	0.72 ± 0.03

and the Hg in mining effluent [10]. The results of our study showed that the chlorophyll and protein content decreased in plant tissues along with the accumulation of mercury in the selected macrophytes. This finding is in a good agreement with those of Gupta and Chandra [32].

Roots are prime sites for accumulation of metals. Metals such as Hg are thought to be attached in an anionic site in the cell wall of plants [36]. They can be the effective traps for immobilizing metals from the contaminated waters. Mercury accumulation in the tissues of experimental plants reached a maximum level after 21 days; after that, it showed a slight decrease, as indicated by Hg concentrations in plant tissues at the end of experiment (28th day). This may be due to the saturation of binding sites in the plant cell wall or a breakdown of the metal regulatory mechanism. This observation suggests that capacity of the plants to retain metal reaches its full limit under the prevailing experimental conditions. Thus, we can suggest that the use of test plants made the best performance in 21 days and that this duration can be recommended as the optimum period for phytoremediation.

3.4. Translocation factor

Tissue concentration has been used as a criterion for identifying hyperaccumulators; however, the translocation factor may give a better idea regarding the metal accumulation capacity of the plants. The translocation factor, defined as metal concentration ratio between shoot and root, indicates relative distribution of absorbed metals in aquatic plants [38]. In this study, the translocation factors were calculated on the 7th, 14th, 21st and 28th days of the experiment for both plants (Table 5). The results indicated consistently that a larger proportion of total accumulated Hg was dominantly retained in roots, as shown by translocation factor value less than unity. The mean translocation factors for *A. pinnata* and *P. stretiotes* were 0.63 and 0.72, respectively. These values are comparable to those previously reported by others [11,38]. The translocation factor can be a major criterion in the judgment of plants for phytoremediation. Plants with a higher translocation factor can be considered as a better candidate for phytoremediation [11]. In this study, *P. stretiotes* with a higher translocation factor removed and accumulated Hg more effectively than *A. pinnata.*

4. Conclusion

In this work, two types of experimental aquatic macrophytes, the water lettuce (*P. stratiotes*) and the small water fern (*A. pinnata)* have been examined for their Hg removal efficiency from coal mining effluents. Both plants were evaluated as a new candidate for phytoremediation of Hg from coal mining effluents. Maximum removal of the Hg was recorded on the 21st day of retention time from both plants; thereafter, the removal capacity decreased moderately. As seen from many previous studies, accumulation of metals in both plants occurred more dominantly in roots (than leaves). Such a high accumulation of mercury however appears to counteract N, P, K, chlorophyll, and the protein contents in the plants, as most of them decrease proportionally with increasing accumulation of Hg. Both aquatic macrophytes investigated in this study were demonstrated to have a great potential to restrict the evasion of effluent Hg to other environmental reservoirs. *P. stratiotes* was a better accumulator of mercury, with higher removal efficiencies. In addition, it was affected less significantly by mercury toxicity, as evidenced by a low decrease in chlorophyll and protein content at the end of the full exposure. Among the two plants studied here, *P. stratiotes* showed the enhanced translocation factor relative to the other. However, both plant species can be utilized for large scale removal of Hg from waste water. Once the mercury entered in the plants, these plants can be used for biogas production, compost production, and solid waste amendments.

Most of the previous phytoremediation studies related to Hg removal from waste water were commonly conducted within a relatively small range of volumes for metal solutions with large plant biomasses. Our study was performed by simulating the real environmental conditions for coal mining effluents with reasonable quantities of biomass, as compared to other workers. However, further studies are required to establish and optimize this methodology for the practical treatment of Hg released into the aquatic ecosystem from the various pollution sources. It is also important to assess the long term feasibility of the methodology along with the identification and selection of new plant species for Hg removal.

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