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Efficiency of Duckweed (*Lemna minor* L.) in Phytotreatment of Tannery Sludge

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ABSTRACT

Lemna minor L. (duckweed) is a free floating small, rapidly growing aquatic plant. It can adjust very easily to several types of aquatic conditions and hence play a central role in extraction and bioaccumulation of toxic pollutants from waters. In the present investigation Duckweed (*Lemna minor* L.), was exposed to different concentrations of tannery sludge i.e., 0, 30 and 60% TS to assess the tolerance and heavy metals uptake. Further, the transfer of these metals from roots to other plant parts was also measured. Results indicate that higher concentration of metals was found in the shoots as compared to the roots. The transfer factor was also greater than 1.0. The enrichment coefficients of the leaves of *L. minor* were found to be lower than 1.0 for all the metals. This study confirmed that *L. minor* could be established as not only as bio-indicator but a bio-accumulator for different sediments and waste water polluted by metals.

Key Words: Bio-indicator, heavy metal pollution, phytoremediation, tannery sludge.

INTRODUCTION

Water contamination with heavy metals is a very important problem in the contemporary world nowadays. There are two important causes of environmental pollution in Pakistan i.e., unhealthy effects of unplanned industrial development and accompanied urbanization. The environmental contamination with heavy metals has become a serious worldwide problem that affects crop overall yields, soil biomass and fertility, and ultimately leads to bioaccumulation of heavy metals in the food chain (Rajkumar *et al*., 2009). Further, leaching or seepage of these heavy metals like Cr, Pb, Cd, Hg, Fe, As, Ni, etc. in the surrounding ecosystem is considered as the major cause of severe pollution (Pandey *et al*., 2009, 2011) and in that way pose a menace to the ultimate receiving habitat.

The disposal of sludge is a source of potentially elements of toxic nature and its removal is considered as a major challenge due to a high metal content. Due to the result of wastewater treatment, sludge production is increasing day-by-day at alarming rate. The nature of toxic sludge that generated from various industries is primarily dependent on the provided raw material used in these industries. The sludge is generally very bulky with high moisture content and its composition may range from highly organic to mineral depending upon its origin. It can be used as fertilizer, provided it is free from toxic metals. Global production of paper-mill sludge was predicted to rise over the next 50 years by between 48 and 86% over the current levels (Mabee & Roy, 2003).

Aquatic plants are well known for accumulation and concentration of a great amount of various substances; among them are the metals, which they up take from the environment and concentrate in the trophic chains. Some aquatic plants in addition have the capability to accumulate the heavy metals with unknown biological function. Among these are the macrophytes greatly capable of accumulating significant amount of different species of metals like Pb, Cd, Hg, Cr, Ar, Cu, Fe, Ni and Zn from variously contaminated waste waters (Upadhyay *et al*., 2007; Horvat *et al*., 2007; Hou *et al*., 2007; Rai and Tripathi, 2009; Rai, 2010; Prasad & Singh, 2011; Rahman & Hasegawa, 2011).

L. minor L. (duckweed) is a free floating, small and rapidly growing aquatic plant, it can adapt very easily to various aquatic conditions. From the literature it shows that *L. minor* play a central role in extraction and bioaccumulation of various pollutants from waste waters (Kaur *et al*., 2010). In particular, different species of *L. minor* are found to accumulate several noxious metals and hence are being considered as experimental model systems to explore heavy metal mediated responses, Bio-availability and bioaccumulation of several heavy metals in aquatic as well as wetland ecosystems is now a day gaining remarkable significance all over the world. In this study, *L. minor* has been used to decontaminate the tannery sludge along with some indigenous microbes (bacteria and fungi) in associated phytoremediation.

MATERIALS AND METHODS

Sludge samples were collected from the sludge lagoon, Kasur Tannery Waste Management

Author's Contribution: S.A.K., Designed, Planned and supervised research work; **F.B**., Performed experimental work, Analyzed data statistically and wrote manuscript; ***Corresponding author: shezakhilji@gmail.com***

Agency (KTWMA) having the primary treatment plant in Kasur, Pakistan. Samples of sludge were collected at depth of approximately 15 cm in clean plastic drums, labeled properly and transported then to the Environmental Biotechnology lab, Botany Department, University of the Punjab, Lahore. The samples were stored in the laboratory in cold rooms at a temperature of 4°C.

L. minor was collected from different ponds and logged areas in the industrial zones of the Kasur and the Sheikhupura Road. The geographical position of the sampling site was (N 31°41.868', E 074°02.038' at an elevation of 666ft).

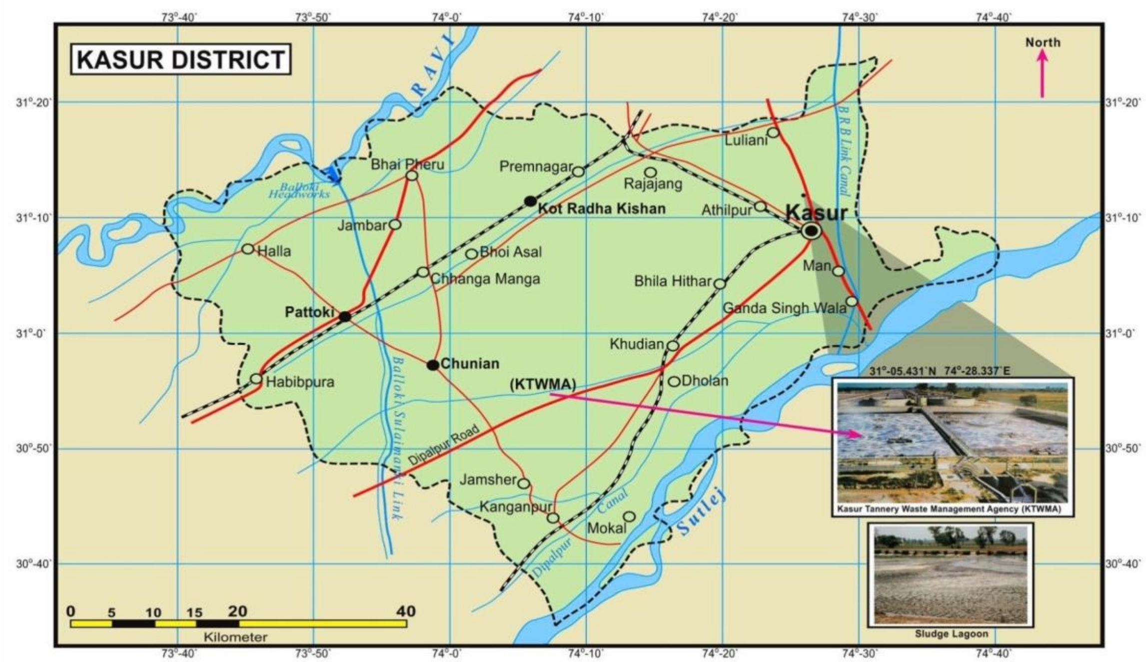

Fig., 1: The map of the Kasur District, showing location of the sampling site.

Nurseries of the transported plants were raised in a green-house in the Department of Botany, University of the Punjab, Lahore Pakistan and maintained in lagoons. The following resistant fungal and bacterial species isolated from the sludge were used for inoculation in experiment namely; *Aspergillus niger* (F1), *Aspergillus terreus* (F2), *Bacillus* sp. (B1) and *Acinetobacter* sp. (B2). They were grown in conical flasks (500 ml) containing potato dextrose broth next for 8 days. The cultures were later on filtered through filter paper (Whatman No. 1) and the mycelial mat was carefully macerated with the help of a Waring blender for 1 min and mixed with 250 ml of 0.1M $MgSO_4.7H_2O$ solution. About 8-10ml of this inoculum containing $5x10^4$ c.f.u./ml was used for inoculating experimental pots. Inoculum was given at least twice in a week after 5-7 days of germination of plants.

The fresh semi-solid tannery sludge was used in the experiments. Different concentrations of sludge were prepared in plastic pots by homogenously sludge mixing with tap water. The actual concentrations selected for the final pot experiments were 0 (control), 30 and 60% sludge because in preliminary experiments it was shown that plants cannot tolerate beyond 60% of sludge concentration. Five plants of same age and uniform size and biomass were grown in each treatment, along with microbial treatment. The experiment was set up in a wire house having a glass roof in the Department of Botany in a "complete randomized design" (Steel *et al*., 1997). The level of sludge mixture in the pots was maintained with tap water for alternate days. Plants and sludge was analyzed at 30-days intervals.

For the analysis of metals, the sample were analyzed on Flame photometer (PFP7, JENWAY, UK) for essential metals while determination of heavy metals Cu, Cr, Cd, Pb, Mg, Ni, Fe and Zn was done Spectrophotometerically (Atomic Absorption, GBC SAVANT AA, Australia). The fully air dried plants after harvest were carefully kept in hot air oven at 80 ◦C for 24 h until a stable mass was noted. The dried plant (roots and shoots) were ground in to a powder in an electric grinder. Acid digestion of this powdered plant material with HNO_3 and $HClO_4$ (1:4) was performed in a digestion and mineralization apparatus (TMD 10, Velp, Italy). The digested samples were subjected to metal analysis by atomic absorption spectrophotometry.

Translocation factor (TF) and Enrichment coefficients (EC) were estimated because both can be employed to determine a plant's probable for phytoremediation purpose (Yoon, 2006). TF and EC are measured when investigating whether a plant is a hyperaccumulator of a metal (Gonzaga *et al*., 2006). In general, Translocation factor was calculated as the ratio of heavy metals in plant shoot to that in plant root (Zhao *et al*., 2002). Enrichment coefficient was determined as the heavy metal element concentration in a plant part above ground divided by this heavy metal concentration in below ground parts (Zhao *et al*., 2003).

Statistical analyses

Univariate analysis was applied to the data for the interpretation of results. Duncan's multiple range test (DMRT) was employed to determine the significance level of the observed means for selected attributes by using SPSS (Version 20.0.0).

RESULTS

The essential metal (Ca, K, Na, and Mg) bioaccumulation by *L. minor* shoot and root after 90 days of growth are presented in Table 1. It was noticed that efficiency of metal accumulation was different in different microbial treatments and in various sludge concentrations. The amount of Ca uptake in shoot was increased as the concentration of tannery sludge increased from 0-60%. The maximum uptake was recorded in combined fungal and bacterial (F1+F2 and B1+B2) treatment than the control and other treatments (F1, F2, B1 and B2). The amount of Na uptake was also highest in 60% concentration of sludge as compared rest of the concentrations. The maximum amount (1,912 mg kg^{-1}) of Na uptake in 60% concentration of TS was also recorded in F1+F2 treatment as compared to other fungal and bacterial treatments at day 90 of plant growth. However, at same concentration of sludge, the minimum uptake of Na (1,823 mg kg^{-1}) was recorded in control treatments. Essential metals in the different concentrations were in the order of Ca> K> Na> Mg while the amounts of heavy metals like Cd, Cu, Cr, Pb and Zn increased in accordance with the increase in the concentration of TS. The amount of heavy metals was considerably higher in 100% as compared to 60, 30 and 0%.

The heavy metal extraction efficiency of *L. minor* from TS concentrations is given in the Table II. The Cr uptake was found to be the maximum in almost all the treatments in 60% TS while the control showed the minimum accumulation. After Cr,

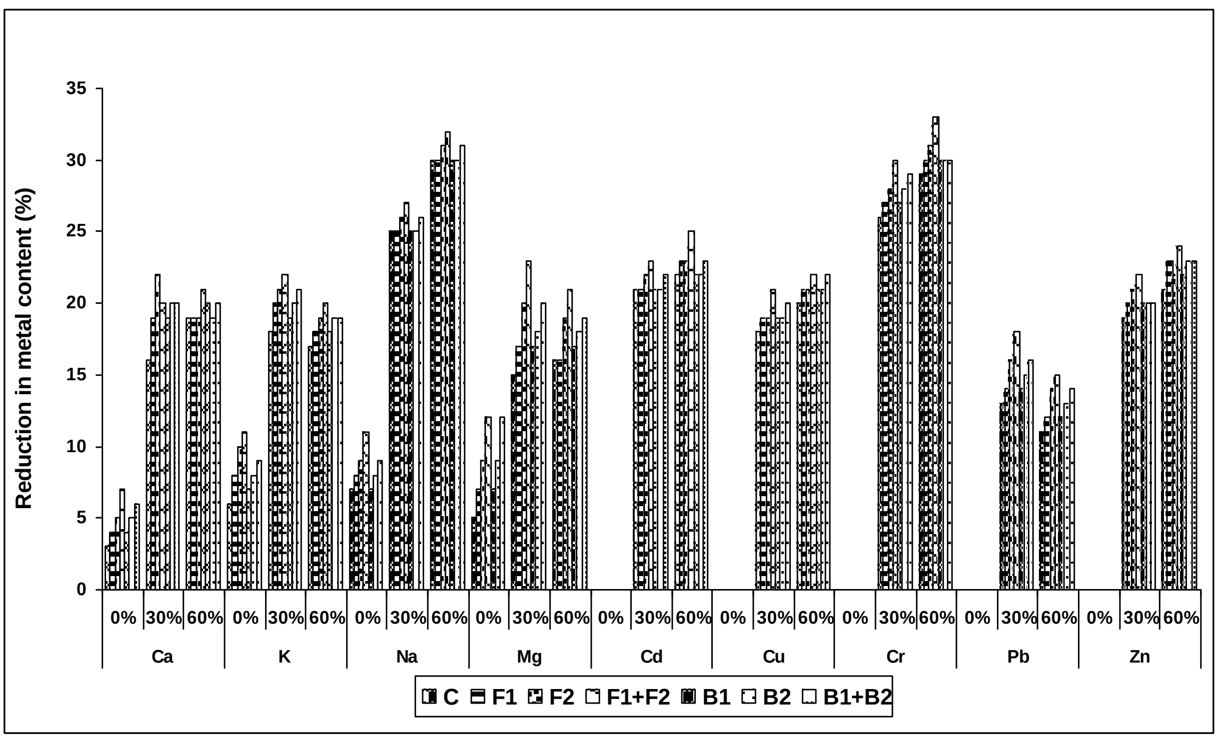

Fig., 2: Percentage reduction in metal content of different TS concentrations after growing *L. minor* treated with different microbial inocula.

Cd uptake was found to be high in shoots of *L. minor* in F1+F2 and B1+B2 treatment as compared to the roots. Pb accumulation in shoots of *Lemna* was significantly less as compared to other heavy metals.

In 30% TS after 90 days, the amount of Cr bioaccumulation was 3,625 mg kg^{-1} and 3,480 mg kg^{-1} of dry weight of shoots in F1+F2 and B1+B2 treatment while in roots, it was 1,378 mg kg^{-1} and 1,370 mg kg^{-1} of dry weight of shoots in after 90 days. In 60% TS, the accumulation of Cr (4,565 mg kg^{-1}), Cd (2,455 mg kg^{-1}) and Zn (2,250 mg kg^{-1}) dry weight of shoots respectively. The amount of Cu (1,185 mg kg^{-1}) and Pb (545 mg kg^{-1}) was comparatively less in shoots and roots as compared to the other metals.

Translocation factor and Enrichment coefficients in metals of 90 days old plant of *L. minor* grown in different concentration of tannery sludge (TS) are shown in the Tables 3 and 4. Translocation Factor (TF) is used to evaluate the effectiveness of *L. minor* and fungal and bacterial inocula in enhancing the capacity of plants to transfer metals from roots to shoots. A marked improvement in the translocation of Cr, Cd, Zn, Cu and Pb from the roots of plants in the sludge was observed by the application of microbial treatment. The TF changed between 1.88 and 2.43. The TFs for the studied elements were generally higher than 1.0 in all the metals. This indicates that *L. minor* accumulates more metals in the shoots than in the roots which has been reported for hyper-accumulators. So, *L. minor* proved to be a metal accumulator species. The bioaccumulation of metals in the plant was in the order of Cu> Zn> Cd> Pb> Cr. Enrichment coefficients of all plants to metals were lower than 1 and was highest in Zn (0.17). The percentage reduction observed in different concentrations of tannery sludge after growing *L. minor* for 90 days is shown graphically in the Fig., 1. The percentage reduction was in the order of Cr> Cd> Zn> Cu> Pb. while the minimum was observed for all metals in control treatment.

DISCUSSION

Industrial development in big cities have direct the way to the detection and increasing understanding of interrelationship between pollution, public health and environment. Rapid industrial development is the major reason of production of industrial effluents and if these are untreated results in water and soil pollution (Fakayode and Onianwa, 2002; Fakayode, 2005). Industrial wastes and emission contain hazardous and toxic substances, most of which are very lethal to human health (Jimena *et al*., 2008; Ogunfowokan *et al*., 2005; Rajaram *et al.*, 2008).

Aquatic plant species including free-floating *Eichhornia*, *Lemna*, *Azolla*, *Salvinia*; submerged *Potamogeton*, *Myriophyllum*, and emergent *Typha*, *Spartina* have shown potential for metal removal from wastewater (Lesage *et al*., 2007; Dhir *et al*., 2009). In the current study, the elemental assay of heavy metals showed that the values of metal bioaccumulation were higher in shoots than roots. According to Dhir *et al*., 2009 and Vymazal *et al*., 2009, aquatic plant biomass represents an abundant biological resource that possesses an immense capacity to accumulate heavy metals and therefore have been exploited world-wide for developing environment-friendly wastewater treatment technologies for removing heavy metals. After Cr, Cd uptake was found to be high in shoots of *L. minor* in combined treatment as compared to the roots. The amount was almost reduced to two third in roots. In agreement with the present research, Shaker *et al*. (2008) and Espinoza-Quinones *et al*. (2005) related it to a plant's ability to absorb abundant metals and accumulated in their tissues. Khellaf & Zerdaoui (2009) have proven through a laboratory experiment the capacity of *L. minor* to tolerant high concentrations of Cu, Cd and Zn. The results of our study in line with the findings of previous studies in terms of the capacity of microphytes on the accumulation of heavy metals and used it as phytoremediator and monitors of heavy metals pollution such as Aziz (2004), Mahmood (2008) and Hanaf (2009). *L. minor* showed a good hyperaccumulation of all the metals, especially important of which was the heavy metal Cr. It showed a significant uptake of all heavy metals and there was quite a good translocation into aerial parts as well.

The contemporary literature shows that several microbes like bacteria, fungi, algae can be used to remove heavy metal pollutants as well as industrial agricultural wastes. It has also been reported that several potential microbes for metal biosorbents may be present in the environment. These consist of several genera of *Bacillus, Streptomyces, Pseudomonas, Aspergillus, Penicilium* and *Rhizopus* (Vijayaraghavan & Yun, 2008). In the current research, four heavy metal resistant microbes were isolated from sludge that was collected from tanning industry i.e., *Aspergillus niger*, *Aspergillus terreus, Bacillus* sp. and *Acinetobacter* sp. All plant species showed the maximum uptake of heavy metals when inoculated with the two fungi in combination as compared to bacterial strains. Among the fungal groups, *Aspergillus* spp. is the most frequently studied and applied in agriculture waste recycling and the biomass energy industry (Gawande & Kamat, 2000). A strain of *Aspergillus terreus* has been shown to take up Cr, Ni and Fe from metallurgical effluents (Dias *et al*., 2002). The bacterial strains also showed significant metal uptake in both tannery and paper sludge. This is in line with the findings of Boswell *et al*. (2001) and Francisco *et al*. (2002) showing that *Acinetobacter* strains play an important role in the removal of heavy metals. According to Idris *et al*. (2004), among herbaceous flora, shoot endophytes of *Thlaspi goesingense* were established to be more tolerant to high Ni concentration than the corresponding rhizosophoric bacteria. Similarly, endophytic bacteria of *Nicotiana tabacum* could reduce Cd phytotoxicity (Mastretta *et al*., 2009).

The present results revealed that in *L. minor*, Translocation Factor was found to be generally higher than 1.0 for all the studied metals. This conforms the findings of Zu *et al*. (2004) who reported that TF value greater than 1.0 were estimated in the metal accumulator species on the other hand TF was in general lower than 1.0 in the metal excluder species. Similar results have been reported by Zhao *et al*. (2002), that TF greater than 1.0 indicates an efficient ability to heavy metal transport from the root to leaf, it is likely due to an efficient metal transporter systems. Further it is probably sequestration of heavy metals in leaf vacuoles as well as in apoplast. The activation of a low metal concentrations affinity transport systems at higher soil metal concentrations. Similarly, the decrease enrichment coefficients was found in all above studied hydrophytes as reported by Zhao *et al.* (2003) who explained that this decrease in enrichment coefficients might be due to high saturation of metal uptake and/or root to shoot transport when internal concentrations of metal were higher.

Generally, sludge is disposed off in nearby landfills, oceans through dumping, incineration and/or solidification. In most of the developing countries, sludge dumping of in landfills and ocean are under growing pressure because of environmental consideration. When the sludge is incinerated, the toxic gases and soluble chemicals are emitted which can cause serious environmental problems like air, soil and water pollution accompanied by high cost. One promising long-term solution appears to be recycling and using this sludge for beneficial purposes by removing hazardous components. The present investigation emphasizes that industrial sludge can be used to grow hydrophytes in treatment lagoons. This can make the sludge non-toxic for application in the field as manure.

Table I: Amount of essential metals in the shoots and roots of 90 days old plants of *Lemna minor* grown in different concentrations of tannery sludge.

Parameters	Conc. of TS	Plant parts	Amount of metal (mg kg^{-1} DW)						
			Treatments						
			C	F1	F2	F1+F2	B1	B2	B1+B2
Ca	0%	Shoots	20^{cE} ±0.20	25^{cD} ±0.33	32^{cBC} ±0.15	40^{cA} ±0.28	26^{cD} ±0.30	30^{cC} ±0.18	35^{cB} ±0.29
		Roots	9^{cE} ±0.11	12^{cD} ±0.15	18^{cBC} ±0.19	27^{cA} ±0.15	10^{cE} ±0.22	15^{cC} ±0.25	20^{cB} ±0.20
	30%	Shoots	423^{bF} ±1.28	450^{bD} ±2.40	512^{bA} ±2.00	454^{bD} ±3.29	434^{bE} ±2.06	460^{bC} ±1.28	472^{bB} ±1.00
		Roots	115^{bE} ±2.24	177^{bCD} ±2.06	191^{bB} ±2.40	206^{bA} ±2.21	172^{bD} ±1.33	181^{bC} ±0.40	193^{bB} ±1.22
	60%	Shoots	938^{aE} ±5.30	952^{aD} ±1.40	970^{aC} ±4.24	1,055^{aA} ±0.36	949^{aD} ±4.00	965^{aCD} ±5.24	980^{aB} ±2.00
		Roots	349^{aD} ±2.21	330^{aE} ±1.30	358^{aC} ±2.15	385^{aB} ±3.28	422^{aA} ±0.33	334^{aE} ±3.24	412^{aAB} ±2.33
K	0%	Shoots	32^{cD} ±0.30	40^{cC} ±0.40	45^{cB} ±0.24	52^{cA} ±0.36	35^{cD} ±0.33	41^{cC} ±0.24	45^{cB} ±0.30
		Roots	18^{cDE} ±0.21	25^{cC} ±0.30	32^{cA} ±0.15	37^{cA} ±0.28	20^{cD} ±0.33	24^{cBC} ±0.24	29^{cB} ±0.33
	30%	Shoots	312^{bG} ±1.19	337^{bE} ±2.28	350^{bC} ±1.33	374^{bA} ±2.20	328^{bF} ±1.24	345^{bD} ±2.36	360^{bB} ±3.21
		Roots	150^{bE} ±1.24	167^{bD} ±0.15	177^{bC} ±1.36	195^{bA} ±2.40	165^{bD} ±4.25	172^{bC} ±4.12	180^{bB} ±2.15
	60%	Shoots	523^{aEF} ±2.28	547^{aD} ±3.00	580^{aB} ±1.21	591^{aA} ±2.25	530^{aE} ±3.11	545^{aD} ±2.15	562^{aC} ±2.09
		Roots	232^{aD} ±4.30	245^{aBC} ±1.24	250^{aB} ±2.33	279^{aA} ±1.28	233^{aD} ±2.15	256^{aB} ±2.40	274^{aA} ±1.10
Na	0%	Shoots	42^{aDE} ±0.24	48^{cD} ±0.40	55^{aB} ±0.15	67^{cA} ±0.21	45^{cCD} ±0.15	50^{cC} ±0.28	55^{cB} ±0.21
		Roots	28^{cEF} ±0.13	35^{cD} ±0.15	40^{cBC} ±0.21	47^{cA} ±0.13	33^{cE} ±0.16	38^{cD} ±0.20	42^{cB} ±0.15
	30%	Shoots	1,010^{bE} ±4.36	1,036^{bD} ±2.00	1,056^{bB} ±6.45	1,110^{bA} ±3.00	1,032^{bD} ±5.36	1,045^{bC} ±5.21	1,056^{bB} ±2.28
		Roots	677^{bE} ±1.30	685^{bD} ±0.37	692^{bC} ±2.21	750^{bA} ±5.40	680^{bD} ±4.36	686^{bD} ±3.33	720^{bB} ±2.10
	60%	Shoots	1,823^{aE} ±4.24	1,834^{aD} ±2.06	1,872^{aB} ±4.45	1,912^{aA} ±2.28	1,830^{aD} ±4.36	1,853^{aC} ±1.40	1,871^{aB} ±2.29
		Roots	633^{aF} ±1.42	665^{aDE} ±2.21	690^{aB} ±4.36	750^{aA} ±3.42	644^{aE} ±5.30	670^{aD} ±3.24	682^{aBC} ±5.35
Mg	0%	Shoots	18^{cD} ±0.28	22^{cC} ±0.30	28^{cB} ±0.24	35^{cA} ±0.12	23^{cC} ±0.22	28^{cB} ±0.21	36^{cA} ±0.13
		Roots	10^{cE} ±0.29	14^{cD} ±0.13	20^{cBC} ±0.17	28^{cA} ±0.11	15^{cD} ±0.20	18^{cC} ±0.22	25^{cAB} ±0.29
	30%	Shoots	202^{bF} ±1.13	230^{bE} ±1.08	256^{bC} ±0.11	277^{bA} ±2.00	238^{bE} ±0.12	245^{bD} ±3.00	267^{bB} ±1.20
		Roots	70^{bDE} ±0.24	85^{bC} ±0.12	102^{bB} ±0.21	146^{bA} ±0.25	77^{bD} ±0.16	85^{bC} ±0.27	100^{bBC} ±0.13
	60%	Shoots	400^{aE} ±2.37	410^{aD} ±1.24	436^{aB} ±1.00	450^{aA} ±0.29	425^{aD} ±2.00	428^{aC} ±0.11	440^{aB} ±1.36
		Roots	168^{aE} ±2.19	174^{aD} ±1.26	200^{aB} ±2.12	245^{aA} ±1.00	177^{aD} ±1.05	185^{aC} ±2.00	197^{aBC} ±1.13

The values given above indicate mean ± SD of 9 replicates. Statistically significance differences are indicated by lowercase letters within columns and by capital letters within rows between concentrations and treatments, respectively. Values with the same letter are not significantly different according to Duncan's multiple range test at P ≤ 0.05.

C = Control, F1 = *Aspergillus terreus,* F2 = *Aspergillus niger*, B1= *Bacillus* sp., B2= *Acinetobacter* sp.

Table II: Amount of heavy metals in the roots and shoots of 90 days old plants of *Lemna minor* grown in different concentrations of tannery sludge.

Metals	Conc. of TS	Plant parts	Amount of metal (mg kg^{-1} DW)						
			Treatments						
			C	F1	F2	F1+F2	B1	B2	B1+B2
Cd	0%	Shoots	1.15^{cG} ±0.17	1.7^{cC} ±0.12	1.9^{cB} ±0.31	2.2^{cA} ±0.21	1.26^{cF} ±0.12	1.52^{cE} ±0.31	1.64^{cD} ±0.25
		Roots	0.11^{cDE} ±0.12	0.15^{cD} ±0.30	0.21^{cBC} ±0.12	0.29^{cA} ±0.31	0.15^{cD} ±0.22	0.19^{cC} ±0.17	0.22^{cB} ±0.21
	30%	Shoots	1,523^{bF} ±2.43	1,550^{bE} ±2.21	1,634^{bB} ±3.00	1,667^{bA} ±4.31	1,546^{bE} ±2.50	1,570^{bD} ±0.43	1,610^{bC} ±5.38
		Roots	628^{bF} ±4.42	645^{bD} ±1.32	660^{bB} ±2.00	678^{bA} ±5.30	634^{bE} ±0.32	646^{bCD} ±5.30	650^{bC} ±4.33
	60%	Shoots	2,145^{aG} ±2.45	2,267^{aC} ±4.43	2,310^{aB} ±1.55	2,455^{aA} ±2.50	2,178^{aF} ±0.37	2,198^{aE} ±3.38	2,234^{aD} ±6.45
		Roots	1,010^{aF} ±5.31	1,058^{aC} ±0.38	1,067^{a} ±4.30	1,126^{aA} ±0.45	1,034^{aE} ±3.38	1,050^{aD} ±2.43	1,077^{aB} ±2.32
Cu	0%	Shoots	1.05^{cG} ±0.31	1.16^{cEF} ±0.17	1.32^{cC} ±0.21	1.36^{cA} ±0.32	1.18^{cE} ±0.30	1.30^{cD} ±0.21	1.35^{cAB} ±0.31
		Roots	0.03^{cE} ±0.21	0.20^{cD} ±0.31	0.22^{cBC} ±0.12	0.29^{cA} ±0.32	0.11^{cD} ±0.17	0.19^{cC} ±0.20	0.23^{cB} ±0.31
	30%	Shoots	710^{bG} ±2.45	735^{bEF} ±4.38	750^{bD} ±2.30	833^{bA} ±4.00	740^{bE} ±2.45	755^{bC} ±3.37	760^{bB} ±2.00
		Roots	302^{bE} ±1.30	330^{abD} ±2.00	332^{abCD} ±1.38	340^{bB} ±3.30	331^{bC} ±2.07	340^{bB} ±1.38	345^{bA} ±2.25
	60%	Shoots	1,120^{aDE} ±4.00	1,145^{aC} ±1.37	1,177^{aB} ±2.45	1,185^{aA} ±1.38	1,122^{aD} ±2.45	1,150^{aC} ±3.30	1,180^{aA} ±2.55
		Roots	314^{aE} ±1.45	333^{aD} ±2.30	340^{aC} ±2.55	368^{aA} ±1.32	343^{aC} ±2.50	355^{aB} ±1.43	358^{aB} ±1.45
Cr	0%	Shoots	2.26^{cEF} ±0.31	2.32^{cC} ±0.43	2.45^{cB} ±0.17	2.53^{cA} ±0.32	2.22^{cF} ±0.12	2.25^{cE} ±0.25	2.28^{cD} ±0.19
		Roots	1.12^{cEF} ±0.21	1.15^{cE} ±0.17	1.20^{cD} ±0.30	1.25^{cB} ±0.21	1.20^{cD} ±0.17	1.24^{cC} ±0.21	1.28^{cA} ±0.33
	30%	Shoots	3,023^{bE} ±5.38	3,134^{bD} ±3.32	3,322^{bC} ±2.55	3,625^{bA} ±2.60	3,130^{bD} ±4.58	3,320^{bC} ±3.32	3,480^{bB} ±6.45
		Roots	1,289^{bE} ±2.45	1,356^{bB} ±3.32	1,378^{bA} ±2.37	1,356^{bB} ±3.30	1,323^{bD} ±2.32	1,344^{bC} ±3.45	1,370^{bA} ±4.43
	60%	Shoots	4,132^{aF} ±4.55	4,289^{aC} ±2.38	4,350^{aB} ±4.31	4,565^{aA} ±5.58	4,162^{aE} ±6.00	4,240^{aD} ±5.45	4,288^{aC} ±5.00
		Roots	1,867^{aG} ±2.55	1,922^{aE} ±3.00	2,026^{aB} ±2.31	2,167^{aA} ±0.58	1,902^{aEF} ±2.55	1,945^{aD} ±2.45	1,970^{aC} ±3.55
Pb	0%	Shoots	0.11^{cDE} ±0.21	0.20^{cC} ±0.25	0.25^{cB} ±0.17	0.30^{cA} ±0.20	0.19^{cD} ±0.23	0.20^{cC} ±0.17	0.23^{cBC} ±0.24
		Roots	0.02^{cE} ±0.12	0.08^{cDE} ±0.20	0.18^{cC} ±0.18	0.24^{cA} ±0.16	0.011^{cD} ±0.12	0.18^{cC} ±0.30	0.22^{cB} ±0.37
	30%	Shoots	216^{bE} ±1.27	234^{bC} ±2.31	256^{bA} ±1.00	227^{bD} ±3.38	222^{bD} ±2.27	239^{bC} ±1.00	250^{bB} ±0.31
		Roots	80^{bDE} ±0.23	87^{bD} ±0.30	106^{bC} ±0.33	122^{bA} ±0.29	86^{bD} ±0.26	96^{bCD} ±0.14	112^{bB} ±0.20
	60%	Shoots	439^{aF}	460^{aE}	512^{aB}	545^{aA}	460^{aE}	477^{aD}	490^{aC}

			±2.32	±3.31	±2.22	±0.45	±4.30	±0.32	±4.00
		Roots	200 ^{aF} ±3.30	222 ^{aE} ±1.25	256 ^{aC} ±0.27	312 ^{aA} ±1.37	237 ^{aD} ±3.01	257 ^{aC} ±1.02	278 ^{aB} ±2.25
Zn	0%	Shoots	1.10 ^{cDE} ±0.27	1.20 ^{cC} ±0.12	1.28 ^{cB} ±2.25	1.33 ^{cA} ±0.30	1.16 ^{cD} ±0.12	1.20 ^{cC} ±0.27	1.26 ^{cBC} ±0.30
		Roots	0.04 ^{cD} ±0.45	0.15 ^{cC} ±0.38	0.20 ^{cBC} ±0.37	0.26 ^{cA} ±0.45	0.15 ^{cC} ±0.38	0.21 ^{cB} ±0.40	0.26 ^{cA} ±0.45
	30%	Shoots	1,223 ^{bE} ±2.25	1,250 ^{bC} ±3.32	1,272 ^{bB} ±4.36	1,342 ^{bA} ±2.45	1,238 ^{bD} ±4.29	1,244 ^{bC} ±3.25	1,276 ^{bB} ±5.32
		Roots	430 ^{bE} ±1.32	446 ^{bD} ±3.40	522 ^{bB} ±1.37	558 ^{bA} ±3.32	443 ^{bDE} ±1.25	450 ^{bD} ±3.37	468 ^{bC} ±1.25
	60%	Shoots	2,123 ^{aE} ±5.40	2,220 ^{aC} ±0.25	2,156 ^{a} ±2.33	2,250 ^{aB} ±2.32	2,133 ^{aD} ±4.39	2,250 ^{aB} ±2.25	2,277 ^{aA} ±4.37
		Roots	823 ^{aF} ±3.40	925 ^{aB} ±2.25	855 ^{aD} ±1.33	938 ^{aA} ±0.32	850 ^{aDE} ±1.19	860 ^{aD} ±1.25	870 ^{aC} ±4.37

The values given above indicate mean ± SD of 9 replicates. Statistically significance differences are indicated by lowercase letters within columns and by capital letters within rows between concentrations and treatments, respectively. Values with the same letter are not significantly different according to Duncan's multiple range test at P ≤ 0.05. C = Control, F1 = *Aspergillus terreus,* F2 = *Aspergillus niger*, B1= *Bacillus* sp., B2= *Acinetobacter* sp.

Table III: Translocation Factor (TF) of metals of 90 days old plant of *Lemna minor* grown in different concentrations of tannery sludge.

Metals	Conc. of TS	Transfer factor (TF) Treatments							Average
		C	F1	F2	F1+F2	B1	B2	B1+B2	
	0%	2.22	2.08	1.78	1.48	2.60	2.00	1.75	
Ca	30%	3.68	2.54	2.68	2.20	2.52	2.54	2.45	2.43
	60%	2.69	2.88	2.71	2.74	2.25	2.89	2.38	
	0%	1.78	1.60	1.41	1.41	1.75	1.71	1.55	
K	30%	2.08	2.02	1.98	1.92	1.99	2.01	2.00	1.94
	60%	2.25	2.22	2.32	2.12	2.27	2.13	2.05	
	0%	1.50	1.37	1.38	1.43	1.36	1.32	1.31	
Na	30%	1.49	1.51	1.53	1.48	1.52	1.52	1.47	1.88
	60%	2.88	2.76	2.71	2.55	2.84	2.77	2.74	
	0%	1.80	1.57	1.40	1.25	1.53	1.56	1.36	
Mg	30%	2.89	2.71	2.51	1.90	3.09	2.88	2.67	2.13
	60%	2.38	2.36	2.18	1.84	2.40	2.31	2.23	
	0%	1.67	1.60	1.55	1.66	1.46	1.40	1.52	
Cd	30%	2.43	2.40	2.48	2.46	2.44	2.43	2.48	2.04
	60%	2.12	2.14	2.16	2.18	2.11	2.09	2.07	
	0%	1.38	1.50	1.41	1.36	1.71	1.45	1.23	
Cu	30%	2.35	2.23	2.26	2.45	2.24	2.22	2.20	2.35
	60%	3.57	3.44	3.46	3.22	3.27	3.24	3.30	
	0%	1.50	1.38	1.29	1.21	1.35	1.25	1.18	
Cr	30%	2.35	2.31	2.41	2.67	2.37	2.47	2.54	1.97
	60%	2.21	2.23	2.15	2.11	2.19	2.18	2.18	
	0%	1.90	1.67	1.72	1.58	1.69	1.50	1.45	
Pb	30%	2.70	2.69	2.42	2.27	2.58	2.49	2.23	2.02
	60%	2.20	2.07	2.00	1.75	1.94	1.86	1.76	
	0%	1.76	2.23	1.25	1.70	1.65	1.60	1.45	
Zn	30%	2.84	2.80	2.44	2.41	2.79	2.76	2.73	2.29
	60%	2.58	2.40	2.52	2.41	2.51	2.60	2.62	

Table IV: Enrichment Coefficient (EC) of metals of 90 days old plant of *Lemna minor* grown in different concentrations of tannery sludge.

Metals	Conc. of TS	Enrichment Coefficient (EC)							Average
		Treatments							
		C	F1	F2	F1+F2	B1	B2	B1+B2	
	0%	0.02	0.03	0.03	0.04	0.03	0.03	0.04	
Ca	30%	0.13	0.14	0.16	0.14	0.13	0.14	0.14	0.10
	60%	0.14	0.14	0.14	0.15	0.14	0.14	0.14	
	0%	0.04	0.05	0.06	0.06	0.04	0.05	0.06	
K	30%	0.12	0.13	0.14	0.15	0.13	0.14	0.14	0.10
	60%	0.12	0.13	0.13	0.14	0.12	0.13	0.13	
	0%	0.04	0.05	0.05	0.06	0.04	0.05	0.05	
Na	30%	0.15	0.15	0.15	0.16	0.15	0.15	0.15	0.14
	60%	0.22	0.22	0.22	0.23	0.22	0.22	0.22	
	0%	0.04	0.04	0.05	0.07	0.04	0.05	0.07	
Mg	30%	0.11	0.13	0.14	0.15	0.13	0.13	0.15	0.12
	60%	0.18	0.18	0.19	0.20	0.19	0.19	0.20	
	0%	-	-	-	-	-	-	-	
Cd	30%	0.15	0.15	0.16	0.16	0.15	0.15	0.16	0.10
	60%	0.15	0.16	0.16	0.17	0.15	0.15	0.15	
	0%	-	-	-	-	-	-	-	
Cu	30%	0.13	0.13	0.13	0.15	0.13	0.13	0.13	0.09
	60%	0.16	0.16	0.17	0.17	0.16	0.16	0.17	
	0%	-	-	-	-	-	-	-	
Cr	30%	0.18	0.19	0.20	0.22	0.19	0.20	0.21	0.13
	60%	0.20	0.21	0.21	0.22	0.20	0.21	0.21	
	0%	-	-	-	-	-	-	-	
Pb	30%	0.10	0.10	0.11	0.12	0.10	0.11	0.11	0.06
	60%	0.08	0.08	0.09	0.10	0.08	0.09	0.09	
	0%	-	-	-	-	-	-	-	
Zn	30%	0.14	0.15	0.15	0.16	0.14	0.15	0.15	0.17
	60%	0.20	0.21	0.20	0.21	0.20	0.21	0.21	

CONCLUSION

L. minor (duckweed) is a hyperaccumulator plant. This plant can also be used for the accumulation of metals. Our experiments revealed that these plants accumulated high levels of heavy metals after 90 days of exposure. Even though the accumulation of certain elements is highest in the plant, it is extraordinarily high when compared to other aquatic plants. Therefore, the plant is considered as accumulators of those elements. In conclusion duckweed shows promise for not only heavy metal removal from tannery sludge but also tolerates maximum concentration of sludge.

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