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TREATMENT OF CADMIUM, LEAD AND COPPER USING PHYTOREMEDIATION ENHANCED BY TITANIUM DIOXIDE NANOPARTICLES.

Harikumar, P.S. and Megha, T.

Water Quality Division, Centre for Water Resources Development and Management, Kozhikode, Kerala, India.
Corresponding author: hps@cwrdm.org; meg8tee@gmail.com

ABSTRACT

Presently, phytoremediation is a beneficial and affordable technique used to extract or remove inactive metals and metal pollutants from contaminated water. Remediation of heavy metal from water and soil is extreme necessary. Phytoremediation ability of two aquatic plants *Eichhornia crassipes* and *Salvinia molesta* for the removal of toxic heavy metals like Pb, Cd and Cu were attempted in the present study under three different conditions. Maximum removal of Pb, Cd and Cu was observed in TiO_2 nanoparticles applied plants used phytoremediation system (C). Results obtained from heavy metal accumulation in plants parts, bioconcentration factor, translocation factor indicates that phytoremediation systems enhanced by TiO_2 nanoparticles showed more tendency to translocate metals to its areal parts. The results showed that, plants applied with TiO_2 nanoparticles exhibited significant increase in physiological response like relative growth and the production of chlorophyll content compared to others. Compared to control (A) and the phytoremediation system in which TiO_2 nanoparticles entrapped calcium alginate beads were applied to plants (B), fast and efficient removal of lead, cadmium and copper was observed in phytoremediation system enhanced by TiO_2 nanoparticles i.e., in C. Phytoremediation technique enhanced by TiO_2 nanoparticles seems to be promising in the treatment of heavy metals from water.

Key words: *Eichhornia crassipes*, *Salvinia molesta*, bioconcentration factor and translocation factor

INTRODUCTION

Heavy metal contamination is a widespread issue that disrupts the environment as a consequence of several anthropogenic activities and their invariably persisting nature in the environment. The ecological balance of the environment and diversity of aquatic organisms have devastatingly affected by heavy metal pollution (Nilantika et al. 2014). Some heavy metals like cadmium, lead and chromium etc. are phytotoxic at both low concentrations as well as very high concentration (Amin, 2012). The prevailing technologies on purification that are used to eradicate these toxic contaminants are costly and sometimes non-ecofriendly also. For the beneficial of community, the research on water purification is focused towards low cost and eco-friendly technologies (Dhote and Dixit, 2009). Phytoremediation is energy efficient, cost-effective, aesthetically pleasing technique of remediation sites with low to moderate levels of contamination.

The excessive concentration of heavy metals in plants can cause oxidative stress and stomatal resistance and can also affect photosynthesis and chlorophyll florescence processes. Copper can inhibit photosynthesis and reproductive processes; lead reduces chlorophyll production; arsenic interferes with metabolic processes, while zinc and tin stimulate the growth of leaves and shoots; ultimately plant growth becomes limited or impossible (Ashraf et al. 2011). The results of some of the experiments indicated that accumulation of some heavy metals such as Cd and Pb may damage chloroplasts in young leaves and the first impact of Cd on plants is the reduction of photosynthetic activity (Ali et al. 2014). Moreover, it has been proved that a decrease in the growth of plants under some stress is due to the restriction of photosynthetic process (Ackerson and Herbert, 1981).

Nanoparticles can be used to increase the supply of elements to plant shoots and foliage and its application can also increase seed germination and seedling growth (Morteza et al. 2013). Furthermore, nanoparticles can facilitate enhanced ability of water and fertilizer absorption by roots, and increase antioxidant enzyme activity such as superoxide dismutase and catalase. Thus, nanoparticles can increase plant resistance against different stresses. Titanium dioxide nanoparticles are used in agriculture to increase growth and can improve yield; improve the rate of photosynthesis and reduce diseases (Morteza et al. 2013). Titanium dioxide (TiO_2) nanoparticles (NPs) are known for its photocatalytic activity, environmental friendly nature, high stability and are found to be safe for human. These particles have been used in pathogen treatments as well as decomposition of phytotoxic compounds (Raskar and Laware, 2013).

The effects of nano-TiO_2 on the germination and growth of spinach seeds were studied by many researchers. These nanoparticles improved light absorbance and promote the activity of rubisco activase thus accelerated spinach growth (Remya et al. 2010). Nano- TiO_2 improved the plant growth by enhanced nitrogen metabolism (Yang et al. 2006) that promotes the absorption of nitrate in spinach and accelerating conversion of inorganic nitrogen into organic nitrogen, thereby increasing the fresh weight and dry weight. It has also been found that nano-anatase TiO_2 promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and enhance the activities of superoxide dismutase, catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increase the evolution oxygen rate in spinach chloroplasts under UV-B radiation (Lei et al. 2008). In this perspective, we tried to enhance efficiency of phytoremediation capacity of *Eichhornia crassipes* and *Salvinia molesta* using titanium dioxide nanoparticles in this study. The aim of our study was to offer an effective approach to enhance the efficiency of the phytoremediation system for the treatment of heavy metals like Pb, Cd and Cu from water.

MATERIALS AND METHODS

Experimental setup

Two free-floating aquatic plants, *Eichhornia crassipes* and *Salvinia molesta* (plate 1) were collected from a natural wetland area, viz. Kottooli wetland in Kozhikode City. In order to simulate the natural environment, plants were placed under natural sun light for one month and the second generations of the plants with similar size were utilized for this study. Before starting the phytoremediation experiment, the epiphytes and insect larvae grown on plants were removed by rinsing with distilled water. The present study was conducted in two stages. During the Stage I, three different conditions (A, B and C) were applied to *Eichhornia crassipes* and *Salvinia molesta* for 10 days. Tanks were setup with different operating conditions and each contained plants and distilled water. Condition A was the control, in which the plants were kept in water under natural conditions. In condition B, TiO_2 nanoparticles entrapped calcium alginate beads (2gm) was applied to the plants and in condition C, TiO_2 powder (2gm) was applied. TiO_2 photocatalyst does not require ultraviolet rays that have an energy level as high as 254nm and are hazardous to humans. It also allows reaction to be initiated by the near – ultraviolet rays with relatively long wavelength contained in sunlight. So, all the experimental setup was placed in the outdoor for maximum sunlight exposure and was conducted during the summer season. Before and after stage I, the chlorophyll content and fresh weight of *Eichhornia crassipes* and *Salvinia molesta* in A, B and C were determined.

In stage II, plants exposed to different conditions were used for the treatment of heavy metals like lead, cadmium and copper from water. Before that, plants were washed and rinsed with distilled water. Thirteen liters of mixed heavy metal (Cd, Pb and Cu) solution was taken in each tank (45 × 30 × 15cm) and the tanks were named A, B and C for both experiments using *Eichhornia crassipes* and *Salvinia molesta*. Thirteen liters of simulated contaminated water was prepared for each tank by dissolving 0.416g of Pb $(NO_3)_2$, 1.022g of $CuSO_4.5H_2O$,

Plate 1: (A) *Eichhornia crassipes* and (B) *Salvinia molesta*

and 0.593g of $(3CdSO_4).8H_2O$ in distilled water. All chemicals used were of the highest purity available and of analytical grade procured from Merck. The heavy metal concentrations were determined by Atomic Absorption Spectrophotometer AAS (Thermo Series) with GF 95. The phytoremediation experiments using *Eichhornia crassipes* was conducted with water having initial concentration of Cd, Pb and Cu were 20.32 ± 0.44 mg/L, 21.45 ± 0.18 mg/L and 21.34 ± 0.22mg/L respectively. The water with initial concentration of Cd, Pb and Cu were 20.99 ± 0.37mg/L, 20.78 ± 0.45 mg/L and 20.56 ± 0.46mg/L respectively, was used in phytoremediation experiments using *Salvinia molesta*. Tank A was the control which comprised of only plants (phytoremediation system). In tank B and tank C, the plants exposed to TiO_2 nanoparticles entrapped calcium alginate beads and TiO_2 nanoparticles powder respectively were used in the treatment of Cd, Pb and Cu.

Experiment was continued for 24 days and water samples were taken in every 3 days. When the experiment was completed, stem, roots and leaves of the plants were separated and dried in a hot air oven. The residual Pb, Cd and Cu concentrations in water and the accumulated metals in different parts of the plants at the conclusion of the experiment were analyzed using Atomic Absorption Spectrophotometer. The effect of the applied conditions on the growth of *Eichhornia crassipes* and *Salvinia molesta* was examined by using relative growth and chlorophyll content. Phytoremediation potential of these native aquatic macrophytes under different applied conditions was also evaluated by metal accumulation, translocation factor and bioconcentration factor.

Plate 2:
TiO_2 nanoparticles entrapped calcium alginate beads

Synthesis of TiO_2 nanoparticles and the encapsulation method for immobilization of nanoparticles in semipermeable alginates beads

TiO_2 nanoparticles were synthesized by simple precipitation method. TiO_2 (Fluka 15%) solution in HCl (10-15%) was stirred in deionized water ($[Ti^{3+}] = 0.15$ mol L^{-1}). A blue-violet solution was obtained at room temperature. The pH was adjusted between 0.5 and 6.5 with sodium hydroxide (NaOH) solution. The solution was then heated at 60^0C in an oven for 24h. The solid obtained was centrifuged, washed with dilute acid (pH=1) and distilled water in order to remove salts (Sophie et al. 2007). A solution containing TiO_2 nanoparticles (0.5wt %) and sodium alginate (0.5wt %) was prepared with 25ml distilled water and stirred for 30 min at 85^0C. Afterwards, the solution was extruded as small drops by means of syringe into a stirred solution of calcium chloride (4.0wt %), where spherical gel beads were formed (plate 2). The gel beads were retained in the calcium chloride solution for 12 h for hardening and then washed with distilled water. Stability of beads depends on pH values of the aqueous solution and the initial physical states of the beads (Harikumar et al. 2013).

Analytical procedures

The plant samples were rinsed with tap water twice and deionized water three times. Rinsed samples were cut into pieces and oven-dried at 70^0C till constant weight was obtained for the dried samples. The oven-dried samples were ground to pass through a 100-mesh sieve and were digested with HNO_3–$HClO_4$ (4:1) mixture and filtered. The filtrates were analyzed for heavy metals using AAS with GF 95 (Xinshan et al. 2011). Chlorophyll (chl.a + chl.b, CHLab) was determined by OD (optical density) values (663 nm and 645 nm using UV-Visible spectrophotometer, Thermo Evolution 201) of extracting solution, which was made by adding 80% acetone into grinding solution of 0.5 g of fresh leaves (Xinshan et al. 2011).

Relative Growth

The relative growth of plants was calculated to assess the effects of applied conditions on the growth of *Eichhornia crassipes* and *Salvinia molesta*.

RG = FFW/IFW

Where RG denotes relative growth of plants during experimental period, dimensionless; FFW denotes final fresh weight in grams of plants taken at the end of each experiment, and IFW denotes the initial fresh weight in grams of plants taken before starting experiment (Gakwavu et al. 2012).

Bioconcentration Factor and Translocation factor

The bioconcentration factor (BCF) was calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment (Zayed et al. 1998). BCF is given by BCF = (P/E)

P represents the trace element concentration in plant tissues (mg kg^{-1} dry wt); E represents the trace element concentration in the water (mg/L). BCF is dimensionless. A larger ratio implies better phytoaccumulation capability. The translocation of heavy metal from roots to aerial part and the internal metal transportation of the plant are generally indicated by Translocation Factor (TF). The translocation factor is determined as a ratio of metal accumulated in the shoot to metal accumulated in the root (Deng et al. 2004).

$TF = (A_s / A_r)$

Where, TF>1 indicates that the plant is capable of effectively translocating the accumulated metals from the root to its aerial part. TF is the translocation factor and is dimensionless. A_r represents the amount of trace element accumulated in the roots (mg kg^{-1} dw), and A_s represents the amount of trace element accumulated in the shoot/leaf (mg kg^{-1} dw). All the tests were conducted in triplicates and the data were statistically analyzed.

RESULTS AND DISCUSSION

The *Eichhornia crassipes* and *Salvinia molesta* grown under different conditions were used for the treatment of heavy metals. TiO_2 nanoparticles that applied to plants were synthesized by chemical precipitation method. The materials were characterized by scanning electron microscopy (Figure 1) and energy dispersive X-ray spectroscopy (Figure 2) spectra. While synthesizing TiO_2 NPs by precipitation method, a mixture of anatase and rutile was precipitated at pH 3. By increasing the pH of the solution, the formation of anatase was favored and at pH 5, only anatase TiO_2 could be formed (Cheng et al.1995). SEM micrograph indicates the presence of nanoparticles below 50nm. The energy dispersive X-ray spectra confirm the presence of TiO_2 and showed no significant levels of impurities.

Phytoremediation of heavy metals

The ability of *Eichhornia crassipes* and *Salvinia molesta* to uptake more than one metal was analyzed by the phytoremediation of the simulated multi metal contaminated water. The residual concentration of Cd, Pb, and Cu were analyzed for each phytoremediation system (A, B and C) during the study, at an interval of 3 days using AAS and the results are summarized in table 1. The metals other than Cu, Cd and Pb were found to be within the permissible limit.

a) Removal of lead from synthetic heavy metal solution

In the case of phytoremediation by *Eichhornia crassipes*, the maximum amount of lead reduction was observed in C with minimum time. Within 3 days, it attained 95.53% reduction, whereas control showed only 10.77% reduction. The TiO_2 nanoparticles applied *Eichhornia crassipes* used phytoremediation system (i.e. C) attained faster removal of Pb compared to control (A) and TiO_2 nanoparticles entrapped calcium alginate beads applied *Eichhornia crassipes* used phytoremediation system (B). Lead was reduced to 50.72% by TiO_2 nanoparticles entrapped calcium alginate beads applied *Eichhornia crassipes* used phytoremediation experiment (B) within 3 days. *Salvinia molesta* also exhibited a similar trend. All the systems attained a reduction of lead ranged from 85.61 to 100% within 24 days. But fast reduction in lead concentration

Figure 1.
SEM micrographs of titanium dioxide nanoparticles

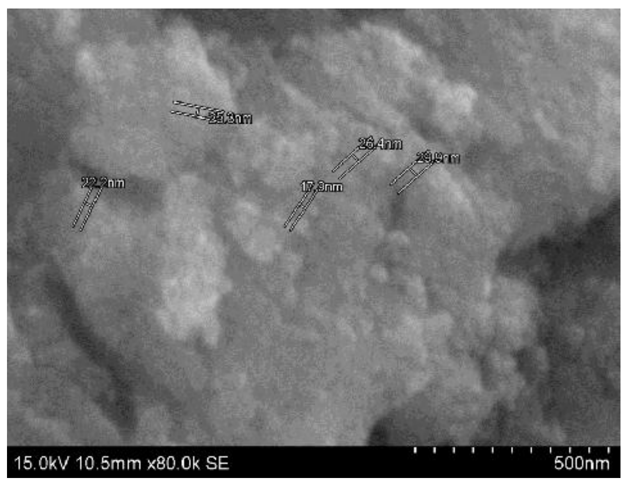
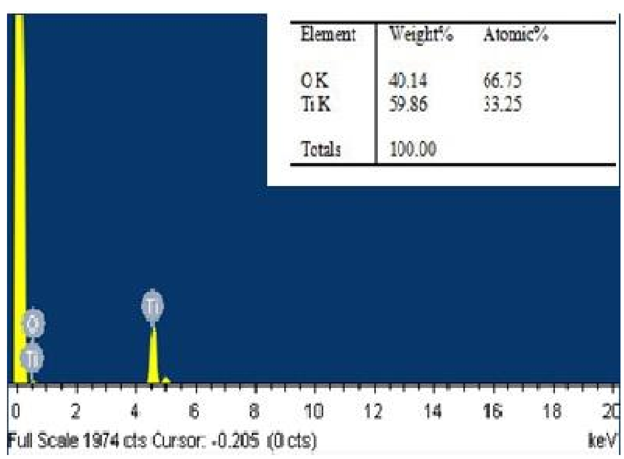


Figure 2.
EDS spectra of titanium dioxide nanoparticles



was observed in C. It showed 77.37% of lead reduction on 3rd day and 100% of reduction on 15th day. The results suggest that the plants exposed to TiO_2 nanoparticles showed efficient phytoremediation ability to remove lead from water.

b) Removal of cadmium from synthetic heavy metal solution

When titanium dioxide nanoparticles applied *Eichhornia crassipes* (C) was used in the phytoremediation of heavy metals, 99.06% reduction of cadmium was observed within 3 days. Only, 10.78% of cadmium was removed in the control experiment after 3 days. Cadmium was completely removed in C within 9 days, while in A and B, the efficiency of cadmium removal was found to be 88.78 and 97.64% respectively after 24 days. Maximum cadmium removal with minimum time period was observed in C; that is the phytoremediation system enhanced by titanium dioxide nanoparticles. Compared to A, better cadmium removal was exhibited by B. B is phytoremediation system, where TiO_2 nanoparticles entrapped calcium alginate beads were applied *Eichhornia crassipes* was used. Titanium dioxide exposed *Salvinia molesta* (C) attained 89.57% cadmium removal within 3 days and 100% of cadmium removal within 12 days. The cadmium removal efficiency was found to be 81.99 and 96.81% after 24 days in A and B respectively. The phytoremediation of cadmium by TiO_2 nanoparticles applied *Eichhornia crassipes* and *Salvinia molesta* showed better performance than control plants. In *Eichhornia crassipes*, 100% of cadmium was removed in C within 9 days, while in *Salvinia molesta* the same reduction was observed only after 12 days. Results from this experiment indicate that, one of the main limitations of phytoremediation of heavy metals, i.e. the long retention period required for remediation by plants can be overcome by, phytoremediation with TiO_2 nanoparticles.

c) Removal of copper from synthetic heavy metal solution

Maximum removal of copper was observed in C within 18 days of exposure to contaminant water by

Table 1. Residual concentration of lead, cadmium and copper in water

Metal	DAY	Residual concentration of heavy metals, mg/L					
		Eichhornia crassipes			*Salvinia molesta*		
		A	B	C	A	B	C
Pb	3	19.14 ± 0.25	10.57 ± 0.07	0.95 ± 0.03	19.64 ± 0.01	12.55 ± 0.11	4.70 ± 0.01
	6	15.48 ± 0.23	7.96 ± 0.03	0.46 ± 0.02	15.89 ± 0.02	9.37 ± 0.06	1.42 ± 0.004
	9	11.57 ± 0.13	5.89 ± 0.12	0.35 ± 0.01	12.41 ± 0.05	7.52 ± 0.02	0.86 ± 0.01
	12	9.75 ± 0.09	3.55 ± 0.05	ND	8.21 ± 0.01	5.72 ± 0.04	0.01 ± 0.0002
	15	6.95 ± 0.04	1.51 ± 0.01	ND	7.04 ± 0.01	3.30 ± 0.04	ND
	18	5.12 ± 0.02	0.82 ± 0.01	ND	5.18 ± 0.23	2.15 ± 0.02	ND
	21	3.01 ± 0.01	0.32 ± 0.01	ND	4.53 ± 0.01	1.48 ± 0.002	ND
	24	2.08 ± 0.01	0.14 ± 0.001	ND	2.99 ± 0.003	0.97 ± 0.001	ND
Cd	3	18.13 ±0.12	14.25 ±0.28	0.19 ± 0.01	18.08 ± 0.05	16.11 ± 0.01	2.19 ± 0.005
	6	14.18 ±0.03	11.24 ±0.05	0.08 ± 0.01	16.13 ± 0.02	14.18 ± 0.08	0.51 ± 0.01
	9	12.11 ±0.11	8.16 ± 0.16	ND	14.16 ± 0.07	10.13 ± 0.01	0.05 ± 0.001
	12	9.88 ± 0.21	4.98 ± 0.21	ND	11.06 ± 0.04	7.96 ± 0.04	ND
	15	5.19 ± 0.11	2.15 ± 0.04	ND	8.11 ± 0.02	5.04 ± 0.05	ND
	18	4.05 ± 0.06	1.01 ± 0.01	ND	6.01 ± 0.05	2.81 ± 0.01	ND
	21	3.59 ± 0.03	0.89 ± 0.04	ND	5.13 ± 0.06	1.02 ± 0.001	ND
	24	2.28 ± 0.03	0.48 ± 0.01	ND	3.78 ± 0.01	0.67 ± 0.03	ND
Cu	3	14.87 ± 0.13	11.59 ± 0.03	4.07 ± 0.02	18.03 ± 0.02	14.45± 0.11	8.05 ± 0.13
	6	11.92 ± 0.06	8.37 ± 0.17	2.67 ± 0.05	14.79 ± 0.04	11.64 ± 0.03	2.51 ± 0.01
	9	8.29 ± 0.02	5.48 ± 0.05	1.41 ± 0.04	11.87 ± 0.03	7.81 ± 0.03	2.51 ± 0.03
	12	7.01 ± 0.09	5.02 ± 0.08	0.36 ± 0.03	7.37 ± 0.13	4.66 ± 0.01	1.21 ± 0.01
	15	4.62 ± 0.05	3.98 ± 0.05	0.26 ± 0.01	5.20 ± 0.03	2.64 ± 0.01	0.90 ± 0.01
	18	3.18 ± 0.05	2.32 ± 0.04	ND	4.91 ± 0.07	1.94 ± 0.02	ND
	21	2.47 ± 0.01	1.14 ± 0.01	ND	3.89 ± 0.04	0.69 ± 0.01	ND
	24	1.64 ± 0.01	0.89± 0.001	ND	2.42 ± 0.01	ND	ND

(Values represent Mean ± SD of three replicates)

ND- not detected

phytoremediation using *Eichhornia crassipes.* In control (A), compared to other metals, more amount of copper was removed by *Eichhornia crassipes* within 3 days. This may be due to the fact that; copper is one of the micronutrient. In the phytoremediation system using *Salvinia molesta*, 95.62% reduction was observed in C within 15 days. On 3rd day, the percentage removal of copper was 60.85% in C and in control (A) it was 12.31%. The residual concentration of copper observed in control (A) during the final stage was 2.42 ± 0.01 mg/L. The effectiveness of two aquatic macrophytes *Eichhornia crassipes* and *Salvinia molesta* grown under three different conditions were tested for their phytoremediation capacity of three metals (Pb, Cd and Cu) and the results indicated that *Eichhornia crassipes* exposed to nanoparticles reported the maximum and fast removal of these heavy metals.

Heavy metal accumulation in plants

The heavy metal analysis of plant segments of both the aquatic native plants, *Eichhornia crassipes* and *Salvinia molesta* were carried out. Distribution of Cd, Pb and Cu in the organs of *Eichhornia crassipes* and *Salvinia molesta* are presented in figure 3 and 4. In A (control), maximum accumulation of metals was observed in root and accumulation of Cd, Pb and Cu in roots of *Eichhornia crassipes* was 6285.04, 5786.30 and 6628.83mg/kg respectively. Many studies revealed that *Eichhornia crassipes* accumulated higher concentrations of heavy metals in the roots than that in the aerial parts. But in C, maximum accumulation of Cd was observed in leaf. More amount of metal accumulation was observed in leaf and stem of C compared to A and B. C was the phyto-remediation systems in which *Eichhornia crassipes* was directly exposed to titanium dioxide nanoparticles. But the present study showed that in C, Cu accumulation is in the order of leaves>roots >stem, Cd accumulation is in the order of leaves> stem > roots and Pb accumulation is in the order of leaves >stem > roots. The results show that, phytoremediation systems enhanced by titanium dioxide nanoparticles showed more tendency to translocate metals to its areal parts.

Salvinia also exhibited the capacity to accumulate high levels of heavy metals. Compared to all phytoremediation systems, a maximum concentration of 8928.74 mg/Kg of Cd was accumulated in leaves of *Salvinia molesta* grown under condition C. Sune et al. 2007 reported that heavy metal uptake in *Salvinia* occurs through a physical process (fast), which involves adsorption, ionic exchange, chelation, while biological processes such as intracellular uptake (transported through plasmalemma into cells) is slow and help in subsequent translocation of metals (Cd) from roots to leaves. The highest amount of lead and copper accumulated in C was 9192.63 and 9888.82mg/Kg respectively. In control plant (A), all the three metals were accumulated at highest level in roots. Roots remain in direct contact with the medium and hence this might result in increased metal concentration in the roots. More amount of metal accumulation was observed in leaf of B compared to A. The results indicate that titanium dioxide nanoparticles applied plants were efficient to translocate the metals from root to its aerial parts.

Bioconcentration factor and translocation factor

The mobility of the heavy metals from the polluted medium to the roots of the plants and the ability to translocate the metals from roots to the harvestable aerial part were evaluated respectively by means of the bioconcentration factor (BCF) and the translocation factor (TF). Bioconcentration factor is a useful parameter to evaluate the potential of the plants in accumulating metals. The BCF values for Cd, Pb and Cu in *Eichhornia crassipes* and *Salvinia molesta* at 24 days' exposure to heavy metals are shown in figure 5 and 6, respectively. Higher BCF values indicates the higher phytoaccumulation capacity. Maximum bioconcentration factors of Cd, Pb and Cu in *Eichhornia crassipes* was observed in C. In C, the highest BCF values found for Cd, Pb and Cu were 852.49, 848.20 and 1047.99 respectively. If the BCF value is more than or equal to 1000 then that plant species is considered as a hyper accumulator. The plant having the abilities to grow in very high concentration of metal and concentrating high heavy metals in their tissues are known as hyperaccumulators (Wahab et al. 2014). The higher BCF value of *Eichhornia crassipes* for Cu (BCF >1000) in C indicates that TiO_2 nanoparticles applied *Eichhornia crassipes* is considered as a good hyper accumulator. The BCFs of control (A) for Cd, Pb and Cu were 615.67, 516.14 and 698.53 respectively. BCF of B for all the metals were higher than control but less than 1000. Phytoremediation experiments using *Salvinia molesta,* a highest BCF values was observed for Cd, Pb and Cu were 750.12, 777.90 and 896.29 respectively in (C). In experiment B of *Salvinia molesta*, BCFs of all the three metals were found higher than that of A. The results indicated that, plants exposed to TiO_2 nanoparticles were accumulated more amount of heavy metals in its upper portions compared to control plants.

The translocation factor for Cd, Pb and Cu in phyto-remediation systems using *Eichhornia crassipes* and *Salvinia molesta* are shown in table 2 and 3 respectively. The value TF > 1 indicated that there is a transport of metal from root to leaf probably through an efficient metal transporter system and the metals sequestration in the leaf vacuoles and apoplast. TF value more than 1 of plant species indicates their hyperaccumulation potential and is identified as hyperaccumulator plants (Marbaniang and Chaturvedi, 2014). For *Eichhornia crassipes,* maximum TF for cadmium was

Figure 3. Accumulation of cadmium, lead and copper in *Eichhornia crassipes*

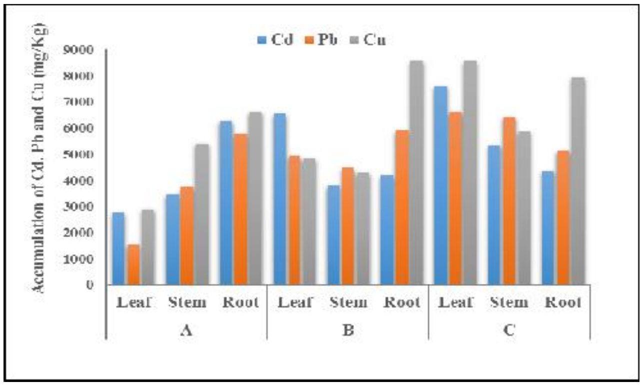


Figure 4. Accumulation of cadmium, lead and copper in *Salvinia molesta*

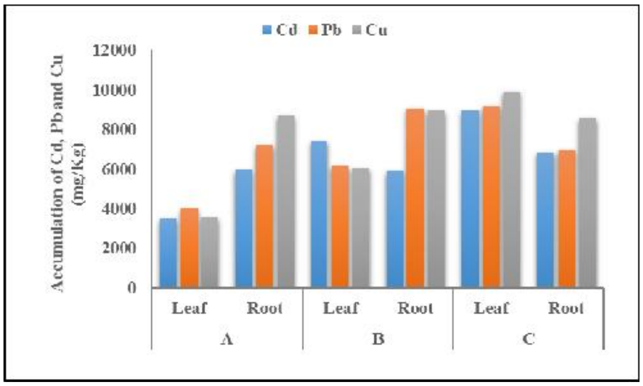


Figure 5: Bioconcentration factors (BCF) of Pb, Cd and Cu in *Eichhornia crassipes*

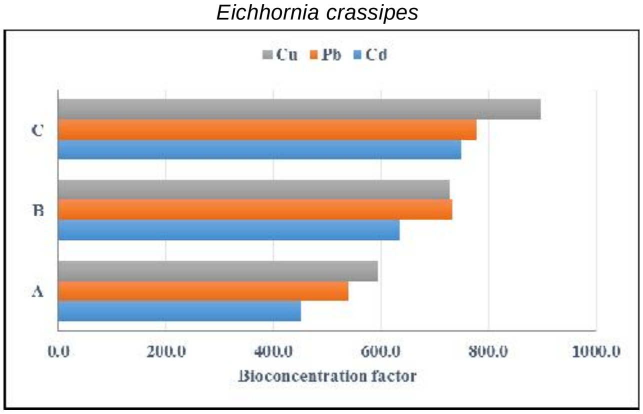
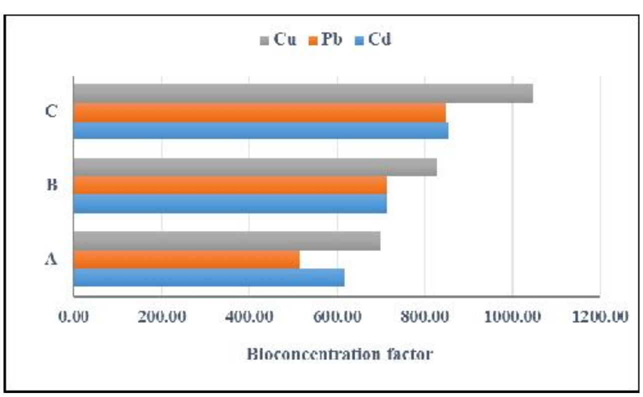


Figure 6: Bioconcentration factors (BCF) of Pb, Cd and Cu in *Salvinia molesta*



observed in C (TF(s/r) = 1.22 and TF(l/r) = 1.74) compared to A and B, which indicates the translocation of high Cd content from roots to its aerial part. Similar trend was observed for Pb also and for Cu, maximum TF observed was TF(l/r) =1.08 in C. These results imply that *Eichhornia crassipes* exposed to nanoparticles accrued an ability to transfer higher metal concentrations from root to its areal part. The lowest translocation factor was observed in the A (control) for Cd and Pb. Similar results were found in phytoremediation systems using *Salvinia molesta* and the translocation factor observed in the C was TF(l/r) = 1.31, TF(l/r) = 1.32 and TF(l/r) = 1.16 for Cd, Pb and Cu respectively. TiO_2 nanoparticles applied plants (C) exhibited better potential to translocate Cd, Pb and Cu to its aerial parts.

Translocation is the movement of metal containing sap from the root to the shoot which was primarily controlled by two processes, root pressure and leaf transpiration. Some metals are accumulated in roots, probably due to some physiological barriers against metal transport to the aerial parts, while the others are easily transported in plants. Translocation of trace elements from roots to shoots could be a limiting factor for the bioconcentration of elements in shoots. It can be proposed that there has mechanism in roots that could detoxify heavy metals or transfer them to aerial parts (Gomati et al. 2014).

Physiological response of plants to the applied conditions

Variation in the production of chlorophyll

In the life activity of green plants, chlorophyll plays the role of absorbing, transferring and transforming energy. The chlorophyll content of plant after experiment was determined using UV-Visible spectrophotometer. Variation in the production of chlorophyll of both the plants is depicted in figure 7. The result shows that, plants from B and C exhibited an increase in the production of chlorophyll compared to A (control) for both *Eichhornia crassipes* and *Salvinia molesta*. Maximum chlorophyll production was observed in C for both the plants.

This may be due to the effects of nano-TiO_2 on the content of light harvesting complex II (LHC II) on thylakoid membranes of plants and nano-TiO_2 has ability to increase LHC II content (Lei et al. 2007 and Hong et al. 2005). These have the ability to promote energy transfer and oxygen evolution in photosystem II (PS II) of plants (Mingyu, 2007). It has also been reported that nano-anatase TiO_2 promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonyldialdehyde content and enhance the activities of superoxide dismutase,

Table 2. Translocation factor of *Eichhornia crassipes*

Metals	TF	A	B	C
Cd	TF(s/r)	0.55	0.91	1.22
	TF(l/r)	0.44	1.57	1.74
Pb	TF(s/r)	0.65	0.76	1.26
	TF(l/r)	0.27	0.83	1.29
Cu	TF(s/r)	0.81	0.50	0.74
	TF(l/r)	0.43	0.56	1.08

Table 3. Translocation factor of *Salvinia molesta*

Metals	TF	A	B	C
Cd	TF(l/r)	0.59	1.26	1.31
Pb	TF(l/r)	0.55	0.68	1.32
Cu	TF(l/r)	0.41	0.67	1.16

Figure 7: Chlorophyll content of plants after treatment

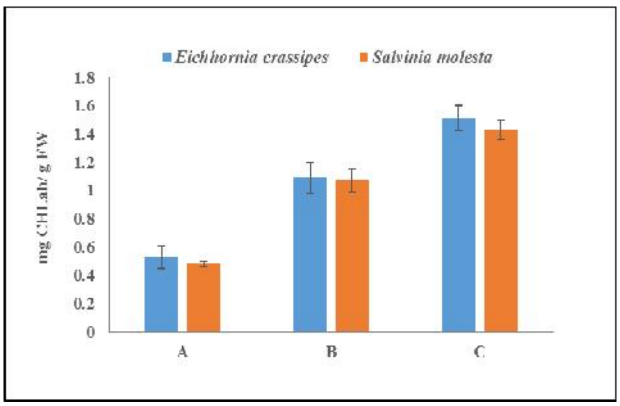
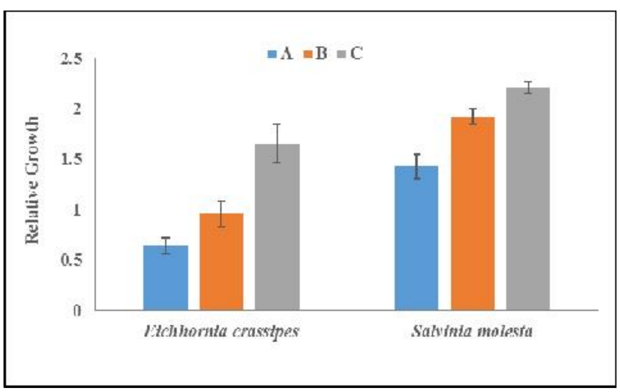


Figure 8. Variations in the relative growth of *Eichhornia crassipes* and *Salvinia molesta*



catalase, ascorbate peroxidase, guaiacol peroxidase and thereby increase the evolution of oxygen rate in plants chloroplasts (Lei et al. 2008). It helps to increase solar energy trapping that might improve the photo synthetic efficiency of plants.

Variations in the relative growth of plants under different conditions

Variations in the relative growth of *Eichhornia crassipes* and *Salvinia molesta* grown under three different conditions are graphically represented in figure 8. The relative growth of control plants showed a significant decrease for both the plants. The plants exposed to TiO_2 nanoparticles (C) exhibited significant increase in relative growth. The plants in C showed high relative growth compared to plants from A and B. In the case of *Salvinia molesta* an increasing trend was observed in all the conditions but maximum increase in weight after treatment was observed in plant from C. Plant in C was the plant exposed to nano - TiO_2. TiO_2 nanoparticles has the ability to improve light absorbance and promote the activity of rubisco activase thus accelerate the growth of plants (Zheng et al. 2005 and Hong et al. 2005).

CONCLUSION

A study was carried out to assess the enhancement of phytoremediation capacity of heavy metals (Cd, Pb and Cu) by *Eichhornia crassipes* and *Salvinia molesta* in the presence of titanium dioxide nanoparticles. TiO2 nanoparticles exposed *Eichhornia crassipes* and *Salvinia molesta* showed 99.06% and 89.57% of phytoremediation efficiency respectively for cadmium removal within 3 days. Maximum phytoremediation efficiency for lead and copper was also observed in TiO_2 nanoparticles exposed plants. It was observed that, in the nano - TiO_2 applied phytoremediation system, more efficient and faster removal of heavy metals was taking place. Results of the study on heavy metal accumulation indicates that titanium dioxide nanoparticles applied plants showed more tendency to translocate metals to its areal parts. Control plant accumulated more metals in their root portion in both the plants. Nano - TiO_2 applied plants exhibited an increase in the production of chlorophyll compared to A (control) and B. TiO_2 nanoparticles exposed plants exhibited significant increase in relative growth compared to others. This may be due to the fact that, the plant exposed to nano –TiO_2 has the ability to improve light absorbance and promote the activity of rubisco activase thus accelerating the growth of plants. The determination of bioconcentration factor indicated that, the uptake potential of metals by plants was increased under nano - TiO_2 applied conditions. Plants exposed to TiO_2 nanoparticles achieved ability to transfer higher metal concentrations from root to its areal part. TiO_2 nanoparticles applied plants (C) showed better performance than TiO_2 nanoparticles entrapped calcium alginate beads applied plants (B). Taking into account the overall results, we can state that TiO_2 nanoparticles applied *Eichhornia crassipes* and *Salvinia molesta* seems to be promising candidate for the phytoremediation of heavy metals from water and exhibited an enhanced phytoremediation ability.

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