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Study the biosorption potential of different macrophytes (*Eichornia crassipes, Pistia stratiotes* and *Lemna minor*) with the special reference of lead heavy metal

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Abstract
Biosorption capacity of three common macrophytes viz., *Eichornia crassipes, Pistia stratiotes and Lemna minor*) was evaluated with respect to the removal of the heavy metal, lead (Pb) from aqueous solution. The influence of various parameters such as pH (5 to 8), biomass weight, initial metal ion concentration (0.25 to1.25 ppm/L) and contact time (3 to 15 days) on biosorption efficiencies were determined. Results indicated that the Maximum metal removal efficiency reached 77.2% for *E. crassipes* at pH 7 due to high sorption of metal, plant fresh weight increased to 26.8g. The biosorption of lead by *P. stratiotes* increased with increasing pH of aqueous solution. At 7.5 pH maximum heavy metal, 53% was removed. *L. minor* shown highest absorption 19.8% at 7 the lead concentration was accumulated to 26% for *L. minor*, 34% for *P. stratiotes* and 36% for *E. crassipes* respectively, after 15 days and reached at equilibrium. These results led to refer that the macroalgal biomass could form a potential, eco-friendly, cost effective and safe alternative biosorbent for fine tuning of waste water treatment.

Keywords: biosorption, macrophytes, lead

Introduction

Heavy metals released into the environment seriously affect the biological system, including human physiology (Kobe *et al*., 2005) [8]. One of the heavy metals, lead contaminates the environment through anthropogenic and natural sources. Plants absorb heavy metals fairly easily, which results in food contamination with high ranking concentrations. The World Health Organization (WHO) declared heavy metals are a human carcinogen (Christobel and Lipton, 2015) [5]. Heavy metal pollution has become a major issue in many countries because their existence in drinking waters and waste waters often exceed the permissible standards. Metal ions in the environment are bio-magnified in the food chain and accumulate in tissues; therefore, toxic effects of heavy metals in particular are especially found in animals of higher tropic levels and also in human. Heavy metals discharged into the aquatic environment will be bound predominantly to suspended materials and finally accumulate, in the sediment (sweetly *et al*., 2014) [13].

Lead (Pb) is one of the metals that are extremely toxic to organisms even at low concentration. It can damage the nervous system, gastrointestinal track, encephalopathy with pretreatment damage, kidneys and reproductive system particularly in children (Adeogun *et al*., 2010) [1].

Biosorption is an innovative technology that employs inactive and dead biomass for the removal and recovery of metals from aqueous solutions. Biomass from various sources such as bacteria, yeast, algae, fungi and plants have been used to adsorb metal ions from the environment (Sulaymon *et al*., 2013) [12].

The aim of the present research is to investigate the experimental and theoretical removal of lead, from simulated wastewater using macrophytes as a biosorbents. Batch experiments were carried out for kinetic studies on the removal of those ions from aqueous solution. The influence of various important parameters such as pH, contact time and initial concentration is investigated.

Materials and Methods

Sample Collection

For this study water samples and macrophytes (Fig. 2) (*Eichornia crassipes, Pistia stratiotes and Lemna minor*) were collected from the Jawahar Baal Udhyaan Lake, Bhopal in different season of 2012-13. The aquatic macrophytes were hand harvested fortnightly using the surface inventory method. The identification was made using a hand book of common aquatic plants (Okayi *et al*., 2011) [9]. It was observed that Jawahar Baal Udhyan pond Fig. 1 is a eutrophic wetland, 13 fish species, 25 phytoplankton and 19 zooplanktonic genera have been recorded (Tamot and Awasthi, 2013) [14].

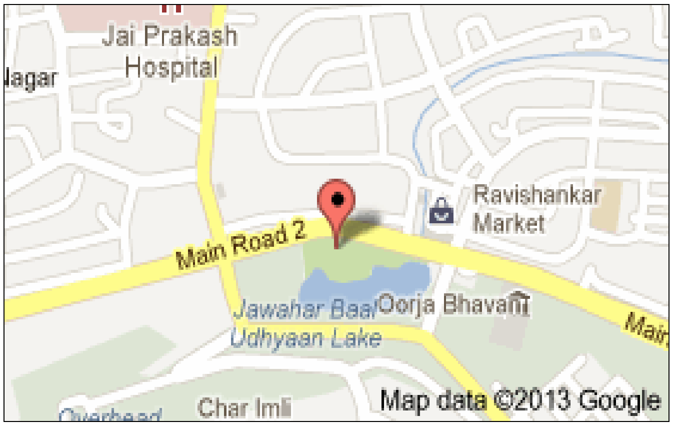

Fig 1: Location map of Jawahar Baal Udhyaan Lake

Floating aquatic macrophytes are defined as plants that float on the water surface, usually with submerged roots. Aquatic plants are suitable for water treatment because they have tremendous capacity of absorbing nutrients and other substances from the water and hence bring the pollution load down. Further noted that the main route of heavy metal uptake in aquatic plants was through the roots in the case of emergent and surface floating plants, whereas in submerged plants roots as well as leaves take part in removing heavy metals and nutrients (Dhote, 2007) [7]. The chemicals which were used in experiment were purchased from authorized dealer of Hi media. All chemicals were of analytical grade.

Fig 2: Samples of Macrophytes (A) *Eichornia crassipes* (B) *Lemna minor* and (C) *Pistia stratiotes*

Preparation of Bioadsorbent

We selected macrophytes in the same size, and then they were washed. After that, they were acclimatized for 15 days with distilled water and used as culture stocks which was ready to be used for further experiments Fig. 3. All macrophytes were hydroponically grown for 15 days in 2 liter of distilled water in 20x25x5 cm plastic tray at 30±2ºC in a polyhouse. They multiply vegetative and produced healthy biomass. The fresh biomass harvested and used as inoculums for further experiment.

Preparation of Chemical Pollutants

All chemicals used in this study were of analytical grade and solutions were prepared using deionized and distilled water. Stock metal solutions were prepared by dissolving appropriate amounts of $Pb(NO_3)_2$ lead nitrate in distilled water. To adjust the pH, 0.1 mol/L HCl and NaOH solutions were used. Measurement of pH was performed using pH meter. The solutions of 0.1 mol/L H_2O, HCl, H_2SO_4, $CaCl_2$ and NaOH were prepared for adsorption experiment. The experimental solution was prepared by diluting the stock solution with distilled water when necessary.

Fig 3: Preparation of biomass (A) *Lemna minor* (B) *Pistia stratiote* (C) *Eichornia crassipes* in lab

Batch Experiments

Experiments were conducted in a container with 2 liter tap water containing different concentration of lead. In one set of the experiments, lead was at concentration of 0.25, 0.50, 0.75, 1.0 and 1.25 ppm/l. There were three replicates of experiments. One control group of plants was also prepared where metal ion solutions were not added. Observed initial concentration of nutrients in raw inlet sample. Collected the samples at regular intervals of one week and observed metal concentration in each. Water quality parameters were analyzed by standard methods given in APHA (1999) 19th edition (Dhote, 2007) [7]. The effect of the initial Pb(II) ion concentration on the biosorption was studied at optimum pH and plants samples were collected on 3rd, 6th, 9th and 15th days of experiments and were subjected to analysis for metal concentrations in the plant tissue and biomass.

Determination of metals sorption

A Garrel-Ash 850 atomic absorption spectrophotometer with a detection range of 0.003-0.01/~g/ml was used to measure metal concentrations. Two blank determinations and four standard readings were taken following each batch of twelve measurements, Metal concentrations in water samples were determined by standard edition of APHA, 1999.

The removal efficiency and adsorption capacity were analyzed as following equations

$\% \text{ Removal} = [(Ci-Ce)/ Ci] \times 100$

Where: Ci and Ce are the initial and final concentrations (mg/L), respectively.

Statistics Analysis

In order to detect quantitative and qualitative differences in the observation made statistical analysis was performed.

Standard deviation was obtained from the variance by extracting the square root and was expressed in the unit in which the measurements were taken. To get better precision to the mean, the standard deviation was used with ± sign after the mean value. From the calculation of standard deviation the extent to which the entire sample is represented by mean can be determined.

The results were analyzed by analysis of variance (ANOVA) using Design Expert Software.

Results and Discussion

Effect of pH on Biosorption of Lead

The effect of pH on the sorption of lead by the macrophytes is presented in Fig. 4, 5 & 6. The pH of the aqueous solution was clearly an important parameter that controlled the biosorption process and physiology of aquatic plants. Uptake of lead by macrophytes was observed during the term of growth changes due to weight gain. In present study, plant growth was measured by biomass production in terms of fresh weight. Maximum metal removal efficiency reached 77.2% for *E. crassipes* at pH 7 due to high sorption of metal, plant fresh weight increased to 26.8g. The biosorption of lead by *P. stratiotes* increased with increasing pH of aqueous solution. At 7.5 pH maximum heavy metal, 53% was removed. *L. minor* shown highest absorption 19.8% at 7 pH (Table 1). It is well known that aquatic biomass irrespective living or dead, exhibits capacity to remove heavy metals from water. The increase in the fresh weight of the plants at different might be due to favorable nature of this essential element in plant growth. It is well known that aquatic biomass irrespective living or dead, exhibits capacity to remove heavy metals from water. The increase in the fresh weight of the plants at different might be due to favorable nature of this essential element in plant growth (Deval *et al.*, 2012). Pennesi *et al.*, 2012 investigate the effect of pH on lead biosorption by marine macrophytes. They observed that *Cymodocea nodosa* sorption ability increases with pH in the investigated range, with the highest performance (0.6 mmol/g) at pH 5 and the lowest at pH 3.3 (0.3 mmol/g). This good lead sorption ability can be attributed to the biochemical composition of the external layer constituents, such as cutin. In fact, carboxylic groups of this amorphous substance likely are responsible for the chemical and physical bond with the lead cation (Sanchez *et al.*, 1999) [11].

Table 1: Effect of pH on biosorption of lead by different Macrophytes

pH	Biosorption of lead (%)		
	L. minor	*E. crassipes*	*P. stratiotes*
5.5	7.99	64	23
6	10.8	68	34
6.5	14.4	72	39
7	19.8	77.2	42
7.5	18.4	60.8	53

$F_{2, 12}$= 58.87, MS= 3740.37, P>.05, Significant

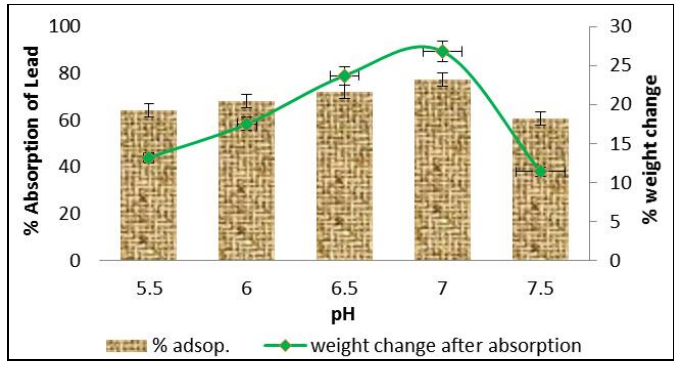

Fig 4: Biosorption of lead by *E. crassipes* at different pH with constant concentration (0.75ppm/L)

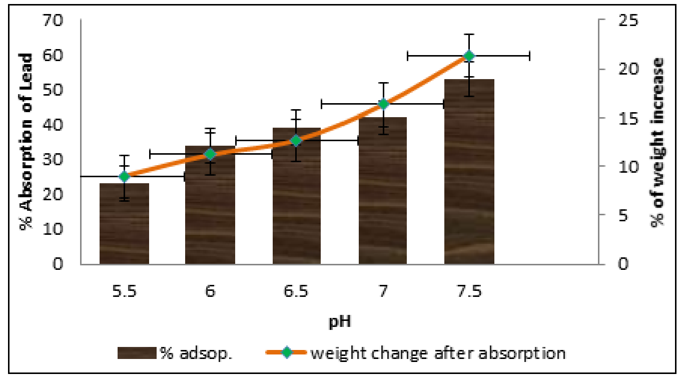

Fig 5: Biosorption of lead by *P. stratiotes* at different pH with constant concentration (1ppm/L)

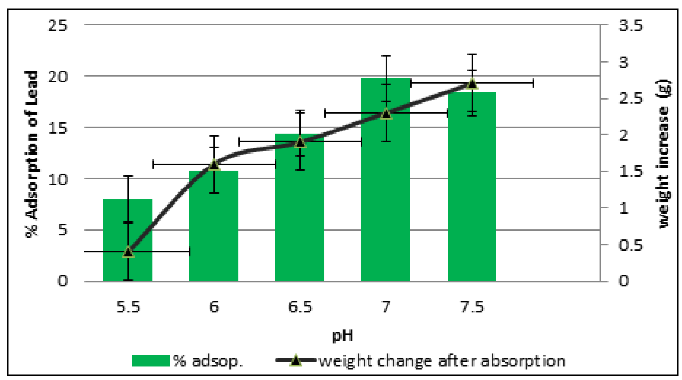

Fig 6: Biosorption of lead by *L. minor* at different pH with constant concentration (0.5ppm/L)

Effect of Contact Time on Biosorption of Lead

Contact time is one of the important parameters for successful use of the biosorbents for practical application and rapid sorption is among desirable parameters. The effect of contact time on the biosorption of Pb (II) onto macrophytes biomass (*L. minor, E. crassipes* and *P. stratiotes*) is shown in Fig. 7.

Uptake of metal ions occurred within 3 days and equilibrium was reached after 12 days for Pb (II). Therefore, the optimum contact time was selected as 15 days for further experiments (Table 2). In the study, the lead concentration was accumulated to 26% for *L. minor*, 34% for *P. stratiotes* and 36% for *E. crassipes* respectively, after 15 days and reached at equilibrium. Contact time is one of the important parameters for successful use of the biosorbents for practical application and rapid sorption is among desirable parameters. The metal uptake increased with increase incubation period (Brahmbhatt *et al.*, 2012) [4]. Interactions between plant and heavy metals have been demonstrated in solution culture (Alia *et al.*, 2004) [3].

Table 2: Effect of Contact Time on biosorption of lead by different Macrophytes

Contact time	% Biosorption of Lead		
Days	***L. minor***	***E. crassipes***	***P. stratiotes***
3	6	12	9
6	19	21	21
9	24	23	28
12	24	31	34
15	26	34	36

$F_{2,12}$=.524, MS= 133.2, P<.05, not Significant

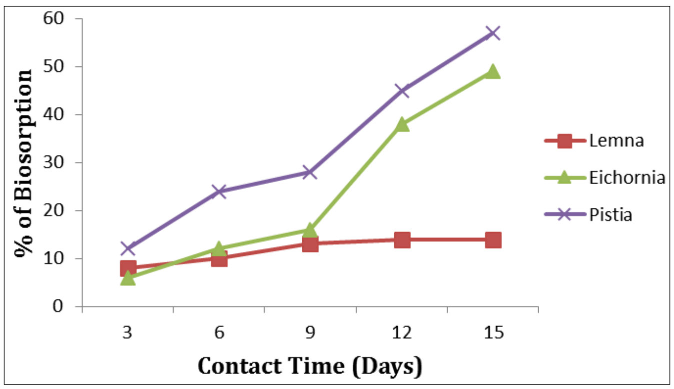

Fig 7: Effect of contact time on biosorption of lead at constant pH and metal concentration.

Effect of Lead concentration on biosorption

The effect of initial concentration on the percentage removal of lead by macrophytes is shows in Fig. 8 & Table 3. It can be seen from the graph that the percentage removal varied with Pb concentration. Macrophytes exhibited different sorption percentage at different concentration of lead. At higher initial concentrations, *P. stratiotes* showed greater percentage removal. Around 84% of lead was absorbed by *P. stratiotes* at 1 ppm concentration, beside this *L. minor* and *E. crassipes* showed maximum sorption at 0.5 and 0.75 ppm concentration of lead. The difference in percentage removal by different macrophytes was found due to the difference in their chemical affinity and ion exchange capacity with respect to the chemical functional group on the surface of the absorbent (Ahalya *et al.*, 2003) [2]. Yasar *et al.*, (2013) [15], reported different concentration of heavy metals on same macrophytes. Their findings concluded that over all *Pistia stratiotes* appeared to be best the accumulator with high bioconcentration factor (>100) for different heavy metals.

Table 3: Effect of concentration of lead on biosorption by different Macrophytes

Concentration	Biosorption of lead (%)		
(ppm)	***L. minor***	***E. crassipes***	***P. stratiotes***
0.25	56	40	32
0.50	79.6	58	60
0.75	74.5	81.33	60
1.0	26	67	84
1.25	25.6	56.8	84

$F_{2,12}$=.397 MS= 632.38, P<.05 not significant

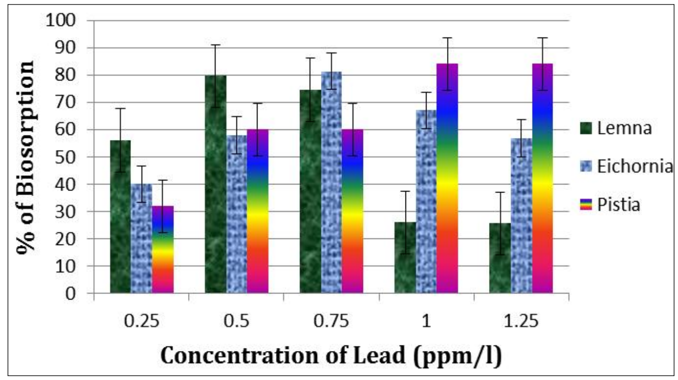

Fig 8: Effect of Pb concentrations on biosorption by macrophytes

Conclusion

Results of experiments could lead to the conclusion that the macrophytes effectively removed the lead ion from aqueous solution. This has tremendous potential as an economic, effective, safe alternative to existing commercial adsorbents. It is easy and effective technique for fine tuning of waste water treatment and removal of heavy metals from the various industrial wastes containing heavy metals. Due to their biosorptive properties and fast adsorption capability, algae biomass could be a potential method for cleaning up surface water or post-treatment of wastewater. The macrophytes biomass have capability to adsorb heavy metals at intermediate pH, ranging from pH 5 to 8.

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