Phytoremediation of various heavy metals (Cu, Pb and Hg) from aqueous solution using water hyacinth and its toxicity on plants

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Abstract
Heavy metals that enter into environment through anthropogenic sources cause adverse effects on living organisms. In this study, various toxic heavy metals (Cu, Pb and Hg) were removed from the aqueous solution using water hyacinth (Eichhornia crassipes) plants at various environmental conditions (retention time, initial concentration of heavy metals, organic loading rate and pH). Heavy metals highly affect the metabolic parameters (Ash, Protein, Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carbohydrate and Crude fibre) of plants. The individual and combined heavy metals were treated with water hyacinth and the metabolic parameters were analyzed, which shows that the reduction of all the essential components of plants. The data were analyzed for the biosorption studies using isotherm and equilibrium kinetic models. The predicted results revealed pseudo second order kinetics was considered as the most appropriate model which predicted the biosorption capacity of both water hyacinth root and shoot with comparison to the experimental results. Langmuir and Freundlich isotherms were used to evaluate the experimental data and their constants were derived.

Key words: Heavy metals, Eichhornia crassipes. Metabolic changes. Langmuir and Freundlich isotherms. Pseudo first and second order kinetic models.

1. Introduction
Since metals plays an important role in industrial applications, removal of them from aquatic environment poses an important environmental issue. Additionally, some of the metals are essential for the growth and development of living organisms. However, many heavy metals are highly toxic when the concentration exceeds certain limits [1]. In recent years pollution in the aquatic environment by heavy metals become acute, because metal ions from natural, domestic and industrial sources tend to concentrate in the organic residue at the sewage treatment works. Several studies have shown that constructs wetlands are very effective in removal of heavy metals from polluted wastewaters [2]. Conventional techniques for removing heavy metals such as chemical precipitation, carbon adsorption, ion exchange, evaporation and membrane processes [3] and are only practical and cost effective when applied to high strength wastes with heavy metal ion concentrations greater than 100 ppm [4, 5]. The laboratory studies demonstrated that the potential use of water hyacinth plants in removing metals from polluted water and have shown that metal concentrations of the plant and the water column are correlated [6].

The sorption characteristics have shown to be influenced by pH, metal ion concentration, temperature and other metal ions [3]. When the water hyacinth plants were treated with heavy metal ions (Hg), the physiological response (chlorophyll, fresh and dry weight, etc.) was decreased [7]. The present study describes the biosorption of Cu(II), Pb(II) and Hg(II) ions by water hyacinth plants at various pH, initial concentrations of metal ions and organic loading rate. The metabolic changes in the plant were investigated through exposure of the plants to various individual and combined heavy metal ions. The biosorption data were analyzed with Langmuir and Freundlich isotherm models and Pseudo – first and second order equilibrium kinetic models.
the present study. Plants with uniform size were selected to reduce errors in the experiment.

2.2. Reagents
A stock solution of Cu, Pb and Hg metal ions (1000 mg l\(^{-1}\)) was prepared by dissolving copper sulphate (CuSO\(_4\cdot5\)H\(_2\)O), lead (II) acetate (Pb(C\(_2\)H\(_3\)O\(_2\))\(_2\).3H\(_2\)O) and mercuric chloride (HgCl\(_2\)) separately and respectively in double distilled water and diluted to get the desired concentration with ddH\(_2\)O. The concentration of metal ions was measured using atomic absorption spectrophotometer (GBC 96-Phorton lamp) [8].

2.3. Experimental setup
Healthy preweighed plants (150 ± 15 g) were exposed to 25, 50, and 100 mg l\(^{-1}\) of Cu(II) and Pb(II) ions and 5, 10 and 15 mg l\(^{-1}\) of Hg(II) ions containing aqueous solution. The experiments were performed as triplicates. Sewage with metal ions was used as negative control and plants dipped in dH\(_2\)O without metal ions was used as positive control. The amount of Cu, Pb and Hg ions from plants (Root and shoot) were estimated at every two days interval by drying in hot air oven, macerated and digested with diacid method (HCl and HNO\(_3\)) followed by standard methods for examination of water and wastewater [9]. The digested samples were analyzed for the estimation of metal ions using AAS. The concentration of metal ions also estimated in both positive and negative control to find out the final concentration of metal ions in plants after the experiment.

2.4. Biosorption studies
The experiments on biosorption of metal ions were conducted in plastic tubs containing 5 liters of heavy metal solution and 150 ± 15 g of fresh water hyacinth plants (batch biosorption experiments). The effect of pH, initial concentrations of metal ions and organic loading rate on the biosorption rate was studied. The effect of pH (3, 5, 7 and 9) and organic loading rate (25, 50 and 75%) on biosorption of metal ions from aqueous solution using water hyacinth was investigated. The effect of initial metal concentrations on biosorption was studied at 25, 50 and 100 mg l\(^{-1}\) for Cu and Pb and 5, 10 and 15 mg l\(^{-1}\) for Hg ions. After the desired retention period (1, 3, 7 and 15 days), plant parts (root and shoot) were acid digested and analyzed in AAS.

2.5. Data analysis
The amount of metal ions per unit biosorbent was obtained by using the following expression [10].

\[ q = [(C_0 - C_1) V / M] \quad (1) \]

where \( q \) is the amount of heavy metal onto the unit amount of biosorbents (mg g\(^{-1}\)) and \( C_0 \) and \( C_1 \) are the concentrations of metal ions from the solution (mg l\(^{-1}\)) before and after biosorption respectively; \( V \) is the volume of the aqueous phase and \( M \) is the amount of the biosorbent (g).

2.6. Kinetic modeling
2.6.1. The Pseudo first order equation
The Pseudo first order equation [11] is generally expressed as follows,

\[ dq_t / dt = K_1 (q_{eq} - q_t) \quad (2) \]

where \( K_1 \) is the rate constant of pseudo – first order biosorption (min\(^{-1}\)) and \( q_{eq} \) and \( q_t \) denote the amount of biosorption equilibrium at time \( t \) (mg g\(^{-1}\)), respectively. After integrating by applying boundary conditions, \( q_t = 0 \) at \( t = 0 \) and \( q_t = q_{eq} \) at \( t = t \), gives

\[ \log (q_{eq} / q_t - q_t) = (K_1 t) / 2.303 \quad (3) \]

The equation (3) can be rearranged to obtain a linear form

\[ \log (q_{eq} / q_t - q_t) = log (K_1 t) / 2.303 \quad (4) \]

A plot of \( \log (q_{eq} - q_t) \) against \( t \) should give a straight line to confirm the applicability of the kinetic model. In a true first – order possess \( q_{eq} \) should be equal to the intercept of a plot of \( \log (q_{eq} - q_t) \) against \( t \).

2.6.2. The Pseudo – second order equation
If the rate of sorption is a second order mechanism, pseudo second order chemisorption kinetics rate equation [12] is expressed as equation (4)

\[ dq_t / dt = K_2 (q_{eq} - q_t)^2 \quad (5) \]

where, \( K_2 \) is the rate constant of Pseudo – second order absorption (g mg\(^{-1}\) min\(^{-1}\)). For the boundary conditions \( t = 0 \) and \( q_t = 0 \) at \( q_t = q_{eq} \) integrated form of the equation (5) becomes:

\[ 1 / (q_{eq} - q_t) = 1 / (K_2 q_{eq}^2) + (1 / q_{eq}) t \quad (6) \]

This is the integrated rate law for pseudo second order reaction. The above equation (6) is rearranged to obtain a linear form:

\[ (t / q) = 1 / (K_2 q_{eq}^2) + (1 / q_{eq}) t \quad (7) \]

A plot of \( t / q \) vs \( t \) should give linear relationship for the applicability of the second order kinetics. The rate constant \( K_2 \) and biosorption at equilibrium \( q_{eq} \) is obtained from the intercept and slope respectively.

2.6.3. Adsorption isotherms
The Langmuir model is described by the following equation

\[ q_e = \frac{K_L C_e}{1 + K_L C_e} \quad (8) \]

where \( C_e \) and \( q_e \) also shows residual metal concentration and the amount of metal on the biosorbent at equilibrium. The \( K_L = K_2 / K_1 \) is the Langmuir constant of the system. The semi reciprocal plot of \( C/q \) vs \( C \) was employed to generate the intercept \( K_L / q \) and the slope \( 1/q \).

The Freundlich equation is the empirical relationship for assumption of biosorption energy of a metal binding site on biosorbent [13, 14]. The Freundlich model is described by,

\[ q_e = K_F C_e^{1/n} \quad (9) \]

\( q_e \) - Metal uptake at equilibrium concentration mg g\(^{-1}\); \( C_e \) – Equilibrium metal ion concentration, mg g\(^{-1}\); \( K_F \) - Freundlich’s constant of biosorption capacity, \( n \) – Freundlich’s constant of biosorption intensity. The \( K_F \) was estimated from intercept and \( n \) from slope.

2.7. Metabolic changes in plants
The metabolic parameters (Ash, Chlorophyll a (Ca), Chlorophyll b (Cb), Total Chlorophyll (TC), Carbohydrates, Proteins and Crude fibre) of the plants before and after exposure to individual (Cu and Pb - 50 mg l\(^{-1}\) and Hg - 10 mg l\(^{-1}\)) and combined metal ions (Cu + Pb, Cu + Hg, Pb + Hg, Cu + Pb + Hg) were studied in laboratory scale [15].

3. Results and Discussion
3.1. Effect of initial concentration of metal ions on biosorption
The time required for the biosorption of metal ions on water hyacinth was studied at various initial concentrations...
Fig. 1: Biosorption of metal ions (Cu, Pb and Hg) at (a) various initial concentrations of metals Cu and Pb, (b) various initial concentrations of Hg, (c) at various organic loading rates and (d) at various pH.

Fig. 2: Changes in physico-chemical properties (Ash, Protein, Crude fibre and Carbohydrate) of water hyacinth before and after exposure of individual and combined heavy metal ions (Cu, Pb and Hg).

Fig. 3: Changes in physico-chemical properties (Chlorophyll a (Ca), Chlorophyll b (Cb) and Total chlorophyll (TC)) of water hyacinth before and after exposure of individual and combined heavy metal ions (Cu, Pb and Hg).

(25, 50 and 100 mg l\(^{-1}\) for Cu and Pb ions and 5, 10 and 15 mg l\(^{-1}\) for Hg ions) with 150 ± 15 g of the biosorbent at different intervals. The biosorption capacity sharply increases with increase in time and attains steady state at 15\(^{th}\) day. The rate of biosorption was increased with increasing initial concentration of metal ions and found to reach steady point at 100 mg l\(^{-1}\) of Cu and Pb ions and 15 mg l\(^{-1}\) of Hg ions. Fig – 1(a) explains the effect of various concentrations of metal ions (Cu and Pb ions) on biosorption of Cu and Pb ions respectively by the plant biomass. The amount of Cu absorbed by water hyacinth was 20, 41 and 72 mg l\(^{-1}\) at the initial concentration 25, 50 and 100 mg l\(^{-1}\) respectively. Similarly the biosorption of Pb ions using water hyacinth was 18, 39 and 60 mg l\(^{-1}\) at the initial concentration 25, 50 and 100 mg l\(^{-1}\) of Pb ions respectively. Fig – 1(b) shows that the effect of various concentrations of Hg ions on the biosorption of Hg ions using water hyacinth. The amount of Hg ions absorbed by water hyacinth was 2, 7 and 8 mg l\(^{-1}\) at the initial concentration 5, 10 and 15 mg l\(^{-1}\) respectively. The result of this study infers that water hyacinth plants have the ability to absorb a large amount of various metal ions present in aqueous solutions. Compared with shoot, root has the ability to absorb a large amount of metal ions. A little quantity of metal ions was absorbed in the shoot of the plant. The rate of biosorption was higher at the beginning due to large available surface area of the biosorbent. At equilibrium, the rate of uptake was controlled by the rate of transported biosorbent from the exterior to the interior sites of the biosorbent particles [16]. Vallisneria showed 70, 84 and 70 % removal for the same initial metal concentrations [17]. Roots of water hyacinth accumulate 2000 mg l\(^{-1}\) of Pb(II) ions but shoots accumulate 700 mg l\(^{-1}\) of Pb(II) ions from aqueous solution [18]. Wheat straw has the ability to absorb 108 mg g\(^{-1}\) of lead ions [19]. Our results coincide with the previous studies and suggested that water hyacinth is the efficient weed that will be helpful for the treatment of various organic and inorganic pollutants containing wastewaters and finally the wastewater containing permissible level of nutrients which can be utilized for irrigation purpose.

3.2. Effect of organic loading rate on biosorption

Biosorption of chosen metal ions (Cu, Pb and Hg) by water hyacinth was analyzed at various organic loading rates (25, 50 & 75%). Fig – 1(c) shows that the biosorption of metal ions at various organic loading rates. Biosorption rate of metal ions was increased at 50% of organic loading rate. The compounds present in the sewage may interfere with the binding sites of metal ions present in the plant material. Because of this interference, the biosorption rate was little bit lower than other optimized parameters. But the organic content present in the sewage enhance the growth of the plants after treatment. One of the earlier work revealed that the physicochemical parameters (pH, conductivity, BOD, COD, Hardness, nitrate nitrogen and ammoniacal nitrogen) of sewage were greatly reduced after the treatment of sewage with water hyacinth at the organic loading rate – 75% [20]. So the organic loading rate may enhance the ability to reduce the physico-chemical and biological parameters of the polluted waters and also to enhance the...
biosorption of metal ions from the aqueous phase.

3.3. Effect of pH on biosorption
pH, an important factor that greatly affects the various biological and biochemical functions of living system. Biosorption of Cu, Pb and Hg ions by water hyacinth was studied at various pH (3, 5, 7 & 9) and the biosorption was increased at pH 7 and gradually decreased at pH 9 (Fig – 1(d)). The plants were dried at 5º day of incubation time at pH – 3, because of the intolerance of acidity and results biosorption of low amount of metal ions. The amount of biosorbed metal ions from water hyacinth plant biomass was 43 mg l⁻¹ of Cu, 45 mg l⁻¹ for Pb and 8.2 mg l⁻¹ of Hg ions at pH 7. The metal biosorption was depended onto the protonation and deprotonation of the carboxyl groups, which had pKa between 3 and 4 [21]. It was suggested that at lower pH value the surface of the biosorbent was surrounded by hydronium ions (H₃O⁺) [22]. The decrease in biosorption at particular pH values was due to the competitiveness of the oxyanion of chromium and OH⁻ ions in the bulk. *Sphaerotilus natans* was able to reach the maximum Pb(II) removal yield at pH 7 [23]. As pH increases deprotonation starts and thereby results in decreased adsorption capacity [24]. The increased pH resulted in the increased biosorption capacity [25]. Grasses were found to show two times higher uptake of lead ions at pH 5 [19]. The final pH of the solutions was less than the initial pH because of the simultaneous release of H⁺ ions with the uptake of metal ions [26].

3.4. Biosorption kinetic modeling
The rapid kinetics had significant practical importance, which facilitated smaller reactor volumes ensuring high efficiency and economy (the first and second order equations), used in assuming measured concentrations. The experimental biosorption capacities and theoretical values for water hyacinth were compared by pseudo - first and second order rate equations (Table – 1). The theoretical q_ₑₒₓₐ values estimated from the first order kinetics gave significant different values when compared to experimental values and the correlation coefficients were found to be slightly lower. The correlation coefficients for the linear plots of t / qt against t for the second order equation were greater than 0.99 of 360 h for the plant biomass. The theoretical q_ₑₒₓₐ values were very close to the experimental values in the case of pseudo second order kinetics. Our results are coincide with the results that suggested the second order mechanism for biosorption of heavy metals using water hyacinth was predominant [27, 28, 29]. The results showed that the biosorption systems were best described by pseudo second order kinetic model than pseudo first order kinetic model. The study of sorption kinetics describe uptake of biosorbent rate and was controls the residence time of biosorbate at the solid – liquid interface [10]. The kinetics of lead ions sorption on bacterial biosorbents was analyzed using different kinetic models, these include, the pseudo first order [11] and pseudo second order [12]. The agreement between the experimental data and the model predicted values for each model was expressed by the coefficient of determination (r²). A relatively high r² value indicates that the model so examined successfully describes the kinetics of metal ions biosorption onto the plant biomass.

| **Table 1**: The Pseudo first and second order kinetics constant for biosorption of lead ions on water hyacinth root and shoot |
|----------------|----------------|----------------|----------------|----------------|
| Heavy Metal | Experimental qₑₒₓₐ (mg g⁻¹) | Pseudo first order | Pseudo second order |
| | | qₑ (mg g⁻¹) | Kᵣ × 10⁻¹ (min⁻¹) | R² | qₑ (mg g⁻¹) | Kᵣ × 10⁻⁹ (min⁻¹) | R² |
| Cu | 49 | 11 | 1.18 | 0.996 | 53 | 0.27 | 0.999 |
| Pb | 48 | 10 | 1.26 | 0.991 | 50 | 0.3 | 0.999 |
| Hg | 9.3 | 10 | 0.67 | 0.979 | 10 | 12.1 | 0.998 |

| **Table 2**: Langmuir and Freundlich isotherm model constant and correlation coefficient for biosorption of lead ions on water hyacinth root and shoot |
|----------------|----------------|----------------|----------------|----------------|
| Biosorbents | Experimental qₑₒₓₐ (mg g⁻¹) | Langmuir constant | Freundlich constant |
| | | qₘ (mg g⁻¹) | Kᵣ × 10⁻⁹ | R² | Kₕ | N | R² |
| Cu | 49 | 50 | 0.18 | 0.943 | 0.19 | 7.4 | 0.996 |
| Pb | 48 | 47 | 0.21 | 0.965 | 0.18 | 6.7 | 0.989 |
| Hg | 9.3 | 9.4 | 10.1 | 0.988 | 0.17 | 4.4 | 0.976 |
3.5. Langmuir and Freundlich sorption isotherms

The Langmuir constants ($q_m$ and $k_L$) along with correlation coefficients ($R^2$) was calculated and results were given in Table – 2. The maximum capacity $q_m$ determined from Langmuir isotherm defined the total capacity of the biosorbents for metal ions. The order of maximum capacity ($q_m$) for the biosorbents of metal removal were found to be roots > shoots of water hyacinth. It is clear that the increase of $q_m$ value was due to sorption sites of biosorbents. The presence of small $k_L$ value indicated that the metal ions had high binding affinity for the biosorbent and $k_L$ values were presented in Table – 2. The $k_L$ values for the biosorption of metal ions were 0.18, 0.21 and 10.1 for Cu, Pb and Hg ions respectively. The sorption isotherm model was used to characterize the interaction of metal ions with the plant biomass. The Langmuir model is based on the assumption that maximum biosorption occurs when a saturated monolayer of solute molecules is present on the biosorbent surface, the energy of biosorption is constant and there is no migration of biosorbate molecules in the surface plane [30, 31]. *Lemna minor* and *Spirodela intermedia* were presented the highest metal removal percentage and the metal biosorption data fitted Langmuir model only for Ni and Cd, evidencing in this case, sorption may be as a monolayer [32].

The Freundlich constants $K_F$ and $n$ showed easy separation of metal ions from aqueous medium and indicated favorable biosorption [33]. The Freundlich plot for the biosorption of metal ions by water hyacinth with intercept $K_F$, 0.19, 0.18 and 0.17 for biosorption of Cu, Pb and Hg ions respectively and the absorption capacity of the biosorbents with slope 1/n, 7.4, 6.7 and 4.4 for the biosorption of Cu, Pb and Hg ions respectively (Table – 2). The $n$ values showed easy uptake of metal ions from aqueous medium with high biosorption capacity. In these systems the metal removal process was based on solid liquid contacting and separation process. In the present investigation, Freundlich model fitted well than Langmuir isotherm model for the biosorption of metal ions from aqueous solutions by water hyacinth. *Lemna minor* and *Spirodela intermedia* were presented the highest metal removal percentage and the metal biosorption data fitted Freundlich isotherm [32].

3.6. Metabolic changes in plants

The changes metabolic properties of water hyacinth were analyzed before and after exposure to individual and combined metal ions (Cu, Pb, Hg, Cu + Pb, Cu + Hg, Pb + Hg and Cu + Pb + Hg). Fig – 2 show the changes in the concentration of ash, protein, crude fibre and carbohydrate. The maximum reduction of ash, protein, crude fibre and carbohydrate was observed during the treatment of plant with Cu, Pb, Hg and Cu + Pb + Hg ions respectively. When compared with other parameters protein shows greater reduction than other parameters (85%). These results revealed that protein was more sensitive than ash, crude fibre, carbohydrate, chlorophyll a, b and total chlorophyll towards the metal ions (Cu, Pb and Hg). Fig – 3 show the changes in the chlorophyll concentration. The maximum reduction in the concentration of chlorophyll a (Ca) and total chlorophyll (TC) was observed in the treatment of plants with Cu + Pb + Hg ions whereas the reduction in the concentration of chlorophyll b (Cb) was observed in the treatment of plants with Cu + Hg ions. The metabolic properties of plants were greatly affected due to the biosorption of metal ions. Metal ions are well known to affect the structure and function of chloroplasts in many plant systems such as *Beta vulgaris* [34], *Spinacea oleracea* [35] and *Zea mays* [36]. Metal ions specifically inhibit chlorophyll biosynthesis through δ-aminolevulinic acid dehydratase (ALA dehydratase) [37] and protochlorophyllide reductase [38] because of the oxidation prone –SH group [39] leading to the lower production of 5-aminolevulinic acid (ALA), the first common precursor for all the tetrapyrroles, thereby impairing chlorophyll biosynthesis. The reduction in chlorophyll content was directly proportional to the concentration of toxicants and period of exposure [40]. The reduction in carbohydrate content due to pollution might be the result of decreased photosynthesis. On the other hand, a decrease in protein could be attributed to both breakdown of existing proteins and reduces de novo synthesis. Such a reduction in protein content may be utilized as a suitable bioindicator of pollution.

4. Conclusions

Water hyacinth has been successfully used as the biosorbent for removal of different metal ions (Cu, Pb and Hg) from metal enriched aqueous solution. The biosorption of metal ions depends on the experimental conditions particularly organic loading rate, pH and concentration of metal ions in the medium. The result from this study shows that the organic loading rate, pH and lead ions concentration highly affects and influence the overall metal uptake capacity of biosorbent. The biosorption rate of metal ions by the biosorbent seems to follow the pseudo second order kinetics. The Freundlich and Langmuir sorption models were employed with the mathematical description of biosorption equilibrium data. The calculated isotherm constants were used to compare the biosorptive capacity at different experimental data for the removal of metal ions. The result of this investigation demonstrates that Langmuir and Freundlich models were fitted well for the biosorption of all metal ions from the aqueous solution. At higher concentration, the individual and combined heavy metals are toxic to the plants and inhibit the synthesis of chlorophyll a, chlorophyll b, total chlorophyll, ash, protein, crude fibre and carbohydrate.

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