Comparative analysis of floating and submerged macrophytes for heavy metal (copper, chromium, arsenic and lead) removal: sorbent preparation, characterization, regeneration and cost estimation

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ABSTRACT
In this study, a comparative evaluation of floating and submerged macrophytes was performed. *Azolla filiculoides* (free floating) and *Hydrilla verticillata* (submerged) aquatic macrophytes were utilized for arsenic, copper, chromium and lead removal from the respective metallic ion solutions. Batch experiments were performed initially with optimization of different physical parameters viz., pH, initial heavy metal concentration, biosorbent dosage, contact time, temperature and agitation speed. Submerged (*Hydrilla verticillata*) had depicted better removal efficiency in comparison to the floating macrophyte (*Azolla filiculoides*). Field emission scanning electron microscopy equipped with energy dispersive spectroscopy and Fourier transform infrared spectroscopy analysis was performed for the characterization of the metal loaded biosorbents. Biosorption of the respective heavy metal was clearly depicted in the FESEM-EDX spectrum, although not much change in the morphology of the biosorbents were examined. FTIR spectra of the biosorbents obtained after the experiments confirmed the involvement of C–H bend, –CH2–(C=O), N–H, –C–O, R2–C= bending and –C–C=O on the biomass. Furthermore, the biosorbent regeneration followed by heavy metal biosorption confirmed the reusability of the prepared biosorbent for at least two consecutive cycles without much significant change in the heavy metal biosorption capacity.

1. Introduction
The rapid increase in the anthropogenic activities such as mining operations, smelting, chemical, paint, fertilizer, pesticide processing plant, leather industries and electronic manufacturing discharge has increased the amount of metal-containing wastewater into the aquatic environment (Goswami, Manikandan, Pakshirajan, & Pugazhenthi, 2017; Yu, Li, & Liu, 2017). The discharge of heavy metals into the environment without any prior treatment is diminishing the water quality to frightening levels, thereby posing its lethal effect on to the living organisms (Martin, Turnbull, Rissmann, & Rieger, 2017). Heavy metals tend to accumulate and concentrate in the living tissues through the food chain, imposing its adverse impact on human health (Etesami, 2018). Henceforth, treatment of wastewater containing heavy metals prior to their discharge is mandatory due to the increasingly restrictive legislation (Arul Manikandan, Alemu, Goswami, Pakshirajan, & Pugazhenthi, 2016).

For the treatment of wastewater contaminated with heavy metals, numerous treatment technologies viz., adsorption, coagulation, chemical precipitation, flocculation, electro-floatation, electrochemical treatment, ion exchange, reverse osmosis, advanced oxidation and membrane separation have been utilized (Anastopoulos & Kyzas, 2015). Among aforementioned techniques, biosorption is found to be the most economical, efficient, passive and widely accepted process, as biosorbents are eco-friendly, effective, readily available in huge quantity and also does not produce any by-products after the treatment (Yu et al., 2017).

Various literatures have been reported with the aim of utilizing different biosorbents, such as chitosans, nano-biosorbents, barks, wastes from food and edible oil industries and agricultural wastes, for the heavy metal removal (Anastopoulos & Kyzas, 2015). Taking into consideration of the above-mentioned aspects, present work was emphasized on heavy metal removal from the simulated aqueous heavy metal solutions individually utilizing macrophytes. Earlier several researches had performed this phenomenon by accounting macrophytes as a potential biosorbent and some have been enlisted in Table 1. An attempt was made for the comparative analysis between two different categories of macrophytes viz., floating and submerged and hence was performed.
to examine the removal efficiencies. The heavy metals tested in this study were copper (Cu (II)), chromium (Cr (VI)), arsenic (As (III)) and lead (Pb (II)). Optimization of the batch study was performed individually to determine the optimal biomass dose, heavy metal concentration, pH, contact time and temperature for heavy metal removal from the aqueous solution. Furthermore, the heavy metal removal process was further investigated employing field emission scanning electron microscopy (FESEM) equipped with energy dispersive spectroscopy (EDX) and Fourier transform infrared spectroscopy (FTIR) analyses.

2. Materials and methods

2.1. Metal stock solution preparation

Analytical grade chemical salts and reagents were utilized throughout the experiment procured from Merck (Mumbai, India). Individual heavy metal stock solutions of Cu (II), Cr (VI), As (III) and Pb (II) of concentration 1000 mg L\(^{-1}\) each were prepared using CuCl\(_2\)·H\(_2\)O, K\(_2\)Cr\(_2\)O\(_7\), As\(_2\)O\(_3\) and Pb(NO\(_3\))\(_2\), respectively, in deionized distilled water. Subsequent volume of metal solution was prepared with proper dilution.

2.2. Collection and pre-treatment of fresh water macrophytes

Freshwater macrophytes, *Azolla filiculoides* (free floating) and *Hydrilla verticillata* (submerged), as the potential biomass were collected from the pond surface near Salori, Allahabad, India (eastern shore of Yamuna River). The samples were washed thrice with deionized water (approximately 18.1 Ω resistance) to remove extraneous materials (each time; 50 mL for 20 min) and was air-dried. *Azolla* and *Hydrilla* species (as the non-living biomass) were then crushed in mortar-pestle and sieved to the powder form (size – 0.075 mm) prior to their utilization. Pre-treatment for the activation of the samples was performed by soaking 2 g of each sample in NaOH (1.0 M) at pH 9.5 for 5 h. Already it is well established that demethylation of pectin results in enhancement of –COOH groups, and may be catalysed using alkali solutions (Rakhshaee, Khosravi, & Ganji, 2006). Pectin is protonated to remove excess cations such as Na\(^+\) or Ca\(^{2+}\) that could interfere with heavy metal sorption and to generate a more distinct and suitable biosorbent. Subsequently, for removing the excess sodium (Na), the samples were washed twice with deionized water (50 mL, 5 min). Further, the samples were soaked in 250 mL of CaCl\(_2\) solution with the total concentration of 2 M and volume ratio of 2:1:1, respectively. pH was adjusted with 0.5 M HCl at 7.0 ± 0.2. These activated samples were oven-dried at 65° ± 2°C for 8 h i.e., until they reached a constant weight. The agitation rate for the activation was fixed at 150 rpm at 25° ± 2°C. Followed by drying, grinding and sieving the samples for their further utilization in the absorption experiments.

2.3. Biosorbent characterization

The prepared biosorbents (after the pre-treatment) were further characterized chemically, physically and morphologically as detailed ahead.

| S. No. | Biosorbent | Heavy metal | Maximum uptake capacity | pH | Isotherm | Removal (%) | References |
|--------|------------|-------------|-------------------------|----|---------|-------------|------------|
| 1.     | *Azolla filiculoides* | Cs | 195 | 8 | Freundlich | – | Mashkani and Ghazvini (2009) |
| 2.     | *Azolla filiculoides* | Sr | 212.1 | 9 | – | 98.2 | Umali, Duncan, and Burgess (2006) |
| 3.     | *Azolla filiculoides* | Au | 98 | – | – | 100 | Antunes, Watkins, and Duncan (2001) |
| 4.     | *Azolla filiculoides* | Ni | 45.32 | 7.8 | Langmuir | – | Ahmady, Mohammadi, Brahimi, Monfared, and Jafari (2011) |
| 5.     | *Azolla filiculoides* (Immobilized) | Cu | 363 | – | – | 75 | Fogarty et al. (1999) |
| 6.     | *Azolla filiculoides* | Cr | 10.6 | 2 | Langmuir, Freundlich | 83.34 | Babu, Sumalatha, Venkateswarulu, Das, and Kodali (2014) |
| 7.     | *Azolla filiculoides* | Zn | 45.2 | 6.0 | – | – | Zhao et al. (1999) |
| 8.     | *Azolla filiculoides* | Ni | 43.4 | 6.5 | Batch and Column study | 60 | Zhao and Duncan (1998) |
| 9.     | *Hydrilla verticillata* | Pb | 2.14 | 5.0 | Pseudo-second-order | 85.7 | Chathuranga et al. (2014) |
| 10.    | *Hydrilla verticillata* | As | 11.65 | 6 | Langmuir | – | Nigam Vankar and Gopal (2013) |
| 11.    | *Hydrilla verticillata* | Cd | 15 | 5 | Langmuir | – | Bunluesin et al. (2007) |
| 12.    | *Hydrilla verticillata* | Cd | 50 | 6 | – | – | Huang et al. (2010) |
| 13.    | *Hydrilla verticillata* | Cr | 29.43 | 4 | Langmuir and Freundlich | – | Mishra, Tripathi, and Rai (2014) |
| 14.    | *Hydrilla verticillata* | Cr | 8–69.9 | 1 | Langmuir | >60 | Pilli, Goud, and Mohanty (2016) |
| 15.    | *Hydrilla verticillata* | Cr | 89.32 | – | – | – | Mishra, Tripathi, and Rai (2016) |
| 16.    | *Hydrilla verticillata* | Cr | 247 | 3 | Langmuir | – | Baral, Das, Chaudhury, and Das (2009) |
The ash content of the two biosorbents was analysed by following the ASTM D1762-84 standard. 1 g of each sample was incinerated at 650 °C in presence of air for 12 h in a muffle furnace (Lab'Tech, India). The elemental compositions of the biosorbents were determined using an elemental analyzer (Eurovector EA3000, Germany). The zeta potential (ZP) and average particle size were measured using a laser particle size analyzer (Delsa™ Nano, Beckman Coulter). For examining the contact angle (CA), the sessile drop method was followed (Bachmann, Goebel, & Woche, 2013).

Surface morphology of the biosorbents was analysed by utilizing field emission scanning electron microscope at ultra-high resolution (FESEM, Zeiss, Sigma, Germany) equipped with energy dispersive spectroscopy (FESEM-EDX). The sample was surmounted onto the copper stub using a carbon tape and a fine doubled-coating of gold were performed prior to the analysis for making the samples more conductive. The sample images were recorded at an operating condition of 3.0 kV (Goswami, Kumar, Arul Manikandan, Pakshirajan, & Pugazhenthi, 2017).

For determining the surface functional groups present in the two biosorbents was analysed using a Fourier transform infrared (FTIR) spectrophotometer (IR Affinity, Shimadzu, U.S.A.). An average of five scans was collected for each analysis (400–4000 cm⁻¹) (Goswami, Tejas Namboodiri, Vinoth Kumar, Pakshirajan, & Pugazhenthi, 2017).

2.4. Experimental set-up

All the batch biosorption experiments were performed in the 250 mL Erlenmeyer flasks with a working volume of 100 mL. For determining the effect of process parameters onto the biosorption phenomena, all the experiments were performed by taking one parameter at a time. The various process parameters were as follows: pH of the solution (2–7), solution temperature (293, 303 and 313 K), initial metal concentration (10, 20 and 50 mg L⁻¹), biosorbent dose (1–5 g L⁻¹), contact time (0–180 min) and agitation speed (50–300 rpm). All the flasks were kept in the rotary shaker with the respective process parameters for determining the biosorption efficiency. A brief schematic of the overall experiment is shown in Figure 1.

2.5. Heavy metal quantification

The quantification of heavy metals after the batch shake experiments was reported in the percentage removal according to the equation (1). Heavy metal concentrations were analyzed by atomic absorption spectroscopy (Perkin Elmer Analyst 400, England) as per the American Public Health Association standards (American Public Health Association [APHA], 2005).

Heavy metal removal (%) = \( \frac{(C_o - C_e)}{C_o} \times 100 \) (1)

where \( C_o \) and \( C_e \), the initial and final heavy metal concentrations (mg L⁻¹) present in the solutions, respectively.

2.6. Regeneration of the exhausted biosorbents and cost estimation

For determining a process to be cost-effective, desorption experiments were performed using 0.5 N HCl and 0.5 N NaOH solutions as the stripping agents. Metal loaded biomass after the biosorption was further transferred to the flasks and kept in an orbital shaker for 24 h. Following this, filtrates were further analysed to examine the percentage fraction of desorbed Cu (II), Cr (VI), As (III) and Pb (II) metal ions to examine the percentage fraction of desorbed. Successive biosorption–desorption cycles were repeated thrice for the similar biosorbents.
(Kaur, Singh, Khare, Cameotra, & Ali, 2013). Desorption efficiency for both the biosorbents was further evaluated from the quantitative amount of metal ions desorbed to the metal ions adsorbed in the desorption medium, as per the expression (2) (Yoonaiwong, Kaewsarn, & Reanprayoon, 2011):

\[
\text{Desorption efficiency (\%)} = \frac{\text{Amount of metal ion desorbed}}{\text{Amount of metal ion adsorbed}} \times 100
\]

### 3. Results and discussion

The raw biomass of both the aquatic macrophytes were washed, dried and crushed to the desired size and both the prepared biosorbents were further physical, chemical and morphological characterized.

#### 3.1. Biosorbent characterization

Table 2 presents the elemental compositions and surface properties of the two prepared biosorbents. FESEM image of the two biosorbent is depicted in Figure 2 that reveals a smooth and clear morphology of the material. Also, the elemental composition of the biosorbent materials with carbon, hydrogen and nitrogen values was evaluated. The zeta potential values of 2.19 and 3.42 mV, for Azolla and Hydrilla species, respectively, indicated that both the biosorbent surfaces were covered with positively charged species.

The average particle size of Azolla and Hydrilla species was found to be 1042.9 and 948.2 nm with a total surface area of 4.29 and 2.23 m² g⁻¹, respectively. Total ash content was found to be 67.12 and 56.28% for Azolla and Hydrilla species, respectively. Further, the contact angle of Azolla and Hydrilla species was found out to be 34.6° and 27.9°, respectively. In general, the hydrophilic surfaces have a contact angle less than 90° and impose positive capillary pressure, thus allowing water to enter into the pores.

FTIR is an imperative to comprehend the chemical nature of individual components which controls the biosorption of the heavy metals. FT-IR spectra of the prepared biosorbent samples which depict various vibrational frequencies due to various functional groups present in the biosorbents. Table 3 presents the respective functional group involved in biosorption phenomenon.

#### 3.2. Effect of different parameters on biosorption capability

##### 3.2.1. Effect of pH

pH, an important parameter for the heavy metal sorption on to the biosorbent from an aqueous solution. It decides the surface charge of biosorbent, the degree of ionization and speciation of absorbate (Gupta, Pathania, Agarwal, & Sharma, 2013; Huang & Zhu, 2013). Functional groups present on the cell wall plays a significant role in heavy metal biosorption (Sarada et al., 2014). Biosorption mechanism involves the complex mechanism of ion exchange, chelation, biosorption by physical forces, and ion entrapment (Sarada et al., 2014). In this study, the biosorption of tested heavy metals was examined in the range of 10–50 mg L⁻¹. Figure 3 shows the effect of pH on biosorption heavy metal through macrophytes. For heavy metal biosorption at low pH, there is competition between heavy metal and H⁺ ions for the absorption sites, affecting the ionization of functional groups onto the biosorbent surface, i.e., at low pH, the acidic surface functional groups tend to be protonated, and henceforth, not considerably contribute to adsorption reactions (Wahab, Jellali, & Jedidi, 2010). As the surface gets positively charged, therefore making (H⁺) ions compete efficiently with metal ions resulting in a decrease in the amount of heavy metal sorption.

The maximum removal of heavy metals was found at a pH of 4 for Pb, 7 for As, 6 for Cu and 2 for Cr, respectively. These pH values were found to be similar to the studies of prior researchers (Sarada et al., 2014; Swarnalatha & Ayoob, 2016). Increase in pH from 2 to 5 had resulted in low percentage removal and above pH 6.0, complex formation of metal ions occurs resulting in precipitation of heavy metals occurs (Sulaymon, Mohammed, & Al-Musawi, 2013a; Swarnalatha & Ayoob, 2016). It can well be related to the fact that the insoluble metal hydroxide precipitation occurs at the higher pH values (Sulaymon et al., 2013a). Whereas biosorption efficiencies of Cr (VI) for both the macrophytes was maximum at low pH (2.0) and have been reported in the literature by many authors. Cr (VI) ions are frequently found in chromates (CrO₄²⁻), dichromates (Cr₂O₇²⁻) and bichromates (H₂CrO₄) forms depending upon the pH (Gupta et al., 2013). At low pH, the concentration of Cr₂O₇²⁻ increases whereas, at higher pH (6.0), Cr (VI) is present in solution in the

### Table 2. Elemental composition, surface area and magnetic properties of the biosorbent.

| Elemental composition (%) | Surface area and pore diameter | Density |
|---------------------------|--------------------------------|---------|
| (a) Hydrilla verticillata  |                                |         |
| C (%)                     | 62.4                           | 4.29    | 0.43    |
| H (%)                     | 3.55                           | 19.7%   | 8.72    |
| N (%)                     | 1.92                           | 0.19    |         |
| H/C                       | 0.057                          |         |         |
| (b) Azolla filiculoides   |                                |         |
| C (%)                     | 53.8                           | 2.23    | 0.39    |
| H (%)                     | 3.15                           | 15.8    | 11.2    |
| N (%)                     | 2.75                           | 0.09    |         |
| H/C                       | 0.037                          |         |         |
other literature for the removal of tested heavy metals (Kaur et al., 2013; Muthusamy, Venkatachalam, Jeevamani, & Rajarathinam, 2014). Beyond the optimum dosage, there was a decline in the removal efficiencies by both the biosorbents due to saturation of active pore sites at the surface of biosorbent (Kamsonlian, Suresh, Majumder, & Chand, 2013). Although the optimum biosorbent dosages of the heavy metals were attained at 2 g L⁻¹ and 2.5 g L⁻¹, respectively, 5 g L⁻¹ of the biosorbent dose was selected for the comparative removal efficiencies of heavy metal at exhaust points. Nevertheless, an optimum biosorbent dose has been utilized for the further studies. Table 1 also clearly depicts the optimized parameters for different heavy metals and macrophytes. pH, contact time and initial heavy metal ion concentration of the aqueous solution were kept invariable at the optimum values for both the biosorbents as attained from the earlier experimental results.

3.2.3 Effect of contact time

Removal of As (III), Cu (II), Pb (II) and Cr (VI) at different contact time were studied for metal concentration (10 mg L⁻¹) at different optimum pH for respective metals with both the macrophytes. The effect of contact time on biosorption of As (III), Cu (II), Pb (II) and Cr (VI) is shown in Figure 5. Uptake of metal ions occurred rapidly within 20 min and equilibrium was attained in 60 min for chromium with Azolla with 88.76% and 40 min for Hydrilla with 91.41% and becomes constant thereafter. (Lim, Priyantha, Tennakoon, & Dahri, 2012; Singha & Das, 2012) Similar fashion was observed with other three heavy metals whose equilibrium time varies: Copper showed equilibrium time of 60 min and 80.14% with Azolla while 30 min and 84.36% with Hydrilla, Arsenic showed 180 min of equilibrium time with 78.94% with Azolla and 240 min with 92.75% Hydrilla and lead showed 83.66% of removal at equilibrium time of 90 min and 90.13% at equilibrium time of 120 min with Hydrilla. Therefore, optimized contact time was taken for all the further experimentation (Gupta et al., 2013).

3.2.2 Effect of biosorbent dosage

Effect of biosorbent dosage on the biosorption efficiencies of tested heavy metals by the two biosorbents were determined in the range of 0.5–5 g (Figure 4). The outcomes clearly revealed that removal (%) of all the four heavy metal ions boosts with an increase in the adsorbent dosage (Kamsonlian et al., 2012). The maximum removal efficiency for Hydrilla verticillata was found at 2 g L⁻¹ of biosorbent dose while Azolla filiculoides shows promising removal at 2.5 g L⁻¹. These outcomes can well be correlated with the increase in absorbent dose leading to increase in the surface area and accessibility of biosorption sites up to 2.5 g (Kaur et al., 2013). Similar kind of trend was depicted by several other literature for the removal of tested heavy metals (Kaur et al., 2013; Muthusamy, Venkatachalam, Jeevamani, & Rajarathinam, 2014). Beyond the optimum dosage, there was a decline in the removal efficiencies by both the biosorbents due to saturation of active pore sites at the surface of biosorbent (Kamsonlian, Suresh, Majumder, & Chand, 2013). Although the optimum biosorbent dosages of the heavy metals were attained at 2 g L⁻¹ and 2.5 g L⁻¹, respectively, 5 g L⁻¹ of the biosorbent dose was selected for the comparative removal efficiencies of heavy metal at exhaust points. Nevertheless, an optimum biosorbent dose has been utilized for the further studies. Table 1 also clearly depicts the optimized parameters for different heavy metals and macrophytes. pH, contact time and initial heavy metal ion concentration of the aqueous solution were kept invariable at the optimum values for both the biosorbents as attained from the earlier experimental results.

Table 3. FTIR absorption bands and their corresponding possible functional groups.

| Frequency (cm⁻¹) | Azolla filiculoides (Free floating) | Hydrilla verticillata (Submerged) | Functional group |
|-----------------|-----------------------------------|----------------------------------|-----------------|
| 3500–3200       | 3280                              | 3277                             | O–H (hydroxyl)  |
| 3000–2850       | 2845                              | 2921                             | –C–H (alkanes)  |
| 1750–1735       | 1737                              | –C=O (aldehyde)                   |
| 1650–1580       | 1641                              | 1610                             | N–H (primary amine) |
| 1400–1370       | 1400                              | 1363                             | –SO₂ (sulphoxide) |
| 1280–1150       | –                                 | 1227                             | –C–O (alcohol, carboxylic acid, esters, ethers) |
| 1250–1020       | 1019                              | –                                | –C=N (aliphatic amines) |
| 900–865         | 873                               | 877                              | –C–H (alkene)   |
| 850–830         | 838                               | –                                | R₂–C= (trisubstituted alkene) |
| 560–510         | 542                               | 526                              | –C=C=O (aldehyde) |

form of CrO₂²⁻ (Samuel, Abigail, & Ramalingam, 2015). The presence of negatively charged sites onto the surface of biosorbent does not favour biosorption owing to the electrostatic repulsions (Gupta et al., 2013). These optimized pH values were chosen as further studies. It might be attributed to the decrease in the solubility of heavy metals at high pH (Huang & Zhu, 2013). Considering these results into account, respective pH at which maximum removal efficiency of biosorbent was attained was selected further for all the experiments.

Figure 2. Micrographs of Azolla filiculoides and Hydrilla verticillata (scale bar = 500 μm).
could be due to electrostatic interactions among active groups and probability of higher interaction among them (Gupta et al., 2013; Muthusamy et al., 2014). However, per cent adsorption of As (III) decreased from 87.5% and 83.5% to 44.23 and 38.8%, for Cu (II); 80.5 and 83.3 to 41.59 and 34.25%, for Pb (II); 71 and 80.7 to 29.9 and 36.6% and for Cr (VI); 91.88 and 89.79% to 51.26 and 43.92% for \( \text{Hydrilla} \) and \( \text{Azolla} \) species, respectively. This increase might be because of rapid saturation of metal functional sites on the biosorbent (Shukla & Vankar, 2012). With the increase in the concentration of heavy metal, the binding sites of the biosorbent are becoming more saturated and after a certain concentration, there will be no further absorption. Furthermore, for the dilute solutions, metal ions have a very high mobility and therefore, the interaction with the biosorbent is very high. Here, the increase in the initial concentration of Cr (VI) and Zn (II) results in the saturation of the biosorbent surface faster that leads to decrease in absorption (Muthusamy et al., 2014).

Also, at a higher concentration, the heavy metals diffuse on the surface of the biosorbent by intra-particle diffusion and also the highly hydrolyzed ions will diffuse...
be several reasons that could be ascribed: the relative increase in escaping tendency from solid to bulk phase of heavy metals and rupture of bonds leads to surface deactivation of biosorbent/weakening of forces involved in bond formation (Samuel et al., 2015). It could be inferred from results that the sorption of heavy metals/metalloids onto macrophytes is an endothermic process. Hence, the submerged aquatic macrophyte, *H. verticillata* might be efficiently utilized for arsenic removal with very promising results (Nigam et al., 2013). Compared to the commercial adsorbents present in the market, the cost of processing this weed i.e., drying, grinding, packaging as well as transportation, incurs the absolutely negligible cost. Previous studies on *H. verticillata* species seems to be very economical as well as an efficient biosorbent for the removal of Cr (VI) from the aqueous solutions.

3.2.6. Effect of agitation speed

The biosorption capacities of the two macrophytes for heavy metal removal in a single component solution at different agitation speeds (50–200 rpm) were evaluated. With the increase in the agitation speed the biosorption of heavy metal increases as there is an enhancement at a slower rate. This specifies the probable monolayer formation of As (III) and Cr (VI) ions on the outer surface.

3.2.5. Effect of temperature

Effect of temperature on the equilibrium sorption potential for Pb (II), Cr (VI), Cu (II) and As (III) ions were investigated in the temperature range 20–50 °C with an initial heavy metal concentration of 10 mg L⁻¹ and at all the previously optimized conditions. Figure 7 depicts the percentage removal of heavy metals at different temperatures. Maximum percentage of removal of the four metals was attained at 30 °C. From the obtained result, it could be inferred that biosorption between macrophyte biomass and the heavy metals might be due to probable involvement of chemical interaction and physical adsorption (Gupta et al., 2013; Sulaymon, Mohammed, & Al-Musawi, 2013b).

With the rise in temperature (up to 30 °C), availability of surface area increases due to enlargement of pore size and leads to decrease in viscosity of the solution. Enlargement of pore size present on macrophyte augments adsorption and diffusion of metal ions within the pores (Sulaymon et al., 2013b). A further rise in temperature (above 35 °C) leads to a decline in the percentage removal efficiency through biosorption. There might be several reasons that could be ascribed: the relative increase in escaping tendency from solid to bulk phase of heavy metals and rupture of bonds leads to surface deactivation of biosorbent/weakening of forces involved in bond formation (Samuel et al., 2015). It could be inferred from results that the sorption of heavy metals/metalloids onto macrophytes is an endothermic process.

Hence, the submerged aquatic macrophyte, *H. verticillata* might be efficiently utilized for arsenic removal with very promising results (Nigam et al., 2013). Compared to the commercial adsorbents present in the market, the cost of processing this weed i.e., drying, grinding, packaging as well as transportation, incurs the absolutely negligible cost. Previous studies on *H. verticillata* species seems to be very economical as well as an efficient biosorbent for the removal of Cr (VI) from the aqueous solutions.

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in the diffusion of heavy metal ions towards the biosorbent surface. These outcomes can well be associated with the fact that the agitation speed in the range of 150–250 rpm is sufficient enough for making the surface binding sites available for heavy metal uptake for both the biosorbents (Srividya & Mohanty, 2009). Therefore, 250 rpm was chosen as the optimum agitation speed for both the biosorbent. Although Azolla took 30, 60, 90 and 180 min for copper, chromium, lead and arsenic, respectively, while Hydrilla have taken 30, 40, 120 and 240 min. Nadeem et al. (2008) reported similar results for different heavy metal biomass system. The attractive forces between heavy metal and the biosorbent viz., vander waal forces, electrostatic forces and rapid pore diffusion into the intra-particle matrix help in attaining the equilibrium (Gupta et al., 2013).

3.3. Characterization of the biosorbents

3.3.1. FESEM-EDX analysis

For analyzing the difference in the morphology and elemental composition of the two biosorbents after the experiments, FESEM–EDX analyzes of the control biosorbent and the metal loaded biosorbent were carried out. From FESEM-EDX spectrum (Figure 8), clearly revealed the occurrence of the additional peak of the respective individual metal ions on the biosorbent. This further confirmed the biosorption phenomena of the heavy metal on the biosorbent surface. There were not many morphological differences were observed during the FESEM analysis of the biosorbent. Figure 9 depicts the micrograph showing FESEM elemental mapping analysis of different metals (for lead experiment with Azolla filiculoides).

3.3.2. FTIR spectroscopy analysis

The two biosorbent depicted wide band spectra at around 3250 cm⁻¹, which is characteristic of the hydroxyl groups (O–H) (Arul Manikandan et al., 2016) (Figure 10). At 2921 cm⁻¹, another band was observed due to –CH₂ followed by a band spectrum at 1737 cm⁻¹ (only in Hydrilla) corresponding to the carbonyl of ester groups; peak at 1641 cm⁻¹ was assigned to the primary amine band spectra. The absorption band in the range 1650–1580 cm⁻¹ signifies the N–H (primary amine). In addition, the FTIR spectra reveal –C=O, –C–C=O, and R₂–C=band stretch corresponding to the functional groups alcohol/carboxylic acid, alkene and aldehyde, respectively.

3.3.3. Regeneration/reusability studies of the exhausted biosorbent

Regeneration studies were further performed to calculate the reusability potential of the bioabsorbent. The efficient desorption of absorbed heavy metals from A. filiculoides and H. verticillata biomass were deemed indispensable to make sure their sustainability for long-term usage for repetitive extraction-elution cycles and reuse potential. (Bunluesin, Kruatrachue, Pokethitiyook, Upatham, & Lanza, 2007; Samuel et al., 2015). Desorption experiments were carried out in 3 cycles and with each cycle the loss in weight of adsorbent was observed. In first two cycles, loss was significant which may be due to washing of soluble material and in the last cycle, no significant weight loss due leftover biosorbent might have resistant material. A steady decrease in sorption with an increase in the number of cycles was also noticed which may due to corrosive nature of acid and washing away of functional group. (Bunluesin et al., 2007; Kaur et al., 2013; Zhao, Duncan, & Van Hille, 1999).

Desorption of adsorbed As (III), Cu (II), Pb (II) and Cr (VI) on A. filiculoides and H. verticillata biomass were studied using 0.1 N H₂SO₄, 0.1 N HCl, 0.1 N HNO₃. The percentage desorption varied between 65.2% for Cu, 59.45% for Pb, 71–72.6% for Cr (VI) and 81.3% for As (III) with H. verticillata. 0.1 N HCl found to be better for desorption of chromium (VI) for H. verticillata, 0.1 N HCl > 0.1 N HNO₃ > 0.1 N H₂SO₄ whereas 0.1 N HNO₃ showed better desorption of adsorbed heavy metal in order of 0.1 N HNO₃ > 0.1 N H₂SO₄ > 0.1 N HCl with A. filiculoides and percentage of desorption was not
Figure 8. FESEM-EDX of *Azolla filiculoides* (a) control biomass and metal loaded biomass (b) Chromium, (c) Arsenic and (d) Lead.

Figure 9. FESEM micrographs along with metal mapping of *Azolla filiculoides* loaded with (at 500 μm) (a) Arsenic, (b) Chromium and (c) Lead; depicting the respective elemental mapping.
4. Conclusions

With the ease and mass availability and low processing cost, the aquatic macrophytes could be among the most promising candidate in the heavy metal removal from the wastewater. Further, the feasibility of the adsorption technology could be explored by up-scaling all the parameters. Henceforth, it might be finally concluded that submerged aquatic macrophyte is a better candidate when compared with its floating counterpart and the present investigation is in sync with the previous studies.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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3.4. Cost estimation

For decades, activated carbons are traditionally utilized for the removal of heavy metal ions from the wastewater which costs around $2000 USD per ton in India (Arul Manikandan et al., 2016). As previously mentioned in the literature, the sorbent utilized for performing this study i.e., Azolla filiculoides and Hydrilla verticillata, aquatic macrophytes, not associated with any economic use. In addition, according to Bhatnagar, Minocha, and Sillanpää (2010), for the processing and transportation of the biosorbent, $100–$200 USD per ton of the biosorbent is predicted. In relation to the cost, the macrophytes are among the most easily accessible, efficient and economic sorbent in comparison to treat a similar volume of heavy metal containing wastewater than the activated carbon. Henceforth, it can well be accomplished that aquatic macrophytes can be utilized as an inexpensive and efficient biosorbent for treating heavy metal-laden wastewater.

Figure 10. FTIR spectra of control biomass and metal loaded biomass (a) Azolla filiculoides and (b) Hydrilla verticillata.
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