



Utilization of Wheat (*Triticum aestivum*) and Berseem (*Trifolium alexandrinum*) Dry Biomass for Heavy Metals Biosorption

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Abstract: Sorption capacity of wheat (*Triticum aestivum*) and berseem (*Trifolium alexandrinum*) biomass was checked against heavy metals i.e. copper (Cu), cadmium (Cd), lead (Pb) and chromium (Cr). Dry biomass was introduced in Erlenmeyer flasks of 250 ml volume to study the effect of varying initial metal concentration, pH and contact time. The filtrates were analyzed using flame atomic absorption spectrophotometer. The adsorption data of wheat and berseem biomass was fitted to Langmuir and Freundlich models. Results exhibited the maximum initial metal concentration (200 mg/L), pH 5 and 9, contact time 60 and 120 min are suitable for biosorption using wheat and berseem plants biomass. It can be recommended from the present study that berseem and wheat biomass can be used for waste water treatment in a cost effective and easy mode.

Keywords: Sorption, Dry biomass, Environment, Green Chemistry, Phytoremediation, Adsorption models

1. INTRODUCTION

Animals and plants have been affected by heavy metal contamination on a global scale. The constant threat imposed by heavy metals on human and animal health can be minimized by utilization of the different crops harvested in the soils affected by heavy metals. Such crops, either food or fodder possess an inherent ability for accumulation of heavy metals by different mechanisms [1, 2]. The potential of fodder crops accumulating heavy metals is influenced by number of factors e.g. pedospheric composition, climatic factors, types of agricultural chemicals used, quality of the irrigation water, the type of plant utilized and the parts of the plants used [3]. Regions located in close proximity to rivers are particularly at risk of pollution due to heavy metals. River water affected by heavy metals when used for irrigation of crops can also influence the soils negatively. Different heavy metals are the essential constituents of different metabolic pathways of plants and animals. Nevertheless, the amount of heavy metals beyond threshold can be lethal and associated with the disruption of the organisms normal physiological functioning [4,5].

Particularly, they can induce extreme toxicity and carcinogenicity on different living organisms [6].

Heavy metal contamination of pedospheric compartment is an ever growing issue and can be attributed to a myriad of anthropogenic activities e.g. agrochemicals, waste dumping etc. [7, 8]. Soil based heavy metal contamination is highly a serious concern since the heavy metals can be translocated into the plants from soils and later transferred to the higher trophic levels of food chain. The translocated heavy metals can be removed and used as a strategy for heavy metal remediation. Such an approach is referred as phytoremediation [9, 10]. The use of energy crops for phytoremediation is an eco-friendly approach marked by plants being inexhaustible energy resource. The integrity of ecosystems can be managed and enhanced by phytoremediation as an effective strategy [11]. Phytoremediation plants are carbon sinks [12] that not only cleans the environment but also reduces the heavier costs associated with other physicochemical modes of remediation. The process of phytoremediation can be further enhanced by utilization of alternating current (AC) [13]. Researches have also been

done by inoculating phytoremediation plants e.g. sunflower with different fungal species for enhancing its heavy metal uptake potential [14, 15].

Industrial wastewater is a source of heavy metals which is responsible for water and soil pollution [16]. The use of this polluted water creates adverse impact on all the living organism and environment and ultimately metal ions can enter into the human food chain from the soil or water, disturb the biochemical processes and finally lead to serious effects on living organisms [17]. Heavy metals are possible to remove from aqueous solution by physical, chemical and biological technologies but conventional methods for removal of metal ions from aqueous solution have been suggested, such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon and evaporation etc. Biosorption is a physico-chemical process and includes absorption, adsorption, ion exchange, surface complexation and precipitation [18]. For the elimination of organics and metals at large scale a huge number of materials have been studied for their capability to be used as biosorbent. On the whole, the experienced biosorbents are categorized as microbial [19] e.g. bacteria, fungi, yeast, algae [20], industrial wastes [21], agricultural wastes [22] and other polysaccharide materials. For this study dry biomass of wheat and berseem were collected from farmer fields. The aim of study was to determine the biosorption capacity of wheat and berseem dry biomass and serve as better biosorbent for environment reclamation.

2. MATERIALS AND METHODS

2.1. Plants sample collection and preparation of biosorbent

Wheat and berseem samples were collected from farmers' fields. After collection wheat and berseem plant samples were washed with tap water and distilled water. Washed samples were air dried and cut into small pieces with the help of knife. Now the plant samples were oven dried by keeping them in oven at 65.8 °C until constant weight. Dried plant samples were grinded with the help of grinding machine. Ground samples were prepared as biosorbents and were ready to use for biosorption experiments. The biosorbents prepared from wheat and berseem plants were packed in air

tight polythene bags and then put into desiccators for use in biosorption experiments.

2.2. Metal biosorption experiment

Biosorption of wheat and berseem dry biomass was checked at different pH (5, 7 and 9), different metals (Cu, Cr, Pb, Cd) concentrations (50 ppm, 100 ppm and 150 ppm and 200 ppm) and different time contact (30, 60, 90 and 120 minutes). Metal stock solutions Cu, Cr, Pb and Cd were prepared in 250ml conical flask. In conical flask 0.25 gm of plant dry biomass was added individually in 60 ml of Cu (II) solutions at different concentrations (50 ppm, 100 ppm, 150 ppm and 200 ppm). The flasks were covered with aluminium foil and were agitated on a rotator shaker for varying time intervals (30, 60, 90, 120 minutes) at 154 rounds per minute, at room temperature and at pH value 7. Similar experiment was conducted with different pH values (5, 7 and 9) while keeping the metal concentration constant at 100 ppm. After each experiment, the mixture was filtered through Whatman filter paper No.1 and the filtrate was analysed for metal concentration by flame atomic absorption spectrophotometer. In order to run the samples in atomic absorption spectrophotometer, the concentration of the metal solution was reduced by doing metal solution dilution after each experiment. At the end of each experiment, triplicates of samples were taken. The samples were stored in plastic bottles and were analysed further in atomic absorption spectrophotometer. Similar procedure was repeated for Cr (III), Pb (II) and Cd (II). Berseem and wheat biosorbents were experimented separately but procedure followed was the same for both biosorbents. The heavy metal concentrations in the filtrate were analysed by flame atomic absorption spectrophotometer. Biosorption capacity i.e. the amount of metal ions (mg) sorbed by plant biomass (g) was calculated by using the equation 1.

$$Q = \frac{(C_i - C_f)}{mV} \quad (1)$$

Where Q = metal ion bioadsorbed (mg g⁻¹) of biomass, C_i = initial metal ion concentration (mg l⁻¹), C_f = final metal ion concentration (mg l⁻¹), m = mass of plant biomass in the reaction mixture (gm) and V = volume of the reaction mixture (l). Statistical analysis of data was done on Microsoft Excel (2013) by using Langmuir isotherm (1916) and Freundlich equation (1909).

3. RESULTS AND DISCUSSION

3.1. Biosorption capacity of Wheat and Berseem biomass at initial metals concentration

For metal removal, agricultural waste supplies are an outstanding source being cellulosic in nature. These have diverse functional groups (hydroxyl, phenolic, carboxyl, amino and, acetamido) and form chelates and metal complexes because of having affinity for metal ions. Lignin and cellulose are the major constituents of agricultural waste material with other polar functional groups (aldehydes, alcohols, carboxylic acid, ketones and ethers) and support metal complexation resulting in uptake of metals. Rice fibre saw dust, wool, orange peel, soya bean and cotton hulls, banana pith, pine bark, wood and peat have been verified to uptake heavy metals from the wastewater. The agro based waste biosorbents are economical, non-risky and vast resources, which are particularly selective for heavy metals and can be simply disposed by burning [23].

3.1.1. Copper (Cu)

Wheat biomass showed a gradual increase in biosorption tendency with increasing initial copper concentration. Maximum biosorption by wheat biomass was noticed 151.4 mg/g (76%) and for berseem biomass 48.03 mg/g (74%) at initial copper concentration of 200 mg/L. The comparison expressed that wheat biomass behaved as better biosorbent as compared to berseem biomass (Figure 1).

3.1.2. Cadmium (Cd)

The wheat biomass showed a gradual increase in biosorption tendency with increasing initial cadmium concentration. Maximum biosorption by wheat biomass was noticed 146.2 mg/g at initial cadmium concentration (200 mg/L). In case of berseem biomass, maximum biosorption (153.8 mg/g) was shown at initial cadmium concentration. This comparison expressed that berseem biomass behaved as a better biosorbent as compared to wheat biomass (Figure 1).

3.1.3. Lead (Pb)

Maximum biosorption by wheat biomass was noticed 164.8 mg/g at initial Pb concentration of

200 mg/L. In case of berseem biomass, optimum Pb removal (62.5%) was observed at 200 mg/L of initial Pb concentration. Comparison of wheat and berseem biomass as biosorbents was done in terms of biosorption capacity when initial Pb concentration was conditioned. This comparison expressed that wheat biomass behaved as better biosorbent as compared to berseem biomass.

3.1.4. Chromium (Cr)

Maximum biosorption by wheat biomass was noticed 177.0 mg/g at initial chromium concentration of 200 mg/L. So, best Cr removal (88.5%) was observed at 200 mg/L (initial Cr concentration). In case of berseem biomass, maximum biosorption (142.34 mg/g) was also shown at initial chromium concentration of 200 mg/L. So, in case of berseem biomass, optimum Cr removal (71%) was observed at 200 mg/L of initial Cr concentration. This comparison expressed that wheat biomass behaved as better biosorbent as compared to berseem biomass. In the present research, biosorption is dependent on initial metal concentration. According to this investigation, there was increase in metal uptake with increase of initial metal concentration. Sorption capacity of wheat and grass biomass gradual increase in Cr (III) removal with increase in the initial concentration of Cr (III).

Biosorption potential of banana peel against Cu, Pb, Zn and Ni increased with initial metal concentration [24]. Biosorption potential of spinach stalk and pawpaw seed against Mn and Pb ions metal uptake increases as the initial metal ion concentration increases. Biosorption potential of agro based waste material maize tassel, for removal of Cd (II) and Cr (VI) increased metal uptake with increase of initial metal concentration up to 300 mg/L. Sorption in this case was also found dependent on increasing initial metal concentration [25].

3.2. Effect of contact time for biosorption capacity of Wheat and Berseem biomass

3.2.1. Cu

Wheat biomass showed different biosorption potential at different time intervals. Initially, at 30 minutes contact time, wheat biomass removed 66% (65.91 mg/g) Cu ions. While at 60 minutes, 77%

(76.77 mg/g) Cu biosorption was being observed. But at 90 minutes time interval, only 62% (62.14 mg/g) Cu removal was shown. However, at 120 minutes, 69% (68.59 mg/g) uptake of Cu was noticed. On the basis of above results, it is obvious that the optimum time for Cu biosorption by the wheat biomass is 60 minutes. In case of berseem biomass; in the beginning, at 30 minutes contact time, berseem biomass removed 43% (42.62 mg/g) Cu ions. While at 60 minutes, only 20% (20.45 mg/g) Cu biosorption was being observed. But at 90 minutes, 44% (43.92 mg/g) Cu removal was shown. However, at 120 minutes, 49 % (48.56 mg/g) uptake of Cu was noticed. On the basis of above results, it can be noticed that the optimum time for Cu uptake by berseem biomass is 120 minutes. Wheat biomass was acting as a better biosorbent as compared to berseem biomass.

3.2.2. Cd

Figure 2 revealed the biosorption tendency of wheat and berseem biomass at varying time intervals (30, 60, 90 and 120 minutes). The results depict that wheat biomass showed different biosorption potential at different time intervals. Initially, at 30 minutes contact time, wheat biomass removed 79% (78.61 mg/g) Cd ions. While at 60 minutes, 82% (81.89 mg/g) Cd biosorption was being observed. At time interval of 90 minutes, 82% (81.54 mg/g) Cd removal was shown. Similarly, contact time of 120 minutes also showed 82% (81.94 mg/g) uptake of Cd.

On the basis of above results, it is obvious that the optimum time for Cd biosorption by wheat biomass is 60, 90 and 120 minutes; because in this case, equilibrium reaches at 60 minutes and it is maintained up to 90 and 120 minutes time interval. In case of berseem biomass; in the beginning, at 30 minutes contact time, it removed 75% (75.16 mg/g) Cd ions. While at 60 minutes, 76% (76.20 mg/g) Cd biosorption was being observed. At 90 minutes, 75% (74.62 mg/g) Cd removal was shown. However, at 120 minutes, 81% (81.37 mg/g) uptake of Cd was noticed. On the basis of above results, it can be noticed that the optimum time for Cd uptake by berseem biomass is 120 minutes. If comparison of biosorption capacity of wheat and berseem biomass is done while considering contact time then it can be noticed by comparing minimum and maximum biosorption values that wheat biomass is acting as better biosorbent as compared to berseem biomass.

3.2.3. Pb

The results depict that wheat biomass showed different biosorption potential at different time intervals. Initially, at 30 minutes contact time, wheat biomass removed 62% (61.99 mg/g) Pb ions. While at 60 minutes, 71% (70.6 mg/g) Pb biosorption was being observed. 90 minutes time interval also showed 71% (71.83 mg/g) Pb removal. However, contact time of 120 minutes resulted in maximum 81% (80.7 mg/g) uptake of Pb. On the basis of above results, it is obvious that the optimum time for Pb biosorption by wheat biomass is 120 minutes. In case of berseem biomass; in the beginning, at 30 minutes contact time, it removed 68% (68.08 mg/g) Pb ions. While 60 minutes time interval gave maximum 80% (79.62 mg/g) Pb removal. Contact time of 90 minutes showed 72% (72.3 mg/g) Pb removal and at 120 minutes time interval, a little more i.e. 73% (72.5 mg/g) uptake of Pb was noticed. On the basis of above results, it can be noticed that the optimum time for Pb uptake by berseem biomass is 60 minutes. If biosorption capacity of wheat and berseem biomass is compared while considering contact time then it can be noticed by comparing minimum and maximum biosorption values that berseem biomass is acting as better biosorbent as compared to wheat biomass.

3.2.4. Cr

The results depict that wheat biomass showed different biosorption potential at different time intervals. Initially, at 30 minutes contact time, wheat biomass removed 65% (65.12 mg/g) Cr ions. While at 60 minutes, 64% (63.74 mg/g) Cr biosorption was being observed. 90 minutes time interval showed maximum 73% (73.06 mg/g) Cr removal. However, at 120 minutes 57% (56.54 mg/g) uptake of Cr was noticed. On the basis of above results, it is obvious that the optimum time for Cr biosorption by wheat biomass is 90 minutes. In case of berseem biomass; in the beginning at 30 minutes contact time, berseem biomass removed 59% (58.53 mg/g) Cr ions. While at 60 minutes, 64% (64.45 mg/g) Cr biosorption was observed. 90 minutes contact time resulted in maximum Cr removal, 79% (79.04 mg/g). While 120 minutes time interval showed 77% (76.67 mg/g) uptake of Cr. On the basis of these results, it can be noticed that the optimum time for Cr uptake by berseem biomass is 90 minutes. If biosorption capacity of

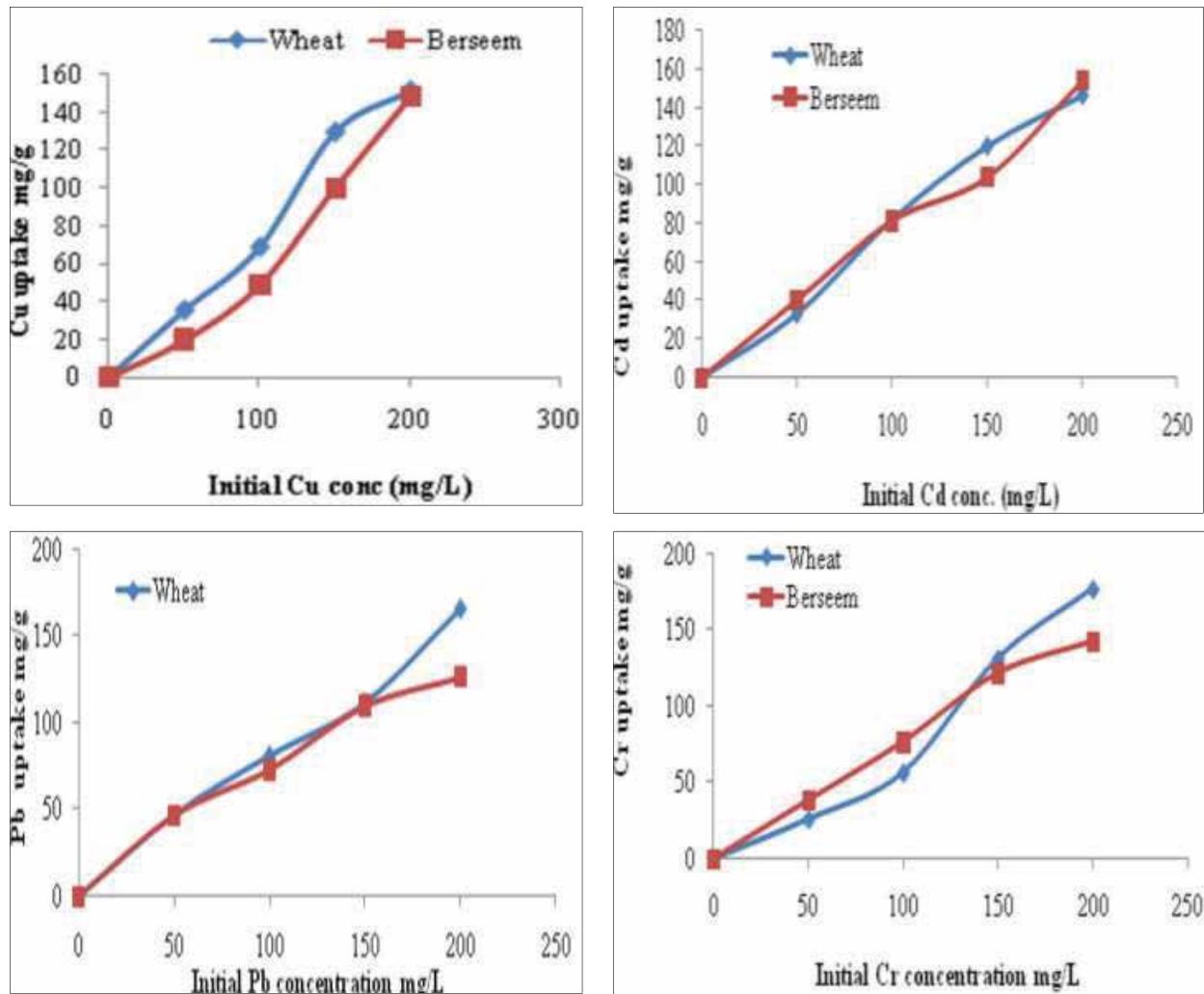


Fig. 1. Effect of initial concentration of metals Cu, Cd, Pb and Cr on the biosorption potential of Wheat and Berseem dry biomass

wheat and berseem biomass is compared while considering contact time; then it can be noticed by comparing minimum and maximum biosorption values that berseem biomass is acting as better biosorbent as compared to wheat biomass.

Optimum contact time for agro based waste material sorption of Pb and Ni was found to be 60 minutes. Finding of this research is similar to finding of this study, which revealed that 60 minutes was optimum contact time for Cu and Cd removal by wheat biomass and 60 minutes was also optimum contact time for Pb removal by berseem biomass. Contact time of 90 minutes was found as optimum time for biosorption of Mn and Pb ions by spinach stalk and pawpaw seed. Contact time of 120 minutes proved to be optimum for biosorption potential of an agro based waste corn cob powder against Cr (VI). This study is similar to results of

present study, which revealed that optimum contact time for Cd and Pb uptake by wheat biomass was 120 minute and 120 minutes contact time was also found as optimum contact time for uptake of Cu and Cd uptake by berseem biomass. Zvinowanda *et al.* [25] investigated optimum time observed for metal removal was 120 minutes for biosorption of agro based waste maize tassel for removal of Cd (II) and Cr (VI).

3.3 Effect of pH for biosorption capacity of Wheat and Berseem biomass for metals concentration

3.3.1. Cu

Biosorption of Cu ions by wheat biomass was maximum 69% (68.59 mg/g) at pH value 5 and was minimum 57 % (56.656 mg/g) at pH value 9.

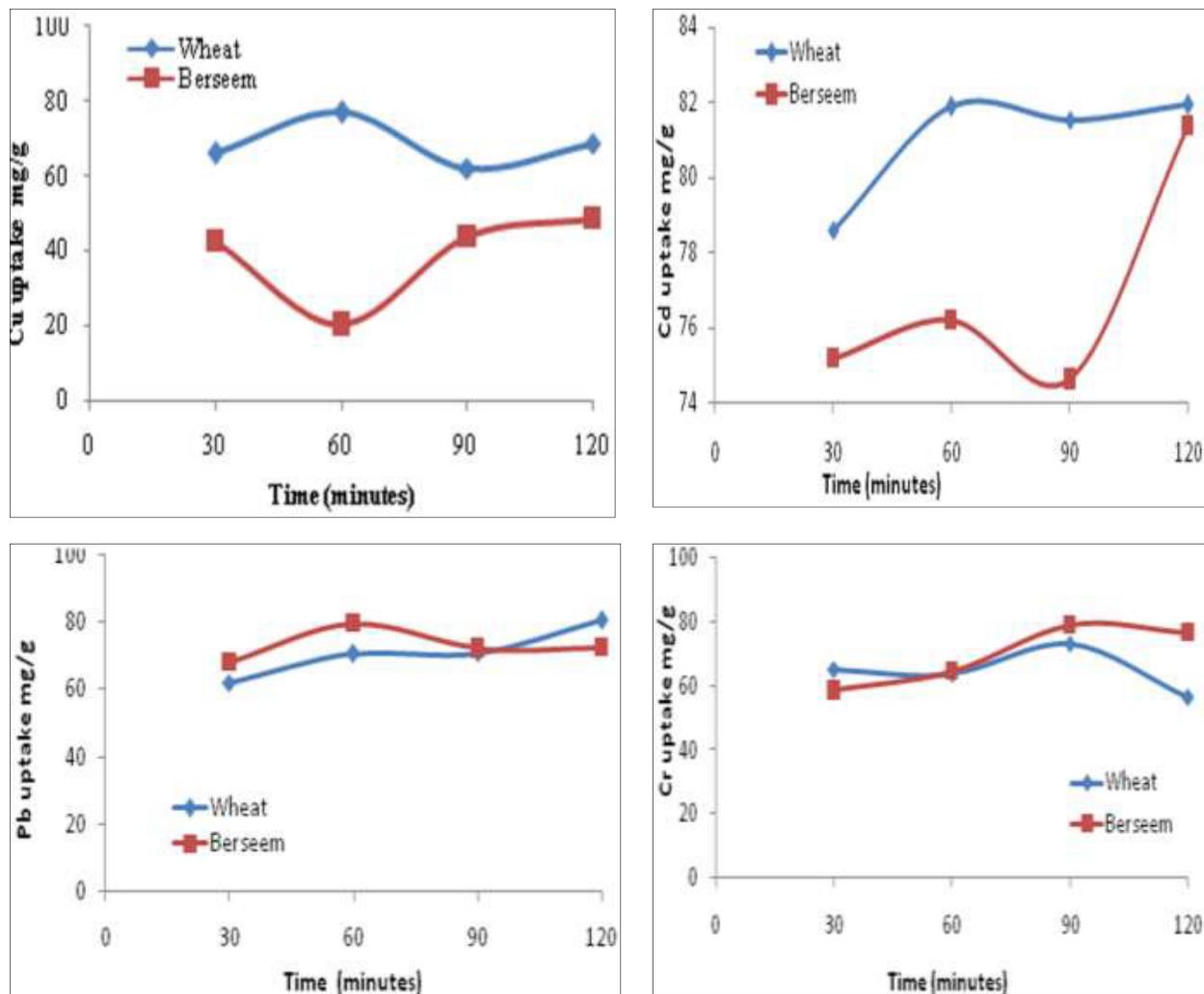


Fig. 2. Effect of contact time on the biosorption of Cu, Cd, Pb and Cr by Wheat and Berseem dry biomass

While at pH value 7, Cu removal percentage was 62% (62.13 mg/g), which is between the other two values. Better Cu removal trend has been shown in acidic medium. This Cu removal trend decreases as the solution turns neutral and it decreases even more in basic solution at pH value 9. Whereas, in case of berseem biomass, biosorption capacity is maximum 79% (78.75 mg/g) at pH value 9 and it was minimum 49% (48.56 mg/g) at pH value 5. It is clear from these results that optimum pH value for Cu removal by berseem biomass is 9. If wheat and berseem biomass are compared in terms of biosorption capacity when varying pH value is conditioned; then it can be noticed by comparing minimum and maximum biosorption values that berseem biomass is acting as a better biosorbent as compared to wheat biomass. Another point to be noticed was that wheat biomass biosorbs Cu ions better in acidic medium whereas berseem biomass biosorbs Cu ions better in basic medium.

3.3.2. Cd

The biosorption of Cd ions by wheat biomass was maximum 82% (81.94 mg/g) at pH value 5 and minimum 61% (61.15 mg/g) at pH value 9. While at pH value 7, Cd removal percentage was 80% (79.97 mg/g) that is between the other two values. Better Cd removal trend has been shown in acidic medium. This Cd removal trend decreases as the solution turns neutral and it decreases even more in basic solution at pH value 9. Whereas, in case of berseem biomass, biosorption capacity is maximum 82% (81.7 mg/g) at pH value 9 and it is minimum 80% (80.43 mg/g) at pH value 7. While at pH value 5, 81% (81.37 mg/g) Cd removal is between the other two values. It is clear from these results that optimum pH value for Cu removal by berseem biomass is 9. In case of berseem biomass, this Cd uptake potential is slightly more in basic medium.

3.3.3. Pb

Biosorption of Pb ions by wheat biomass was maximum 87% (86.89 mg/g) at pH value 9 and minimum 32% (32.45 mg/g) at pH value 7. While at pH value 5, Pb removal percentage was 81% (80.7 mg/g), which is between the other two values. These results depicted that optimum pH value for Pb uptake by wheat biomass is 9. Better Pb removal trend has been shown in basic medium. This Pb removal trend decreases as the solution turns neutral but it increases again in basic solution at pH value 5. Whereas, in case of berseem biomass, biosorption capacity is maximum 82% (81.58 mg/g) at pH value 9 and it is minimum 44% (44.19 mg/g) at pH value 7. While at pH value 5, 73% (72.51 mg/g) Pb removal is between the other two values. It is clear from these results that optimum pH value for Pb removal by berseem biomass is 9. Like wheat biomass, berseem biomass also shows

better Pb removal trend in basic medium. If wheat and berseem biomass are compared as biosorbents in terms of biosorption capacity when varying pH value is conditioned then it can be noticed by comparing minimum and maximum biosorption values that wheat biomass is acting as better biosorbent as compared to berseem biomass.

3.3.4. Cr

Figure 3 shows that biosorption of Cr ions by wheat biomass was maximum 68% (68.37 mg/g) at pH value 9 and minimum 50% (49.87 mg/g) at pH value 7. While at pH value 5, Cr removal percentage was 57% (56.54 mg/g), which is between the other two values. These results depict that optimum pH value for Cr uptake by wheat biomass is 9. Better Cr removal trend has been shown in basic medium. Whereas, in case of berseem biomass, biosorption capacity is maximum 79% (78.86 mg/g) at pH

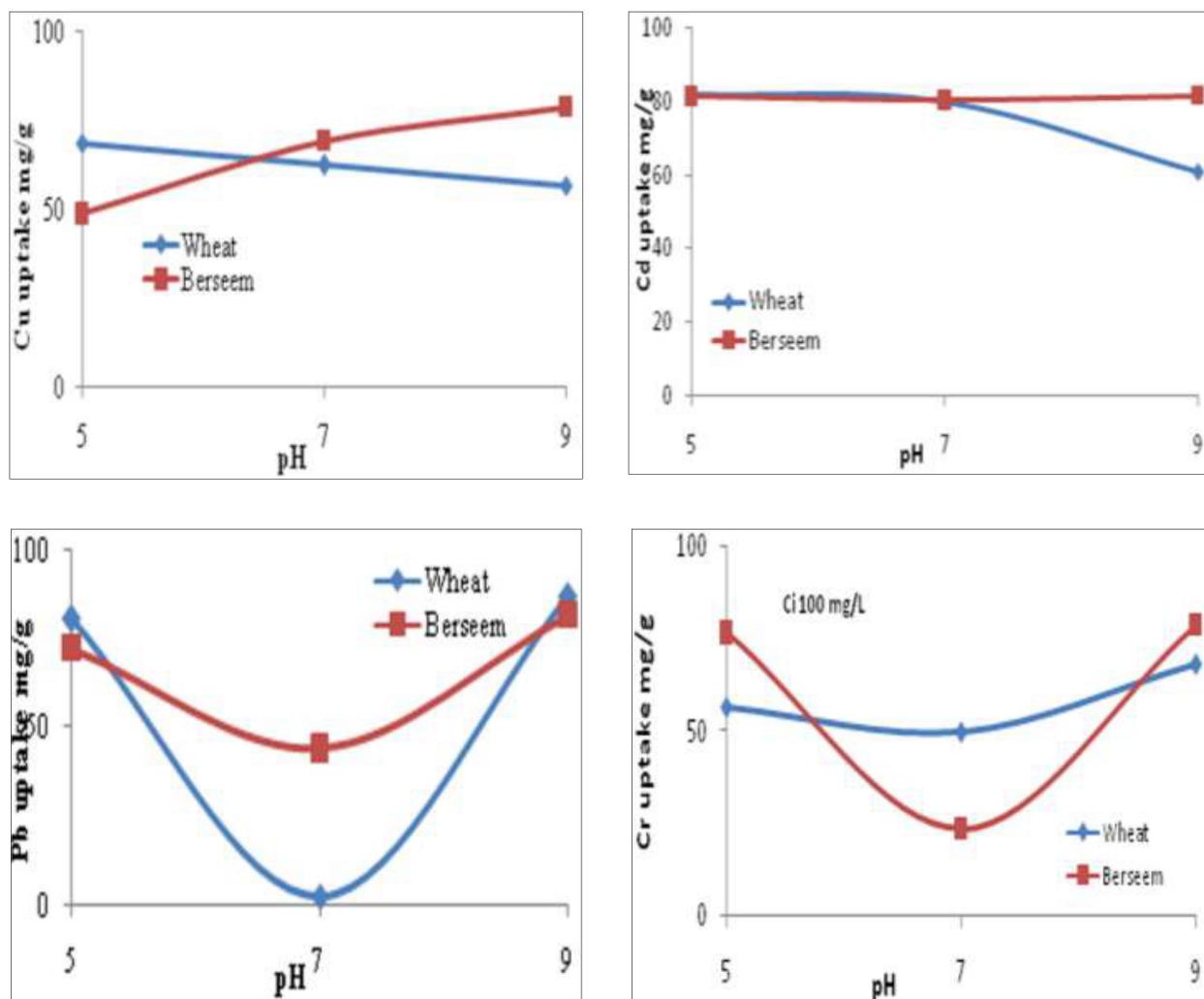


Fig. 3. Effect of pH on the biosorption of Cu, Cd, Pb and Cr by Wheat and Berseem dry biomass

Table 1: Kinetic modelling expressing regression coefficient R2 of biosorption by wheat and berseem biomass via Langmuir and Freundlich isotherm

	Langmuir isotherm								Freundlich isotherm							
	Wheat biomass				Berseem biomass				Wheat biomass				Berseem Biomass			
	Cu	Cd	Pb	Cr	Cu	Cd	Pb	Cr	Cu	Cd	Pb	Cr	Cu	Cd	Pb	Cr
pH	0.99	0.9 9	0.99	0.9 9	0.98	1	0.99	0.99	0.9 9	0.9 9	0.9 6	0.9 8	0.9 6	0.9 9	0.96	0.99
Contact time	0.99	1	0.99	0.9 9	0.99	0.9 9	0.99	0.99	0.9 8	0.9 9	0.9 6	0.9 8	0.9 8	0.9 9	0.98	0.98

value 9 and it is minimum 24% (23.55 mg/g) at pH value 7. While at pH value 5, 77% (76.67 mg/g) Cr removal is between the other two values. It is clear from these results that optimum pH value for Cr removal by berseem biomass is 9. In contrast to wheat biomass, better Cr removal trend has been shown in both acidic and basic medium. In case of berseem biomass, this Cr uptake behaviour decreases as the solution turns neutral. Another point to be noticed is that wheat biomass biosorbs Cr ions better in acidic medium whereas berseem biomass biosorbs Cr ions with almost equal efficacy in both acidic and basic mediums. It has been generally agreed that metal removal intensity of biosorbents can be strongly influenced by pH value of metal solution indicating that the process is governed by an ion-exchange method. At high acidic pH values of the solution, there is increased concentration of hydrogen (H^+) and hydronium (H_3O^+) ions, which compete efficiently with metal ions in binding to negatively charged groups on the biosorbent surface. Therefore, enhancement in heavy metal removal with increasing pH values could be credited to less ionic competition [14].

A number of researchers have also investigated the effect of pH value on biosorption of heavy metals by using different biomass and found similar results with the this study. Han *et al.* [26] used cereal chaff as a biosorbent for Pb and Cu adsorption and found that there was an increase in the Pb uptake when the pH value of the medium was increased and had optimum pH value ranging 5-6 but maximum pH value for Cu and Pb uptake was 9. These findings are similar to the findings of the current study where maximum biosorption was attained at pH value of 9 by berseem biomass against all the four metals under consideration (Cu, Cd, Pb and Cr). Whereas maximum biosorption was also attained at pH value 9 by wheat biomass against Pb and Cr.

Agro based by product *Tamarindus indica* seeds (Indian date) also used as biosorbent for uptake of Cr (VI) and applied at pH range 4-9. Maximum metal removal was observed at pH 9. Wheat shell biomass act as biosorbent for Cu (II) from aqueous solutions and adsorption capacity was maximum at pH value 5. Metal chemistry and sorbent surface binding sites are influenced by the pH value and sorption increased at pH 5 [27]. Agricultural waste olive pomace has biosorption potential against Pb, Cd and Cu and has pH influenced for metal ions uptake and maximum biosorption been achieved at pH 5 for all three metals [28-33].

3.4 Statistical analysis

Biosorption of Cu, Cd, Pb and Cr were investigated for kinetic parameters via Langmuir and Freundlich isotherm that expressed varying degrees of sorption (Table 1). Both the models fitted well with isotherms applied however, $R^2 = 1$ was obtained for Cd biosorbed by both wheat and berseem biomass that signifies the perfect fitting of data in Langmuir model. The adsorptive interactions indicated by both isotherms developed between metals and biosorbents can either be due to transfer of metallic mass over the boundary or the metallic ions can also be sorbed onto the biomass material. However there are chances of intra-particle diffusion as well. Either mechanism occurring for metals removal via wheat and berseem biomass marks the potential of these biosorbents in pollution remediation via facile, cost effective and eco-friendly mode. Such favourability of biosorbents is also indicated by the linearity.

4. CONCLUSION

Heavy metals contaminated soils can be sustainably remediated by utilization of plant varieties that

are inherently provided with the ability of heavy metal uptake. The positive results of the current research for heavy metal i.e. copper (Cu), cadmium (Cd), lead (Pb) and chromium (Cr) removal via Wheat (*Triticum aestivum*) and Berseem (*Trifolium alexandrinum*) biomass is indicative of the future prospects of these plants in environmental cleanup. Phytoremediation done via Wheat and Berseem plant is not only an environmental friendly strategy but it also serve as an economical alternative to the conventional physicochemical methods of remediation. Energy crops after heavy metal uptake can be used as a biodiesel and estimations regarding its effectiveness can be done.

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