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# The use of *Phanerochaete chrysosporium* for modification of bentonite for preconcentration and determination of heavy metals

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## Abstract

In this study, a solid phase extraction method was successfully applied in the preconcentration and determination of trace levels of Cu(II) and Cd(II) ions and *Phanerochaete chrysosporium* (white rot fungus) modified bentonite was used as adsorbent. After the biosorption of Cu(II) and Cd(II) ions, metal concentrations in the samples were determined by atomic absorption spectrophotometry. pH, adsorbent amount, eluent type, sample volume, and flow rate, which are effective in the adsorption of metal ions, have been studied. 1 M HCl was used for desorption of these metal ions retained (recovery 95–100%). In addition, the effect of interfered ions has also been investigated. Sorption data were examined according to Langmuir and Freundlich adsorption equations. The results obtained show that the applied method has a high metal biosorption capacity, and Cu(II) and Cd(II) ions are successfully recovered. It was also successful in applying the proposed enrichment method to real water samples. Recovery values between 92.3% and 97.3% were obtained for the studied metal ions. According to the results, the proposed method can be successfully applied to water analysis at 95% confidence interval.

**Keywords:** Heavy metals, Bentonite, *Phanerochaete chrysosporium*, Bioseparation, Solid phase extraction, Environmental protection

## Introduction

Environmental pollution has increased with the increase in industrialization in recent years. Solid and liquid wastes released to the environment present danger due to the toxic substances they contain (Costa and Duta 2001). The spread of heavy metals into the environment as a result of various industrial processes causes serious ecological and health problems for all living things. In addition, they are not easily degraded in their environment and have the property of accumulation (Lyer et al. 2005).

Chemical precipitation, adsorption, ion exchange, liquid and solid phase extraction, phytoextraction, electrochemical

processes, reverse osmosis, advanced filtration, evaporation, and oxidation/reduction reactions are commonly used techniques for removing heavy metals. Within these methods, ion exchange and adsorption appear to be more preferred methods (Kocaoba 2003; Teutli-Sequeira et al. 2009; Fu and Wang 2011; Shirvani et al. 2015; Kaur et al. 2018; Ma et al. 2019; Sun et al. 2019).

Accurate and precise detection of heavy metals are extremely important for analytical chemistry and environmental science. In studies of determination of heavy metal ions using instrumental techniques, the low amount of analyte, interference effects of samples with high salt content make the determination difficult and it is necessary to use separation and preconcentration techniques before determination.

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Liquid and solid phase extraction, cloud point extraction, membrane filtration, and electrochemical deposition are widely used for the separation and preconcentration of heavy metals (Ghaedi 2006; Yin 2007; Turker 2007; Askun et al. 2008; Refiker et al. 2008; Yan et al. 2017; Kocaoba and Arisoy 2018; Carvajal-Flórez and Cardona-Gallo 2019).

In recent studies, natural materials, rock minerals, industrial products, or agricultural wastes that are locally available as low-cost adsorbents with high-metal uptake capacities are frequently used.

Cheaper alternatives such as silica gel, fly ash, coal, zeolites, chitosan, agricultural waste, wood waste, and natural clays have been studied by many researchers (Babel and Kurniawan 2003; Gode and Pehlivan 2005; Pehlivan et al. 2006; Kocaoba 2007; Rangel et al. 2009; Imamoglu et al. 2015; De Gisi et al. 2016; Yusuff et al. 2018; Onutai et al. 2019).

Biosorption is preferred as an alternative method because of its environmentally friendly structure, excellent performance, and low cost. Biosorption is a highly effective method for separation of inorganic and organic species, including metals, dyes, and a physical parameter of odor, using live or dead biomass or derivatives: fungi, algae, and by-products in the fermentation industry; sludges from biological waste water treatment plants, forest debris, and seaweed; or agricultural substances such as rice husk, rice, and wheat bran use as biomass. Biosorption depends on the type of biomass and sorbate, and the other ions in the medium, the method of preparation, and various physicochemical parameters (Goksungur et al. 2005; Baysal et al. 2009; Febrianto et al. 2009; Srivastava et al. 2015; Anastopoulos et al. 2019; Freitas et al. 2019).

*Phanerochaete chrysosporium* is a white rot fungus and a powerful reducer of various xenobiotics. It is understood from previous biosorption studies that it has environmental importance due to its high efficiency for removal of heavy metals from aqueous systems (Iqbal and Edyvean 2004; Li et al. 2004; Huang et al. 2008; Zeng et al. 2015; Dzionek et al. 2016; Noormohamadi et al. 2019).

There are many studies on this white rot fungus, but there are not enough studies using immobilized *Phanerochaete chrysosporium* to remove heavy metals.

In this study, adsorption and preconcentration of Cu(II) and Cd(II) ions of *Phanerochaete chrysosporium* immobilized bentonite was investigated. The effects of some parameters such as pH, adsorbent amount, eluent type, and volume on the retention of metal ions were searched. In addition, equilibrium studies were carried out and applied to isotherm models.

## Experimental

### Apparatus

Heavy metal determinations were done with atomic absorption spectrometer (AAS) (Analytical Jena-Nova 300

model). pH measurements were done with WTW Series Inolab pH 720 digital pH meter. Column experiments were performed by pumping of metal solutions with Heidolph Pumpdrive 500I model peristaltic pump.

### Growth conditions of microorganisms

White rot fungus *Phanerochaete chrysosporium* was applied for preconcentration and other experiments. Stock cultures of fungus were protected in malt agar extracts. The culture medium was prepared by a variety of the method applied by Eaton (1985) and Kocaoba and Arisoy (2011).

### Chemicals

Analytical grade chemicals were supplied from Merck. 1000 ppm stock solutions of Cu(II) and Cd(II) were prepared from  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{CdCl}_2 \cdot \text{H}_2\text{O}$  salts by using Milli-Q water. pH adjustments were made with 0.1 M NaOH and HCl solutions. The solutions used in all studies were prepared fresh from stock solutions.

### Materials

Natural bentonite used in these studies was obtained from Kütahya in Turkey. The chemical composition of the bentonite clay was determined by X-ray diffraction (XRD), differential thermal, and IR spectrophotometric analysis. Chemical analysis of the sample was carried out by GE-SPG 7, X-ray fluorescence spectrophotometer (XRF). The results are given in Tables 1 and 2. To remove impurities on bentonite clay, it was washed with Milli-Q water and dried at 40°C for 4–5 h. The clay samples were 200 mesh particle size.

## Experimental procedure

### Immobilization of *Phanerochaete chrysosporium* onto bentonite

Immobilization of the *Phanerochaete chrysosporium* on bentonite was done with respect to the method proposed by Mahan and Holcombe (1992).

**Table 1** Chemical analysis of natural bentonite

Component	%
SiO <sub>2</sub>	53.72
Al <sub>2</sub> O <sub>3</sub>	19.12
CaO	5.28
Fe <sub>2</sub> O <sub>3</sub>	4.93
Na <sub>2</sub> O	3.64
MgO	3.29
TiO <sub>2</sub>	0.85
K <sub>2</sub> O	0.44
L.O.I.	8.75

L.O.I loss of ignition

**Table 2** Mineralogical analysis of bentonite

Component	%
Montmorillonite	77
Cristobalite-Opal C	18
K-Feldspar	5
Plagioclase	Trace

A 200 mg of dried *Phanerochaete chrysosporium* was taken and mixed with 2 g of bentonite. This mixture was wetted with 2 mL of Milli-Q water and then stirred well. It was dried at oven at 80°C for 24 h. The wetting and drying steps were repeated 3 times for better contact of the fungus and bentonite and to improve the immobilization efficiency.

#### Column preparation

A mini glass column (10 mm i.d and 200 mm length with a porous disc and a stopcock) was used for the purpose of preconcentration. A small amount of glass wool was placed under the column to prevent loss of bentonite during flow before filling 0.2 g of *Phanerochaete chrysosporium* immobilized bentonite into the column. Before using bentonite and biomass loaded column, 1 mol/L HCl solution and Milli-Q water were passed for conditioning and cleaning steps. The column was then adjusted to working pH with HCl or NaOH solutions. The studies were performed with the aid of a peristaltic pump from a column in the downstream mode of metal solutions.

#### General preconcentration procedure

The effectiveness of *Phanerochaete chrysosporium*-loaded bentonite was investigated with model and real samples. A 100 mL of aqueous solutions containing 10 µg Cu(II) and Cd(II) was taken, and the pH of the solutions was adjusted. Metal solutions were passed through the column using a peristaltic pump, and after the entire solution was passed, it was washed twice with 10 mL of Milli-Q water.

The retained metal ions in the column were eluted with 10 mL of 1 M HCl. Then, metal ions were analyzed with AAS by diluting the solution as required. Optimum conditions (pH, amount of solid phase, initial concentration, etc.) have been determined in the studies.

#### Results and discussion

The studies were carried out primarily under conditions where biomass was not available, only bentonite was used as an adsorbent. In these studies, removal of 70.53% for Cu and 74.38% for Cd was achieved at 10 ppm metal concentration in the determined optimum conditions. With 0.2 g, which was found as the optimum amount of adsorbent, 70.64% removal for Cu and

73.81% for Cd was achieved. Percentages of removal at pH: 5 for both metals were 70.74 for Cu and 72.51 for Cd, respectively. After the bentonite clay was modified by biomass (*Phanerochaete chrysosporium*), the % removal values increased above 90% as can be seen in the following studies. This shows that biomass modifications are extremely effective in heavy metal removal.

#### Effect of pH

The pH of the solutions is an important factor in the studies of preconcentration of trace metal ions by solid phase extraction (Tuzen et al. 2007; Yang et al. 2009; Yahaya et al. 2009; Duran et al. 2009; Kocaoba and Arisoy 2011). In the column containing *Phanerochaete chrysosporium* immobilized bentonite, retention of metal ions depending on pH was investigated. The pH of the metal solutions was adjusted between 1 and 8 with acid or base solutions. The flow rate of the prepared sample solutions from the column was determined as 2.5 mL/min. Then, 10 mL of 1 M HCl was passed through the column and washed, and the concentrations of metals were determined by AAS. From the results given in Fig. 1, it was monitored that adsorption for Cu(II) and Cd(II) ions have risen with rising pH values and attained the highest level in pH: 5. The retained metal ions amount at this pH was 90–95%.

Microbial cells such as polysaccharides and metabolites are very strong for metal accumulation, as there are many attachment sites in the cell wall of microorganisms and diversity in these attachment sites. The low pH values between the metal ions and functional groups on the cell wall and the positive charge of the cell surface have an effect on reducing the sorption (Bayramoğlu and Arica 2008). It has been observed that when the cell surface is loaded more negatively and at higher pH values (pH: 5), sorption increases. The observed increase of biosorption capacity of biosorbent could be attributed to the change of biosorbent surface charges, which determines the dissociation of functional groups. For pH values higher than the optimum values, the retention decreases again due to the competition between the hydroxylated complexes of the metal and active sites of the cell. For avoiding precipitation of metals, high pH values were not preferable and the optimum pH was chosen as pH 5 for all metals. The solution pH affects the solubility of metal ions and the ionization state of the functional groups (–COOH, –NH, –OH) of the fungal surfaces (Say et al. 2001). At a pH of 3, it has 10 times more H<sup>+</sup> in the solution than at a pH of 4. H<sup>+</sup> would compete with metal cation for adsorption site on the fungal cell surface, resulting in poor biosorption effect (Iqbal and Edyvean 2004). At pH higher than the optimum value, sorption was reduced again due to the possibility of precipitation of metals (Gabriel et al. 2001; Pardo et al. 2003; Perez et al. 2014; Ummalyma et al.

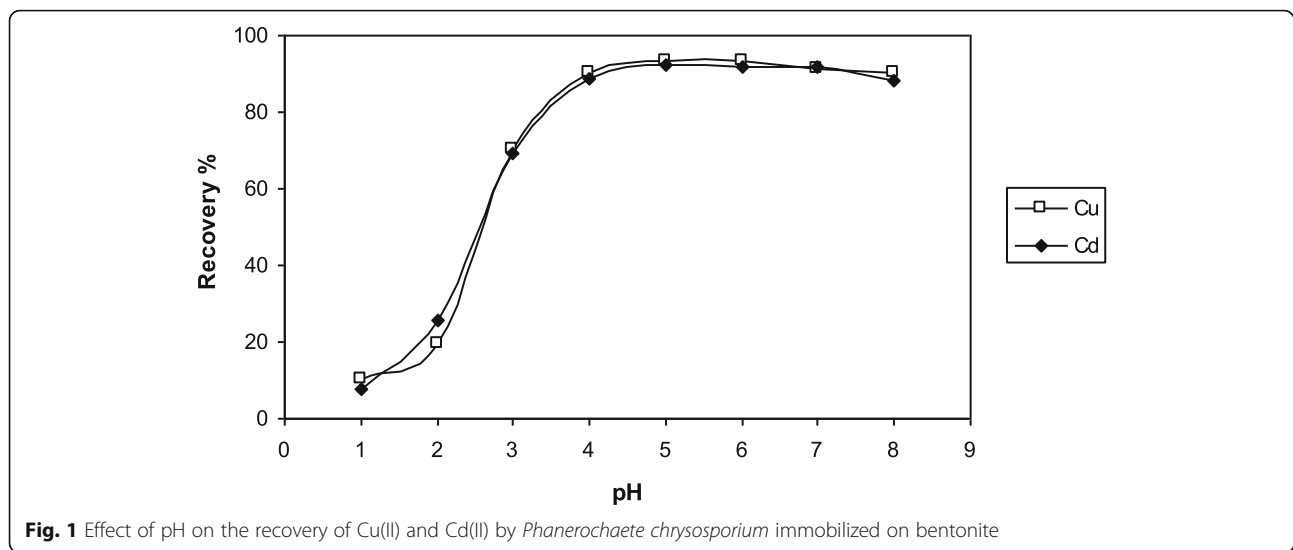


Fig. 1 Effect of pH on the recovery of Cu(II) and Cd(II) by *Phanerochaete chrysosporium* immobilized on bentonite

2014; Oh and Seo 2019). The active sites on clay surface have been known to be weakly acidic, and these sites are gradually deprotonated at comparatively higher pH resulting in larger uptake of metal ions. It has been known that the stability of the hydrated hydroxide ion in aqueous solution is less than that of the hydrogen ion. This is due to the fact that the hydroxide ion is larger than the proton, and therefore, more water molecules are required to surround this ion for hydration. This results adsorption of much more OH<sup>-</sup> ions on clay compared to H<sup>+</sup> ions. With increasing pH, more and more OH<sup>-</sup> ions will be adsorbed on clay making it negatively charged and consequently, and adsorption of metal ions will also increase (Kocaoba 2020).

**Effect of adsorbent amount**

Metal retention was investigated in adsorbent amounts ranging between 0.05 and 0.4 g. From the results shown

in Fig. 2, it was found that the retention of both metals showed a slight increase for adsorbent amounts up to 0.2g. For this reason, 0.2 g was determined as the optimum adsorbent amount in the studies.

**Effect of volume and type of elution solutions**

Another significant parameter affecting the preconcentration is the volume, type, and concentration of the eluate used to remove metal ions. The elution solution was optimized to achieve maximum recovery with the minimum concentration and volume of the elution solution. HNO<sub>3</sub> and HCl solutions prepared in different volumes in water, and acetone was tested for elution of metal ions retained in the column. As given in the Table 3, for both metals, HNO<sub>3</sub> and HCl solutions were prepared in different concentrations and volumes of water and organic solvent (in acetone). The recovery percentages of

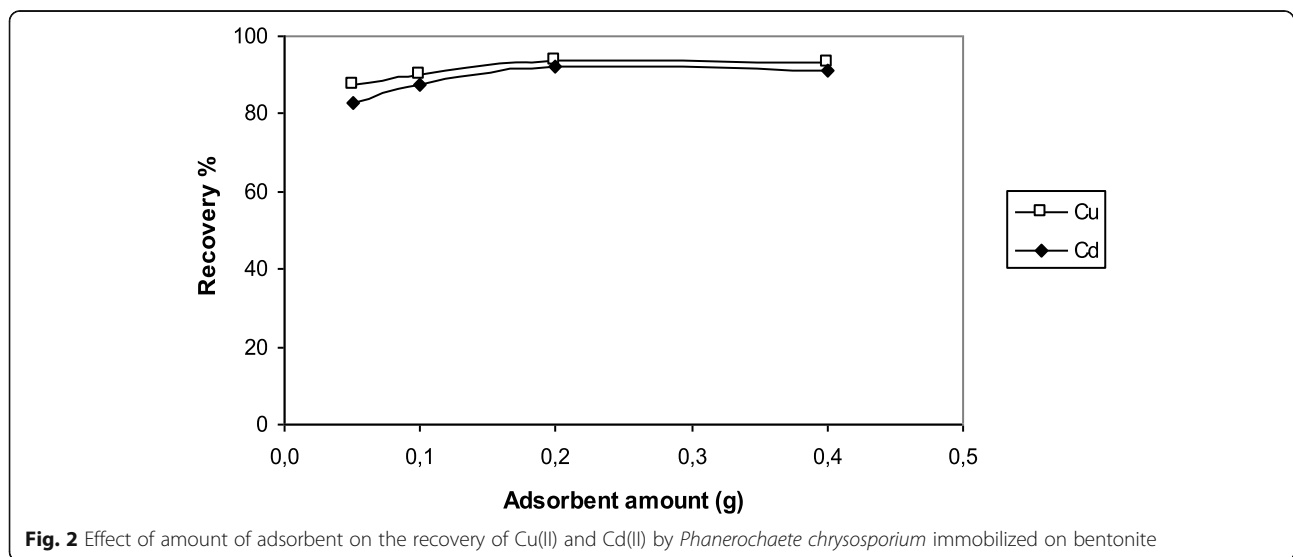
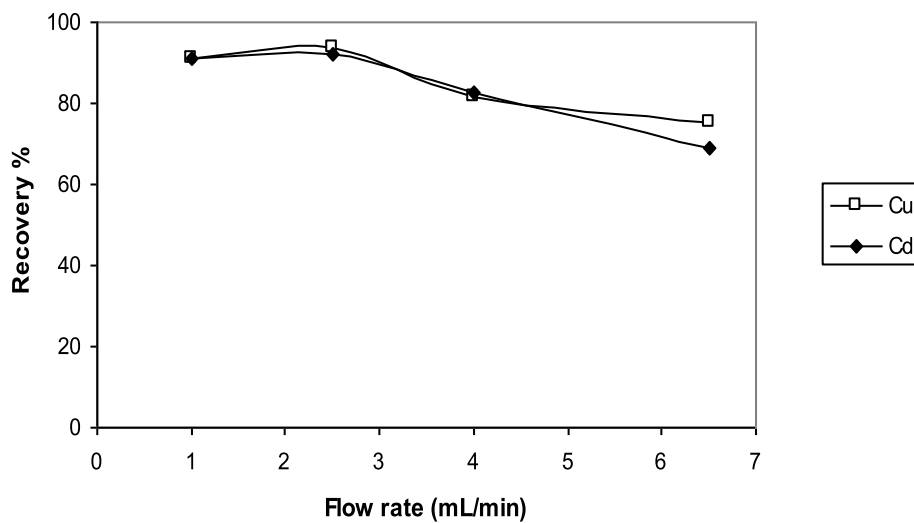
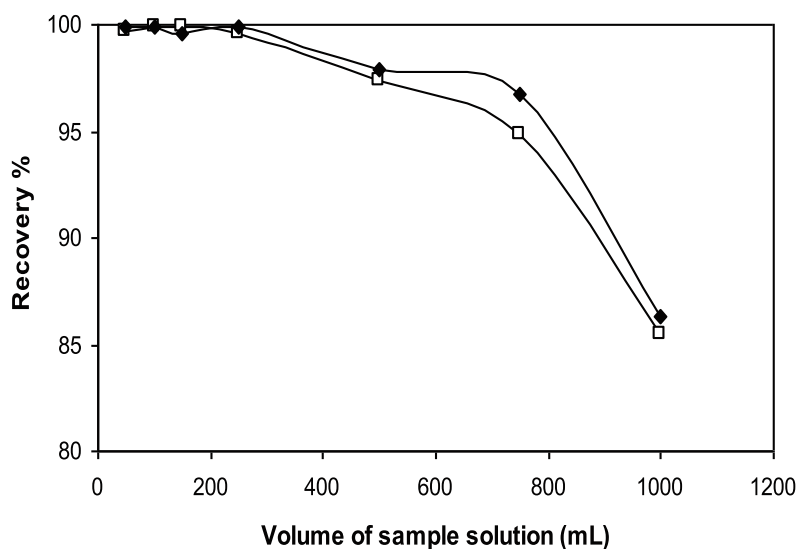


Fig. 2 Effect of amount of adsorbent on the recovery of Cu(II) and Cd(II) by *Phanerochaete chrysosporium* immobilized on bentonite

**Table 3** Effect of the type and volume of elution solutions on the recovery of metals

Element	Type of elution solution	Volume (mL)	Concentration (mol/L)	Recovery (%)
Cu(II)	HCl (in water)	5	1	96.16
	HCl	10	1	99.97
	HCl	15	1	99.90
	HNO <sub>3</sub> (in water)	5	1	96.02
	HNO <sub>3</sub>	10	1	99.96
	HNO <sub>3</sub>	15	1	99.74
	HCl (in acetone)	5	1	96.43
	HCl	10	1	99.65
	HCl	15	1	99.91
	HNO <sub>3</sub> (in acetone)	5	1	96.60
	HNO <sub>3</sub>	10	1	99.67
	HNO <sub>3</sub>	15	1	99.96
Cd(II)	HCl (in water)	5	1	95.04
	HCl	10		99.94
	HCl	15		99.99
	HNO <sub>3</sub> (in water)	5		94.75
	HNO <sub>3</sub>	10		99.82
	HNO <sub>3</sub>	15		99.96
	HCl (in acetone)	5		94.81
	HCl	10		99.25
	HCl	15		99.99
	HNO <sub>3</sub> (in acetone)	5		94.67
	HNO <sub>3</sub>	10		99.42
	HNO <sub>3</sub>	15		99.87

**Fig. 3** Effect of flow rate on the recovery of Cu(II) and Cd(II) by *Phanerochaete chrysosporium* immobilized on bentonite



**Fig. 4** Effect of sample volume on the recovery of Cu(II) and Cd(II) by *Phanerochaete chrysosporium* immobilized on bentonite

HCl and HNO<sub>3</sub> used as elution solutions are quite high, and the results are close to each other. It was found that 10 mL of 1 mol/L HCl eluent in both metals was sufficient to use HCl instead of HNO<sub>3</sub>, which has a strong oxidizing property, and to avoid the use of organic

solvent and excess eluent volume. As given in Table 3, 10 mL of 1 mol/L HCl solution was found to be sufficient in both metals (more than 95% recovery).

**Table 4** Recovery values of Cu(II) and Cd(II) metal ions for the reusability study of the column

Cu		Cd	
Cycle	% Recovery	Cycle	% Recovery
1	99.93	1	99.94
2	99.96	2	99.89
3	99.92	3	99.96
4	99.92	4	99.92
5	99.93	5	99.92
6	99.90	6	99.92
7	99.95	7	99.87
8	99.92	8	99.89
9	99.91	9	99.90
10	99.88	10	99.77
11	99.90	11	98.94
12	99.73	12	98.18
13	98.83	13	97.01
14	98.83	14	96.28
15	98.74	15	94.98
16	97.48	16	95.22
17	97.65	17	92.45
18	96.50	18	92.22
19	94.88	19	91.85
20	93.75	20	90.90

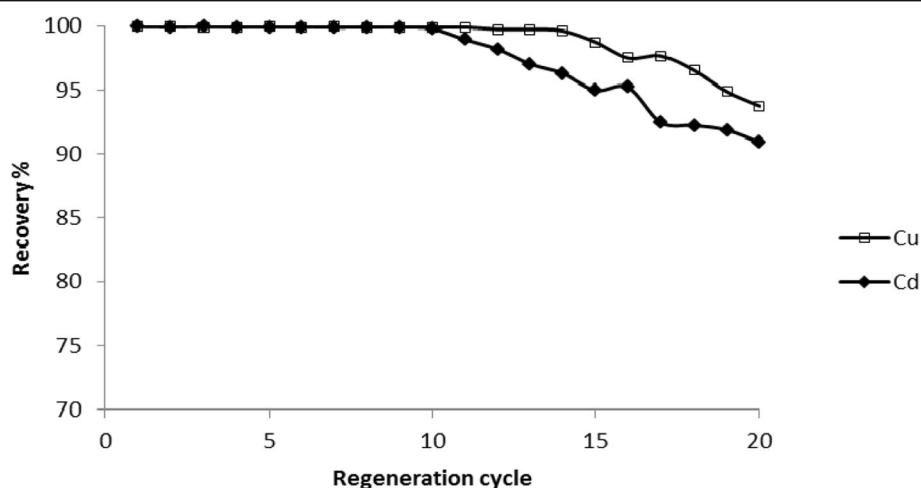
#### Effect of flow rates of sample solutions

Flow rate is another significant parameter affecting adsorption. Since the mass transfer from the solutions to the biomass-bentonite adsorbent is influenced by the flow rate, the effect of this parameter has been studied under optimum conditions such as pH, adsorbent amount, and eluent type. Flow rates from 1 to 6.5 mL/min were examined in column studies. As can be seen in Fig. 3, a decrease in metal recoveries was observed at flow rates higher than 2.5 mL/min. This reduce is probably due to the metal ions not getting enough contact and the adsorbent reaching equilibrium. Therefore, 2.5 mL/min flow rate was applied to studies for both metals.

#### Effect of sample solution volume

Since trace elements are in very low concentrations in water, soil, and biological samples, it is necessary to know the appropriate sample solution volume in their determination. This effect was investigated to determine the maximum volume or minimum analyte concentration of the sample solution passing through the column. Under optimum conditions (pH: 5, adsorbent amount: 0.2 g, flow rate: 2.5 mL/min.), at 20 µg constant metal concentration, 50, 100, 150, 250, 500, 750, and 1000 mL sample solutions were passed through the column and then the column was eluted with 10 mL of 1 M HCl solution. As can be seen from Fig. 4, it has been found that both Cu(II) and Cd(II) can be quantitatively recovered up to 250 mL sample volume (> 99 %). When high volumes are reached, there is a decrease in the recovery of





**Fig. 5** Reusability of the columns for Cu(II) and Cd(II) recoveries

metals. Preconcentration factor was found to be 25 for 10 mL elution volume.

#### Effect of column reuse

Several adsorption elution cycles were performed with metal ions to investigate the stability and reproducibility of the column, and changes in its recovery were examined. In each cycle, 50 mL of the sample was passed through the column at an analyte concentration of 10 mg/L and then eluted with 10 mL of 1 M HCl. This process was performed five times a day for several days. The column appears relatively stable until 15 studies. The results are given in Table 4 and Fig. 5. Therefore, it is possible to use the column repeatedly.

#### Capacity studies

The capacity of the adsorbent was investigated to find the amount of metal adsorbed into the *Phanerochaete chrysosporium* modified to bentonite. Solutions of 50 mL volume, adjusted to the appropriate pH at various concentrations ranging from 5 to 100 mg/L, were preconcentrated and eluted. Amounts of metal ions retained in different concentrations were calculated according to the following equation:

$$C = cV/w \quad (1)$$

C: capacity of adsorbent (mg/g), c: eluted metal concentration (mg/L), V: solution volume (L), and w:

**Table 5** Capacity for the metal ions and recovery

Metal ion	Capacity (mg/g)	Recovery
Cu(II)	4.573	97
Cd(II)	4.542	95

amount of immobilized material (g). The results are given in Table 5.

#### Effect of interfering ions

The effect of interference elements on adsorption was investigated. For this purpose, while these elements are in the same environment as the metals studied, the recovery of metals is examined. In this study, some metal ions like Fe(III), Co(II), Mn(II), and Zn(II) and some anions like Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, and SO<sub>4</sub><sup>2-</sup> were chosen as interfere ions. Interfered ions were prepared at 10 mg/L concentrations. As can be understood from Table 6, where the results are given, the effect of interference ions can be negligible. According to these results, Cu(II) and Cd(II) can be quantitatively determined in environmental samples containing such ions.

#### Kinetic studies

Kinetic experiments were performed depending on the contact time using *Phanerochaete chrysosporium* immobilized bentonite (0.5 g) in systems containing two metals at a concentration of 100 mg/L.

As given in Fig. 6, the biosorption of metal ions tended to increase in contact times between 0 and 30 min and then became almost constant until the end of the experiments (180 min). The biosorption rate was very fast during the first 30 min contact time, and most of the metal adsorption occurred during this time, and a linearity was

**Table 6** The effect of interfering ions on recovery

Interfering ions	Concentration (mg/L)	Recovery
Fe(III), Mn(II), Zn(II), Co(II), Cd(II)	10	93.09 (Cu)
Fe(III), Mn(II), Zn(II), Co(II), Cu(II)	10	91.05 (Cd)
Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> (mixed ions)	10	97.40 (Cu)
Cl <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> , SO <sub>4</sub> <sup>2-</sup> (mixed ions)	10	98.25 (Cd)

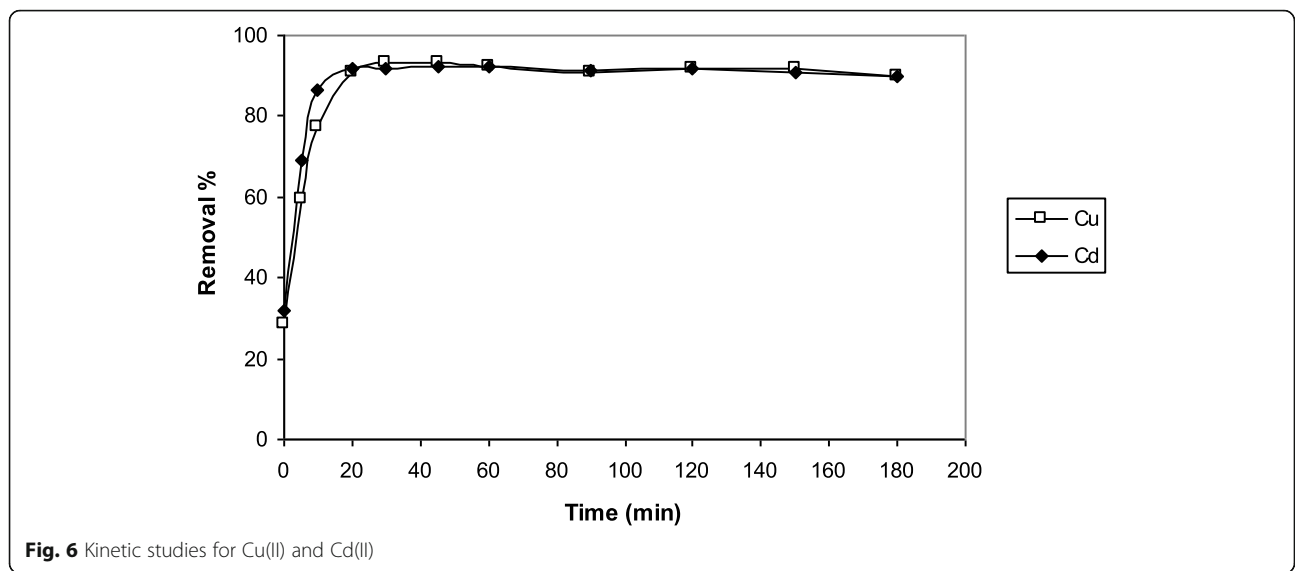


Fig. 6 Kinetic studies for Cu(II) and Cd(II)

sighted between the percentage of Cu(II) and Cd(II) bio-sorption and contact time (> 90%).

**Adsorption isotherms**

There are many models that explain the adsorption equilibrium, but the most important factor to consider here is that they can be applied under all working conditions. Among these, Langmuir and Freundlich isotherms are the most frequently used models to define the adsorption properties of adsorbents used especially in water systems (Freundlich 1906; Langmuir 1918; Donat et al. 2005; Kocaoba 2007; Chen et al. 2017; Barquilha et al. 2019). For equilibrium investigation, 0.5, 1, 1.5, 2, and 3 g of biomass modified bentonite samples were put into Erlenmeyers. Metal solutions of 200 mL at fixed concentration (100 mg/L) were added and contacted on

a shaker at room temperature (20 ± 2°C) for 5 days. The amount of metal retained on the adsorbent was determined from the equation below:

$$Q_e = (C_o - C_e) / m.V \tag{2}$$

C<sub>o</sub>: initial; C<sub>e</sub>: equilibrium concentrations of metal ions (meq/L); m: amount of adsorbent (g); and V: volume of the solution (L). Sorption data were examined according to Langmuir and Freundlich adsorption equilibriums as given in the reference (Kocaoba 2007). The results are shown in Figs. 7 and 8. The adsorption coefficients were obtained from the graphics and the results are given in Table 7.

In both cases, the plots have good linearity at room temperature (Freundlich plots, R<sup>2</sup> = 0.932–0.954,

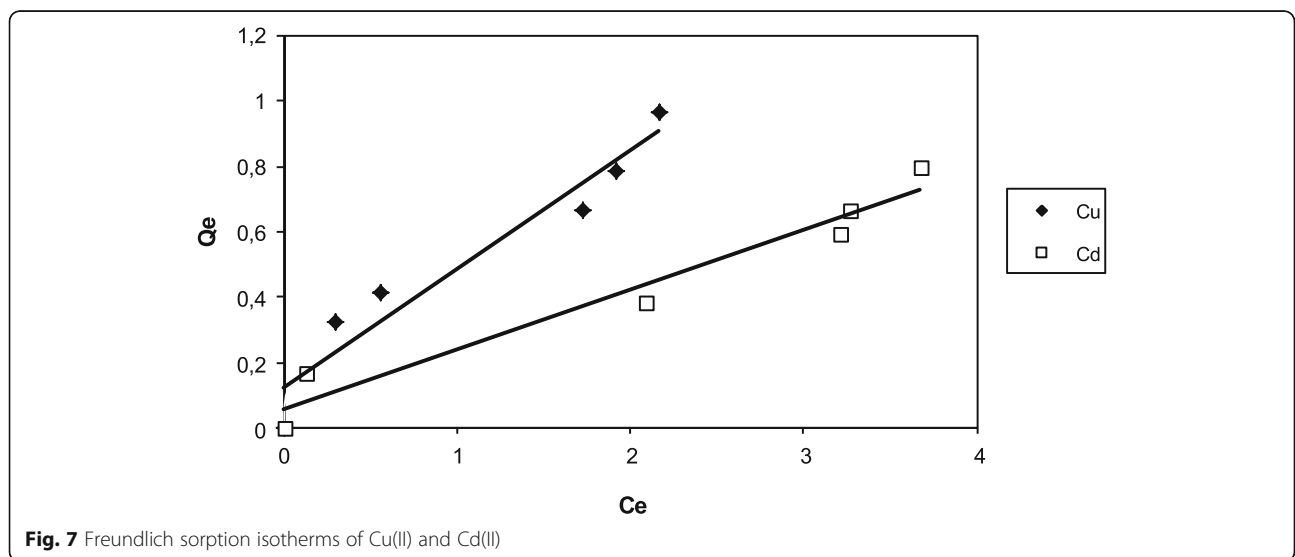


Fig. 7 Freundlich sorption isotherms of Cu(II) and Cd(II)



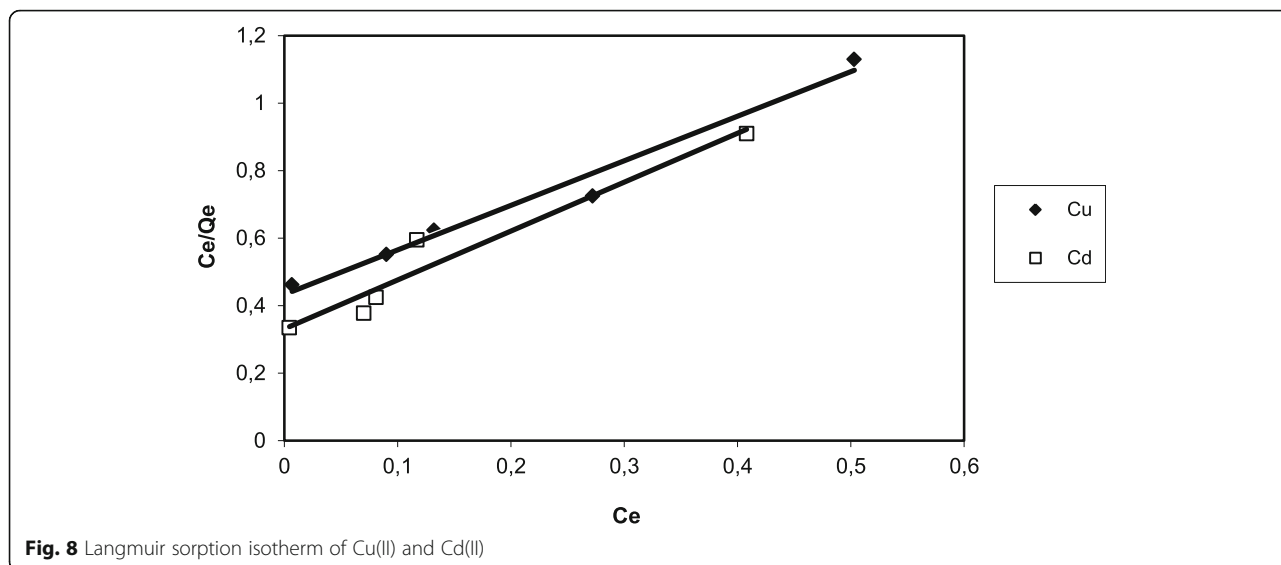


Fig. 8 Langmuir sorption isotherm of Cu(II) and Cd(II)

Langmuir plots,  $R^2 = 0.943-0.977$ ). Langmuir model,  $R^2$  values compared Freundlich model can be seen to provide a better fit. As given in Table 7, Langmuir  $Q_0$  (Langmuir maximum adsorption capacity) values were between 0.331 and 0.433 meq/g, and  $b$  (Langmuir constant) values were between 1.321 and 1.450. The separation factor,  $R_L$  0.745–0.806 values also supported positive adsorption of metal ions on *Phanerochaete chrysosporium* immobilized bentonite. From the Freundlich constants,  $K_f$  indicates the degree of adsorption, and  $n$  is the degree of nonlinearity between solution and concentration. For a positive adsorption,  $n$  value should be  $0 < n < 1$ . As  $n$  value approaches zero, all samples appear to have a very high affinity for the adsorption of heavy metals. The value of  $n$  is in any case between 0.058 and 0.123 and  $< 1$ . The  $K_f$  constant showing the adsorption capacity of the adsorbent used was 0.362 for Cu(II) and 0.188 for Cd(II).

The adsorption coefficients show the appropriate structure of the adsorption of both metal ions on *Phanerochaete chrysosporium* immobilized bentonite. In addition, compared to previous studies with heavy metals, the results obtained from the studies were found to be quite compatible with the Langmuir and Freundlich isotherms (Babel and Kurniawan 2003; Kocaoba 2007; Saeeda et al. 2009; Torres et al. 2014).

Table 7 Parameters of Langmuir and Freundlich isotherms for sorption of metal ions on *Phanerochaete chrysosporium* immobilized bentonite

Metal ion	Langmuir isotherm method				Freundlich isotherm method		
	b	$Q_0$	$R^2$	$R_L$	$K_f$	n	$R^2$
Cu <sup>2+</sup>	1.321	0.433	0.977	0.806	0.362	0.123	0.932
Cd <sup>2+</sup>	1.450	0.331	0.943	0.745	0.188	0.058	0.954

### Analytical figures

The precisions of the column method evaluated as the relative standard deviation obtained after analyzing a series of 10 replicates for 5 mg Cu(II) and 10 mg Cd(II) in 100 mL of model solutions were 2.5% and 3.6%, respectively. The limit of detection (LOD) of the proposed method for the determination of Cu(II) and Cd(II) was studied under optimum experimental conditions. The detection limits based on three times the standard deviations of the blank ( $N=10$ ,  $X_L = X_b + 3s$ ;  $X_L$ : LOD,  $X_b$ : blank) for Cu(II) and Cd(II) were found to be 0.5 and 0.7 mg/L, respectively.

Table 8 Determination of copper and cadmium in water samples

Water samples	Element	Added ( $\mu$ g)	Recovery <sup>b</sup> %
Tap water	Cu	10	94.5 $\pm$ 0.3
		20	95.6 $\pm$ 0.4
		30	96.4 $\pm$ 0.3
	Cd	10	92.3 $\pm$ 0.4
		20	94.2 $\pm$ 0.3
		30	95.5 $\pm$ 0.4
Spring water	Cu	10	96.5 $\pm$ 0.4
		20	96.8 $\pm$ 0.3
		30	97.2 $\pm$ 0.4
	Cd	10	95.7 $\pm$ 0.4
		20	96.4 $\pm$ 0.3
		30	97.3 $\pm$ 0.4

<sup>b</sup>Mean of 5 determinations with 95% confidence level ( $N=5$ )

## Application

Cu (II) and Cd (II) determinations were made by applying the proposed method to some water samples. Samples of 100 mL volume of water were taken and then filtered to remove suspended substances. Then, the pH of the 50 mL water samples taken was adjusted and passed through the column under optimum conditions and metal ions were analyzed according to the predetermined general method. According to the results given in Table 8, the recommended method can be successfully applied to water analyzes in a 95% confidence interval. Analyte concentrations in all samples were determined with a relative error of less than 10%.

## Conclusion

This method has been successfully applied to preconcentrate copper and cadmium. The recovery of the analytes examined was quantitative ( $\geq 95\%$ ). It has been observed that each column can be successfully used to recovery of metal ions without undergoing significant changes up to 15 times. *Phanerochaete chrysosporium* modified bentonite can be effectively applied as a new adsorbent for the preconcentration and determination of Cu(II) and Cd(II) ions in water samples due to its low detection limit and relative standard deviation and high resistance to interfered ions. These good properties of the proposed method have shown that this is a useful and cost-effective method. In addition, the preconcentration step and the use of AAS increase its sensitivity and selectivity of the method.

## Abbreviations

AAS: Atomic absorption spectrometer; L.O.I: Loss of ignition; i.d.: Inner diameter

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## Authors' contributions

SK designed the study and directed all the experiments and wrote the manuscript. The experiments were carried out by MDP. MA prepared the biomass. The authors read and approved the final manuscript.

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## Availability of data and materials

Research data have been provided in the manuscript.

## Declarations

## Competing interest

The authors declare that they have no competing interests.

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