

In Vitro Bioadsorption of Cd²⁺ Ions: Adsorption Isotherms, Mechanism, and an Insight to Mycoremediation

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Date Submitted: 2021-02-22

Keywords: mycoremediation, isotherms, heavy metals, cadmium, bioadsorption, *T. fasciculatum*, bioadsorption mechanism, *T. longibrachiatum*

Abstract:

The objective of this paper is to establish the significance of the mycoremediation of contaminants such as Cd²⁺ to achieve sustainable and eco-friendly remediation methods. Industries such as electroplating, paint, leather tanning, etc. release an enormous amount of Cd²⁺ in wastewater, which can drastically affect our flora and fauna. Herein, we report on the in vitro bioadsorption of Cd²⁺ ions using fungal isolates obtained from different contaminated industrial sites. The detailed studies revealed that two fungal species, i.e., *Trichoderma fasciculatum* and *Trichoderma longibrachiatum*, were found to be most effective against the removal of Cd²⁺ when screened for Cd²⁺ tolerance on potato dextrose agar (PDA) in different concentrations. Detailed adsorption studies were conducted by exploring various experimental factors such as incubation time, temperature, pH, inoculum size, and Cd²⁺ salt concentrations. Based on optimum experimental conditions, *T. fasciculatum* exhibited approximately 67.10% removal, while *T. longibrachiatum* shows 76.25% removal of Cd²⁺ ions at pH 5.0, 120 h incubation time, at 30°C. The inoculum sizes for *T. fasciculatum* and *T. longibrachiatum* were 2.5% and 2.0%, respectively. Finally, the morphological changes due to Cd²⁺ accumulation were examined using scanning electron microscopy (SEM). Further, Fourier transform infrared spectroscopy (FTIR) spectroscopy reveals the presence of various functional groups (-CH, ?C=O, NH and ?OH), which seem to be responsible for the efficient binding of Cd²⁺ ions over the fungal surfaces.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):

LAPSE:2021.0076

Citation (this specific file, latest version):

LAPSE:2021.0076-1

Citation (this specific file, this version):

LAPSE:2021.0076-1v1

DOI of Published Version: <https://doi.org/10.3390/pr8091085>

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Article

In Vitro Bioadsorption of Cd²⁺ Ions: Adsorption Isotherms, Mechanism, and an Insight to Mycoremediation

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Received: 1 August 2020; Accepted: 29 August 2020; Published: 2 September 2020



Abstract: The objective of this paper is to establish the significance of the mycoremediation of contaminants such as Cd²⁺ to achieve sustainable and eco-friendly remediation methods. Industries such as electroplating, paint, leather tanning, etc. release an enormous amount of Cd²⁺ in wastewater, which can drastically affect our flora and fauna. Herein, we report on the in vitro bioadsorption of Cd²⁺ ions using fungal isolates obtained from different contaminated industrial sites. The detailed studies revealed that two fungal species, i.e., *Trichoderma fasciculatum* and *Trichoderma longibrachiatum*, were found to be most effective against the removal of Cd²⁺ when screened for Cd²⁺ tolerance on potato dextrose agar (PDA) in different concentrations. Detailed adsorption studies were conducted by exploring various experimental factors such as incubation time, temperature, pH, inoculum size, and Cd²⁺ salt concentrations. Based on optimum experimental conditions, *T. fasciculatum* exhibited approximately 67.10% removal, while *T. longibrachiatum* shows 76.25% removal of Cd²⁺ ions at pH 5.0, 120 h incubation time, at 30°C. The inoculum sizes for *T. fasciculatum* and *T. longibrachiatum* were 2.5% and 2.0%, respectively. Finally, the morphological changes due to Cd²⁺ accumulation were examined using scanning electron microscopy (SEM). Further, Fourier transform infrared spectroscopy (FTIR) spectroscopy reveals the presence of various functional groups (-CH, -C=O, NH and -OH), which seem to be responsible for the efficient binding of Cd²⁺ ions over the fungal surfaces.

Keywords: *T. longibrachiatum*; *T. fasciculatum*; bioadsorption; cadmium; heavy metals; isotherms; bioadsorption mechanism; mycoremediation

1. Introduction

The emerging worldwide environmental problems of the past decades are mostly related to the superfluous increase in metallic contaminants in the environment [1–6]. Inorganic contaminants such as heavy metals released into the environment through various industrial, agricultural, and domestic practices [5,6]. Heavy metals may also enter into the environment by natural sources, but the extent of such exposures is non-significant. Heavy metals are often present together with organic pollutants in industrial wastewater [7]. The industrial effluents are rich in dissolved salts of Cr, Cu, Ni, Zn, and Cd, etc., and can cause a serious problem to the surrounding environment. Heavy metals pollution causes serious problems due to their non-biodegradable nature [8,9]. Although these metals are vital for the proper functioning of biological systems but only in trace concentrations, higher concentrations may lead to disturbed functioning of bio-geo-chemical cycles [10–12]. In addition, serious health issues may arise if such metal ions can enter into the food chains and their corresponding products. Thus, even 1.0–10 mg/L can cause health problems in human beings such as jaundice, facial edema, blue lungs, kidney damage, hearing disorder, skin cancer, asthma, protein metabolism, and bronchial cancer, etc. [13,14]. Therefore, a number of traditional physiochemical treatment techniques have been reported to remove the heavy metals from wastewater, especially from the industrial wastewater, to name a few, electro-coagulation, solvent extraction, ion-exchange, electro-reduction, reverse osmosis, adsorption, membrane separation, chemical precipitation, and so on [15–29]. Even though used widely, these above-mentioned techniques exhibited several disadvantages which include the utilization of high-cost equipment and monitoring systems, the use of various expensive chemicals, the discharge of toxic sludge, long processing time, the production of toxic waste products that need further processing, and so on [23–31]. Thus, it is the necessity of the time to develop a new, simple, and inexpensive method for heavy metal removal from wastewater. Recently, by utilizing living/non-living microorganisms and their derivatives to remove the heavy metals from industrial wastewater is considered a promising technique [32–34]. Accordingly, the bioremediation can be considered as one of the most important and advantageous process that can efficiently remove the heavy metals from industrial wastewater. The entrapment of the heavy metal ions and their corresponding sorption on the binding sites of the cellular structures leads to the bioadsorption of heavy metal ions from the industrial wastewater [35,36]. As a result of the faster adsorption process, the microbial cells are considered as an important and advantageous scaffold for the biosorption of heavy metals and wastewater treatment.

In this study, different fungal isolates extracted from various contaminated sites (Haryana, India) were cultured and screened for tolerance to Cadmium (Cd^{2+}). Various process factors, i.e., inoculum size, pH, initial metal ion concentration, temperature, and incubation time on the Cd^{2+} removal by highly efficient Cd^{2+} tolerant fungal isolates have been investigated and presented in this article. In addition, mechanisms of Cd^{2+} removal by efficient Cd^{2+} tolerant fungal isolates have also been studied.

2. Materials and Methods

2.1. Chemicals Used

Potato dextrose broth (PDB) and nutrient media, potato dextrose agar (PDA), were procured from Hi-Media, Mumbai India. $\text{Cd}(\text{NO}_3)_2$, was provided by Fine-Chem Limited, Mumbai, India. The solutions were prepared in triply distilled water (conductivity = $0.5 \mu\text{S cm}^{-1}$), and the reagents were used without any further purification.

2.2. Sample Collection and Isolation of Fungi

Samples from various sites such as industrial effluents, sewerage discharge, and sludge were collected from Karnal, Panipat, and Sonapat districts of Haryana (India). The samples were stored at 4°C prior to further processing. Fungal isolates were isolated from samples by the PDA serial dilution method. The serial dilutions were made up to 10^6 and 1 mL of each dilution i.e., 10^4 and 10^6

were poured over PDA plates. An incubation of cultured petri plates was performed at 28 °C for 96 h. The prominently grown fungal colonies were regenerated and purified using the streak plate method.

2.3. Screening and Identification of Efficient Cd²⁺-Tolerant Fungal Isolate

The isolated fungal isolates were tested for their potential to remove Cd²⁺ at 25, 50, and 75 mg/L concentrations. The concentrations were prepared from a sterilized 100 mg/L solution of Cd(NO₃)₂ containing PDA. A loop full of purified isolates was positioned at the Petri plates' center comprising of PDA (pH 5.0) and the above-mentioned Cd²⁺ concentrations. The growth of fungal isolates on the pure PDA medium was labeled 'Control'. All plates were sealed with parafilm and subjected to incubation of 5 days. Based on the visual observation, the growth of fungal isolates was labeled as absent growth (−), least growth (+), less than normal growth (++) , slightly less than normal growth (+++), and normal growth (++++). The highly efficient Cd²⁺-tolerant fungal isolates were sent for identification to the Indian Agricultural Research Institute, New Delhi, India.

2.4. Optimization of Batch Cultures

The highly efficient Cd²⁺-tolerant fungal culture was tested at different pH, i.e., 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 for uptake and percentage removal of Cd²⁺ from PDA containing 20 mg/L of Cd²⁺. First, 1M HCl and 1M NaOH were used to adjust the pH of PDB. Then, 1mL spore suspension of efficient fungal culture containing 10⁴ spores (1%) was inoculated into 250 mL Erlenmeyer conical flasks containing 100 mL of PDB enriched with 20 mg/L of Cd²⁺ followed by shaking these flasks for 120 h at 30 °C. For the control, a non-inoculated flask having 20 mg/L of Cd²⁺ and PDB was used. After 120h, fungal growth was collected through filtration using Whatman Filter Paper No. 1, which was later dried for 48 h at 80 °C in an oven and then weighed. Atomic absorption spectrophotometer (AAS, GBC 932, Dandenong, Australia) was used to evaluate the Cd²⁺ concentration left in the filtrate. To evaluate the incubation time effect, the incubation time was varied between 24 and 144h under optimum pH. Under optimum pH and incubation time, the effect of inoculum size was investigated by performing batch experiments at different inoculum sizes i.e., 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%. Temperatures of 20, 25, 30, and 35 °C were studied to examine the temperature effect at optimum pH, incubation time, and inoculum size. Under optimum pH, the effect of initial Cd²⁺ concentration, inoculum size, and incubation time was investigated by changing the concentrations of Cd²⁺ ions from 5 to 50 mg/L. The process to perform all the experiments was the same as mentioned above. All experiments were performed in triplicate, and data were statistically examined by *t*-test with the help of SPSS 11.5.

The Cd²⁺ uptake by fungal biomass was calculated as follows:

$$Q = \frac{v(C_0 - C_f)}{M} \quad (1)$$

where Q is the Cd²⁺ uptake (mg/g); C₀ and C_f are the initial and leftover concentrations of Cd²⁺ (mg/L), respectively; v is the total volume of the solution (L); and M the dried biomass of fungus (g).

The removal of Cd²⁺ (%) was calculated using following equation:

$$\% \text{ Removal of Cd}^{2+} = \frac{C(\text{initial}) - C(\text{final})}{C(\text{initial})} \times 100 \quad (2)$$

2.5. Equilibrium Isotherms

2.5.1. Langmuir Isotherm

Langmuir isotherm is a mathematical deduction of kinetic equilibrium between the condensation and loss of adsorbed molecules.

$$C_e/q_e = 1/q_{\max} b + C_e/q_{\max} \quad (3)$$

where C_e is equilibrium concentration of Cd^{2+} (mg/L), q_e is amount of Cd^{2+} adsorbed per gram of fungal biomass (mg/g), q_{\max} is Langmuir constant, and b is the energy of adsorption. The values of b and q_{\max} were evaluated by the intercept and slope of the graph, respectively.

2.5.2. Freundlich Isotherm

This isotherm is the result of empirical consideration and expressed as

$$q_e = K_f C_e^{1/n} \quad (4)$$

where q_e is the Cd^{2+} adsorbed (mg/g), C_e is the equilibrium concentration of Cd^{2+} (mg/L), K_f is the adsorption coefficient (mg/g) and directly related to the standard free energy change, and n is the empirical constant.

The linear logarithmic form of the isotherm is:

$$\text{Log } q_e = (1/n) \text{ log } C_e + \text{log } K_f \quad (5)$$

From the straight-line plot between $\text{log } q_e$ and $\text{log } C_e$, the values of K_f and $1/n$ were found.

2.6. Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The morphological changes in the fungi without and after incubating with Cd^{2+} were visualized using Scanning Electron Microscopy (SEM; JSM-6100, JEOL, Tokyo, Japan). The changes in surface chemical profiling were analyzed and interpreted by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spectrum BXII, Waltham, MA, USA, in 4000–400 cm^{-1} frequency range with a resolution of 1 cm^{-1}).

3. Results and Discussion

3.1. Isolation, Screening, and Identification of Cd^{2+} -Resistant Fungal Biomass

Twenty-five sludge, industrial effluents and sewage samples were collected from Panipat, Sonapat and Karnal districts of Haryana, India to isolate fungal isolates. All the fungi were maintained on PDA slants (pH 4.5) at 30 °C. Screening of these fungal isolates on PDA containing 25, 50, and 75 mg/L of Cd^{2+} showed that 13 fungal isolates were tolerant to 25 mg/L Cd^{2+} , 7 fungal isolates were tolerant to 50 mg/L, and 3 fungal isolates were tolerant to 75 mg/L (Table 1). Such phenomena indicate that some fungal isolates exhibited inhibition at higher metal ions concentrations. Out of three fungal isolates tolerant to 75 mg/L Cd^{2+} , the highly Cd^{2+} -tolerant fungal isolate FS7 and FS16 were identified as *Trichoderma fasciculatum* (ITCC 7547) and *Trichoderma longibrachiatum* (ITCC 7062) by the Indian Culture Collection, Indian Agricultural Research Institute. IARI, New Delhi, India.

Table 1. Growth profile of all the fungal isolates on potato dextrose agar (PDA) (pH 5.0, temperature 30 °C) containing 25, 50, and 75 mg/L of Cd²⁺ after five days.

Sr. No.	Fungal Isolate Code	Control	Cd ²⁺ Ion Concentration (mg/L)		
			25	50	75
1	FS1	++++	++	++	+
2	FS2	++++	++	++	+
3	FS3	++++	++	++	+
4	FS4	++++	++++	+++	++
5	FS5	++++	++	++	+
6	FS6	++++	++	++	+
7	FS7	++++	++++	++++	+++
8	FS8	++++	–	–	–
9	FS9	++++	++++	+++	++
10	FS10	++++	++++	+++	++
11	FS11	++++	–	–	–
12	FS12	++++	++++	+++	+++
13	FS13	++++	–	–	–
14	FS14	++++	++	+	–
15	FS15	++++	–	–	–
16	FS16	++++	++++	++++	+++
17	FS17	++++	+	–	–
18	FS18	++++	+	–	–
19	FS19	++++	–	–	–
20	FS20	++++	+++	++	+
21	FS21	++++	+++	++	+
22	FS22	++++	++++	+++	++
23	FS23	++++	+++	++	+
24	FS24	++++	–	–	–
25	FS25	++++	++	+	–

Note: Absent growth (–), least growth (+), less than normal growth (++) , slightly less than normal growth (+++), and normal growth (++++).

3.2. Optimization of Process Parameters

Various surface and physiochemical properties—for instance, pH, incubation time, inoculum size, temperature, metal ion concentrations, quantity of biosorbents, etc. are affecting the process for the bioaccumulation of heavy metal ions in fungal biomass.

3.2.1. pH Effect

In the process to remove the heavy metal ions from fungi, the pH plays an important and significant role. The observed results demonstrated that there was an enhancement in the Cd²⁺ ion uptake and percentage removal up to pH 5.0 from the liquid medium by *T. fasciculatum* and *T. longibrachiatum*. After pH 5.0, the uptake and percentage removal was significantly decreased with the increasing pH (Figure 1a). The outcomes showed that the ideal pH for uptake and percentage removal of Cd²⁺ + from the liquid medium by *T. fasciculatum* and *T. longibrachiatum* was 5.0.

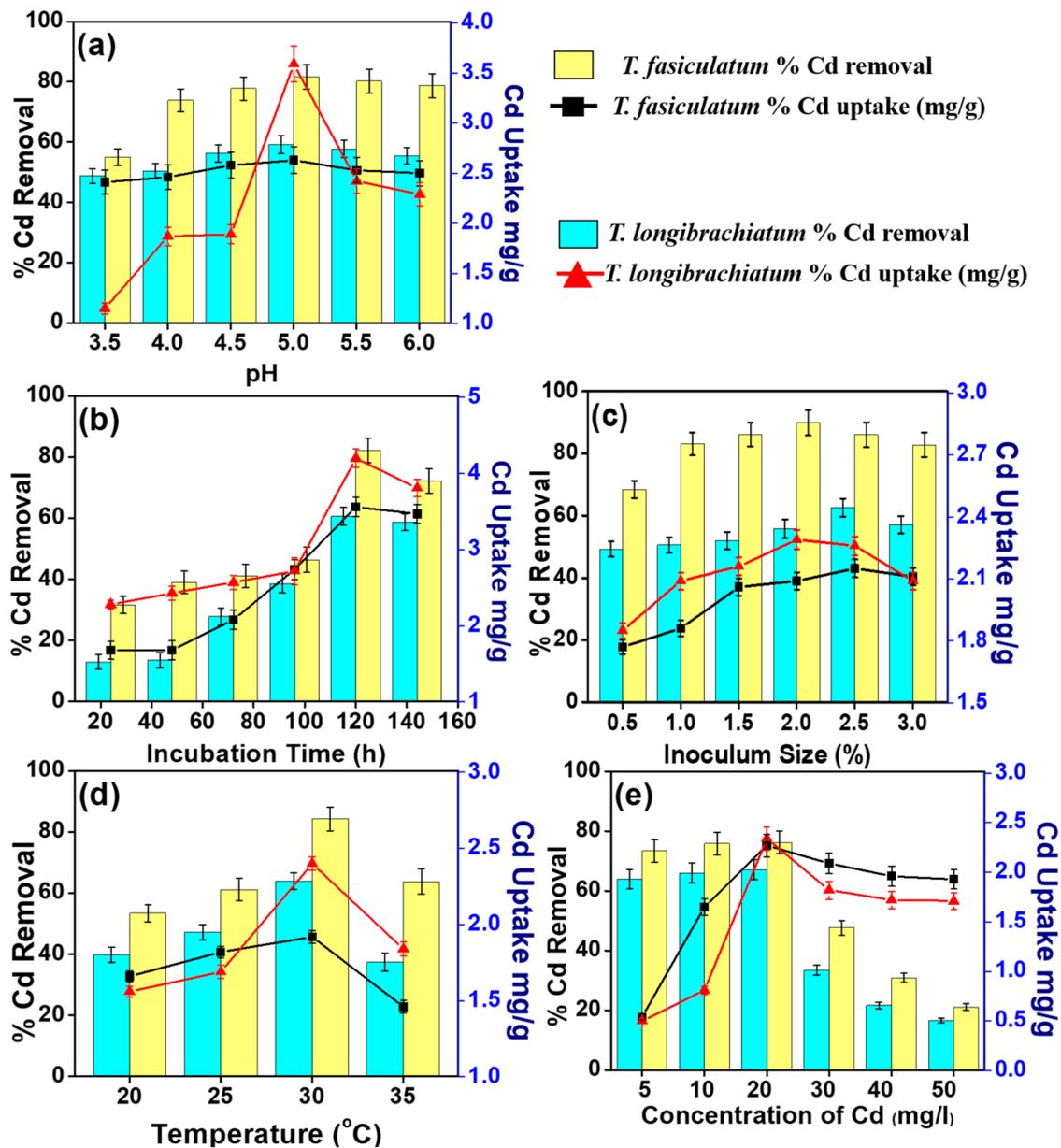


Figure 1. Effect of operational parameters on the Cd²⁺ removal efficiency by *T. fasciculatum* and *T. longibrachiatum* (a) pH (b) Incubation Time (c) Inoculum Size (d) Temperature (e) Concentration of Cd.

The observed uptake and percentage removal of Cd²⁺ from the liquid medium by *T. fasciculatum* at pH 5.0 were 2.63 mg/g and 59.20%, respectively. In addition, the uptake and percentage removal of Cd²⁺ by *T. longibrachiatum* were 3.59 mg/g and 81.60%, respectively. Interestingly, it has been reported that the optimum pH is important to get better results regarding the removal of heavy metal ions using fungal isolates [37,38]. In many cases, the optimal value of pH was 5.0, which shows a maximum removal of heavy metal ions (Pb(II), Cd(II), As(III), and Hg(II)) by fungus isolates such as *Penicillium purpurogenum* [37,38].

3.2.2. Incubation Time Effect

Figure 1b exhibits the incubation time effect on the Cd uptake and percentage removal from the liquid medium by *T. fasciculatum* and *T. longibrachiatum*. It was observed that at optimum pH 5.0, the uptake and percentage removal of Cd²⁺ ions from the liquid medium by *T. fasciculatum* and *T. longibrachiatum* were increased with increasing the incubation time. Interestingly, it was seen

the uptake and percentage removal were enhanced from 24 h to 120 h; however, they reduce after 120 h. The maximum uptake and percentage removal of Cd^{2+} by *T. fasciculatum* at 120 h incubation time were 3.56 mg/g and 60.55%, respectively. Nevertheless, at 120 h incubation time, the uptake and percentage removal of Cd^{2+} ion by *T. longibrachiatum* were 4.2 mg/g and 82.2%, respectively.

It is believed by most of the researchers that ion exchange or physical adsorption at the surface of the cell might relate with the initial rapid phase [39]. However, in the presence of salt, the metal ion transport into the cytoplasm across the cell membrane via an active metabolism-dependent transport is responsible for the slower phase [40]. Moreover, in the absence of salt, other biosorption mechanisms that consist of complexation, microprecipitation, etc. due to the diffusion of metal ions into the cell debris are responsible for the second slower phase [41].

3.2.3. Inoculum Size Effect

Under the optimized conditions (pH 5.0, incubation time 120 h), with increasing the inoculum size from 0.5% to 3.0%, the Cd^{2+} ion uptake and percentage removal was enhanced up to a particular size and then reduced. Figure 1c depicts the relation between the Cd^{2+} ion uptake and percentage removal and inoculum size. The experimental observations confirmed that the uptake and percentage removal of the Cd^{2+} ion was increased with increasing the inoculum size from 0.5% to 2.5% for *T. fasciculatum* and 2.0% for *T. longibrachiatum*, while it declined afterwards. The maximum uptake and percentage removal of Cd^{2+} from the liquid medium by *T. fasciculatum* was approximately 2.15 mg/g and 62.60%, respectively. Moreover, under the same optimized experimental conditions, the Cd^{2+} ion uptake and percentage removal in the liquid medium by *T. longibrachiatum* were 2.29 mg/g and 89.85%, respectively. Interestingly, after the inoculum size 2.5% for *T. fasciculatum* and 2.0% for *T. longibrachiatum*, the uptake and percentage removal of the Cd^{2+} ions were decreased significantly. It has been reported that the percentage removal of heavy metal ions increases with enhancing the adsorbent dose due to the fact that it increases the adsorption sites, which leads to high adsorption and high removal efficiency [42–44].

3.2.4. Temperature Effect

Interestingly, the adsorption medium temperature is also substantially important in the biosorption of the metal ions by microbial cells for energy-dependent mechanisms. As reported in the literature, mostly, the adsorption is considered as an exothermal process [45]. To understand the effect of temperature on the uptake and percentage removal of Cd^{2+} ions, temperature-dependent experiments were performed by keeping all other optimized experimental conditions the same, i.e., pH 5, incubation time 120h, inoculum size 2% for *T. longibrachiatum*. Figure 1d exhibits the relation between the Cd^{2+} ion uptake and percentage removal and adsorption temperature. The observed results revealed that under optimized conditions, the maximum Cd^{2+} ion uptake and percentage removal from the liquid medium by both fungi were obtained at 30 °C. It was interesting to note that for *T. fasciculatum*, the maximum Cd^{2+} uptake and percentage removal was 1.92 mg/g and 63.85%, respectively while for the *T. longibrachiatum*, the uptake and percentage removal was 2.40 mg/g and 84.25%, respectively.

3.2.5. Initial Metal Concentration Effect

Initial metal ion concentrations also have a particular effect on the adsorption characteristics of heavy metal ions. Figure 1e shows the effect of initial metal ion concentration on the uptake and percentage removal of Cd^{2+} ions using fungal isolates. To check the initial ion concentrations, several concentrations (5–50 mg/L) were tested, and all the experiments were performed at optimized experimental conditions, i.e., pH 5.0, incubation time (120 h), inoculum size (2.5% for *T. fasciculatum*, and 2.0% for *T. longibrachiatum*), and temperature 30 °C. The observed results confirmed that under optimized reaction conditions, for both fungal isolates, the Cd^{2+} uptake and percentage removal increased with the increasing the Cd^{2+} concentrations from 5 to 20 mg/L, and afterwards, a decline was seen. At an optimum concentration of 20 mg/L, the uptake and percentage removal of Cd^{2+}

from liquid medium was 2.27 mg/g and 67.10% for *T. fasciculatum* and 2.34 mg/g and 76.25% for *T. longibrachiatum*, respectively. This enhancement may be because of an increase in the electrostatic associations (comparative with covalent interactions), including sites of continuously lower affinity for metal ions [46].

3.3. Adsorption Isotherms

The availability of a finite number of binding sites can be expected in the Langmuir model, which equally distributed to the sorbent surface. It presents the same attraction for single-layer sorption with no interaction between sorbed species. The Freundlich equation provides the adsorption data for a limited concentration range and proposed an adsorption sites heterogeneity on the biomass [47]. By changing the pH from 3.5 to 6.0 for Cd^{2+} ion removal from the liquid medium using *T. fasciculatum* and *T. longibrachiatum*, the obtained results were examined, and sorption isotherms were calculated. Figure 2 exhibits the calculated Langmuir and Freundlich isotherms for Cd^{2+} removal by *T. fasciculatum* and *T. longibrachiatum*. As a result of the higher value of correlation coefficients $R^2 > 0.90$, the observed results revealed that the Cd^{2+} removal using both the fungal isolates, i.e., *T. fasciculatum* and *T. longibrachiatum*, better fit with the Langmuir isotherm models compared with the Freundlich isotherm. Various parameters of Langmuir and Freundlich models for the Cd^{2+} ion sorption on the live cells of *T. fasciculatum* and *T. longibrachiatum* are presented in Table 2. As observed, the maximum Cd^{2+} ion adsorption capacities (q_{max}) for *T. fasciculatum* and *T. longibrachiatum* fungal isolates were 1.90 mg/g and 0.80 mg/g, while the calculated correlation coefficients (R^2) were 0.99 and 0.99 for both the fungal isolates, respectively.

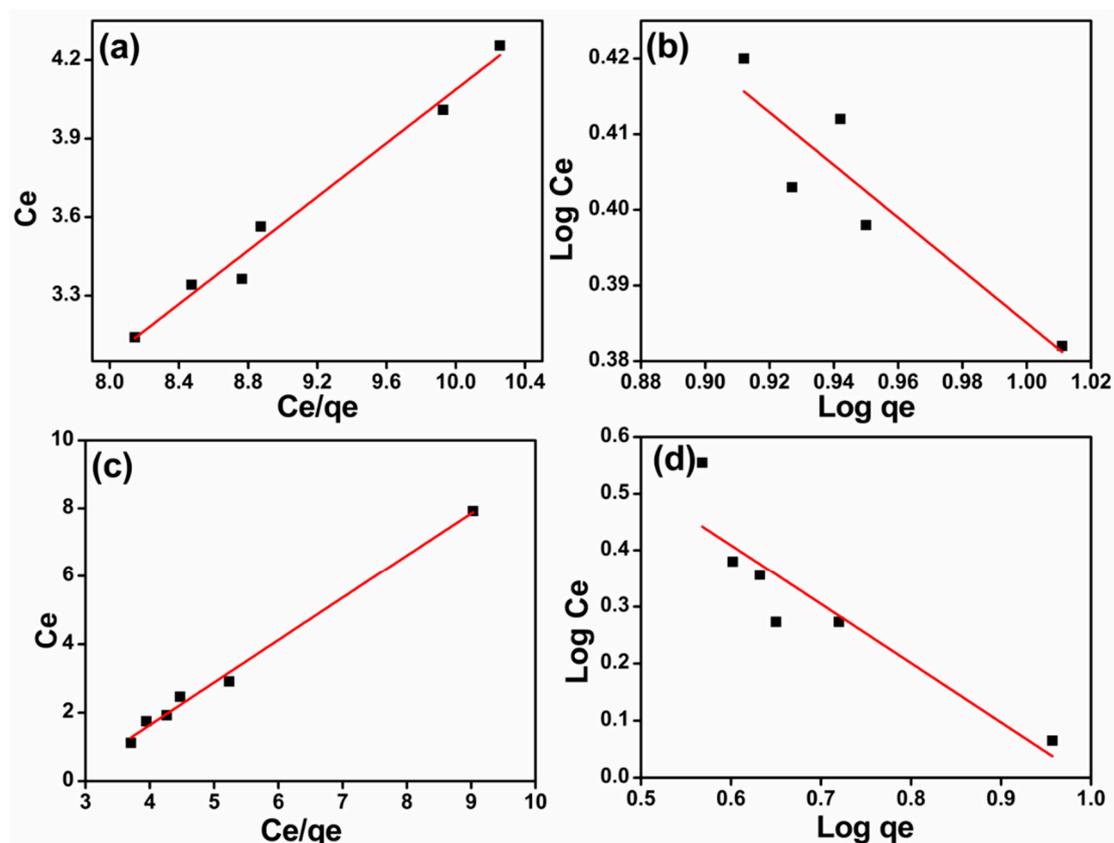


Figure 2. (a,c) Langmuir and (b,d) Freundlich isotherms for Cd^{2+} removal by *T. fasciculatum* and *T. longibrachiatum*, respectively.

Table 2. Langmuir and Freundlich isotherm constants for removal of Cd^{2+} by *T. fasciculatum* and *T. longibrachiatum*.

Isotherms	Parameters	<i>T. fasciculatum</i>	<i>T. longibrachiatum</i>
Langmuir	q_{max} (mg/g)	1.90	0.80
	b (L/mg)	−2.23	−2.75
	R^2	0.99	0.99
Freundlich	K_f (L/g)	−0.15	0.02
	n	−3.11	−0.95
	R^2	0.84	0.84

3.4. SEM and FTIR Analysis

The morphological changes before and after the Cd^{2+} ions accumulation in the fungal isolates, i.e., *T. fasciculatum* and *T. longibrachiatum*, were further analyzed by scanning electron microscopy (SEM), and the results are presented in Figure 3.

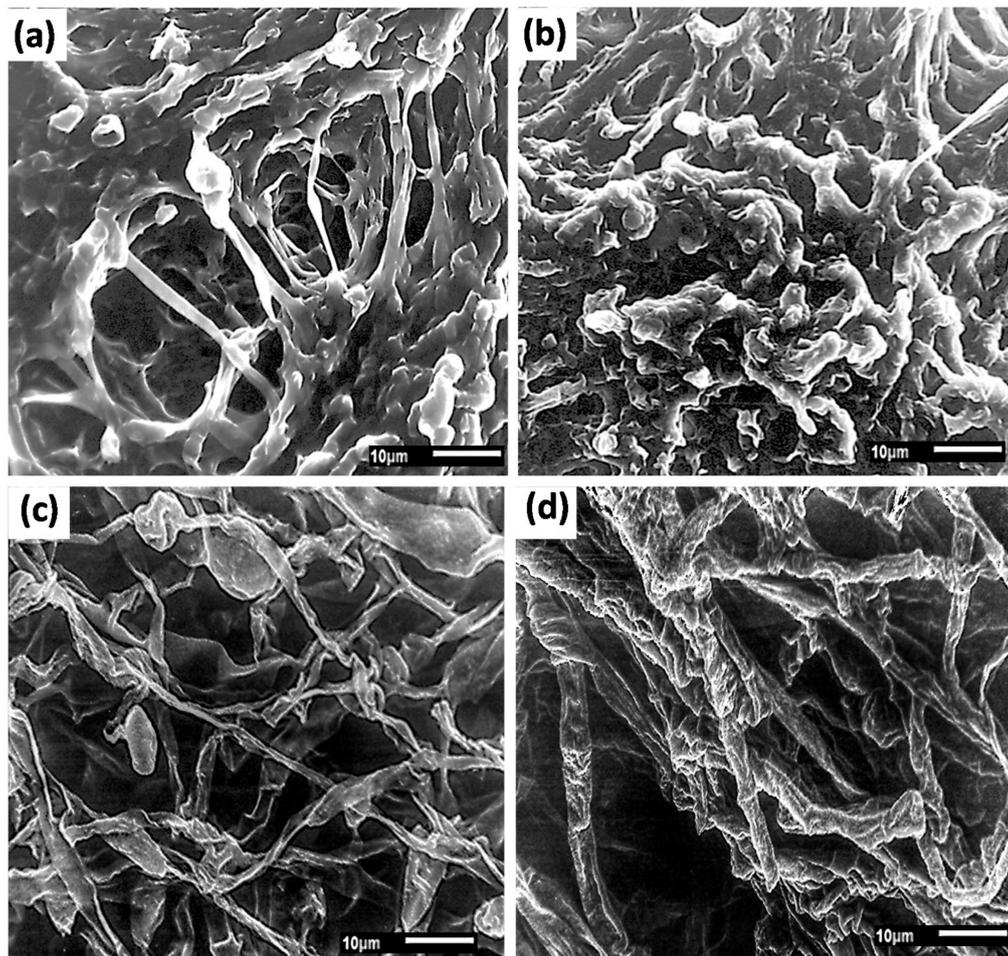


Figure 3. SEM images of (a) *T. fasciculatum* control and (b) after treatment with Cd^{2+} , (c) *T. longibrachiatum* control and (d) after treatment with Cd^{2+} .

The observation revealed that after 120 h, the hyphae of both the fungi was tube shaped, septate, and diverged with no metal treatment. However, after treatment with 20 mg/L of Cd^{2+} after 120 h, there was a thorough disruption and dissolution of mycelium of *T. fasciculatum* and *T. longibrachiatum*.

These metals were consistently intact to the fungal mycelium, and a higher absorption of Cd^{2+} ions along with flocculation in mycelium was seen. The formation of such complexes was most probably because of the detoxification mechanism, in which normally fungal isolates are used in order to manage the lethal concentrations of heavy metal ions [48].

To understand the metal ions bindings, the attachment of various functional groups on the fungal surface are important to study. To examine the functional groups available on the surfaces of fungal isolates, FTIR studies were performed for both the fungal biomass, i.e., *T. fasciculatum* and *T. longibrachiatum* before and after Cd^{2+} ions treatment. Figure 4 exhibits the typical FTIR spectra in the range of $450\text{--}4000\text{ cm}^{-1}$ for both the fungal biomass, i.e., *T. fasciculatum* and *T. longibrachiatum* before and after Cd^{2+} ions treatment. The Cd^{2+} free biomass of *T. fasciculatum* and *T. longibrachiatum* exhibited several absorption peaks, which show the complex nature of the biomass. The peak that appeared in the range of $3500\text{--}3200\text{ cm}^{-1}$ was due to stretching of the N–H bond of the amino groups and the O–H bond of the hydroxyl group. Upon Cd^{2+} loading to the biomass of *T. fasciculatum* and *T. longibrachiatum*, a significant change in the peak positions was seen, which confirmed the Cd^{2+} binding with hydroxyl and amino groups (Table 3). The peaks that appeared at 2853 cm^{-1} and 2922 cm^{-1} exhibited the presence of C–H methyl groups stretching. Furthermore, the peak shown at 1747 cm^{-1} represents the native carbonyl stretching, while the peak at 1373 cm^{-1} represented as CH symmetric/symmetric band. The peaks that appeared at 2853 cm^{-1} , 2922 cm^{-1} , and 1747 cm^{-1} did not show any significant change in samples exposed to Cd^{2+} except for a peak exhibited at 1373 cm^{-1} , which showed a slight change from its original position.

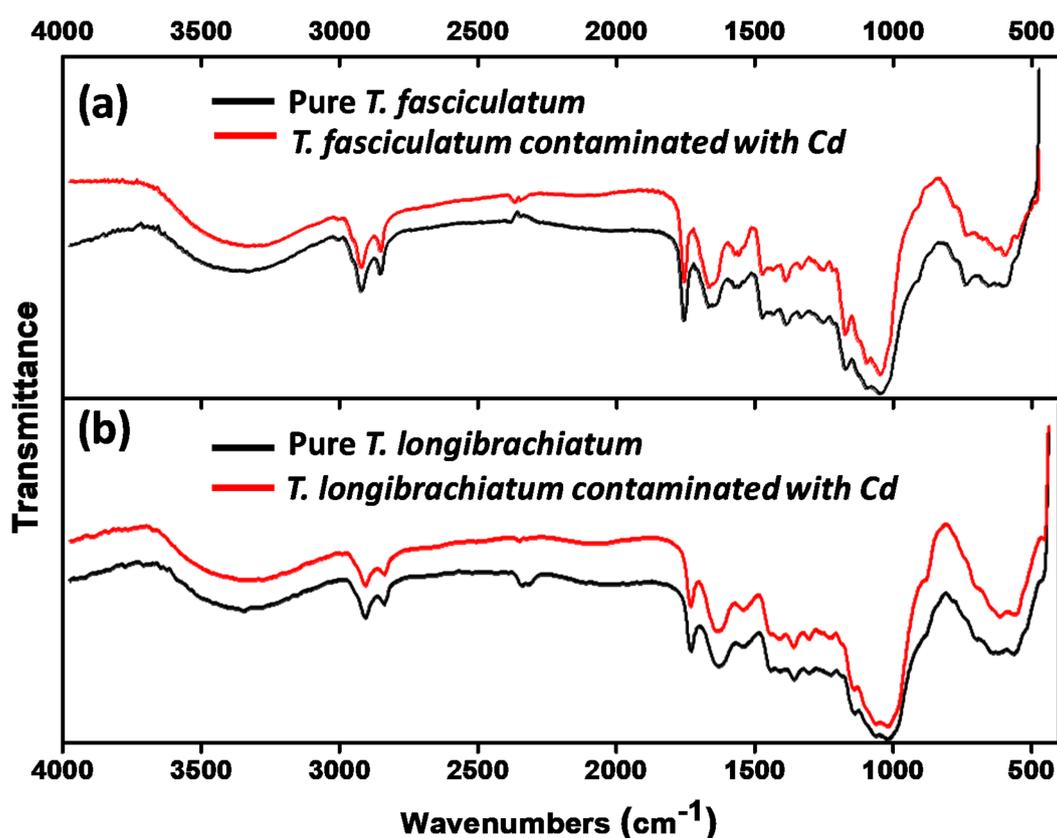


Figure 4. Fourier transform infrared spectroscopy (FTIR) spectra of (a) *T. fasciculatum* and (b) *T. longibrachiatum* fungal biomass before and after treatment with Cd^{2+} .

Table 3. FTIR peaks and their corresponding functional groups of *T. fasciculatum* and *T. longibrachiatum* before and after the addition of Cd^{2+} .

Sr. no.	<i>T. fasciculatum</i>		<i>T. longibrachiatum</i>		Assigned Groups
	Control	Cd^{2+} Addition	Control	Cd^{2+} Addition	
1	3332.6	3320.1	3363.5	3325.2	-OH, -NH
2	2923.6	2924.3	2923.3	2923.3	-CH, -OH
3	2853.3	2853.2	2854.6	2854.9	-CH
4	1745.4	1746.0	1744.9	1745.1	-C=O, of ester group
5	1654.8	1654.3	1645.5	1648.1	-C=O, COO
6	1560.2	1563.0	1554.5	1558.8	-NH
7	1373.8	1375.6	1372.3	1373.8	-CH

In case of Cd^{2+} -unloaded spectra of *T. Fasciculatum* and *T. longibrachiatum*, the peaks appeared at 1650 and 1544 cm^{-1} are related with the C=O of amide I and the NH/C=O blend of the amide II bond, respectively confirming the availability of the carboxyl groups [49,50]. It is interesting to see that the peak positioned at 1544 cm^{-1} expanded in the presence of Cd^{2+} ions, which confirms the interaction of Cd^{2+} with carboxyl groups. The above observations revealed the presence of various functional groups such as -CH, -OH, -C=O, and -NH in the binding of Cd^{2+} ions with the fungal biomass, i.e., *T. fasciculatum* and *T. longibrachiatum*. The observed results are in agreement with the reported literature [49–51].

3.5. Proposed Mechanism for Cd^{2+} Removal

Based on the findings of this work, we proposed the possible mechanism of *T. fasciculatum* and *T. longibrachiatum* response to Cd^{2+} stress (Figure 5). The bioaccumulation of Cd^{2+} in *T. fasciculatum* and *T. longibrachiatum* is a cumulative result of three pathways. First the Cd^{2+} get adsorbed over the fungal outer membrane [52]. It has been shown in the FTIR results that various functional groups available on the fungal surface showed significant changes in their peak positions, indicating the chemical adsorption of Cd^{2+} over the membrane. Then, the ions are engulfed in the fungal cytoplasm where they are assimilated and sequestered by the cellular reactions [53]. In addition, the fungi can release exopolysaccharides (high-molecular-weight polymers composed of sugar residues) to entrap or adsorb the extracellular Cd^{2+} ions [54].

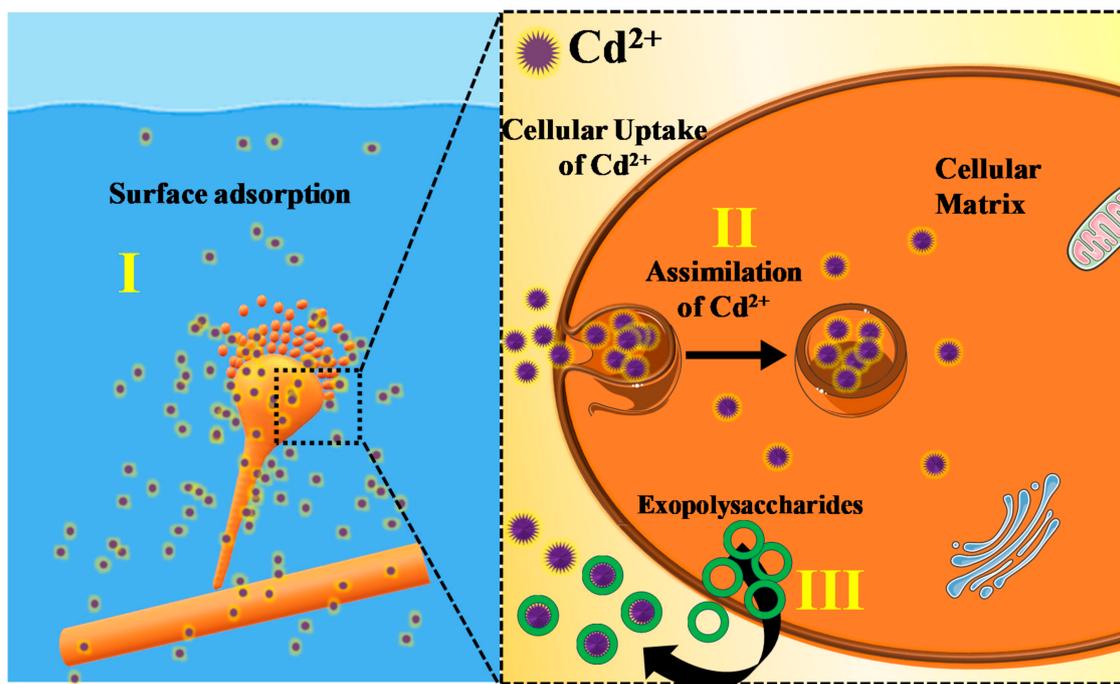


Figure 5. Pictorial illustration of Cd^{2+} removal by *T. fasciculatum* and *T. longibrachiatum*.

4. Conclusions

In summary, 25 sludge, industrial effluents and sewage samples were collected to isolate fungal isolates and screened to their tolerance to Cd^{2+} in PDA medium containing Cd^{2+} from 25 to 75 mg/L. There was a decline in the number of fungi for their tolerance to Cd^{2+} with the increment in concentration of Cd^{2+} from 25 to 75 mg/L. The removal of Cd^{2+} by highly effective fungi *T. fasciculatum* and *T. longibrachiatum* was dependent on various experimental parameters such as temperature, initial metal ion concentrations, pH, incubation time, inoculum size, etc. Under optimized conditions, i.e., pH 5.0, inoculum size (2.5% for *T. fasciculatum* and 2.0% for *T. longibrachiatum*), incubation time (120 h), initial Cd^{2+} concentration (20 mg/L), and temperature (30 °C), the maximum removal of Cd^{2+} was 67.10% for *T. fasciculatum* and 76.25% for *T. longibrachiatum*. The observed data were well fitted with the Langmuir and Freundlich isotherm models. The SEM examinations confirmed the morphological changes in response to the Cd^{2+} aggregation in *T. fasciculatum* and *T. longibrachiatum*. Both the fungal isolate exhibited Cd^{2+} binding on the cell wall surface as a mechanism of metal tolerance. The FTIR studies confirmed the presence of various functional groups such as $-\text{CH}$, $-\text{OH}$, $-\text{C}=\text{O}$ and $-\text{NH}$ in the binding of Cd^{2+} ions with the fungal biomass, i.e., *T. fasciculatum* and *T. longibrachiatum*. The observed results revealed the potential biosorption tendency of *T. fasciculatum* and *T. longibrachiatum* to remove Cd^{2+} from wastewater.

Author Contributions: Conceptualization, R.K. (Raman Kumar), N.S. and A.U.; methodology, R.K. (Raman Kumar), N.S. and A.U.; software, R.K. (Raman Kumar), N.S., P.S., A.U., R.K. (Rajeev Kumar), P.K.J., F.A.A., A.A.A. and N.A.-Z.; validation, R.K. (Raman Kumar), P.S., A.U., R.K. (Rajeev Kumar), P.K.J., F.A.A., A.A.A., and N.A.-Z.; formal analysis, R.K. (Raman Kumar), N.S. and A.U.; the investigation, R.K. (Raman Kumar), and A.U.; resources, R.K. (Raman Kumar), P.S., A.U., R.K. (Rajeev Kumar), N.S., P.K.J., F.A.A., A.A.A., and N.A.-Z.; data curation, R.K. (Raman Kumar), P.S., A.U., R.K. (Rajeev Kumar), N.S., P.K.J., F.A.A., A.A.A., and N.A.-Z.; writing—original draft preparation, R.K. (Raman Kumar) and A.U.; writing—review and editing, R.K. (Raman Kumar) and A.U.; visualization, R.K. (Raman Kumar), P.S., A.U., R.K. (Rajeev Kumar), N.S., P.K.J., F.A.A., A.A.A. and N.A.-Z.; supervision, R.K. (Raman Kumar); N.S. project administration, R.K. (Raman Kumar), N.S. and A.U. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group No. RGP-1441-305.

Acknowledgments: The authors are thankful to Haryana State Council of Science and Technology, Panchkula, Haryana for providing financial support for carrying out research work (Grant No. HSCST/R&D/2018/209R). The authors are thankful to Maharishi Markandeshwar (Deemed to be University), Mullana, India for providing the lab facilities. The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group No. RGP-1441-305.

Conflicts of Interest: The authors declare no conflict of interest.

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