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# Removal of heavy mercury(II), cadmium(II) and zinc(II) metal ions by live and heat inactivated *Lentinus edodes* pellets

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**Abstract**

The live and heat inactivated forms of *Lentinus edodes* pellets were used for the biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions. The maximum adsorption of metal ions on the live and heat inactivated pellets of fungus was observed at pH 6.0 for all the used metal ions. The effect of temperature on the biosorption capacity was negligible in the range of 15–45 °C. The biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on the live and heat inactivated pellets of fungus was studied in aqueous solutions in the concentration range of 25–600 mg/L. The metal biosorption capacities of the live fungal pellets $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ were $336.3 \pm 3.7$, $78.6 \pm 2.6$ and $33.7 \pm 1.6$ mg/g, respectively, while $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ the biosorption capacities of the heat inactivated pellets were $403.0 \pm 2.9$, $274.3 \pm 3.6$ and $57.7 \pm 1.1$ mg/g, respectively. The adsorption capacities of the heat inactivated fungus for metals were markedly increased compared to native form. For both forms the same affinity order on a molar basis were observed for single or multi-metal ions ($Hg^{2+} > Cd^{2+} > Zn^{2+}$). The Langmuir and Freundlich equilibrium models represent well the experimental data. The experimental kinetic data were analyzed using the first- and second-order kinetic models and the second-order kinetic model described the biosorption kinetics accurately for each metal ions.


*Keywords:* White rot fungus; *Lentinus edodes*; Heavy metal ions; Biosorption; Kinetics

## 1. Introduction

The presence of toxic heavy metals in water resulting from rapid industrialization and technological advances is a world wide environmental problem [1–4]. Removal of these pollutants from wastewater has conventionally been accomplished through a range of chemical and physical processes [4–8]. However, these processes can be expensive and not fully effective. Biosorption with microbial biomasses has become an alternative to traditional methods of industrial wastewaters treatment, such as precipitation, adsorption, coagulation, etc. [9,10] and it is relatively inexpensive, non-hazardous, and may permit recovery of the metals from the adsorbing biomass [11–13]. Biosorption is the non-specific term used to denote the complex process whereby biomass, usually microbial, is utilized to remove solutes during water treatment. Biosorption may involve one or more of several processes depending on physicochemical conditions and the origin and physiological state of the biomass. These include metal ion coordination complex, ion exchange and covalent linkage to biomass components [14–16]. Live or inactivated microbial cells can be used to remove heavy metal ions, but maintaining the survivability of the microbial cells during biosorption process is difficult, because they require a continuous supply of nutrients and metal toxicity might take place for microbial cells [17–19]. Therefore, the use of non-living microbial cells can eliminate these problems and can be regenerated and reused for many cycles [20,21].

Among heavy metals, $Hg^{2+}$ and $Cd^{2+}$ are in most widespread concern to human health. Mercury in its organic form (e.g., fish containing high levels) attacks the central nervous system, causing mental and motor dysfunction. Inorganic mercury, such as that found in the water column impairs kidney function [22]. Cadmium has been classified by U.S. Environmental Protection Agency as a probable human carcinogen. At very low levels ingestion causes vomiting; chronic exposure results in kidney dysfunction; high levels of exposure will result in death [14].

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*Lentinus edodes* is a white rot fungus and its biomass may be a good source for removal of toxic heavy metal ions from aqueous solutions. *L. edodes* grows readily on an easily acquired carbon sources (i.e., cellulose) and has several extra cellular enzymes for bioremediation of various xenobiotics. There are a few studies with this fungal biomass. In the recent studies, *L. edodes* was used as a biosorbent for bioremediation of chromate and textile dyes contaminated wastewaters [23–25]. In this study, the agricultural by-product of the live and heat inactivated pellets of *L. edodes* was used as a biosorbent for the recovery of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ from aqueous solution. The effects of contact time, initial concentration of metal ions and pH on the adsorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions have been investigated.

## 2. Materials and methods

### 2.1. Microorganism and media

Pure culture of *L. edodes* (MAFF 430012) was obtained from MAFF GENE BANK Culture Collection (Kannondai, Tsukuba, Ibaraki, Japan), and was maintained by subculturing on malt dextrose agar slants. The growth medium and growth conditions for white rot fungi were previously described elsewhere [2]. The cultivated fungus pellets of *L. edodes* were washed with sterile physiological saline solution several times to remove dirt particles. Some of them were heated at 90 °C for 15 min and referred as heat inactivated fungal pellets. These resulting products were directly used as biosorbent.

### 2.2. Biosorption studies

The biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on the live and heat inactivated pellets of *L. edodes* was investigated in a batch reactor and the batch reactor was made from Pyrex glass with a water jacket (inner volume: 150 mL). The reactor temperature was controlled with a thermo circulator. The stock solutions of metal ions (i.e., $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$: 1.0 g/L) were prepared using nitrate salts in double distilled water. A known quantity of wet live and heat inactivated pellets of *L. edodes* was used in the adsorption tests (about 0.1 g biosorbent in 100 mL metal ions solution). After adsorption process, the adsorbents were dried in an oven at 50 °C overnight and the dry weight of the preparations was used in the calculations.

The effect of pH on the biosorption rate was investigated in the pH range 3.0–7.0 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled afterwards) at 25 °C. Solution containing 100 mg/L of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions and each live and heat inactivated pellets of *L. edodes* was combined and the samples were stirred at 400 rpm. The effect of the initial $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions concentration on biosorption was studied at pH 6.0 and the concentration of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions in the adsorption medium was varied between 25 and 600 mg/L.

The competitive biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions from their mixture was investigated in the same manner. The medium containing 0.2 mmol/L of each metal ion was incubated with the biosorbents in batch fashion.

### 2.3. Analytical procedure

Biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions from aqueous solutions were studied in batch systems. After the desired incubation period (up to 120 min) the aqueous phases were separated from the biosorbents and the concentration of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions in these phases were measured. A flame atomic absorption spectrophotometer (Shimadzu, Model AA-6800) was used for the determination of metal ions. Deuterium background correction was used and the spectral slit width was 0.5 nm. The working current/wavelength values for $Cd^{2+}$ and $Zn^{2+}$ were 8.0 mA/228.8 nm and 8.0 mA/213.9 nm, respectively. Mercury determinations were realized by using Mercury Vapor Unit (MVU-1A). The working current/wavelength was 6 mA/253.6 nm. The instrument response was periodically checked with metal ion standard solutions. For each set of data reported, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

The amount of metal ions adsorbed per unit live and heat inactivated pellets of *L. edodes* (mg metal ions/g dry mass) was obtained by using the following expression:

$$q_{\text{exp}} = \frac{(C_{\text{o}} - C)V}{M} \quad (1)$$

Where $q_{\text{exp}}$ is the amount of metal ions biosorbed onto the unit mass of the biosorbent (mg/g), $C_{\text{o}}$ and $C$ are the concentrations of the metal ions before and after biosorption (mg/L), $V$ is the volume of the aqueous phase (L), and $M$ is the amount of the biosorbent (g).

### 2.4. Characterization studies

The surface area of the live and heat inactivated fungal pellets was determined by Brunauer–Emmett–Teller (BET) equation using a surface area apparatus (Model Flowsorb II 2300; Micromeritics Instrument Corporation, Norcross, USA). The dried fungal pellets were coated with gold under reduced pressure and their scanning electron micrographs were obtained using a JEOL (JSM 5600) scanning electron microscope. FT-IR spectra of powdered live and heat inactivated fungal pellets were obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). The dry fungal biomass (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

## 3. Results and discussion

### 3.1. Properties of the fungal biomass

Cell walls of fungal biomass can be regarded as a mosaic of different functional groups where coordination complexes and/or ion exchange with metal ions can be formed. The functional groups for heavy metal ions binding on the fungal cell walls are carboxyl (–COOH), phosphate ($PO_4^{3-}$), amide ($–NH_2$), thiol (–SH), and hydroxide (–OH). In fungal cell walls,

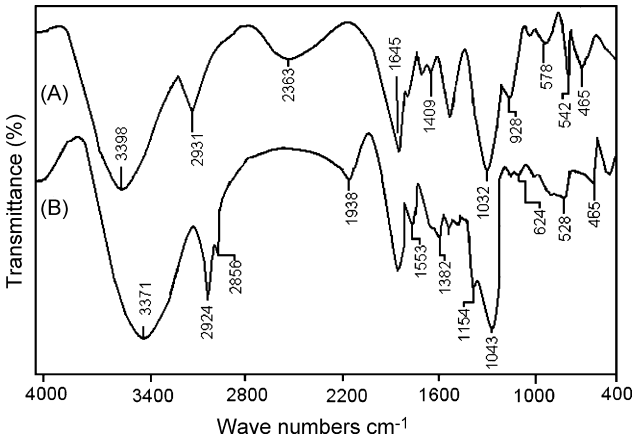


Fig. 1. FT-IR spectra of live (A) and heat inactivated (B) fungal pellets.

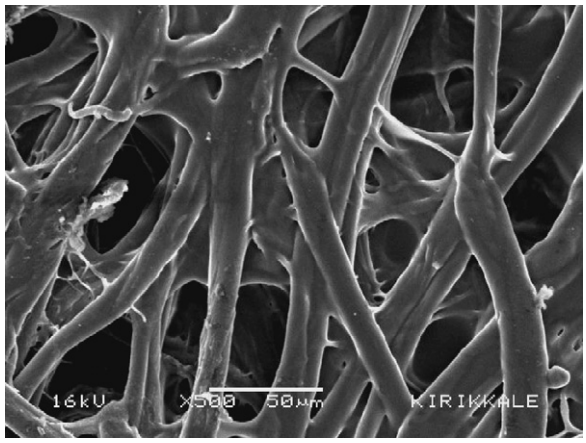


Fig. 2. Representative SEM micrograph of the fungus.

chitin and its associated proteins contain many carboxyl groups with p$K_a$ values in the range of 4.0–5.0 [14,26–28]. Phosphate groups are present mainly in glycoproteins and are believed to play an important role in biosorption because they can exhibit a negative charge above pH 3.0 [29,30].

The mechanism of metal ions biosorption (i.e., $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions) by live and heat inactivated fungus pellets was elucidated on the basis of heat treatment; FT-IR, SEM and BET method. Heat treatment can produce additional binding sites via denaturation of proteins on the cell wall structures. The changes in the functional groups and the surface properties of the fungal pellets are confirmed by the FT-IR spectra before and after heat treatment (Fig. 1). FT-IR spectra of native and heat inactivated fungus confirm the biosorbents heterogeneity and evidence the presence of different characteristics peaks in agreement with the possible presence of amino, carboxylic, hydroxyl and carbonyl groups are presented in Fig. 1. In general, the FT-IR spectra of the live and heat inactivated fungal biomass have intense peaks at the frequency level of 3400–3200 $cm^{-1}$ representing –OH stretching of carboxylic groups and also representing stretching of –NH groups. The strong peaks at around 1645 $cm^{-1}$ are caused by the bending of N–H groups of chitin on the cell wall structure of fungal pellets. The peaks at 1938 $cm^{-1}$ is observed in the fingerprint region representing aromatic ring substitution overtones. The peaks at 2924, 1553, 1382 and 1043 $cm^{-1}$ representing C–H stretching vibrations, N–H bending (scissoring), $–CH_3$ wagging (umbrella deformation) and C–OH stretching vibrations, respectively, are due to the several functional groups present on the fungal cell walls. On the other hand, the peaks of N–H stretching vibrations at around 1000 $cm^{-1}$ are also masked with the broad band of C–O stretching and the peak at 578 and 542 $cm^{-1}$ representing O–C–O scissoring and C=O bending vibrations are only observed for the live fungal biomass and these peaks was not seen for the heat inactivated counterpart can be due to resulted of the removal of lipid compounds after heat treatment. The band at 485 $cm^{-1}$ representing C–N–C scissoring is found in polypeptide structure.

The surface morphology and bulk structure of the dried live fungal pellets is exemplified by the scanning electron micrograph in Fig. 2. As clearly seen here, the fungal pellets have fibrous and porous surface structures. These surface properties can be considered as a factor providing an increase in the total surface area. In addition, the fibrous and pores structures of the fungal pellets could reduce the diffusional resistance and facilitate mass transfer because of their high internal surface area.

The surface areas of the live and heat inactivated fungal pellets were measured by BET method and were found to be 0.89 and 1.18 $m^2/g$ fungal biomass, respectively. The surface areas of the fungal biomass were increased after heat treatment compared to native form. Noted that heat treatment appears to provide more surface area for the fungal pellets would favour higher adsorption capacity for metal ions due to the increase in the surface area after heat treatment.

### 3.2. *Effect of contact time and temperature on biosorption*

The biosorption rates of heavy metal ion species on both live and heat inactivated pellets of *L. edodes* were obtained by following the decrease in the concentration of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions within the adsorption medium with time. The biosorption rate of metals ions is exemplified for heat inactivated fungal pellets at 25 °C and at pH 6.0 (Fig. 3). The biosorption capaci-

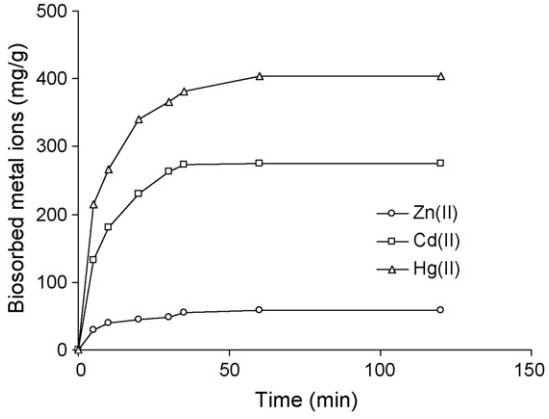


Fig. 3. Equilibrium biosorption time of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions by heat inactivated fungal pellets. Adsorption conditions: initial concentration of metal ions: 500 mg/L, pH: 6.0, temperature: 25 °C.

ties increased with increasing contact time and larger amount of metal ions were removed by both live and heat inactivated pellets of *L. edodes* in the first 20 min of contact time. Equilibrium was established in about 120 min. This trend in binding of metal ions suggests that the binding may be through interactions with functional groups located on the surface of the biosorbents. On the other hand, the equilibrium biosorption of metal ions to the both live and heat inactivated pellets of *L. edodes* was not affected by temperature and suggesting possible monolayer coverage. The observed rapid kinetics has also significant practical importance as it will facilitate the scale-up of the process to smaller reactor volumes ensuring efficiency and economy. Data on the adsorption rates of heavy metal ions by various biosorbents have shown a wide range of adsorption time. The $Hg^{2+}$ biosorption rate of *Phanerochaete chrysosporim* is fast and reached saturation value within 1 h [31]. Pagnanelli et al. [32] have studied $Pb^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Cd^{2+}$ biosorption in single and multi-metal systems on *Sphaerotilus natans* biomass and the biosorption equilibrium was established within 30 min.

### 3.3. Effect of pH

It is well known that biosorption of heavy metal ions by biosorbents depends on the pH of the solution. The pH affects the speciation of metal ions in solution and the metal binding sites on biosorbent surface. Fig. 4 shows the effect of pH on the biosorption of metal ions where the maximum biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on both live and heat inactivated fungal pellets were observed at around pH 6.0. There was an increase in the adsorbed amount of heavy metal ions per unit weight of fungal pellets with increasing pH from 3.0 to 6.0. The observed increase in the biosorption levels with pH can be explained by the strong relation of biosorption to the number of surface negative charges, which depends on the dissociation of functional groups. The low biosorption capacity at pH values below 4.0 was attributed to hydrogen ions that compete with metal ions on the sorption sites [33,34].

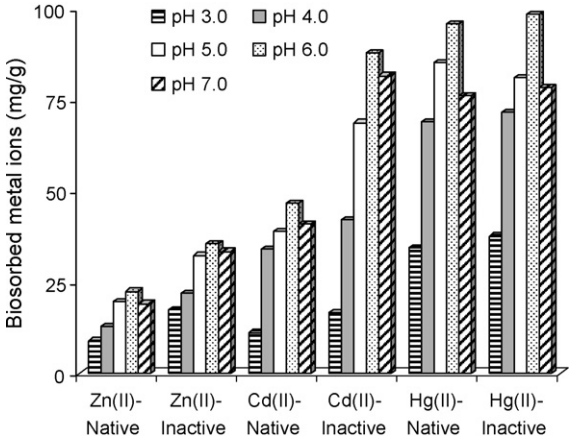


Fig. 4. Effect of pH on the biosorption capacity of metal ions on the live and heat inactivated fungal pellets. Biosorption conditions: initial concentration of metal ions: 100 mg/L; volume of biosorption medium: 100 mL; temperature: 25 °C; biosorption time: 120 min.

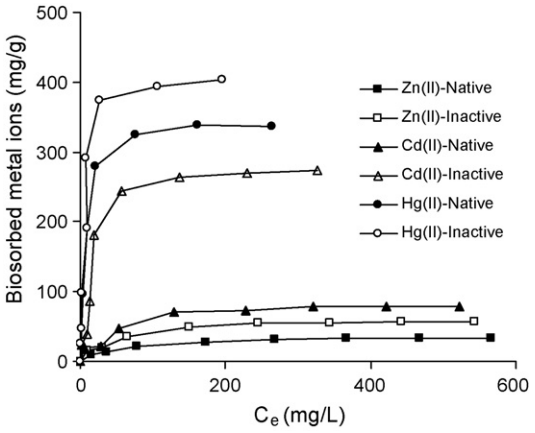


Fig. 5. Equlibrium biosorption of live and heat inactivated fungal pellets for $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions. Biosorption conditions: volume of the biosorption medium: 100 mL; pH: 6.0; temperature: 25 °C; biosorption time: 120 min.

### 3.4. Biosorption equilibrium studies

As seen from Fig. 5, the maximum of $Hg^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ ions adsorbed on the live fungal pellets were $336.3 \pm 3.7$, $78.6 \pm 2.6$ and $33.7 \pm 1.6$ mg/g dry fungal biomass, respectively. On the other hand, maximum biosorption for the heat inactivated pellets of fungus were found to be $403.0 \pm 2.9$ mg/g for $Hg^{2+}$, $274.3 \pm 3.6$ mg/g for $Cd^{2+}$ and $57.7 \pm 1.1$ mg/g for $Zn^{2+}$. The heat inactivation of the fungal pellets was resulted an increase in the biosorption capacity for all the tested metal ions compared to live counterpart. Similar observations were reported for other biomasses, including fungi and yeast [2,35,36] and the low biosorption capacity of live fungal pellets can be attributed to a variety of mechanisms such as extracellular complexation with metal binding proteins such as metallothionein and phytochelatins which are proteins that contain large amounts cysteine and bind heavy metal ions and/or efficient pumping out metal ions from the living cell [14].

The electro-negative values of $Hg^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ metal ions are 2.00, 1.69 and 1.65, respectively. The more electronegative metal ions will be more strongly attracted to the fungal cells surfaces. $Hg^{2+}$ has the highest affinity for both immobilized live and inactivated fungal pellets and it has a greater electronegativity than both $Cd^{2+}$ and $Zn^{2+}$. The sorption capacity of both biosorbents for $Cd^{2+}$ ions is also greater than $Zn^{2+}$ ions and the same trend was observed by their respective electronegativities.

### 3.5. Adsorption isotherms

In order to optimize the design of a biosorption system to remove metal ions it is important to establish the most appropriate correlations for the equilibrium curves. Two isotherm equations have been tested in the present study, namely Langmuir [37] and Freundlich [38].

The most widely used equation for modeling equilibrium data is the Langmuir equation, which for dilute solutions may be

represented as:

$$q_e = \frac{Q_o b C_e}{1 + bC_e} \quad (2)$$

where $q_e$ is the amount of biosorbed metal ions at time $t$ (mg/g), $C_e$ is the equilibrium concentration (mg/L). $Q_o$ (mg/g) and $b$ (L/mg) are the maximum biosorption capacity and energy of adsorption, respectively.

$K_a = 1/K_d = b$, $\ln K_a = -\Delta G_{max}/RT$ ($R$ is the gas constant, 8.314 J/mol K). The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor called the equilibrium parameter, $R_L$, which is used to predict if an adsorption system is "favorable" or "unfavorable. It is by the following relationship:

$$R_L = \frac{1}{1 + bC_o} \quad (3)$$

where $R_L$ and $C_o$ are the dimensionless constant separation factor or equilibrium parameter and initial metal ions concentration, respectively. The value of $R_L$ indicates the shape of isotherm to be either unfavorable ($R_L > 1$) or linear ($R_L = 1$) or favorable ($0 < R_L < 1$) or irreversible ($R_L = 0$).

The Freundlich expression is an empirical equation based on adsorption on a heterogeneous surface. The equation is commonly presented as:

$$q_e = nC_e K_F \quad (4)$$

where $q_e$ is the amount of adsorbed metal ions at time $t$ (mg/g), $C_e$ is the equilibrium concentration (mg/l). $K_F$ (mg/g) and $n$ (g/L) are the equilibrium constants indicative of biosorption capacity and biosorption intensity.

The Langmuir and Freundlich constants along with the correlation coefficients ($R^2$) have been calculated from the corresponding plots for biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on the biosorbents and the results are presented in Table 1. The Langmuir model was able to describe the experimental equilibrium data for biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on both live and heat inactivated fungal pellets under given experimental conditions (Table 1). The model parameters were also largely dependent on the type pellets (i.e., native or heat treated) and metal ions species. For example, $Hg^{2+}$ biosorption by the heat-treated fungal biomass at 25 °C was greater than that of the native counterpart with a maximum capacity value of 419.1 mg/g, while the maximum biosorption capacities of heat-treated fungal pellets for $Cd^{2+}$ and $Zn^{2+}$ ions were 299.4 and 63.3 mg/g, respectively. All the values of $Q_o$ appeared to be significantly lover for the metal ions-native fungal pellets in comparison with the maximum biosorption of heat-treated form. The other Langmuir constant $b$ is related to the free energy change of adsorption, $\Delta G$ ($b \propto e^{-\Delta G/RT}$) and indicates the affinity of biosorbent for the binding of metal ions. Its value is the reciprocal of the metal ions concentration at which half of the saturation of the adsorbent is attained (or amount of metal ions is bound $Q_o/2$) so a high value of $b$, indicates a steep desirable beginning of the isotherm which reflects the high affinity of the biosorbent for the sorbate (i.e., metal ions) resulting in a stable adsorption product. In our case, the $b$ values for heat inactivated fungal pellets were found to be higher than those of the native counterpart for all the tested metal ions.

The values of standard Gibbs free energy ($\Delta G_o$) were calculated for each fungal biosorbent and presented in Table 1. The negative values of $\Delta G_o$ confirm the feasibility and spontaneous nature of the fungal biosorption processes at 25 °C with a high degree of affinity of the metal ions for both biosorbent surface.

The highest values of $K_F$ were determined to be 36.5 and 61.7 for $Hg^{2+}$ ions for native and heat-treated fungal pellets. As expected, the value of $K_F$ for the biosorption of all the tested metal ions on the native fungal pellets is significantly lover than that of biosorption on heat-treated counterpart. The $n$ value, the other Freundlich constant, is an empirical parameter that varies with the degree of heterogeneity indicating the degree of nonlinearity between metal ions biosorption capacity and equilibrium concentration of metal ions in aqueous phase and is related to the distribution of bonded ions on the sorbent surface. Table 2 also indicated that $n$ is greater than unity, indicating that each tested metal ions is favorably adsorbed by the biosorbents.

Based on the effect of separation factor $R_L$ values are in the range of $0 < R_L < 1$, which indicates that the live and heat inactivated fungal pellets of *L. edodes* are favorable biosorbents for $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ metal ions removal from aqueous solution (Table 2).

### 3.6. Biosorption kinetics modelling

In order to examine the controlling mechanism of biosorption process such as mass transfer and chemical reaction, kinetic models were used to test the experimental data. The large number and different chemical groups on the cell wall of the fungal

Table 1
The Langmuir and Freundlich isotherm models constants and correlation coefficients for biosorption of $Hg^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ metal ions from aqueous solution

| Metal ions | Fungal forms | Freundlich isotherm model | | | | Langmuir isotherm model | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $q_{exp}$ (mg/g) | $n$ (g/L) | $K_F$ (mg/g) | $R^2$ | $b \times 10^1$ (L/mg) | $Q_o$ (mg/g) | $R^2$ | $\Delta G$ (kJ/mol) |
| $Zn^{2+}$ | Native | 33.7 ± 1.6 | 2.57 | 3.5 | 0.977 | 0.18 | 37.7 | 0.999 | −17.52 |
| | Inactive | 57.7 ± 1.1 | 2.61 | 6.2 | 0.970 | 0.21 | 63.3 | 0.998 | −17.78 |
| $Cd^{2+}$ | Native | 78.6 ± 2.6 | 2.67 | 9.2 | 0.950 | 0.23 | 86.4 | 0.996 | −19.46 |
| | Inactive | 274.3 ± 3.6 | 2.03 | 24.2 | 0.918 | 0.39 | 299.4 | 0.992 | −20.78 |
| $Hg^{2+}$ | Native | 336.3 ± 3.7 | 2.08 | 36.5 | 0.898 | 0.77 | 358.1 | 0.998 | −23.90 |
| | Inactive | 403.0 ± 2.9 | 2.29 | 61.7 | 0.917 | 1.45 | 419.1 | 0.997 | −25.47 |

Table 2
RL values based on Langmuir equation for biosorption of $Hg^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ metal ions from aqueous solution with live and heat inactivated fungal pellets

| Initial concentration of metal ions (mg/L) | $R_L$ values | | | | | |
|---|---|---|---|---|---|---|
| | $Zn^{2+}$ native | $Zn^{2+}$ inactive | $Cd^{2+}$ native | $Cd^{2+}$ inactive | $Hg^{2+}$ native | $Hg^{2+}$ inactive |
| 25 | 0.6896 | 0.6667 | 0.6494 | 0.5063 | 0.3419 | 0.2162 |
| 50 | 0.5263 | 0.5001 | 0.4808 | 0.3390 | 0.2062 | 0.1212 |
| 100 | 0.3571 | 0.3333 | 0.3165 | 0.2041 | 0.1149 | 0.0645 |
| 200 | 0.2174 | 0.5021 | 0.1880 | 0.1136 | 0.0610 | 0.0333 |
| 300 | 0.1562 | 0.1429 | 0.1337 | 0.0787 | 0.0415 | 0.0225 |
| 400 | 0.1219 | 0.1111 | 0.1037 | 0.0602 | 0.0315 | 0.0169 |
| 500 | 0.1001 | 0.0909 | 0.0848 | 0.0488 | 0.0253 | 0.0136 |
| 600 | 0.0847 | 0.0769 | 0.0716 | 0.0410 | 0.0212 | 0.0114 |

mycelia (e.g., –COOH, $–NH_2$, =NH, –SH, –OH) imply that there are many types of fungal mycelia–metal ions interactions. The kinetic models (the first-order, second-order and intra-particle diffusion equations) can be used in this case assuming that measured concentrations are equal to cell surface concentrations.

The first-order rate equation of Lagergren is one of the most widely used for the sorption of solute from a liquid solution. It may be represented as follows:

$$\frac{dq_t}{dt} = k_1(q_{eq} - q_t) \tag{5}$$

where $k_1$ is the rate constant of the first-order biosorption ($min^{-1}$) and $q_{eq}$ and $q_t$ denote the amounts of biosorption at equilibrium (mg/g) and at time $t$, respectively.

Ritchie proposed a second-order rate equation for the kinetic adsorption of gases on solids [39].

$$\frac{d\theta}{dt} = k(1-\theta)^n \tag{6}$$

The integration form of Eq. (6) becomes second order for $n = 2$.

$$\frac{q_{eq}}{q_{eq} - q_t} = kt + 1 \tag{7}$$

In order to assess the nature of the diffusion process reasonable for the adsorption of metal ions onto the fungal pellets attempts were made to calculate the pore diffusion coefficients. When the water sample is shaken, the metal ions species (i.e., $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$) are transported to the solid phase by the intra-particle transport phenomenon. The intra-particle transport is supposed to be the rate-controlling step. The rate of particle transport through this mechanism is slower than adsorption on the exterior surface site of the adsorbent. The amount of adsorbed species can lead varies proportionately with a function of retention time. The intra-particle diffusion model was proposed by Weber and Morris [40], the initial rate of intra-particular diffusion is calculated by linearalization of the curve $q = f(t^{0.5})$:

$$q = K_i t^{0.5} \tag{8}$$

where $q$ (mg/g) is the amount of biosorbed metal ion on the mycelia at time $t$, and $K_i$ the diffusion coefficient in the solid (mg/g $min^{0.5}$) and $t$ is the time (min). $K_i$ has been determined by a plot $q = f(t^{0.5})$ taking account only of the initial period.

In order to analyze the biosorption kinetics of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions, the Lagergren first-order and the Ritchie second-order kinetics models were applied to the experimental data [41–44]. The second-order equation fitted well with the experimental data. The comparison of experimental biosorption capacities and the theoretical values estimated from the above two equations and are presented in Table 3. The theoretical $q_{eq}$ values estimated from the first-order kinetic model gave significantly different values compared to experimental values, and the correlation coefficients were also found to be slightly lower. These results showed that the biosorption systems were not well described by the first-order kinetic model.

The correlation coefficients for the linear plots of $1/q_t$ against $1/t$ for the second-order equation are greater than 0.985 for the biosorbents for contact times of 120 min. The theoretical $q_{eq}$ values for all the tested biosorbent systems were very close to the experimental $q_{eq}$ values in the case of second-order kinetics. The second-order kinetics best described the data. This suggests

Table 3
The first-order kinetic, second-order kinetic and intra-particle diffusion models for biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on the native and heat inactivated fungal pellets of *Lentinus edodes*

| Metal ions | Fungal forms | First-order | | | Second-order | | | Intra-particle diffusion | |
|---|---|---|---|---|---|---|---|---|---|
| | | $k_1 \times 10^1$ ($min^{-1}$) | $q_{eq}$ (mg/g) | $R^2$ | $k_2 \times 10^1$ (g/mg/min) | $q_{eq}$ (mg/g) | $R^2$ | $K_i$ (mg/g $min^{0.5}$) | $R^2$ |
| $Hg^{2+}$ | Native | 0.92 | 174.2 | 0.993 | 2.89 | 357.1 | 0.996 | 12.21 | 0.769 |
| | Inactive | 0.69 | 270.2 | 0.989 | 1.97 | 416.7 | 0.993 | 21.11 | 0.851 |
| $Cd^{2+}$ | Native | 1.05 | 51.3 | 0.983 | 2.50 | 84.8 | 0.994 | 3.18 | 0.769 |
| | Inactive | 1.41 | 405.5 | 0.946 | 1.49 | 308.4 | 0.985 | 15.19 | 0.798 |
| $Zn^{2+}$ | Native | 0.65 | 16.6 | 0.985 | 2.56 | 35.5 | 0.998 | 1.41 | 0.826 |
| | Inactive | 1.15 | 95.5 | 0.942 | 1.96 | 58.8 | 0.992 | 3.13 | 0.880 |

that the rate-limiting step may be the chemical adsorption not the mass transport limitation.

The $K_i$ values are calculated for intra-particle model and tabulated in Table 3 for all the tested fungal preparations. The intra-particle diffusion rate equation does not fit well to the biosorption process for all the tested metal ions with the live and heat inactivated fungal pellets of *L. edodes*. These results indicate that the metal ions diffused quickly among the sorbents at the beginning of the biosorption process, then intra-particle diffusion slowed down and stabilized. The deviation of straight lines from the origin indicates that intra-particle transport is not the rate-limiting step.

### 3.7. Multi-metal ions biosorption studies

The biosorption capacities of the live and heat inactivated pellets in the multi-metal ions system were 0.58 and 0.81 mmol for $Hg^{2+}$, 0.43 and 0.52 mmol for $Cd^{2+}$, and 0.21 and 0.35 mmol for $Zn^{2+}$ per g of dry biosorbents, respectively. The biosorption order under multi-metal ions conditions was $Hg^{2+} > Cd^{2+} > Zn^{2+}$ in mmol basis for all the tested biosorbents. This affinity order is the same as in the single metal biosorption studies. The total biosorption capacities of the fungal preparations in the multi-metal system were lower than those of the single metal ions system. The presence of other heavy metal ions slightly decreased the total biosorption capacity of the native and heat-treated fungal biomass pallets under given experimental conditions. The heat-treated *L. edodes* exhibits the highest biosorption ability for $Hg^{2+}$ ions. The differences in the biosorption affinities could also be contributed to differences in the electrode potentials of various ions. In competitive biosorption, the complex interactions of several factors such as ionic charge, ionic radii and electrode potential would affect the biosorption of metal ions on the live and heat inactivated fungal pellets of *L. edodes*.

## 4. Conclusion

The kinetics of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions biosorption on the fungal biomass depend on the experimental conditions particularly medium pH and metals ion concentrations. The biosorption capacity of the treated fungal pellets was enhanced greatly when biosorption took place following heat inactivation. As the pH increased, the metal biosorption capacity increased significantly up to pH 6.0. The distribution of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions between liquid phase and solid phase was analyzed by the Langmuir and the Freundlich isotherm models. The characteristic biosorption parameters for each isotherm were determined. Comparing the equilibrium capacities ($q_{eq}$) of the kinetic models "namely first and second order" with the experimental equilibrium capacities of the biosorbents, the calculated maximum capacities from second-order equation seems to describe best the experimental data. Since biosorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions cannot be described very well by the intra-particle diffusion model. It therefore means that intra-particle diffusion is not the rate-limiting step for the sorption of $Hg^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ ions on to the live and heat inactivated fungal pellets of *L. edodes*.

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