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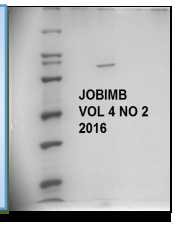

Mechanism of Biosorption of Pb (ii) and Cu (ii) ions using Dead Biomass of *Fusarium equiseti* strain UMAS *and Penicillium citrinum* strain UMAS B2

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ABSTRACT

Filamentous fungi such as *Fusarium equiseti* KR706303 and *Penicillium citrinum* KR706304 are capable of sequestering heavy metals from aqueous solutions. In the present study, the role play by various functional groups present in the cell wall of *F. equiseti* KR706303 and *P. citrinum* KR706304 during lead and copper ions biosorption was investigated. The fungal biomass was chemically treated to modify the functional groups present in their cell wall. These modifications were studied through biosorption experiments. It was found that an esterification of the carboxyl and phosphate groups, methylation of the amine groups and extraction of lipids significantly decrease the biosorption of both lead and copper ions studied. Therefore, the carbonyl, hydroxyl and amide groups were recognized as important in the biosorption of metal ions by the tested fungi. The study showed that there was no release of any metal ions from the biomass after biosorption, indicating that ion exchange may not be a key mechanism in the biosorption of lead and copper ions by these fungi but complexation of metal ions within the fungal cell wall.

INTRODUCTION

Lead pollution is a serious environmental problem in many countries, as a result of industrial and technological activities, and poses a significant threat to the environment and public health [1-3]. Copper is an essential micronutrient for living organisms, involved in biochemistry processes such as detoxification and oxidation [4]. Excessive ingestion of copper in humans and animals are very serious and prolonged exposure may result into liver damage and gastrointestinal catarrh [5,6]. Due to the toxic nature of lead and copper ions as it occurred in some industrial effluents and wastewaters, their removal become indispensable.

Biosorption is a process, which represents a biotechnological innovation as well as a cost effective excellent tool for sequestering heavy metals from aqueous solutions [3,7], and not only cost effective, but also provides an opportunity for the recycling of waste materials [8]. Biosorption, based on the interactions between living and non-living microorganisms and metallic ions in the system, offers advantages such as low operating cost and high efficiency for removing low concentrations of heavy metal from wastewater [9,10,11]. The removal of toxic metals from wastewaters by biosorption is cost effective and the economic feasibility can be obtained by optimization of the environmental conditions [12]. Various biomaterials have been examined for their biosorptive potentials and different types of biomass have shown high level of metal

uptake [13]. Fungi has been reported by many authors as versatile biosorbents as they can withstand extreme environmental conditions [11, 14].

Fungal cell walls and their components have a major role in biosorption, the mechanism seems to be species dependent [11,15,16]. The mechanism of fungal metal biosorption is complicated and not fully understand [3,17,18]. Based on the cells' metabolism, biosorption mechanisms can be grouped into: 1) Metabolism dependent (active metal uptake) which includes transport across cell membrane and this is energy-driven process [17] and 2) Non-metabolism dependent (passive metal uptake) which includes ion exchange, physical adsorption, complexation, and precipitation [3,19, 20, 21]. The mechanisms involved in metal sequestration has been extensively discussed by various authors [22]; however, it is generally accepted that the metal ion binds through the formation of a "pending complex" vs " bridge" model that suggests that the metal ion is bound with two or more nitrogen atoms from the same or vicinal chains [23].

The aim of this work is to study the biosorption potentials of Dimethyl sulfoxide (DMSO) treated *Fusarium equiseti* and *Penicillium citrinum* and to determine the mechanisms of biosorption processes through the modifications of functional groups present in the fungal cell wall.

MATERIALS AND METHODS

Organism and culture conditions

The newly isolated *Fusarium equiseti* KR706303 and *Penicillium citrinum* KR706304 were grown on Potato Dextrose broth for seven days, then, harvested for biomass treatment. The live harvested biomass (20 g wet weight) of both fungi were pretreated with dimetyhl sulfoxide (DMSO) solution by boiled for 15 min in 200 ml of 50% (v/v) DMSO. After pretreatment, the biomass was washed thoroughly with sterilized distilled water until the pH of the washing solution reached the neutral range (pH 6.9- pH7.1), and then dried for 48hrs at 60˚C. The dried biomass was powdered using a mortar and pestle. In order to examine the roles played in the performance of metal biosorption by functional groups; such as carboxyl, amine, phosphate groups and lipids fraction present in the biomass, various chemicals were used to modify the dimethyl sulfoxide pretreated biomass.

Chemical modification of the biomass

The harvested fungal biomass, grown for 7 days on potato dextrose broth and pretreated with DMSO, were chemically treated to determine which functional group/groups on the fungal biomass may be involved in binding of Pb (ii) and Cu (ii) ions. The modifications performed were as reported by [3,24] and include the followings;

Esterification of the carboxylic groups

A volume of 2 g of dried DMSO-pretreated biomass was added to 130 mL of methanol with 1.2 mL of concentrated hydrochloric acid (HCl) added to the suspension. The mixture was agitated on an orbital rotary shaker for 6h at 125 rpm. This treatment of biosorbent with methanol results in the esterification of carboxylic acids present on the cell wall of biosorbent and the reaction can be represented as follows:

$$RCOOH+CH_3OH \xrightarrow{H^+} RCOOCH_3+H_2O$$

where R denotes the organic network of biomass molecules. Metal binding capacity of carboxyl groups will be reduced, because of this esterification. The biomass residue obtained was referred to as chemical modification 1 (CM1).

Methylation of amino groups

2g of dried DMSO-pretreated biomass was added with 40 mL of formaldhyde (HCHO) and 80 mL of formic acid (HCOOH). The mixture was shaken at 125 rpm for 6 h and the resulting reaction takes place as follow:

$$RCH_2NH_2 \xrightarrow{HCHO,HCOOH} RCH_2N(CH_3)_2+CO_2+H_2O$$

Due to the methylation of amino groups, their participation in metal biosorption was expected to be inhibited, resulting in the reduction in metal biosorption capacity on residual biomass. The obtained biomass residue was referred to as chemical modification 2 (CM2).

Esterification of the phosphate groups

A volume of 2 g of dried DMSO-pretreated biomass was heated under reflux and stirring conditions with 80 mL of triethyl phosphite and 60ml of nitromethane for 6h. The obtained biomass residue was referred to as chemical modification 3 (CM3).

Extraction of lipids

2g of dried DMSO- pretreated biomass were heated separately with 150 mL of acetone and benzene under reflux and stirring conditions. This treatment will extract the lipid fraction from the biomass. The obtained biomass residues were referred to as chemical modifications 4 (CM4) and 5 (CM5). Upon the completion of chemical modifications, all biomass samples were then washed with dH_20, dried at 60˚C for 24h, and stored until further use.

Batch biosorption experiment using free fungal biomass

The experiment of batch biosorption of *Fusarium equiseti* and *Penicillium citrinum* were conducted in triplicates**.** Each 100 mL Erlenmeyer flask containing 0.04 g of the powdered biosorbent chemically modified (designated as CM1- CM5) of *Fusarium equiseti* and *Penicillium citrinum* in 20 mL of Cu (ii) and Pb (ii) (50 mgL^{-1}) separately at 30^0C on an orbital rotary shaker at 150 rpm. These conditions were maintained in all of the experiments. The metal solutions were centrifuged (for 10 mins at 10,000 rpm) after the desired contact time, i.e equilibrium time, and metal ion concentrations in the supernatant were determined. The initial and final metal concentrations of the solution were recorded for each experiment. For each experiment, a control, with 20 mL of both Cu (ii) and Pb (ii) and 0.04 g of DMSO treated biosorbent without chemical modifications was also shaken to be used as a basis for comparison with chemically modified biosorbents. Each experiment was repeated three times and the results given are the average values.

Fourier transforms infrared spectroscopy (FTIR) analysis

For surface functional groups characterization, Fourier Transform Infrared (FTIR) spectra of the powdered DMSO-pretreated fungal biomasses before and after biosorption (for 2 h in 50 mgL^{-1} initial metal concentration) were carried out. The samples were dried overnight to remove any water remained in the sample, which could interfere with observation of hydroxyl groups on the surface. This was followed by encapsulation into dry potassium bromide (KBr) discs. The discs were then scanned into transmission mode using a Fourier transform infrared (FTIR) spectrometer (Nexus 670, Nicolet, USA), through a wavelength range from 4000 to 400 cm^{-1}. IR spectra of control and metal loaded biomasses were recorded.

RESULTS

Effect of chemical modification on biosorption of lead (II) ion

The chemical modification effect of *F. equiseti* KR706303 and *P. citrinum* KR706304 pretreated biomasses on biosorption of Pb (ii) ions is illustrated (**Figs. 1** and **2**). The biosorption of Pb (ii) decreases in comparison with the control (DMSO pretreated without chemical modification). From the results, it is evident that the biosorption capacity of Pb (ii) significantly decreases in comparison with pretreated biomass without chemical modification ($p< 0.05$). The Pb (ii) biosorption value reduced from 17.06±1.09 to 3.56±0.81, 7.13±2.64, 7.55±1.10, 8.66±3.84 and 9.1±0.73 mg g^{-1} d.wt by CM2, CM4, CM5, CM1, and CM3 chemically modified biomasses of *F. equiseti* respectively (**Fig. 1**). The Pb (ii) biosorption efficiency values reduced from 67.7 to 34.9, 33.2, 38.7, 29.3 and 36.6%, respectively (**Fig. 2**).

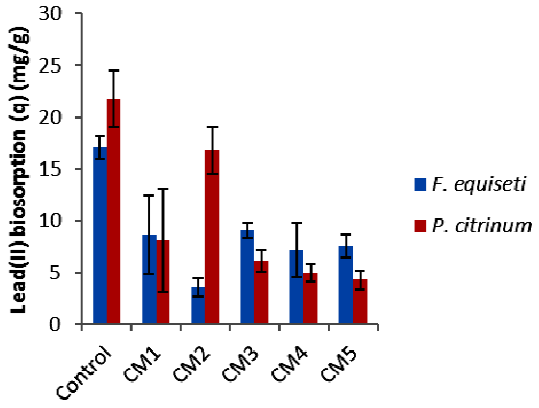

Fig. 1. Effect of biomass chemical modification on Pb (ii) biosorption (mg g^{-1}) by *Fusarium equiseti* and *Penicillium citrinum*. The data are the mean values of 3 replicates, and the bars indicate the standard error of the mean.

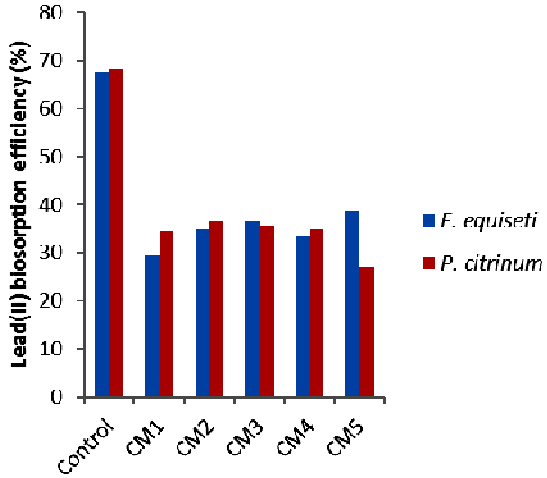

Fig. 2. Effect of biomass chemical modification on Pb (ii) biosorption efficiency (%) by *Fusarium equiseti and Penicillium citrinum.*

The chemically modified biomass of *P. citrinum* with CM1, CM3, CM4 and CM5 significantly reduces as well, in comparison with DMSO pretreated biomass without chemical modification. The biosorption capacity reduces from 21.78±2.67 to 8.12±4.97, 6.13±1.06, 4.98±0.83 and 4.27±0.93 mg g^{-1} d.wt, with Pb (ii) biosorption efficiencies from 68.3 to 34.4, 35.7, 34.9 and 27.1 %, respectively. The CM2 chemically modified biomass of this fungus is not significantly different from the control ($p>0.05$). The Pb (ii) biosorption value dropped from 21.78±2.67 to 16.87±2.27 mg g^{-1} d.wt, with Pb (ii) biosorption efficiencies from 68.3 to 36.6%. In general, the results indicated that Pb (ii) biosorption reduction order was as follows: CM2 < CM1 < CM3 < CM4 < CM5 for *P. citrinum*, whereas the order was CM2 < CM4 < CM5 < CM1 < CM3 exhibited by *F. equiseti* .

Effect of chemical modification on copper (II) biosorption.

The chemical modification effect of *F. equiseti* and *P. citrinum* pretreated biomasses on biosorption of Cu (ii) ions is presented in **Figs. 3** and **4**. The biosorption of Cu (ii) decreases in comparison with the control (DMSO pretreated without chemical modification). From the result, it was observed that the biosorption capacity of Cu (ii) significantly decreases in comparison with the control except CM2 and CM3 ($p< 0.05$).

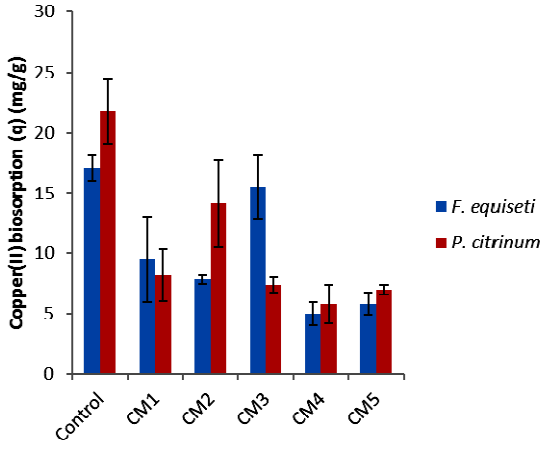

Fig. 3. Effect of biomass chemical modification on Cu (ii) biosorption (mg g^{-1}) by *Fusarium equiseti* and *Penicillium citrinum.* The data are the mean values of 3 replicates, and the bars indicate the standard error of the mean.

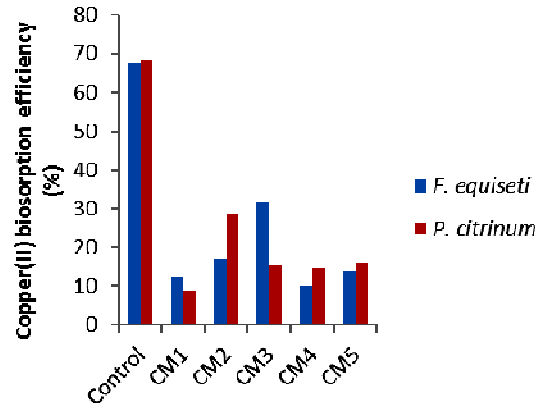

Fig. 4. Effect of biomass chemical modification on Cu (ii) biosorption efficiency (%) by *Fusarium equiseti and Penicillium citrinum*. Control, biosorbent without chemical modification, CM1: esterification of carboxylic group, CM2: methylation of amino group, CM3: esterification of phosphate group, CM4: extraction of lipids with acetone and CM5: extraction of lipids with benzene.

The Cu (ii) biosorption values dropped from 17.06±1.09 to 9.52±3.54, 7.83±0.34, 5.80±0.92 and 4.98±0.94 mg g^{-1} d.wt, by CM1, CM2,CM5 and CM4 chemically modified biomasses of *F.equiseti*, respectively (**Fig. 3**). The Cu (ii) biosorption efficiency values reduces drastically from 67.7 to 12.3, 16.9, 13.9 and 9.9%, respectively (**Fig. 4**).

The chemically modified biomass of *P. citrinum* with CM1, CM3, CM4 and CM5 significantly reduced, in comparison with the control (DMSO, pretreated biomass without chemical modification). The Cu (ii) biosorption value reduced from 21.78±2.67 to 14.11±3.60, 8.18±2.16, 7.37±0.66, 6.97±0.38 and 5.78±1.55 mg g^{-1} d.wt, with Cu (ii) biosorption efficiencies from 68.3 to 28.4, 8.8, 15.3, 16.1 and 14.8% respectively (Figures 3 and 4). In general, the results indicated that Cu (ii) biosorption reduction order was as follows: CM2 >, CM1>, CM3>, CM5> and CM4 for *P. citrinum*, whereas the order was CM3 > CM1 > CM2 > CM5 > CM4 exhibited by *F. equiseti*.

FTIR spectral analysis

The FTIR spectra of free and metal loaded fungal biomass in the range 4000-400 cm^{-1} were taken and compared with each other to obtain information on the nature of the possible biomass-metal ions interactions. The results obtained is presented in **Figs. 5** and **6**. The FTIR spectra of unloaded DMSO-treated *Fusarium equiseti* and *Penicillium citrinum* biomass display a number of absorption peaks which reflect the complexity of examined biomasses. The broad adsorption bands at 3700-3000 cm^{-1}, representing –OH groups of the glucose and the –NH stretching of the protein and chitosan. In the range 3000-2800 cm^{-1} the bands are representative of symmetric and asymmetric vibrations of stretching of CH_3 and CH_2 groups. The peaks at 1643.80 and 1557.76 cm^{-1} of *F. equiseti* (**Fig. 5**) and 1646.02 and 1540.63 cm^{-1} of *P. citrinum* (**Fig. 6**) can be assigned to a carbonyl group (C=O) stretching in carboxyl or amide groups. The following observations were noted, after metal biosorption:

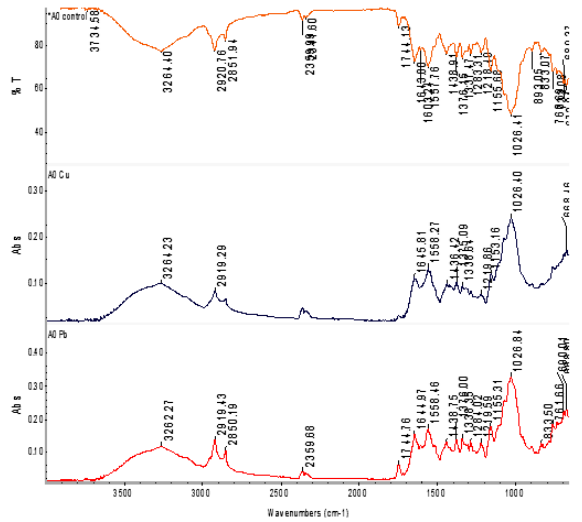

Fig. 5. FTIR spectra of dried unloaded (A0 control), Pb (ii)-loaded (A0 Pb), and Cu (ii)-loaded biomass of *F. equiseti* (A0 Cu).

Little shifting of peaks (corresponding to –OH and –NH groups) from 3264.40 to 3262.27 and 3264.23 cm^{-1} after Pb (ii) and Cu (ii) biosorption by *F. equiseti*, respectively, and from 3273.15 to 3273.38 after Pb (ii) biosorption by *P. citrinum*. This shifting indicates that these groups were involved in Pb (ii) and Cu (ii) biosorption. Changed absorption bands (corresponding to symmetric and asymmetric vibrations of stretching of CH_3 and CH_2 groups) at 2920.76 to 2919.43 and 2919.29 cm^{-1} after Pb (ii) and Cu (ii) biosorption by *F. equiseti,* respectively, and from 2922.72 to 2922.83 after Pb (ii) biosorption by *P. citrinum*.

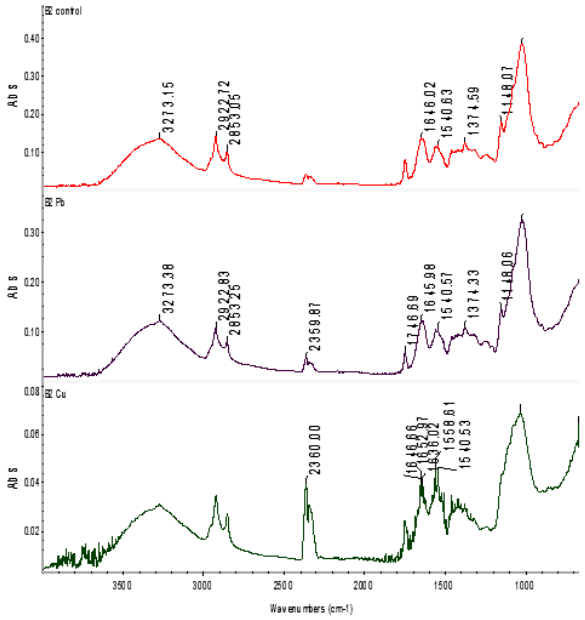

Fig. 6. FTIR spectra of dried unloaded (B2 control), Pb (ii)-loaded (B2 Pb), and Cu (ii)-loaded biomass of *P. citrinum* (B2 Cu).

This slight shifting indicates the participation of these groups in the biosorption of Pb (ii) and Cu (ii) by both *F. equiseti* and *P. citrinum*. Another little change in the spectrum was the carbonyl group (C=O) stretching in carboxyl or amide groups. The shift was from 1643.80 to 1644.97 and 1645.81cm^{-1} after Pb (ii) and Cu (ii) biosorption by *F. equiseti*, respectively. The shift for amide was from 1557.76 to 1558.46 and 1558.27 cm^{-1} after biosorption of Pb (ii) and Cu (ii) by *F. equiseti*. These indicated that carbonyl groups of the fungal cell wall are active in metal removal. The same finding was observed in the spectrum of *P. citrinum* and a slight shifting was occurred from 1646.02 to 1645.98 and 1652.97 cm^{-1} after Pb (ii) and Cu (ii) biosorption respectively.

DISCUSSION

Biosorption is one of among many types of metal-microbe interactions. Different parts of the microbial cell may sequester metals via process such as complexation, chelation, coordination, ion exchange, precipitation, and reduction. These mechanisms are collectively known as sorption and the overall phenomenon as biosorption [25]**.** The mechanism of metal biosorption is complicated and not fully well understood. The status of biomass (living or non-living), types of biomaterials, properties of metal-solution chemistry, and ambient/environmental conditions such as pH, will all influence the mechanism of metal biosorption [3,18].

Two types of metal sequestering mode were identified which includes; the passive mode by dead or inactive cells and active mode by living cells. Passive mode is independent of

energy, mainly through chemical functional groups of the material, comprising the cell and particularly cell wall. Active mode is metabolism-dependent and related to the metal transport and deposition [3, 18]. Fourier transform infrared analysis (FTIR) is an important tool to identify the functional groups [26,27]. The FTIR spectra of the fungal biomasses after Pb (ii) and Cu (ii) biosorption showed various changes, especially in hydroxyl (–OH), amide (-NH), and carboxyl (–COO^-) indicating the involvement of these groups for Pb (ii) and Cu (ii) binding to *F. equiseti* and *P. citrinum* biomasses. The changes in the peak as observed in this study could be attributed to the chemical interactions between the metal ions and the functional groups present in the fungal cell walls [28]. The similar FTIR results were previously reported for the biosorption of Pb (ii), Cd (ii), and Cu (ii) onto *Botrytis cinerae* fungal biomass [29], Pb (ii) and Cd (ii) onto *Lactarius scrobiculatus* biomass [30] and Pb (ii) and Co (ii) onto *R. oryzea* and *S. cerevisiae* biomasses [3].

DMSO-treated *F.equiseti* and *P. citrinum* biomasses were chemically modified in a different way to understand better the role of functional groups in the biosorption of Pb (ii) and Cu (ii) ions. The chemical treatments applied to the fungal biomasses were described previously. From the results, it was found that esterification (using methanol and HCl) of carboxyl and methylation (using formaldehyde and formic acid) of amine groups present in the cell wall of fungi greatly decreases the biosorption of Pb (ii) and Cu (ii) ions. These findings suggest that carboxyl and amine groups are important in Pb (ii) and Cu (ii) biosorption on *F. equiseti* and *P. citrinum* biomasses. Also, these results may be sufficient to indicate the important participation of carboxyl and amine groups in the biosorption of Pb (ii) and Cu (ii) ions. Similar results were reported by [3], where carboxyl and amine groups were involved in the biosorption of Pb (ii) and Cobalt(II) by *R. oryzae* and *S. cerevisiae*. [31] showed that, after the carboxyl groups were esterified, Pb (ii) binding on cell walls of *S. cerevisiae* decreases to about 96.3%. Furthermore, reduction in the biosorption of Cd, Cu and Pb ions by *Aspergillus niger* when subjected to esterification of its carboxyl groups have been reported by [32].

Copper biosorption has been reported to be affected due to modification of carboxyl and amine groups present in the cell wall of *S. cerevisiae* [33]. In the biosorption of Cu^{2+}, Ni^{2+}, Zn^{2+}, and Cr^{3+} by *Penicillium chrysogenum*. [34] found that the main chelating sites in the mycelium are amine groups of chitosan. The treatment of biomass with acetone and benzene extracts the lipid fraction of the biomass [35]. Therefore, the reduction in biosorption efficiency when acetone and benzene treated biomasses were used, reveals that the lipids in the cell wall of *F. equiseti* and *P. citrinum* contributed to Pb (ii) and Cu (ii) biosorption. [32] observed a slight decrease in biosorption of lead, cadmium, and copper when lipids were extracted from *Aspergillus niger*. They attributed that the decrease to either lipid extraction or the probable structural changes that may have resulted due to the harsh conditions of the extraction process. The carboxyl, amine, and phosphate groups and lipids are negatively charged. However, the interaction between amine group and Pb (ii) or Cu (ii) ions could be seen as complexation.

This study showed that the electrostatic attraction and complexation seem to be the most important mechanism of biosorption of Pb (ii) and Cu (ii) ions as reported for Pb (ii) and cobalt(II) ions biosorption by [3]. In conclusion, this study identified carbonyl, hydroxyl, amide and lipids portion as key functional groups in the fungal cell wall that participated in the biosorption mechanisms of this fungi.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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