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Removal of Cu(II) and Zn(II) Using Lignocellulosic Fiber Derived from *Citrus reticulata* (Kinnow) Waste Biomass

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Abstract: The biosorption of Cu(II) and Zn(II) using dried untreated and pretreated *Citrus reticulata* waste biomass were evaluated. The Cu(II) and Zn(II) sorption were found to be dependent on the solution pH, the biosorbent dose, the biosorbent particle size, the shaking speed, the temperature, the initial metal ions (800 mg/L), and the contact time. Twenty-eight physical and chemical pretreatments of *Citrus reticulata* waste biomass were evaluated for the sorption of Cu(II) and Zn(II) from aqueous solutions. The results indicated that biomass pretreated with sulphuric acid and EDTA had maximum Cu(II) and Zn(II) uptake capacity of 87.14 mg/g and 86.4 mg/g respectively. Moreover, the Langmuir isotherm model fitted well than the Freundlich model with $R^2 > 0.95$ for both metal ions. The sorption of Cu(II) and Zn(II) occurred rapidly in the first 120 min and the equilibrium was reached in 240 min. FTIR and SEM studies were also carried out to investigate functional groups present in the biomass and the surface morphological changes of biomass.

Keywords: *Citrus reticulata*, Cu(II), isotherms, pretreatments, Zn(II)

INTRODUCTION

Heavy metal solutions are widely used in industrial activities such as metal finishing, electroplating, painting, dyeing, photography, surface

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treatment, printed circuit board manufacture, etc. Most of the heavy metal ions are well-known toxic and carcinogenic agents, while metal residues in the environment pose a threat not only for human health, but also have serious detrimental effects for the aquatic ecosystem (1–5). The presence of heavy metals in the aquatic environment has forced international environmental agencies to introduce strict regulations with regard to metal discharge, especially from industrial activities (6). Zn(II) may be found in wastewater discharges from acid mine drainage (AMD), galvanizing plants, and natural ores, and from municipal wastewater treatment plant discharges (7). Although Zn(II) proved to be one of the less toxic metals for anaerobic digestion as well as human metabolism, mainly in children and in patients with metabolic dysfunctions, people are surely affected at high concentrations (8). Too much Zn(II) can lead to respiratory incapacitation, as indicated by increased respiratory activity (i.e., breathing rate, coughing). Cu(II) is a widely used material; there are many actual or potential sources of copper pollution (9,10). The presence of Cu(II) ions causes serious toxicological concerns, and it is usually known to deposit in brain, skin, liver, pancreas, and myocardium (11).

Therefore, several technologies for treating heavy metal bearing wastewater, such as precipitation reduction, solvent extraction (12,13), and membrane processes (14) have been used until recently. However, these methods have several disadvantages, which include incomplete metal removal and toxic sludge generation. On the other hand, the use of artificially prepared ion exchange resins is effective, but too expensive to be applied on an industrial scale. For this reason, the potential of a new method of removing heavy metals by biosorbents presents an important break through (15,16). The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on the metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake. This phenomenon, defined as biosorption, seems to be a good alternative to the existing methods since it does not produce chemical sludge, it could be highly selective, more efficient, easy to operate, and hence cost effective for the treatment of large volumes of wastewaters containing low pollutant concentrations (17,18). The use of dead, dried aquatic plants, for metal removal as a simple biosorbent material has advantages in its high efficiency in detoxifying dilute effluents, minimization of the volume of chemical and/or biological sludge to be disposed off, no nutrient requirements, low cost, conservation, transport, handling, etc. (4,19,20).

Citrus reticulata (Kinnow) is a widely grown variety of citrus fruit. Citrus juice is one of the most favorite drinks worldwide. Biomass left after extraction of juice from *Citrus reticulata* fruit is a waste material. This work is aimed at evaluating the possibility of using lignocellulosic fibers derived from *Citrus reticulata* (Kinnow) as biosorbent for removal of Cu(II) and Zn(II) from aqueous solution. The effect of pH, the biosorbent dosage and size, the initial Cu(II) and Zn(II) concentration, the contact time, the agitation speed, the temperature and the pretreatment of cellulosic fibers on both sorption kinetics and equilibrium were also looked at.

MATERIALS AND METHODS

Citrus reticulata (Kinnow) Biomass

Citrus reticulata biomass used in this work was harvested from the Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. The lignocellulosic fiber was derived from *Citrus reticulata* (Kinnow) fruit by cold pressing. The derived lignocellulosic fiber was soaked overnight in distilled water to remove particulate matter and other waste soluble residues. Biomass was first sun dried and then oven dried at 60°C till constant weight. *Citrus reticulata* waste biomass was analyzed for Na, K, Li, Cu, Zn, Mn, Pb, Cr, Co, Fe, and Mg using a flame photometer (Sherwood 410) and Atomic Absorption Spectrophotometer (Perkin-Elmer AAnalyst 300). The concentration of each metal determined is shown in Table 1. The results demonstrated that *Citrus reticulata* waste biomass contains minute amounts of these metals. The dried biomass (1 kg) was ground using a food processor and then sieved through an Octagon siever (OCT-DIGTAL 4527-01) to obtain the biosorbent with a homogenous known particle size. The fraction

Table 1. Mineral composition of the *Citrus reticulata* waste biomass

Minerals	Amount (mg/g)
Na	3.01
K	5.27
Li	2.69
Cu	0.01
Zn	0.05
Pb	0.01
Cr	0.26
Co	0.02
Fe	0.36
Mg	0.13

with 0.250–1 mm size was selected for use in the sorption tests. The sieved sorbent was stored in an air tight plastic container for further experiments.

Reagents

All chemical reagents used in the present study were of analytical grade. HCl and NaOH were obtained from Merck while $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, and standard copper and zinc solutions (1000 mg/L) were purchased from Merck Company (Darmstadt, Germany). Glassware and polypropylene flasks used were immersed overnight in 10% (v/v) HNO_3 and rinsed several times with distilled deionized water (DDW).

Cu(II) and Zn(II) Solutions

To prepare stock Cu(II) and Zn(II) solutions (1000 mg/L), 3.95 g of $CuSO_4 \cdot 5H_2O$ and 4.41 g of $ZnSO_4 \cdot 7H_2O$ were dissolved in 100 mL of DDW and diluted quantitatively to 1000 mL using DDW. Different concentrations were prepared by adequate dilutions of the stock solution with DDW.

Batch Biosorption Studies

A fixed volume (100 mL) of each metal solution was used to determine the effects of various influencing parameters such as the pH, the biosorbent dose, size, the initial metal concentration, the contact time, the shaking speed, and the temperature. Different conditions of pH (3, 4, 5, 6, 7, and 8), biosorbent dose (0.05, 0.1, 0.2, and 0.3 g), biosorbent size (0.250–1 mm), initial metal concentration (25, 50, 100, 200, 400, and 800 mg/L), contact time (up to 24 hours), shaking speed (0, 50, 100, and 150 rpm), and temperature (30, 40, 50, 60, and 70°C) were studied. For adjusting the pH of the medium 0.1N solutions of NaOH and HCl were used. The flasks were placed on a rotating shaker (PA 250/25. H) with constant shaking. At the end of the experiments, the flasks were removed from the shaker and the solution was separated from the biomass using Whatman No. 40 ashless filter.

Pretreatments of the Biomass

The biosorbent was physically modified by heating (60°C in an electrical oven for 30 minutes) and boiling (5 g biosorbent/100 mL water for

30 minutes). For chemical pretreatment, 5 g of finely divided biomass was soaked in 100 mL of HCl (0.1N), H_2SO_4 (0.1N), HNO_3(0.1N), NaOH (0.1N), $Al(OH)_3$ (0.1N), C_6H_6 (0.1N), HCHO (0.1N), CH_3OH (0.1N), C_2H_5OH (0.1N), $Ca(OH)_2$(0.1N), $(NH_4)_2SO_4$(0.1N), acetone (0.1N), $CaCl_2$(1%), EDTA (1%), potash alum (1%), Ƙ-carragnen (1%), Triton X-100 (1%), *Moringa Olifera* seed powder (1%), Na-alginate (2%, 50 mL), Ca-alginate (2%, 50 mL), glutaraldehyde (25%, 50 mL), PEI (1%, 50 mL) and PEI + glutaraldehyde (2%, 100 mL). CO_2 and H_2S gas was passed through 5 g of biomass soaked in 100 mL of DDW at the rate of 10 mL/min for 10 minutes. The pretreated biosorbents were extensively washed with DDW. Finally, the resulting biomass was oven dried for 48 h to constant weight. The dried samples were grounded with a laboratory grinder and passed through a laboratory vibro-sieving machine (Octagon siever model: OCT-DIGITAL 4527-01) prior to their testing for metal biosorption. The fraction of particles of 0.250 to 1.0 mm was used in all the experiments.

Determination of Cu(II) and Zn(II) Contents in the Solutions

Cu(II) and Zn(II) contents in the sample and control assays before and after the equilibrium were analyzed by flame atomic absorption spectrometry (FAAS), using a Perkin-Elmer AAnalyst 300 atomic absorption spectrometer equipped with an air–acetylene burner and controlled by Intel Pentium-4 personal computer. The hollow cathode lamp was operated at analytical wavelength of 342.8 nm for Cu (II) and 213.9 nm for Zn (II).

Determination of Metals Uptake Capacity and % Removal

The Cu(II) and Zn(II) uptake were calculated by the simple concentration difference method (20). Adsorption capacity "q" is the amount of metal ion (mg) biosorbed per g (dry weight) of biomass. The following equations were used to compute the percent metal uptake by the sorbent (Eq. (1)) and sorbent uptake capacity at equilibrium q_e (mg/g) (Eq. (2)).

$$\%\text{sorption} = (C_i - C_e)100/C_i \tag{1}$$

$$q_e = (C_i - C_e)V/1000\,W \tag{2}$$

where C_i (mg/L) is the initial metal concentration, C_e (mg/L) is the equilibrium concentration, V is the volume of the solution in mL, and W is the mass of the sorbent in g.

FTIR and SEM Studies

The chemical characteristics of *Citrus reticulata* waste biomass were analysed and interpreted by Bruker Tensor 27 Fourier transform infrared spectrometer with the samples prepared as KBr discs. The surface structure of *Citrus reticulata* waste biomass was analyzed by JEOL JMT 300 scanning electron microscope (SEM).

Statistical Analysis

All data represent the mean of three independent experiments. Error bars shown in figures represent standard deviation values.

RESULTS AND DISCUSSION

Effect of pH

pH is considered as an important parameter affecting the sorption of metal ions on adsorbents as it not only affects metal species in solution, but also influences the surface properties of the adsorbents in terms of dissociation of functional groups and surface charge (21,22). Depending on the solution pH, metal cations and protons could compete for the active sites of the biomass (23). For the *Citrus reticulata* waste biomass the adsorption capacity increased with an increase in solution pH. The metal uptake increased with increase in pH from 1–5 for Cu(II) and 1–6 for Zn(II) at fixed biomass concentration (0.1 g/100 mL) (Fig. 1). *Citrus reticulata* showed the maximum biosorption capacity at pH 5 for Cu(II) and 6 for Zn(II) beyond which precipitation occurred. At lower pH, the positively charged hydrogen ions may compete with the metal ions for binding on the functional groups on the biomass surface thereby decreasing the interaction of metal ions with the cells of the biomass surface. The competing effect of hydrogen ions decreased when the pH was increased and the positively charged metal ions took up the free binding sites. The metal uptake was hence increased. As the pH was increased, the ligands would be exposed, increasing the negative charge density on the biomass surface, resulting in greater attraction between metallic ions and ligands. The effect of pH on biosorption of Cu(II) and Zn(II) have been investigated by various investigators using a variety of different biomass types and similar results have been reported in literature for different metal-biomass systems (24–28).

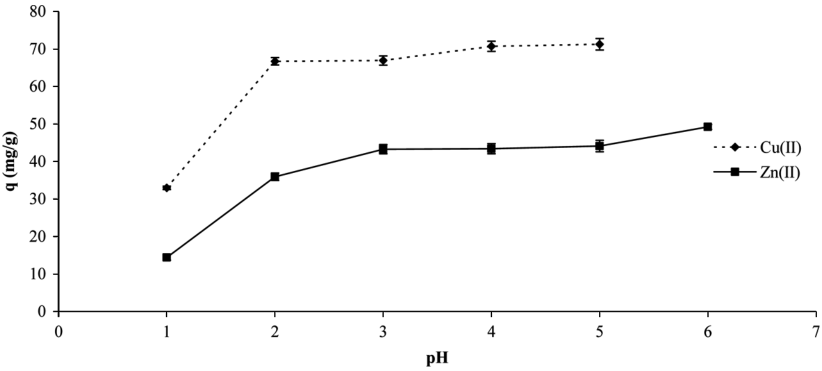

Figure 1. Effect of pH on sorption of Cu(II) and Zn(II) by *Citrus reticulate* waste biomass.

Effect of Biosorbent Dose

The metal sorption process is significantly influenced by the amount of the biosorbent added. The amount of biomass added into the solution determined the number of binding sites available for the adsorption (27). The effect of the biomass dose on the amount of Cu(II) and Zn(II) ions removed were studied by the application of varying biosorbent doses (0.05, 0.1, 0.2, 0.3, and 0.4 g) at a Cu(II) and Zn(II) concentration of 100 mg/L (Fig. 2). The maximum adsorption by *Citrus reticulata*

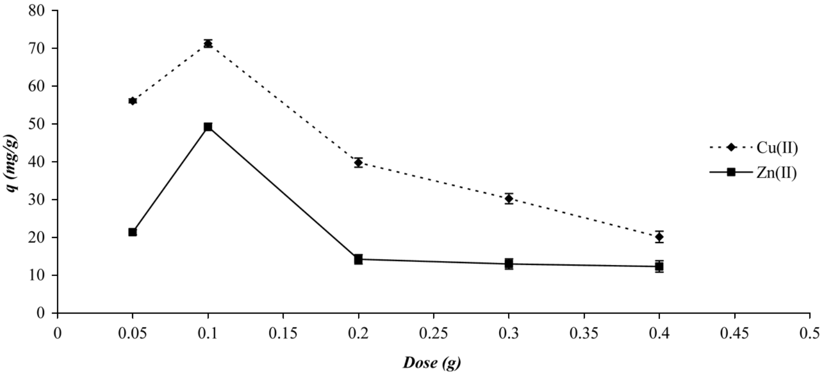

Figure 2. Effect of biosorbent dose on sorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

was achieved with a biomass dose of 0.1 g and the sorption capacity declined sharply with the increase in the amount of biomass to 0.2 g and continued decreasing up to 0.4 g. The initial increase in the Cu(II) and Zn(II) adsorption capacity was due to the availability of more adsorption sites. On increasing the biomass concentration further, the binding of Cu(II) and Zn(II) steadily decreased. This effect might be attributed to overlapping or aggregation of adsorption sites of biomass resulting in a decrease in the total surface area of the biosorbent. This is in line with the reports by other researchers who reported a similar trend of biosorbent dosage on the biosorption process of heavy metals (27, 29). The optimum *Citrus reticulate* biomass dose selected was 0.1 g for the rest of the experimental studies.

Effect of Biosorbent Particle Size

The particle sizes of the biosorbent greatly influenced the sorption capacity due to change in the total surface area provided for sorption of metal ions. The effect on the sorption capacity, q (mg/g) due to change in the sorbent particle size showed that higher Cu(II) and Zn(II) removal were achieved by the smaller particles. Figure 3 illustrated that finely grounded biomass adsorbed Cu(II) and Zn(II) ions more rapidly. Equilibrium was reached faster with finer biosorbent particles than with larger ones. This was most probably due to the increase in the total surface area, which provided more sorption sites for the metal ions (27,28,30).

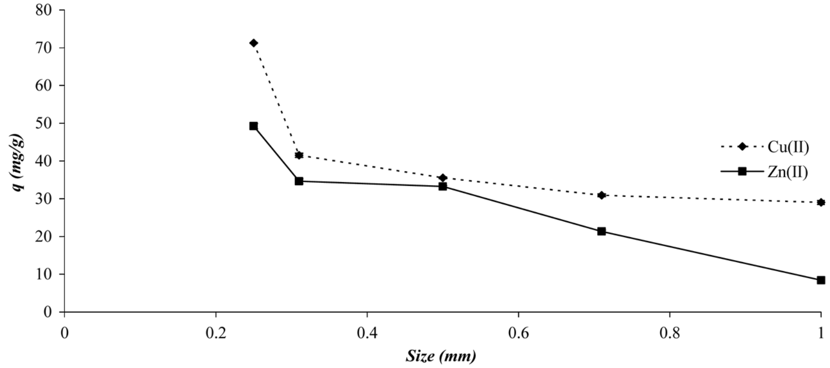

Figure 3. Effect of particle size on sorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

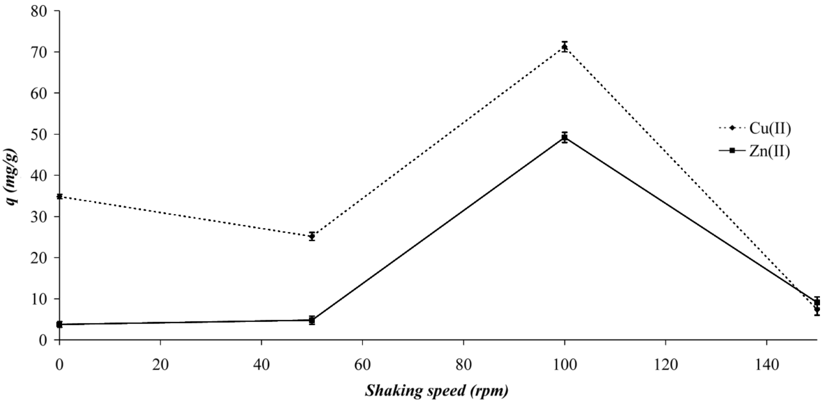

Figure 4. Effect of shaking speed on sorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

Effect of Shaking Speed

The uptake of Cu(II) and Zn(II) ions by *Citrus reticulata* waste biomass was evaluated by varying the agitation rate from 0 (without agitation) to 150 rpm (Fig. 4). The adsorption capacity reached the maximum value at 100 rpm for both metal ions and then decreased. These results can be associated to the fact that the increase of the agitation speed improved the diffusion of Cu(II) and Zn(II) ions towards the surface of the *Citrus reticulata* (10). As at very high shaking speeds, the desorption of the adsorbed metals can occur.

Effect of Temperature

The effect of temperature on the removal of Cu(II) and Zn(II) in aqueous solution by *Citrus reticulata* waste biomass was studied by varying the temperature between 30 and 70°C. As seen from Fig. 5, the maximum equilibrium uptakes were found to be at 30°C. The results indicated that an increase of the temperature leads to decrease in the equilibrium uptake capacity of *Citrus reticulata* for Cu(II) and Zn(II) ions. The decrease of the equilibrium uptake capacity with the increase in temperature means that the biosorption processes of these metal ions by *Citrus reticulata* were exothermic. This decrease at higher temperatures may be due to the damage of active binding sites in the biomass. According to Aksu and Kutsal (31), at high temperature, the thickness of the boundary layer decreased, due to the increased tendency of the metal ion to escape from the biomass surface to the solution phase, which results in a decreased

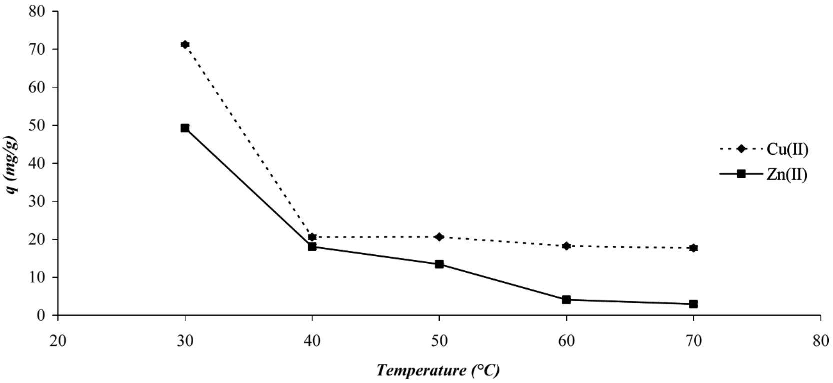

Figure 5. Effect of temperature on sorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

adsorption. The decrease in adsorption with increasing temperature, suggested weak adsorption interaction between the biomass surface and the metal ion, which supported physisorption. This is in line with the reports by Jnr and Spiff (32) and Shafqat et al. (33) who indicated that the biosorption of metals decreased with an increase in the temperature of the medium.

Effect of Contact Time

In Fig. 6, the Cu(II) and Zn(II) uptake capacity (mg/g) of *Citrus reticulata* waste biomass has graphically been shown as a function of time at

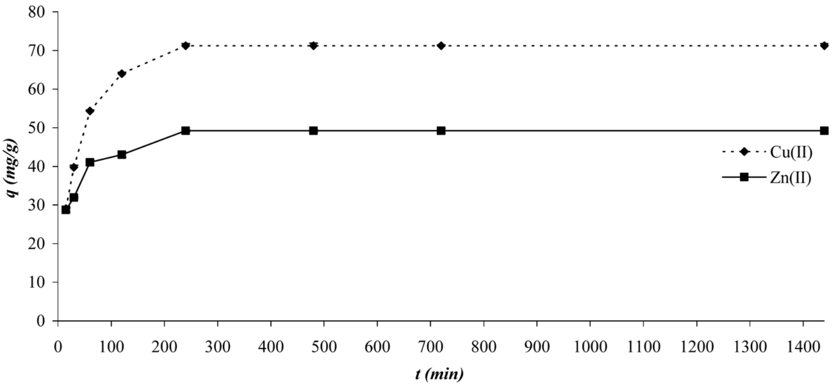

Figure 6. Effect of contact time on sorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

Table 2. Comparison between Lagergren pseudo first order and pseudo second order kinetic models for Cu(II) and Zn(II) sorption by *Citrus reticulata* waste biomass

	Pseudo first order kinetic model			Experimental value	Pseudo second order kinetic model		
Metal	q_e (mg/g)	$K_{1,ads}$ (min^{-1})	R^2	q_e (mg/g)	q_e (mg/g)	$K_{2,ads}$ (g/mg min)	R^2
Cu (II)	35.48	2×10^{-2}	0.963	71.24	76.92	1.05×10^{-3}	0.999
Zn (II)	21.63	3×10^{-2}	0.934	49.21	52.63	2.59×10^{-3}	0.999

100 mg/g initial metal concentration. The removal rate was rather fast in the first 120 min and equilibrium was reached in 240 min.

The mechanism of biosorption and the potential rate controlling steps of the biomass employed as a free suspension, must be investigated to properly understand adsorption kinetics (34). A comparison between the Lagergren pseudo-first-order and pseudo-second-order kinetic models are tabulated in Table 2. The form of the first-order Lagergren equation is given as (Eq. (3)):

$$\log(q_e - q) = \log q_e - \frac{k_{1,adst}}{2.303} \tag{3}$$

The pseudo-second-order equation (Eq. (4)) is:

$$\frac{t}{q} = \frac{1}{k_{2,ads} q_e^2} + \frac{t}{q_t} \tag{4}$$

where q_e is the mass of metal adsorbed at equilibrium (mg/g), q_t the mass of metal adsorbed at time t (min.), $k_{1,ads}$ the pseudo-first-order reaction rate of adsorption (per min.), $k_{2,ads}$ the pseudo-second-order rate constant of adsorption $\mathrm{mg/g\,min^{-1}}$. The obtained kinetic data suggest that the sorption of Cu(II) and Zn(II) ions followed the second kinetic model which relies on the assumption that biosorption may be the rate limiting step. In addition to the high value of the coefficient of determination, R^2 (Table 2), a close agreement between the experimental and the estimated q_e values suggests that the sorption process followed the second-order mechanism. These results are in close agreement with the reports by Javed et al. (30) and Zubair et al. (34) who observed that the biosorption of metals by rose waste biomass and *Citrus reticulata* biomass followed pseudo-second-order kinetics.

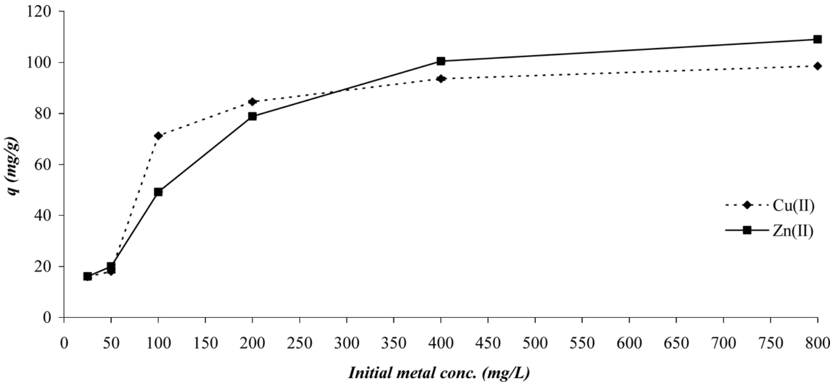

Figure 7. Effect of different initial metal concentration on biosorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass.

Effect of Initial Metal Concentration

The data revealed that the sorption capacity of the biosorbent increased with increase in initial metal ion concentrations at fixed biomass concentration (0.1 g/100 mL) (Fig. 7). This sorption characteristic represented that the surface saturation was dependent on the initial metal ion concentrations. At low concentrations, the adsorption sites take up the available metal ions more quickly. However, at higher concentrations, metal ions need to diffuse to the biomass surface by intraparticle diffusion and more hydrolyzed ions will diffuse at a slower rate (33,35).

The Langmuir and Freundlich adsorption constants evaluated from the isotherms with correlation coefficients are presented in Table 3. The Langmuir parameters can be determined from a linearized form of the equation (Eq. (5)), represented by:

$$C_e/q_e = 1/X_m K_L + C_e/X_m \tag{5}$$

Table 3. Comparison of Langmuir and Freundlich isotherm parameters for Cu(II) and Zn(II) uptake by *Citrus reticulata* waste biomass

	Langmuir isotherm parameters			Experimental value	Freundlich isotherm parameters			
Metal	q_{max} (mg/g)	K_L (L/mg)	R^2	q_{max} (mg/g)	q_{max} (mg/g)	K (mg/g)	1/n	R^2
Cu II)	94.33	2.3×10^{-2}	0.9673	98.54	128.47	8.77	4×10^{-1}	0.6314
Zn (II)	125	1.1×10^{-2}	0.9818	109.02	138.42	5.57	4.9×10^{-1}	0.8894

where q_e is the metal ion sorbed (mg/g), C_e the equilibrium concentration of the metal ion solution, and X_m and K_L are Langmuir constants. The sorption of both metals i.e., Cu(II) and Zn(II) followed the Langmuir isotherm well, which suggested that the monolayer of the sorbate was formed on the biosorbent. The adsorption-partition constants were determined for Cu(II) and Zn(II) using the following log form of the Freundlich isotherm (Eq. (6)):

$$\log q_e = (1/n)\log C_e + \log K \tag{6}$$

where q_e is the metal ion sorbed (mg/g), C_e the equilibrium concentration of the metal ion solution (mg/L), and K and n are the Freundlich constants.

The constants K and $1/n$ were determined by linear regression from the plot of log q_e against log $\mathrm{C_e}$. K is a measure of the degree or strength of adsorption, while $1/n$ is used as an indication of whether the adsorption remains constant (at $1/n = 1$) or decreases with increasing adsorbate concentrations (with $1/n = 1$). The q_{max} value is the maximum value of q_e, which is important to identify which biosorbent has the highest metal uptake capacity. It is very useful value in scale-up considerations of metal removal by a particular biosorbent. The magnitude of the experimental q_{max} for *Citrus reticulata* waste biomass were found to be 98.54 mg/g for Cu(II) and 109.0 mg/g for Zn(II). The theoretical q_{max} for *Citrus reticulata* waste biomass were found to be 94.33 mg/g and 125 mg/g for Cu(II) and Zn(II) with respect to the Langmuir isotherm model. While according to the Freundlich isotherm the magnitude of theoretical q_{max} was 128.47 mg/g and 138.42 mg/g for Cu(II) and Zn(II) respectively. By comparison, it was found that the value of q_{max} calculated from Langmuir isotherm model was close to the experimental value, hence the biosorption of Cu(II) and Zn(II) by *Citrus reticulata* waste biomass was better described by the Langmuir isotherm model. The comparison of theoretically calculated q_{max} values from Langmuir and Freundlich isotherm models is presented in Table 3, thus indicating that Langmuir isotherm model fitted well to the data.

Effect of Pretreatments

To evaluate the effect of pretreatment on *Citrus reticulata* waste biomass, 100 mg/L Cu(II) and Zn(II) solution were shaken at 100 rpm with 0.1 g/L of pretreated biosorbent having size 0.250 mm at pH 5 for Cu(II) and 6 for Zn(II) for 24 h. The q values (mg/g) of non treated, physically and chemically modified *Citrus reticulata* waste biomass for Cu(II) sorption are shown in Fig. 8. Boiling of the biomass removed mineral matter from

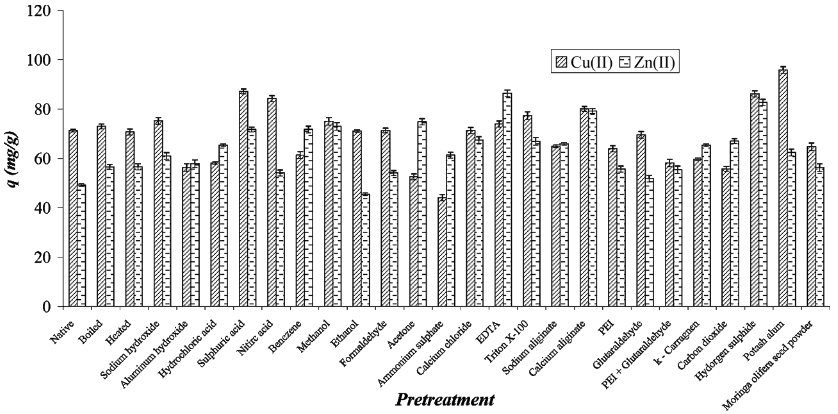

Figure 8. Cu(II) and Zn(II) sorption capacity ‘q’ (mg/g) of pretreated *Citrus reticulata* waste biomass. Cu(II) and Zn(II) solution (100 mg/L); shaking speed 100 rpm; biosorbent dose (0.1 g/L); biosorbent size 0.250 mm; optimum pH 5 for Cu(II) and 6 for Zn(II); time 24 h.

biomass and introduced more sorption sites on the biomass surface hence the sorption capacity has increased. Heating of biomass decreased the metal uptake due to the loss of intracellular uptake (36).

After acidic pre-treatment there are two factors that play key roles in the determination of the sorption capacity of a particular biomass:

(i) the polymeric structure of biomass surface exhibiting a negative charge due to the ionization of organic groups and inorganic groups (37–39) and
(ii) at a certain concentration, acids can enhance the uptake capacity of biomass by increasing the surface area and porosity of the original sample (35,40).

Similarly the biosorbent uptake capacity after basic pretreatment was determined by two factors:

(i) it may destroy autolytic enzymes that cause putrefication of the biomass and remove lipids and proteins that mask reactive sites (39,41–45) and
(ii) after certain concentration of an alkali, the number of protein amino groups that can be engaged in metallic ion binding markedly decreased. Deproteination should, theoretically, reduce metal retention (35–36,39, 40–45).

The difference in results after alginate pretreatment might be due to change in the cell wall composition of *Citrus reticulata* caused by these chemicals. Calcium stabilizes the biomass by binding the alginate and converting it to the gel state, resulting in increased sorption capacity. Calcium alginate is a polymer and carboxylate group of this alginate has been identified as the main binding site (46). By comparison, pretreatment using Na-alginate lowered the metal sorption capacity and it could be explained as after treatment Na^+ might have remained on the cell wall of the biomass and in competition with the investigated heavy metal on the binding sites of the cell wall. Heavy metal uptake decreased with increasing Na^+ ion concentration in the cell suspension (47,48).

Pretreatment with potash alum increased the sorption capacity. Potash alum is a synthetic coagulant and it caused the precipitation of inorganic substances present in the biomass. Due to the removal of inorganic species, more vacant sites were created on the biomass at which more sorption took place. *Moringa Olifera* Lam is a natural coagulant. It not only contained polyelectrolytes (responsible for coagulation) but also organic compounds (can adsorb on biomass surface) and minerals. These minerals and organic compounds might mask the functional groups responsible for a particular metal biosorption, hence the sorption capacity decreased.

H_2S forms sulphonic acid in aqueous medium. Sulphonic acid is a weak acid causing a negative charge on the polymeric structure of the biomass surface due to ionization of organic and inorganic groups. More negatively charged biomass has more ability to adsorb metals. Thus the Cu(II) sorption capacity of *Citrus reticulata* biomass increased after pretreatment with H_2S. The treatment with H_2S increased the porosity of biomass resulting in increased surface area and thus, enhanced uptake capacity. On the other hand, CO_2 form carbonic acid in aqueous phase. Carbonic acid thus formed during pretreatment of the biosorbent might affect the metal uptake capacity of the biosorbent being treated. The net increase or decrease in the sorption capacity of the biomass after weak acid treatment could be attributed to effect, played by the polymeric structure of the biomass and the concentration of weak acid.

Triton X-100 removed oily phase/lipids from the cell surface; hence the sorption capacity increased. Triton X-100 is a non-ionic surfactant. Surfactants are the substances with lyophilic and lyophobic groups capable of adsorbing at interfaces. The adsorption of heavy metals onto the biomass from solutions could be enhanced in the presence of surfactants due to reduced surface tension and increased wetting power (5). Similarly, the sorption capacity of *Citrus reticulata* was found to be increased after treatment with methanol, EDTA, formaldehyde, and $CaCl_2$ whereas with ethanol, glutaraldehyde, PEI, benzene, K-carragnen, PEI + glutaralehyde, acetone, ammonium sulphate was decreased. Ethanol treatment of biosorbent reduced the Cu(II) biosorption capacity of the biomass. When *Citrus reticulata* was treated with ethanol, the carboxylic acids present on the cell wall esterified as follows:

$$\mathrm{RCOOH + CH_3CH_2OH \rightarrow RCOOCH_2CH_3 + H_2O}$$

The reduction in Cu(II) biosorption with ethanol pretreatment, reveals that the carboxylic group on the cell wall of *Citrus reticulata* contributes to biosorption. Beveridge and Murray (49) observed that the modification of carboxyl groups on *Bacillus subtilis* decreased copper biosorption. Similar results have also been reported in another study (50). Pretreatment of *Citrus reticulata* with benzene resulted in reduction in Cu(II) biosorption. The treatment of biosorbent with benzene extracted the lipid fraction of biosorbent (50,51). Hence the reduction in the biosorption capacity when benzene-treated biosorbent was used, showed that the lipids in the cell wall of *Citrus reticulata* contributed to Cu(II) biosorption. Kapoor and Viraraghvan (39) observed slight decreases in biosorption of lead, cadmium, and copper when lipids were extracted from *Aspergillus niger*.

Methanol pretreated biomass resulted in increase in the sorption capacity because methanol is a polar solvent. It caused the polarization in the biomass due to which the metal sorption capacity of biomass increased. Pretreatment of the biomass with $CaCl_2$ only slightly enhanced the removal efficiency of Cu(II). This enhancement was due to cleaning of the biomass surface by calcium ions. Park et al. (52) also reported a similar increase in Cr(VI) biosorption after $CaCl_2$ treatment of *Ecklonia* sp.

EDTA pretreated biomass resulted in increase in the sorption of Cu(II). EDTA is a chelating agent. Metals present in biomass (Table 1) may form a complex with EDTA. Therefore more sorption sites were created on the biomass which resulted in increase in the sorption capacity. Pretreatment of biomass using formaldehyde resulted in increase in Cu(II) biosorption. Ilhan et al. (53) also reported an increase in copper biosorption by *Penicillium lanosa-coeruleum* after formaldehyde treatment. Gluteraldehyde, PEI, and PEI + glutaralehyde pretreatment resulted in decrease in Cu(II) sorption. Gluteraldehyde is a cross-linking reagent with multifunctional groups. According to Jianlong (54), gluteraldehyde pretreated *Saccharomyces cerevisiae* biomass retained almost all its original biosorption capacity whereas Deng and Ting (22) reported a marked increase in the adsorption capacity after PEI treatment. The differences in the results might be due to differences in the cell wall structure of the biomass used.

Pretreatment of biomass with acetone resulted in decrease in Cu(II) adsorption capacity of *Citrus reticulata* waste biomass. Due to high polarity of acetone, the cell wall structure of biomass becomes disturbed. It might change the nature of functional groups responsible for metal biosorption. Pretreatment using K-carragnen resulted in the decrease in the sorption capacity as it caused the dissolution of the cell wall of the biomass (55). Cu(II) adsorption capacity *of Citrus reticulata* waste biomass pretreated with ammonium sulphate decreased. Ammonium sulphate is a protein salting out agent. It can denature proteins and cause their precipitation. Ammonium sulphate precipitation may increase or decrease the sorption capacity of a biosorbent. The decrease in sorption capacity can be attributed to disturbance of those functional groups which were involved in Cu(II) biosorption. By comparison, Sar et al. (56) found that NaOH pretreatment enhanced the metal loading capacity, whereas detergents and acetone were inhibitory.

Figure 8 illustrates that pretreatment with all of the chemicals except ethanol enhanced the sorption of Zn(II) ions. These results are different from that of Cu(II) where some chemicals showed an increase in sorption capacity while others showed a decrease. So we can say that biosorption mechanisms could vary, depending on the metal species. However, the factors contributing in determining the Cu(II) and Zn(II) sorption capacity in case of acidic, basic, gaseous, and some other chemicals are similar and had been discussed earlier in the current section. Heating of biomass, resulted in decomposition of organic matter, creating more sorption sites on the biomass surface, hence the Zn(II) sorption capacity of biomass was increased (34). The alginates exhibited the best overall ability to remove Zn(II). Carboxylate groups of alginates have been identified as the main binding sites (46).

Table 4. Cu(II) adsorption capacities for some adsorbents reported in the literature

Adsorbent	q_{max} (mg/g)
Citrus reticulata waste biomass pretreated using H_2SO_4 (Present Study)	87.75
Aspergillus oryzae (57)	4.448
Lignite (57)	6.355
Wheat shell (58)	8.26
R. arhizus (57)	15.89
Pseudomonas aeruginosa (57)	19.06
Padina sp (57).	50.84
S. fluitans (57)	61.00
E. radiate (57)	70.54
L. japonica (57)	76.26

Table 5. Zn(II) adsorption capacities for some adsorbents reported in the literature

Adsorbent	q_{max} (mg/g)
Citrus reticulata waste biomass pretreated using EDTA (Present Study)	86.40
Biosolids (59)	36.88
Powdered waste sludge (60)	82.00
Heat inactivated *Botrytis cinerea* biomass (61)	12.98
Nonliving *Streptomyces rimosus* (62)	30.00

Pretreatment of biomass with coagulants increased its Zn(II) sorption capacity as they caused the precipitation of inorganic substances present in the biomass. Due to this precipitation, vacant sites were

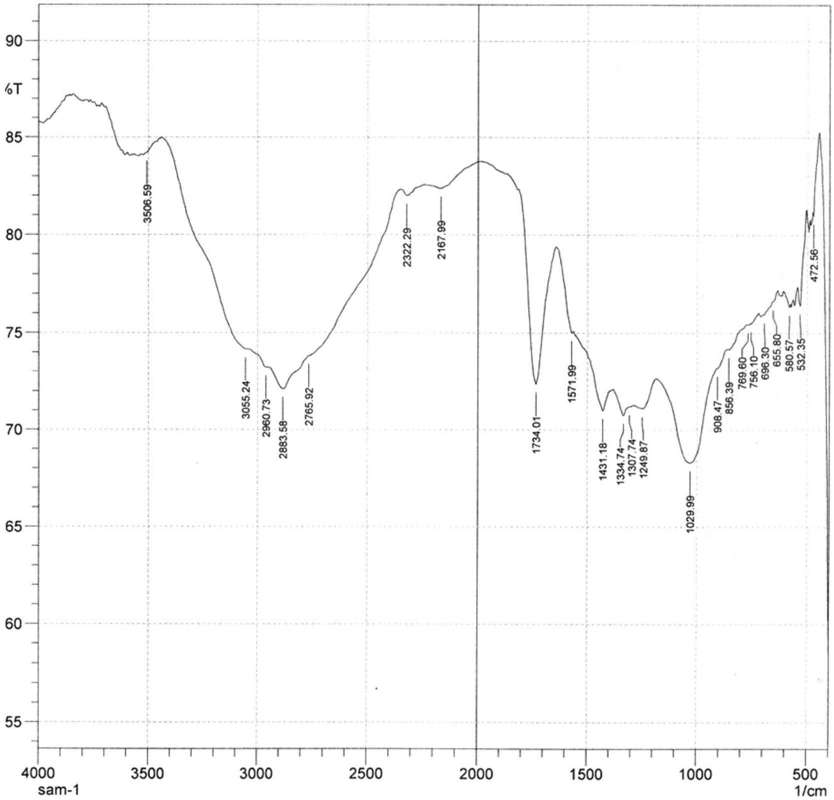

Figure 9. FTIR spectra of *Citrus reticulata* waste biomass.

created on the biomass at which more sorption could take place, thus indicating that biomass pretreated with sulphuric acid and EDTA had maximum Cu(II) and Zn(II) uptake capacity of 87.14 mg/g and 86.4 mg/g respectively. *Citrus reticulata* waste biomass is compared with the adsorption capacities for some adsorbents reported in the literature (Tables 4 and 5).

FTIR Spectral Analysis

The FTIR spectra of *Citrus reticulata* waste biomass in the range of 400–4000 cm^{-1} was obtained to determine main functional groups present on biomass cell surface (Fig. 9). The strong asymetrical stretching band at 3506.59 cm^{-1} is indicative of –OH and –NH groups. The broad, intense –OH stretching absorption from 3300 to 2500 cm^{-1} suggests the presence of carboxylic group on cell surface of *Citrus reticulata* waste biomass. The presence of carbonyl groups on the biomass cell surface can be confirmed from a band situated at 1734.01 cm^{-1}. The absorption peaks observed from 1450 to 1240 cm-1 may be assigned double bond containing groups. The bands below 800 cm^{-1} suggest the presence of aromatic amino acids on the biomass cell surface (61).

CONCLUSIONS

The present study demonstrated the removal of Cu(II) and Zn(II) by *Citrus reticulata* (Kinnow) waste biomass. Biosorption of Cu(II) and Zn(II) was strongly dependent on process parameters such as the biosorbent size, the biosorbent dose, the pH of the medium, metal concentration, and the time to reach equilibrium. The experimental data fitted well to the Langmuir sorption isotherm and the pseudo-second-order kinetic model. The results indicated that the biomass pretreated with sulphuric acid and EDTA had maximum Cu(II) and Zn(II) uptake capacity of 87.14 mg/g and 86.4 mg/g respectively. This suggested that *Citrus reticulata* waste biomass could be useful for the removal of heavy metals from wastewaters for industrial applications.

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