

# Fourier Transform Infrared Spectroscopic characterization and optimization of Pb(II) biosorption by fish (*Labeo rohita*) scales

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## Abstract

The present study reports the use of locally available fish (*Labeo rohita*) scales for Pb(II) removal from aqueous solutions under different experimental conditions. Maximum Pb(II) adsorption ( $196.8 \text{ mg g}^{-1}$ ) occurred at pH 3.5. Pb(II) sorption was found to be pH, dose, initial metal concentration, contact time and shaking speed dependent while particle size and temperature independent. Experimental data of Pb(II) biosorption onto fish scales fitted well to Freundlich isotherm model in comparison to the model of Langmuir. The fast adsorption process in first 30 min followed by subsequent slow adsorption rate was suitably described by pseudo-second order model. In addition, this study was designed to evaluate the effect of physical and chemical pretreatments on surface properties of fish scales by the application of Fourier Transform Infrared (FTIR) Spectroscopic analysis. Physical pretreatments resulted in partial degradation of some functional groups. Alkaline pretreatments of fish scales did not have any significant influence on the nature of functional groups responsible for Pb(II) uptake, while acidic pretreatments resulted in degeneration of the most of functional groups on biosorbent cell wall. FTIR analysis confirmed the involvement of amino, carboxylic, phosphate and carbonyl groups in Pb(II) biosorption by fish scales.

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**Keywords:** Pb(II); FTIR; Biosorption; Fish scales; Isotherms

## 1. Introduction

Pb(II) is a highly toxic and cumulative poison. Once in the environment, it is like other metals, difficult to recover and can adversely affect human health. Pb(II) has toxic effects on the nervous system and on the function of brain cells. The health hazards due to the presence of Pb(II) in water are of extreme concern to the public, government and industry [1]. Industries such as smelters, metal refineries, and mining operations have been indicated as major sources of metal release into the environment [2–4]. From the standpoint of environmental pollution control,

there is significant need for developing methods for effective removal of Pb(II) from water at least below the regulatory level. In environmental restoration areas conventional techniques used to eradicate heavy metals from wastewater include filtration, precipitation, flocculation, ion exchange resins and reverse osmosis [5]. Some of these methods, apart from being expensive, create secondary problems with metal bearing toxic sludge, are the thwart towards their prolific utility [6]. As a consequence, the search for effective new technologies has directed attention to biosorption, a technically feasible and economically attractive approach using biological material as sorbents. Biosorption has distinct advantages over conventional methods: it is non-polluting and it can be highly selective, more efficient, easy to operate and hence cost-effective for treatment of large volumes of wastewaters containing low metal concentrations [7]. Biosorption of heavy metals occurs as a result of physicochemical interactions, mainly ion exchange or complex formation between metal ions and the functional groups present on the

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cell surface. Various functional groups involved are phosphates, carboxyl, amines, carbonyl, sulfhydryl, sulphonates, phosphonates and amides. Recently various biomass materials including microbial and non-microbial have been identified and documented as effective metal-removing agents [8]. Although waste biomasses from animal sources are abundant in nature, but they still need to be explored for their metal sorption ability.

The present work explores the unexploited property of fish (*Labeo rohita*) scales, available in abundance as a waste material from Fishermen's Market, as a new biosorptive approach to remove Pb(II) from aqueous solutions. In this study, the influence of different environmental parameters such as pH, biosorbent dosage, biosorbent particle size, initial Pb(II) concentrations, temperature, agitation speed and contact time on Pb(II) biosorption was investigated, and the role played by the functional groups was determined by Fourier Transform Infrared (FTIR) Spectroscopy.

## 2. Materials and methods

### 2.1. Reagents

All the chemicals/reagents used in these studies were of Analytical Reagent Grade, including Pb(NO<sub>3</sub>)<sub>2</sub> (BDH, England), concentrated HNO<sub>3</sub>, concentrated H<sub>2</sub>SO<sub>4</sub>, concentrated HCl, concentrated H<sub>3</sub>PO<sub>4</sub>, Al(OH)<sub>3</sub>, Ca(OH)<sub>2</sub>, NaOH. (Merck) and Pb(II) Atomic Absorption Spectrometry standard solution 1000 mg L<sup>-1</sup> (Fluka Chemicals) supplied by Analytical Measuring Systems (AMS) Lahore, Pakistan.

### 2.2. Biosorbent preparation

Fish (*Labeo rohita*) scales were collected from the Fishermen's Market located in Jhang Bazar, Faisalabad, Pakistan. Mature fish scales were washed repeatedly with water to remove adhering dust and soluble impurities from their surface. The fish scales were allowed to dry in sunshine for 2 days. The scales were then kept in an oven at 70 °C till the fish scales became crispy. The dried scales were then converted into fine powder by grinding in a mechanical grinder (Retsch Germany). The powder was then sieved through octagon sieve (OCT-DIGITAL 4527-01) to obtain biosorbent with homogenous known particle size. The fractions with 40, 63 and 150 μ were separated for use in sorption tests and preserved in polyethylene containers for further use as biosorbent.

#### 2.2.1. Chemical pretreatment of fish scales

For chemical pretreatment, 10 g of powdered sample was soaked in 150 mL of 0.1 M HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, NaOH, Ca(OH)<sub>2</sub> and Al(OH)<sub>3</sub> for 2 h at 30 °C and 100 rpm at an orbital shaker while for physical pretreatment 10 g powdered sample was heated at 100 °C for 24 h, boiled and autoclaved at 121 °C and 15 Psi for 30 min in 150 mL of deionized water. The pretreated fish scales (except heat treated) were filtered and washed with generous amount of deionized water until a pH 7 ± 0.1 was attained. Resulting biomass was dried in oven till constant weight. Pretreated samples were ground in pestle and mortar

and sieved to optimized size of 150 μm prior to their testing for Pb(II) biosorption.

### 2.3. Pb(II) solutions

Stock Pb(II) solution (1000 mg L<sup>-1</sup>) was prepared by dissolving, 1.598 g of Pb(NO<sub>3</sub>)<sub>2</sub> in 100 mL deionized water shaking it for 15 min on a magnetic stirrer to obtain complete dissolution and then diluting quantitatively to 1000 mL using deionized water. Stock solution was adequately diluted with deionized water to obtain the different concentrations. Glassware and polypropylene flasks used were washed with detergent, then immersed in 10% (v/v) HNO<sub>3</sub> for 2 h and rinsed several times with deionized water.

### 2.4. Determination of the Pb(II) contents in the solutions

The Pb(II) concentrations in the solutions before and after the equilibrium were determined by Flame Atomic Absorption Spectrometry (FAAS) using a PerkinElmer AAnalyst 300 Atomic Absorption Spectrometer using an air-acetylene flame at the fixed flow rate of fuel and oxidant. The spectrometer was controlled by Intel Pentium IV Personal Computer using AA Win Lab Analyst software. Hollow cathode lamp was operated at 15 mA current, 217.0 nm wavelength with 0.7 nm slit width. The instrument was calibrated with series of Pb(II) standard solutions in the range of 1–20 mg L<sup>-1</sup>.

### 2.5. Shake flask biosorption studies

A fixed volume of Pb(II) solution (100 mL) was mixed with predefined mass of fish scales, except, for fish scale dosage experiment at 30 °C up to 24 h having fixed initial concentrations. All contact investigations were carried out in an orbital rotary shaker (IFS-1-W Kuhner Switzerland) set at 200 rpm. After shaking the flasks for 24 h the reaction mixtures were filtered through 0.45 μm membrane filter paper. Filtrates were analyzed by FAAS after 10× dilutions.

### 2.6. Metal uptake

The Pb(II) uptake was calculated following the concentration difference method [9]. The initial concentration  $C_i$  (mg L<sup>-1</sup>) and leftover metal concentration at different time intervals,  $C_e$  (mg L<sup>-1</sup>), were determined and the metal uptake  $q_e$  (mg Pb(II) adsorbed/g adsorbent) was calculated from mass balance equation (Eq. (1)) as follows:

$$q_e = \frac{C_i - C_e}{M} \times V \quad (1)$$

where  $V$  is the volume of the solution in  $L$  and  $M$  the mass of sorbent in  $g$ .

The extent of sorption in percentage is found from the relation [5].

$$\text{Sorption(\%)} = \frac{C_i - C_e}{C_i} \times 100 \quad (2)$$

### 2.7. Fourier Transforms Infrared (FTIR) Spectroscopy

FTIR Spectroscopy was used to determine the frequency changes in the functional groups in the biosorbent fish scales. The spectra were collected using PerkinElmer spectrum BX FTIR system (Beaconsfield Bucks, England) equipped with diffuse reflectance accessory within the range of 400–4000  $\text{cm}^{-1}$ . The samples were ground in an agate pestle and mortar (Merck, for spectroscopy), immersed in small amount, of DDW, vortexed for 5 min. A small amount of sample was transferred to diffuse reflectance rods in the form of thin film. The samples were dried for 6 h in a heating oven. With regard to recording the spectra, 16 scans and 8  $\text{cm}^{-1}$  resolutions were applied. The background obtained from a scan of empty rod was automatically subtracted from the sample spectra. All spectra were plotted using the same scale on the absorbance axis.

### 2.8. Statistical analysis

All data represents an average of three independent experiments. All statistical analysis was done using Microsoft Excel 2004 version office XP and slide write.

## 3. Results and discussion

### 3.1. Biosorption conventional experimental variables

In this section effect of different experimental variables such as pH, biosorbent dosage, biosorbent particle size, temperature, initial metal concentration, shaking speed and contact time which are conventionally being used to optimize the suitable experimental conditions for the maximum metal uptake by the selected biosorbent is described comprehensively.

pH of the aqueous solution is an important affecting parameter in the adsorption process. The effect of pH on Pb(II) uptake by *Labeo rohita* scales is shown in Fig. 1. Sorption experiments beyond pH 5 were not conducted since insoluble precipitates appeared beyond this pH. The highest removal capacities 196.80  $\text{mg g}^{-1}$  of Pb(II) ions by fish scales were obtained at pH 3.5 and the overall removal capacity of Pb(II) decreased to 86.96  $\text{mg g}^{-1}$  as pH increased up to 5 [10]. The

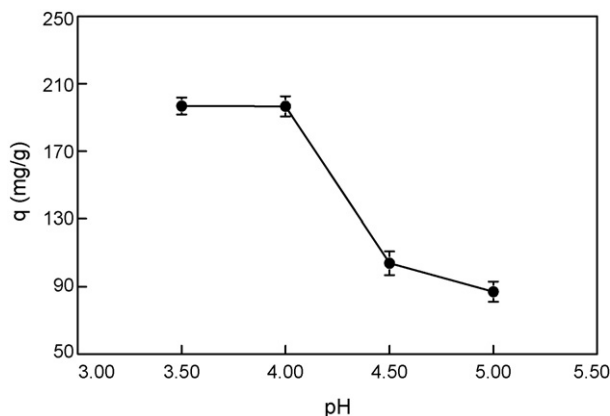


Fig. 1. Effect of pH on Pb(II) biosorption.

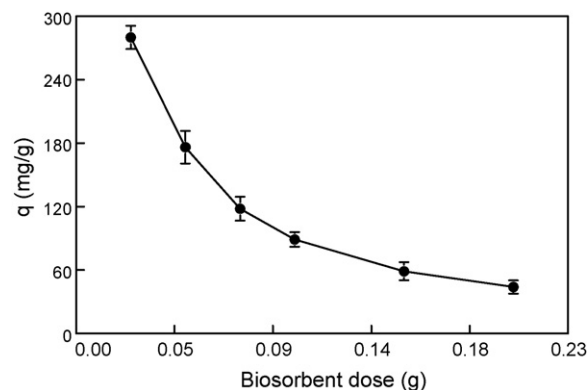


Fig. 2. Effect of biosorbent dose on Pb(II) biosorption.

pH dependence of metal uptake could be related to the functional groups of the biomass and also to solution chemistry [11]. This leads to the hypothesis that the cell wall functional groups and their associated ionic states are responsible for the extent of adsorption. Biosorbent materials primarily contain weak acidic and basic functional groups. It follows from the theory of acid-base equilibria that the binding of heavy metal cations is determined primarily by the state of dissociation of the weak acidic groups [12]. The results given in Fig. 2 indicate that the specific metal uptake values decreased with increments in biosorbent concentrations. About 84% decrease was recorded, as the dry biosorbent increased from (0.025–0.200 g per 100 mL). Higher uptake at low biosorbent concentrations could be due to an increased metal-to-biosorbent ratio, which decreases upon an increase in dry biomass concentrations [2,13]. Results indicated that particle size of fish scales does not influence on the adsorption capacity of Pb(II) cation (Fig. 3). Since  $q$  values remains constant for all the three selected particle sizes. No effect of altering sorbent particle size on  $q_e$  ( $\text{mg g}^{-1}$ ) is consistent with other reports [14]. All three particle sizes showed the similar efficacy. It is always preferable to use rigid and slightly larger particles in sorption process [15]. So particle size of 150  $\mu\text{m}$  was subsequently employed in all biosorption experiments. Fig. 4 shows that the temperature in the range 25–45  $^{\circ}\text{C}$  did not produce any significant difference in Pb(II) adsorption capacity. The temperature independent biosorption process of Pb(II) by fish scales is

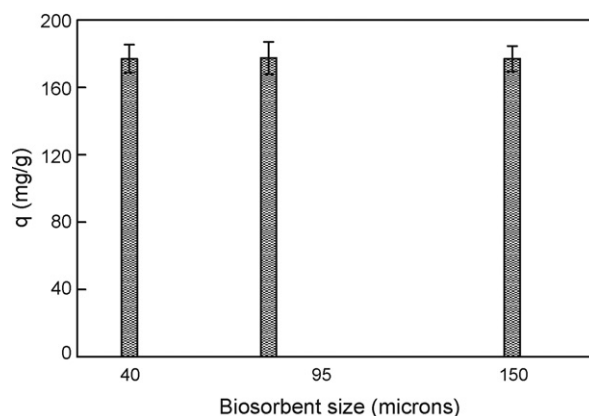


Fig. 3. Effect of particle size of biosorbent on Pb(II) biosorption.

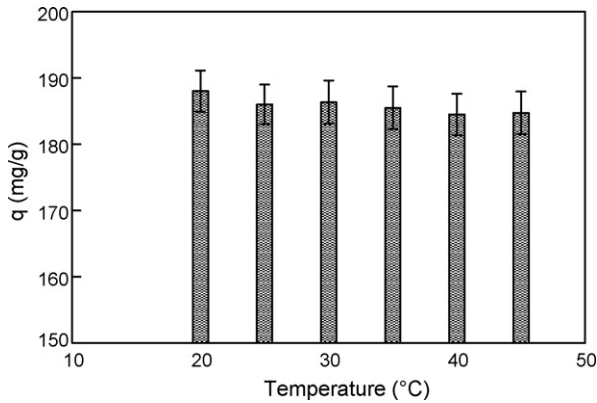


Fig. 4. Effect of temperature (°C) on Pb(II) biosorption.

in accordance with [16,17]. Experimental data points obtained for the biosorption of Pb(II) by fish scales biomass at several Pb(II) concentrations are represented in Fig. 5. These results revealed that specific metal uptake increased with an increase in the Pb(II) ion concentration. The highest uptake of the fish scales was around  $472 \text{ mg g}^{-1}$  at an initial Pb(II) concentration of  $800 \text{ mg L}^{-1}$ . The enhancement in metal sorption could be due to an increase in electrostatic interactions involving the sites of progressively lower affinity for metal ions [18]. However, percent adsorption of Pb(II) decreased from 70 to 29% with an augmentation of Pb(II) concentration due to rapid saturation of the metal binding sites of the biosorbent [19,20]. Fig. 6 shows that Pb(II) uptake increases with the increase in shaking rate ( $q = 159 \text{ mg g}^{-1}$  in the absence of agitation and  $197.64 \text{ mg g}^{-1}$  at 400 rpm) for a contact time of 24 h. These results can be associated to the fact that the increase of agitation speed, improves the diffusion of Pb(II) ions towards the surface of the adsorbent. This also depicts that a shaking rate in the range 50–400 rpm is sufficient to assure that all the surface binding sites are made readily available to Pb(II) uptake. Then the effect of external film diffusion on adsorption rate can be assumed not significant, being ignored in any engineering analysis. So it was concurred to select 200 rpm as the optimum speed for all the experiments. Similar results were reported in literature for different metal-

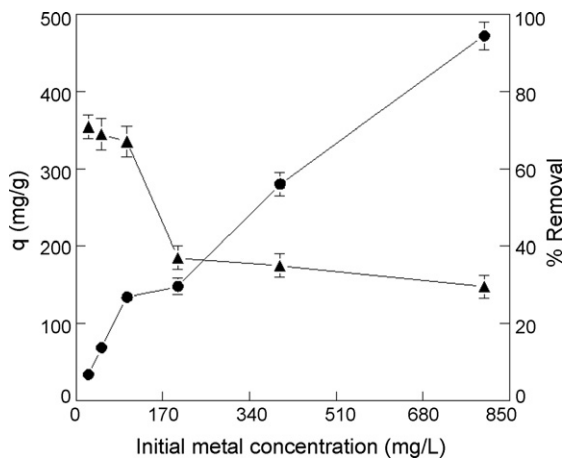


Fig. 5. Effect of initial metal ion concentration ( $q$ , ● and % removal, ▲) on Pb(II) biosorption.

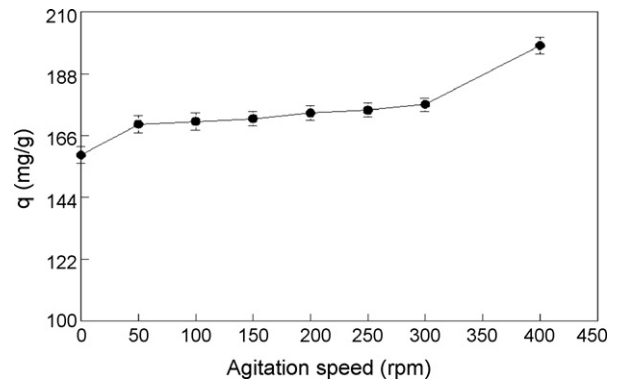


Fig. 6. Effect of agitation speed on Pb(II) biosorption.

biomass systems [21–23]. Kinetic data depicted that in the first time interval (15 min) up to 84% metal was removed indicating sorption took place rapidly, then it continued at a relatively slower rate up to maximum (90%) sorption at 120 min and nearly equilibrium was achieved (Fig. 7a). Thus, it can be con-

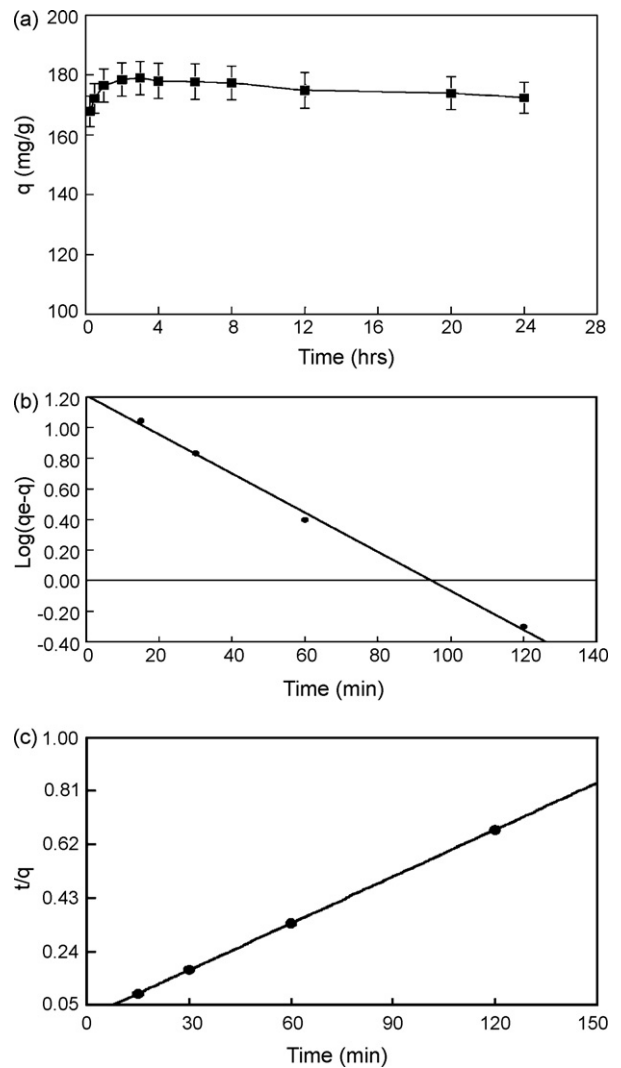


Fig. 7. (a) Effect of time interval on Pb(II) biosorption. (b) Pseudo-first order kinetic model for Pb(II) biosorption on fish scales at different time intervals. (c) Pseudo-second order kinetic model for Pb(II) biosorption on fish scales at different time intervals.

cluded that biosorption phenomena occurred in two phases, a very rapid surface adsorption and a slow intracellular diffusion. Similar conclusions were obtained by Yin et al. [24]. However, for higher contact time 12–24 h, a slight decrease was observed in biosorption capacity, possibly due to a vortex phenomenon [25].

### 3.2. Theoretical modeling of data

#### 3.2.1. Kinetic modeling

To understand the practical application of adsorption and design of the batch reactor, batch rate analysis can be used. Several kinetic models with varying degree of complexity are available to describe the kinetics of heavy metal biosorption in batch systems [26,27]. To understand the controlling mechanism of Pb(II) biosorption on fish scales pseudo-first order equation of Lagergren [28] and pseudo-second order equation of Ho and McKay [29] were used in this study. The pseudo-first order kinetic model assumes that “metal sorption process is first order in nature as it is only dependent on the number of metal ions present at the specific time in the solution”. In contrast to pseudo-first order kinetic model, the pseudo-second order kinetic model assumes that “the metal biosorption process is dependent on the number of metal ions present in the solution as well as the free biosorption sites on the biosorbent surface”.

The pseudo-first order Lagergren model (Fig. 7b) is expressed as:

$$\log(q_e - q_t) = \log q_e - \frac{K_1 t}{2.303} \quad (3)$$

where  $K_1$  ( $\text{min}^{-1}$ ) is the rate constant of pseudo-first order adsorption model,  $q_e$  and  $q_t$  ( $\text{mg g}^{-1}$ ) denote the amounts of adsorbed metal ions on fish scales at equilibrium and at any time  $t$  (min).

Pseudo-second order rate expression (Fig. 7c) can be presented as follows:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

where  $K_2$  is the rate constant of pseudo-second order biosorption model ( $\text{g mg}^{-1} \text{min}^{-1}$ ).

A comparison between pseudo-first order and pseudo-second order kinetic parameters (Table 1) suggested that Pb(II) biosorption by fish scales followed the pseudo-second order kinetics rather than pseudo-first order kinetics since the value of  $q_e$  obtained from pseudo-second order kinetic model was in close agreement with that of experimental value whereas the value of  $q_e$  computed from pseudo-first order kinetic model did not agree with the experimental value. This indicates that pseudo-first

order kinetics might be insufficient to interpret the mechanism of Pb(II) biosorption [30]. This kinetics information is of significant practical value for technological applications, since kinetic modeling successfully replaces time and material consuming experiments, necessary for process equipment design [31] and can be used to find important parameters for a bioreactor design [32].

#### 3.2.2. Sorption isothermal modeling

The analysis of equilibrium sorption data is important in the design of biosorption systems and can be employed to compare the efficacy of different biomaterials under varying operational conditions to optimize an operating procedure [33–35]. Several isotherm equations have been used for the equilibrium modeling of biosorption systems, among these, two most frequently used, the Langmuir and Freundlich equations have been adopted for this study to fit the data. The Langmuir monolayer sorption isotherm is based on the following assumptions [32]:

- The solid surface presents a finite number of energetically uniform identical sites.
- There is no interactions among adsorbed species, i.e. the amount adsorbed has no influence on the rate of adsorption.
- A monolayer is formed when the solid surface reaches saturation.

The linearized Langmuir equation refers to [34].

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}} \quad (5)$$

where  $q_e$  ( $\text{mg g}^{-1}$ ) is equilibrium sorption capacity and  $C_e$  ( $\text{mg L}^{-1}$ ) is Pb(II) equilibrium concentration.  $q_{\max}$  ( $\text{mg g}^{-1}$ ) is the maximum amount of metal ion per unit weight of adsorbent to form a complete monolayer on the surface and  $b$  is a constant related to the affinity of binding sites with the metal ions ( $\text{L mg}^{-1}$ ).

The Freundlich equation is an empirical equation based on adsorption on heterogeneous surface [36] and its linearized form can be given as:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (6)$$

where  $K_F$  and  $1/n$  are Freundlich constants related to adsorption capacity and intensity of adsorption, respectively.

The linearized form of Langmuir and Freundlich adsorption-isotherms obtained at different concentrations are given in Fig. 8a and b, respectively, whereas Table 2 presents the correspondent constants along with the coefficients of correlation ( $R^2$ ) associated to each linearized model. These results depict

Table 1  
A comparison between pseudo-first order and pseudo-second order models kinetic parameters

Pseudo-first order			Experimental value	Pseudo-second order		
$q_e$ ( $\text{mg g}^{-1}$ )	$K_1$ ( $\text{min}^{-1}$ )	$R^2$	$q_{\max}$ ( $\text{mg g}^{-1}$ )	$q_e$ ( $\text{mg g}^{-1}$ )	$K_2$ ( $\text{g mg}^{-1} \text{min}^{-1}$ )	$R^2$
16.33	$2.95 \times 10^{-2}$	0.9969	178.5	181.81	$4.384 \times 10^{-3}$	1.00

Table 2  
A comparison between Langmuir and Freundlich isotherm parameters

Langmuir isotherm parameters			Experimental value	Freundlich isotherm parameters			
$q_{\max}$ (mg g <sup>-1</sup> )	$K_L$ (L mg <sup>-1</sup> )	$R^2$	$q_{\max}$ (mg g <sup>-1</sup> )	$q_{\max}$ (mg g <sup>-1</sup> )	$K$ (mg g <sup>-1</sup> )	$R^2$	$1/n$
555.55	0.0059	0.8096	472	438.92	14.418	0.9447	0.5392

Freundlich model (Fig. 8b) better fits the adsorption process of Pb(II) cations by fish scales in comparison to the Langmuir model (Fig. 8a) due to high value of correlation coefficient. The value of  $q_{\max}$  as calculated from Freundlich model was in good agreement with that of experimental value. The magnitude of experimental  $q_{\max}$  for fish scales was found to be 472 mg g<sup>-1</sup>. The well fitting of experimental data to Freundlich model clearly demonstrated that Pb(II) sorption onto fish scales is heterogeneous in nature.

### 3.3. Specific surface area calculation

Monolayer coverage of the surface by metal ions can be used for the calculation of the specific surface area  $S$  according to following equation [37].

$$S = q_{\max} \frac{NA}{M} \quad (7)$$

$S$  is the specific surface area m<sup>2</sup> g<sup>-1</sup> adsorbent;  $q_{\max}$  is Monolayer sorption capacity g (metal) per g (biosorbent);  $N$  is

Table 3  
A comparison of biomass specific surface area for Pb(II)

Biosorbent	$q_{\max}$ (mg g <sup>-1</sup> )	$S$ (m <sup>2</sup> g <sup>-1</sup> )	Reference
Fish scales	472	76.32	This study
Tree fern	39.8	6.43	[37]
<i>M. spicatum</i>	46.49	7.51	[38]
Neem leaf powder	300	48.50	[45]

Avogadro number  $6.02 \times 10^{23}$ ;  $A$  is cross section area of the metal ion m<sup>2</sup>;  $M$  is molecular weight of the metal.

For Pb(II) ions the molecular weight is 207 and cross sectional area of Pb(II) is 5.56 Å<sup>2</sup> (Pb(II) radii is 1.33 Å in a close packed monolayer) [38]. The maximum specific surface area for fish scales for binding Pb(II) is 76.32 m<sup>2</sup> g<sup>-1</sup>.

Table 3 shows a comparison of specific surface areas for Pb(II), calculated on the same basis using different biosorbents.

### 3.4. Pretreatment on fish scales and their characterization using FTIR

The effect of physical and chemical pretreatments on fish scales biomass is shown in Fig. 9. The FTIR Spectroscopy is an important analytical technique which detects the vibration characteristics of chemical functional groups in a molecule. On interaction of an infrared light with the matter, chemical bonds will stretch, contract and bend. As a result, chemical functional group tends to absorb infrared radiation in a specific wavelength range regardless of the structure of the rest of the molecule. Based on these principles, effect of pretreatment on the surface

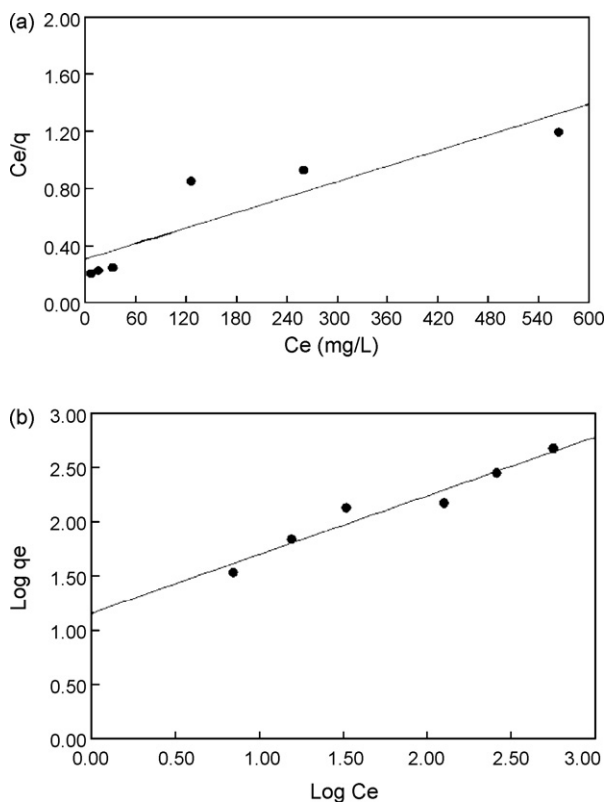


Fig. 8. (a) Langmuir isotherm plot for Pb(II) biosorption on fish scales at different initial metal concentrations. (b) Freundlich isotherm plot for Pb(II) biosorption on fish scales at different initial metal concentrations.

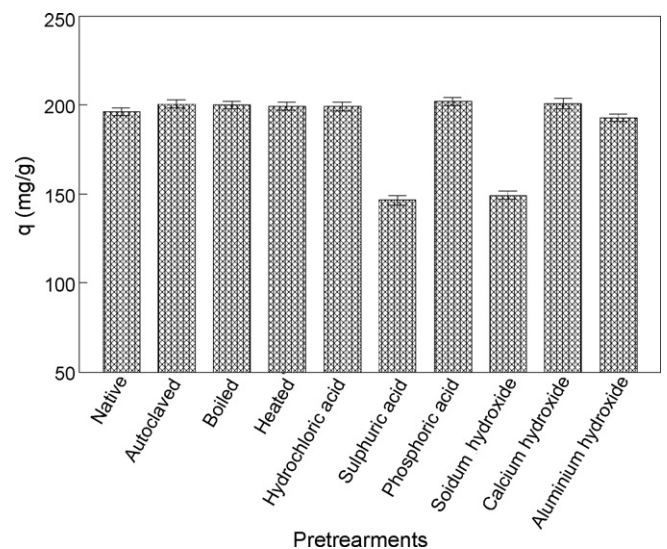


Fig. 9. Effect of pretreatment of fish scales on Pb(II) biosorption.

functional groups of fish scales was adequately studied in the range of  $400\text{--}4000\text{ cm}^{-1}$  (Fig. 10a–t). FTIR analysis for fish scales and differently pretreated fish scales were taken before and after Pb(II) uptake. The main functional groups present on surface of fish scales were amino, carboxylic, phosphate and carbonyl. The spectra of non-treated fish scales showed peaks at  $3500\text{--}3000\text{ cm}^{-1}$  due to  $\text{--NH}$  stretching and  $\text{--NH}$  bending between  $1550$  and  $1500\text{ cm}^{-1}$  presenting amino groups while  $\text{C=O}$  stretching at  $1641\text{ cm}^{-1}$  [39]. The peaks in the region of

wave number  $1470\text{--}1365\text{ cm}^{-1}$  are representative of amino substituted alkyl group. The peaks produced from  $3000\text{--}2500\text{ cm}^{-1}$  are due to carboxylic group. There is a clear disappearance of the band at  $1335\text{ cm}^{-1}$  in (Fig. 10a) when compared with that of Pb(II) loaded fish scales (Fig. 10b). This observation indicates the involvement of amino group in biosorption process. A distinctive peak near  $1079.33\text{ cm}^{-1}$  indicative of stretching phosphate was observed in fish scales spectra. After contact with Pb(II) solution, the fish scales exhibit spectra with clear

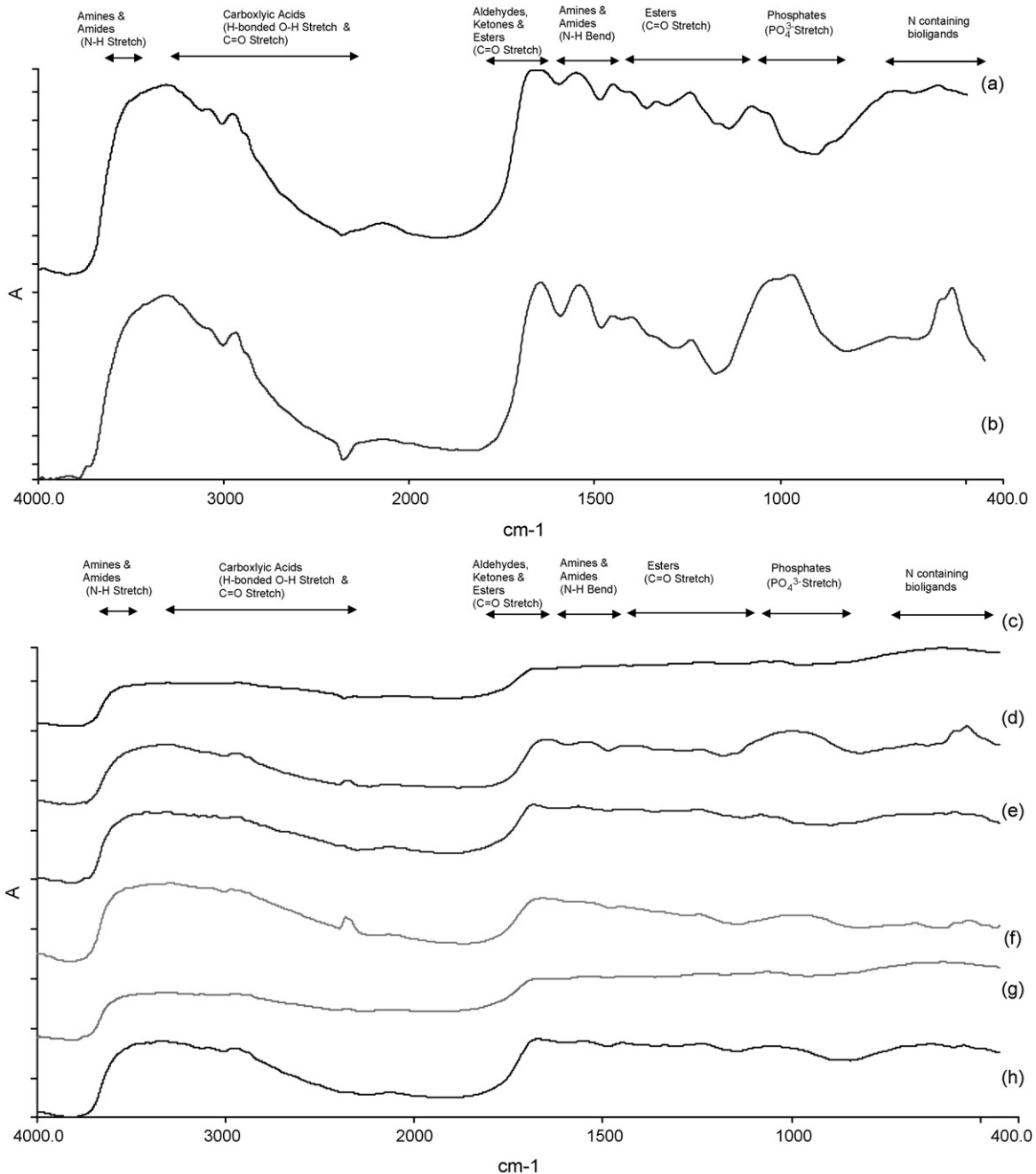


Fig. 10. (a–t) FTIR spectra of fish scales. (a) Non-treated fish scales. (b) Pb(II) loaded non-treated fish scales. (c) Autoclaved fish scales. (d) Pb(II) loaded autoclaved fish scales. (e) Boiled fish scales. (f) Pb(II) loaded boiled fish scales. (g) Heated fish scales. (h) Pb(II) loaded heated fish scales. (i) NaOH pretreated fish scales. (j) Pb(II) loaded NaOH pretreated fish scales. (k)  $\text{Ca}(\text{OH})_2$  pretreated fish scales. (l) Pb(II) loaded  $\text{Ca}(\text{OH})_2$  pretreated fish scales. (m)  $\text{Al}(\text{OH})_3$  pretreated fish scales. (n) Pb(II) loaded  $\text{Al}(\text{OH})_3$  pretreated fish scales. (o) HCl pretreated fish scales. (p) Pb(II) loaded HCl pretreated fish scales. (q)  $\text{H}_2\text{SO}_4$  pretreated fish scales. (r) Pb(II) loaded  $\text{H}_2\text{SO}_4$  pretreated fish scales. (s)  $\text{H}_3\text{PO}_4$  pretreated fish scales. (t) Pb(II) loaded  $\text{H}_3\text{PO}_4$  pretreated fish scales.

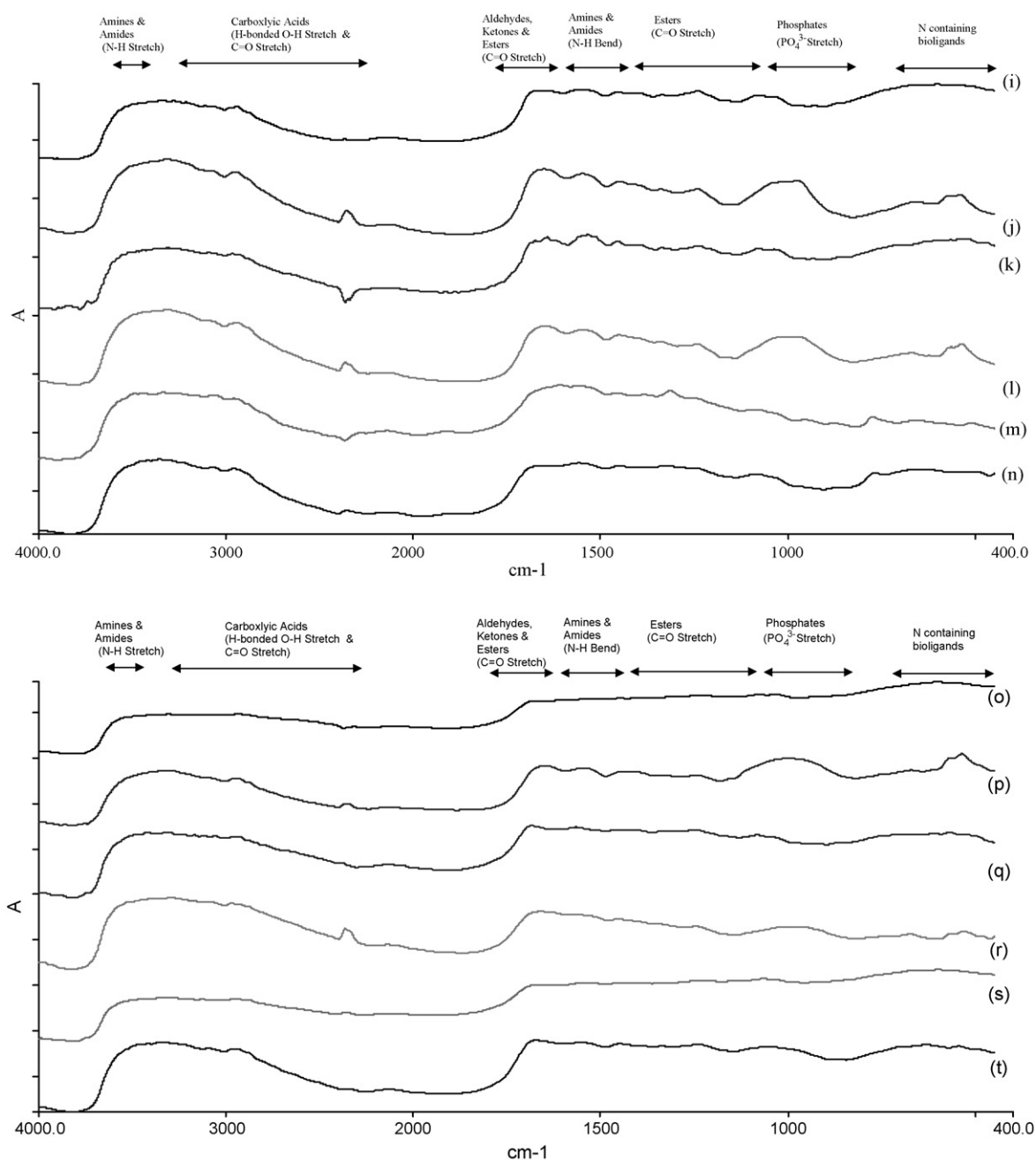


Fig. 10. (Continued)

shift of phosphate band to the lower frequency  $968\text{ cm}^{-1}$ . Since phosphate has a major region of absorption between  $950$  and  $1100\text{ cm}^{-1}$  [40]. This shift is typical for the complexation of phosphate groups by coordination with metal ions [41]. Finally, it should be noted that in the region of lower wave number (under  $700\text{ cm}^{-1}$ ) a sharp peak at  $537\text{ cm}^{-1}$  appeared after Pb(II) biosorption in comparison with that of unloaded fish scales, which contained multiple absorption peaks in this region. This change could be attributed to an interaction between Pb(II) ions and N-containing bio-ligands. Thus, the peak at  $968$  and  $537\text{ cm}^{-1}$  are characteristics of the stretching vibrations of Pb(II) ions [42]. Moreover, FTIR spectral analysis of fish scales loaded with Pb(II) indicated elongation of all these bands after Pb(II)

biosorption, suggesting the role of these groups during biosorption process.

All physical modifications increased the sorption capacity of fish scales. The  $q$  values ( $\text{mg g}^{-1}$ ) of non-treated and physically modified fish scales were in following order: Autoclaved ( $200.47$ ) > boiled ( $199.88$ ) > heated ( $199.1$ ) > non-treated ( $196.8$ ). Physical modifications of biomass remove mineral and organic matter from biomass. Boiling remove mineral matter by dissolving it whereas autoclaving and heating remove organic and mineral matter by decomposing it, the subsequent result is introduction of more sorption sites on biomass surface [1]. Fig. 10c and d shows the surface functional groups of autoclaved fish scales before and after Pb(II) uptake. From the figure



it can be noted, there is no effect of autoclaving on the fish scales functional groups (i.e. amino, carboxylic and carbonyl) absorbing in the range 4000–1500  $\text{cm}^{-1}$ . However, autoclaving have an impact on phosphate and alkylated amino groups. These functional groups were mainly responsible for Pb(II) biosorption by autoclaved fish scales. The FTIR spectra of boiled fish scale (Fig. 10e and f) revealed that functional groups (i.e. amino and carboxylic groups) absorbing in the range 4000–2000  $\text{cm}^{-1}$  are undisturbed after boiling pretreatment. Pb(II) biosorption by boiled fish scale was mainly due to involvement of phosphate functional group in sorption process. Preheating of fish scales did not influenced the surface functional groups, however, it changed the role of functional groups in the Pb(II) sorption process (Fig. 10g and h). Alkylated amino groups and carboxylic groups are significant contributors for the Pb(II) sorption by heated fish scales.

Sorption capacities ( $\text{mg g}^{-1}$ ) of fish scales pretreated using bases were in the following order:  $\text{Ca(OH)}_2$  (200.76) > non-treated (196.8) >  $\text{Al(OH)}_3$  (192.76) > NaOH (149.14). The two factors are responsible for increase or decrease in sorption capacity of a particular biomass after basic pretreatment. The net increase or decrease will be due to the contribution played by following two factors (i) alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites. Removal of impurities from surface and rupturing of cell-membrane is reason behind the increase in metal uptake capacity of biomass after basic pretreatment [43], (ii) however, after certain concentration of an alkali used for pretreatment of biomass, the number of protein amino groups that can be engaged in metallic ion binding markedly decreased. Deproteinization should, theoretically, reduce metal retention [44]. Basic pretreatment (NaOH,  $\text{Ca(OH)}_2$  and  $\text{Al(OH)}_3$ ) of fish scales did not have any significant influence on the nature of functional groups responsible for Pb(II) biosorption, when compared to non-treated fish scales (Fig. 10i–n). However, acidic pretreatment of fish scale explored very interesting results on functional groups of biosorbent. Sorption capacities ( $\text{mg g}^{-1}$ ) of fish scales pretreated with mineral acids were in the following order:  $\text{H}_3\text{PO}_4$  (201.86) > HCl (199.18) > non-treated (196.8) >  $\text{H}_2\text{SO}_4$  (146.42). The difference in results after a specific pretreatment may be attributed to the specific type of interaction between the biomass and chemical used for pretreatment. The two factors are responsible for increase or decrease in sorption capacity of a particular biomass after acidic pretreatment. The net increase or decrease can be attributed to the portion played by two factors. These are (i) the polymeric structure of biomass surface exhibits a negative charge due to the ionisation of organic groups and inorganic groups. Biomass having the higher electronegativity will have the greater attraction and adsorption of heavy metal cations. However, after a certain concentration of an acid the electronegativity of biomass will decrease due to the remaining  $\text{H}^+$  ions on the acidic pretreated biomass, may change the biomass electronegativity, resulting in a reduction in biosorption capacity [45–47], (ii) at a certain concentration, acids can enhance uptake capacity of biomass by increasing the surface area and porosity of original sample [3,48]. The pretreatment with HCl,  $\text{H}_2\text{SO}_4$

and  $\text{H}_3\text{PO}_4$  showed markedly different results from each other Fig. 10(o–t). Pretreatment of fish scales using HCl resulted in degeneration of the most of functional groups present on cell wall of fish scales. Amino, alkylated amino and carbonyl groups were completely denatured by this pretreatment (Fig. 10o and p) whereas, fish scales pretreatment with  $\text{H}_2\text{SO}_4$  generated more sorption sites on cell surface of biomass.  $\text{H}_3\text{PO}_4$  also affected functional groups sorption behavior but to a lesser extent in comparison to HCl pretreatment of fish scales.

#### 4. Conclusions

The following conclusions can be withdrawn from present study:

- On studying the effect of different experimental variables on the uptake of Pb(II) using fish scales it was found that pH, dose, initial Pb(II) concentration, contact time and shaking speed were dependent variables while particle size and solution temperature were independent variable during Pb(II) biosorption by fish scales.
- Pb(II) isothermal equilibrium sorption using fish scales was necessarily heterogenous in nature and was well described by Freundlich equation which followed pseudo-second order kinetic mechanism.
- FTIR analysis confirmed the involvement of amino, carboxylic, phosphates and carbonyl groups in Pb(II) biosorption by non-treated fish scales.
- Pretreatment of biomass using autoclaving, boiling, heating, HCl,  $\text{H}_3\text{PO}_4$  and  $\text{Ca(OH)}_2$  resulted in enhancement of sorption capacity of fish scales while reverse was true for  $\text{H}_2\text{SO}_4$ , NaOH and  $\text{Al(OH)}_3$ .
- An important conclusion which can be withdrawn from the present research is that the different pretreatments of fish scale affected the surface functional groups in a differential way.
- These findings open up new avenues in the toxic metals sequestration by locally available fish scales from water bodies as cheap and eco-friendly safe technology.

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