Bimodal applications of LDH-chitosan nanocomposite: water treatment and antimicrobial activity

W Kamal¹, Waleed M A El Rouby ², Ahmed O El-Gendy³, A A Farghali²*

¹ Department of chemistry, Faculty of Science, Beni- Suef University (BSU), Beni-Suef, Egypt.
² Material Science and Nano technology Department , Faculty of Postgraduate Studies for Advanced Sciences (PSAS), Beni-Suef University (BSU), Beni-Suef, Egypt.
³ Microbiology and immunology department, Faculty of pharmacy, Beni-Suef University (BSU), Beni-Suef, Egypt.
* Corresponding author, e-mail: d_ farghali@yahoo.com

Abstract. Chitosan-LDH (layered doubled hydroxide) composite was prepared for removal heavy metals and antimicrobial activity. It was characterized by Fourier transform infrared spectrometer (FTIR), X-ray diffraction (XRD), Transmission electron microscopy (TEM), Scanning electronic microscope (SEM). Studying various physicochemical parameters such as the effects of pH, adsorbent amount, and contact time for removing heavy metals were performed. The adsorption kinetics and adsorption isotherm model were done to understand the adsorption process. The prepared composite exhibited strong antimicrobial activity against different gram positive (Lactobacillus sakei LMG 2313, Corynebacterium sp, Staphylococcus aureus 5247) and gram negative bacteria (Escherichia coli AO5). Results were recorded in terms of minimum inhibitory concentration (MIC), which is the lowest concentration of antimicrobial/antifungal agent that will inhibit the visible growth of a microorganism after overnight incubation.

Key Words: Chitosan, LDH, nanocomposite, adsorption, copper, iron, antimicrobial activity, waste water treatment.

1. Introduction

Water is the lifeblood and the most important component in the living cell (50-60% of the weight of the cell) [1]. Many human activities in the environment cause pollution of water due to production of chemical and physical contaminants [2]. Sources of water pollution include industrial waste, metal cleaning, electronic devices manufacturing, mining, fertilizer,… etc [2,3]. Toxic elements such as iron, copper, lead, nickel, mercury, chromium, cobalt and cadmium at high concentrations are the main source of industrial waste water and cause serious concern because of its negative effects on the environment, health and society [4,5]. These toxic elements deposit in the soil and reach to the plant and then to the animal and human causing many diseases [6]. For instance Arsenic (III) and (V), chromium (V1), Cadmium, Copper are carcinogens, whilst Mn (II), Fe (II) and (III) are toxics at high levels [7]. Water treatment and removal of heavy metals were performed by using different ways of chemical precipitation, coagulation, ion exchange, membrane separation, electro deposition and other methods [8]. All of these methods have high efficiency and good results in removal, but the high cost limit its use [1,4,9]. On the other hand, adsorption is one of the most common physical or chemical methods used for water treatment due to its use with small concentrations of heavy metals [1,4,5,9] in addition to cost-effective, simplicity and rapidness of operation, minimal sludge production, high efficiency and reusability.

Water pollution problems include presence of many microorganisms such as bacteria colon group, which is the main source of intestinal diseases, and Salmonella bacteria, which causes diseases of typhoid fever and intestinal infections and Shigella bacteria that cause diarrhea and post-operative wound infections [10,11]. It is necessary to pay attention to the heavy metal removal and microbial
activity causing all these health problems and diseases using absorbent materials owing to their economic efficiency, environmental and low cost [12]. Nanostructures have high efficiency and speed in handling contaminated water compared to traditional materials, by a condition that, nanomaterials must meet many qualities to be non-toxic, natural substances with a high ability to remove heavy metals [13].

Chitosan is a natural polymer that has polymorphic properties as it is nontoxic, antibacterial, biocompatible and bio-degradable [14,15]. It is a linear polysaccharide composed of randomly distributed β—linked D-glucosamine and N-acetyl-D-glucosamine. Treating the chitin shells of shrimp and other crustaceans with an alkaline substance, like sodium hydroxide is an economic and beneficial substance for the environment for extracting Chitosan [16]. Being contain amine and hydroxide groups, Chitosan able to make strong bonds with ions [17]. Layered double hydroxides (LDHs) are an anionic or hydrotalcite-like clays, a class of lamellar compounds that comprise positive charged brucite as layers and hydrate exchangeable anions [18]. LDHs were remarkable because of their ability to adsorb heavy metals and use them as catalysts, catalyst supports, ion exchangers [19,20]. Intercalation process between LDHs and biomolecules creates bio-nano composite [21]. In this study, we focused on the synthesis of LDHs and the intercalation between Chitosan molecules. We used Chitosan-LDH Nano-composite as a bimodal, first for heavy metal removal—the main target-, second, as antimicrobial activity.

2. Materials and method

2.1. Chemicals and Strains

Shrimp peel were bought from market, FeSO₄.7H₂O, NaOH and Acetone were purchased from Piochem for chemicals (Egypt), HCl, Al(NO₃)₃.9H₂O, 1-Propanol, β- alanine, CuCl₂.2H₂O were ordered from Oxford lab (India). Zn(NO₃)₂.6H₂O was provided by SDFCL (India) while Methanol, Acetic Acid was purchased from Adwic (Egypt). All chemicals were used without purification and double-distilled water was used for preparation. Micro-organisms: Gram-positive organisms used in the study were Bacillus subtillis, Lactobacillus sakei LMG 2313, Corynebacterium sp, Listeria inocua Lmg 2710 Mycobacterium pheli, Enterococcus faecalis, MRSA, Staphylococcus aureus 5247. Gram-negative organisms used were Escherichia coli ATCC 8739, Escherichia coli AO5, Proteus Vougaris and Pseudomonas auroginosa. Yeast used was Candida albicans.

2.2. Preparation of chitosan

The method of preparation of the Chitosan is reported as found in the literature with some modifications [3,5,14]. Starting with Demineralization process for removing minerals, primarily calcium carbonate then Deproteinization process due to disruption of chemical bonds between chitin and proteins and using mechanical stirring in Decolouration process.

2.3. Synthesis of Al Zn-NO₃ LDH–Chitosan

Chitosan-LDH nanocomposite was formed by composite by intercalation of Chitosan into the Al-Zn LDH by the co-precipitation method. 35ml of Al(NO₃)₃.9H₂O (0.0004M) and Zn(NO₃)₂.6H₂O were mixed with 25 ml distilled water, 10 ml 1-propanol and 30 ml of Chitosan (0.25g/l) using β-alanine (0.005 M) to increase Chitosan dissolution. NaOH solution (0.5 M) was added drop-wise with high magnetic stirring till pH (7.5); then, the solution was transferred into a Teflon-lined autoclave and heated at 120 for 18 hours. The precipitate was filtered, washed several times with distilled water to remove inorganic ions, and then dried in a vacuum at 60°for 24 hours [22–25].

2.4. Characterization of chitosan, LDH and chitosan-LDH
X-ray diffraction XRD patterns were determined on a 202964 PANalytical Empyrean X-ray diffraction using Cu Kα radiation scan angle 5°–80° range. Fourier transformation infra-red FTIR spectra were recorded using the KBr pellet technique on a Burker Vertex 70 FT-IR spectrometer in the range from 400 to 4000 cm⁻¹. The analysis of metals was measured by the atomic absorption spectroscopy AAS 200 series AA Agilent Technologies. High resolution transmission electron microscope HRTEM images were taken using (JEM-2100, JEOL, Japan) with an acceleration voltage of 200 kV. Field emission scanning electron microscope FESEM micrographs was obtained by Quanta FEG 250 (Switzerland).

2.5. Antimicrobial sensitivity test using agar diffusion method (Cup Technique)

Cup plate method is one of the official methods where the test samples diffuse from the cup through an agar layer in a Petri dish or plate until the growth of added microorganisms is restricted to a circular area or zone around the cavity containing an antibiotic solution [26]. The antimicrobial activity was expressed as zone diameter in millimeters [27].

2.6. Experimental procedure

Each overnight culture of the tested microorganisms was mixed with Muller Hinton agar media to give a final concentration of 1% microorganism (about 0.5 McFarland) and poured into sterile Petri dishes in a fixed amount of 20 ml using aseptic conditions. The sterile cork borer was used to prepare cups of 10 mm diameter. The tested samples and standard drugs with volumes of 60 μl were introduced into cups with the help of a micropipette. All the plates were kept at room temperature for effective diffusion of the test drug and standard and incubated at 37 ± 1°C for 24 hours. The inhibition zones around the cup indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded [28].

2.7. Determination of minimum inhibitory concentration (MIC)

Determination of minimum inhibitory concentration (MIC) was performed using agar dilution method according to Clinical Laboratory Standards Institute (CLSI) [29]. For each sample, different concentrations were diluted with Muller Hinton agar to give a final concentration ranging from (800 μg/ml–6.25 μg/ml). Dimethyl sulfoxide (DMSO) was used as a negative control plate. All isolated bacteria were subcultured on Brain Heart Infusion agar (B.H.I.A.) and incubated at 37 °C for 24 hours [30]. Three colonies of each microorganism were suspended in 5 ml saline, and the suspension was adjusted to a 0.5 McFarland standard and then diluted 10-fold with saline to give organism suspension of (1×10⁶ to 5×10⁶ CFU/ml). This suspension was then further diluted by putting 1 ml suspension to 9 ml saline to give a final suspension volume of 1×10⁵ to 5×10⁵ CFU/ml. A multiple inoculator was used to inoculate the prepared agar plates. A 100 μl (i.e. 10⁴ CFU) of the prepared inoculums were put in the well of multi-inoculator, where each inoculation time by multi-inoculator gave about 10 μl of prepared inoculums to the plate (i.e. 103 CFU). Each experiment was performed in duplicates. All plates were incubated at 35°C for 48 hrs.

2.8. Metal adsorption study

Stock solutions (100 ppm) of copper (II) and iron (II) were diluted to give standard solutions of appropriate concentrations. Batch experiments were conducted in 100 ml conical flasks containing 50 ml of various concentrations of Cu²⁺ and Fe²⁺ at 300 rpm at room temperature. These experiments were conducted under different conditions. These conditions include: (i), the impact of pH (from 1 to 5) using 30 ppm of adsorbate and 40 mg of adsorbent for 20 hrs. (ii), the impact of adsorbent amount (from 10 to 50 mg) using pH 5 and 30 ppm of adsorbate for 20 hrs. (iii), the impact of time (from 30 to 360 minutes) using pH 5 and 40 mg of adsorbent and 30 ppm of adsorbate. After that, the adsorbent was separated by centrifugation and the supernatant was collected. The metal ion content was
The adsorbed amount of metal ions \( Q_e \) onto the adsorbent was calculated by equation (1)

\[
Q_e = \frac{C_0 - C_e}{M} V
\]

Where \( C_0 \) and \( C_e \) (mg/l) are the initial and final concentration of the metal ion, \( V \) (Litre) is the volume of the aqueous solution and \( M \) (gm) is the weight of the adsorbent. The total ion removal efficiency was determined by equation (2).

\[
(\%) \text{ Removal} = \frac{C_0 - C_e}{C_0} \times 100
\]

3. Results and discussion

3.1. Materials characterization

The synthesis of three materials Chitosan, LDH, Chitosan-LDH composite for waste water treatment and antimicrobial activity. FTIR spectra of all prepared materials are illustrated in Figure (1). Chitosan nanoparticles (Figure 1a), the band at 3455 cm\(^{-1}\) is related to O-H and NH stretching vibration [28]. The peaks at 1384 cm\(^{-1}\), 1654 cm\(^{-1}\), 1174 cm\(^{-1}\) and 1629 cm\(^{-1}\) can be attributed to the CH\(_3\) symmetrical in an amide group, bending vibration of N-H group, carbonyl group and amide I, respectively [31]. As shown in Figure 1b, a strong broad absorption band appears at 3448 cm\(^{-1}\) which can be attributed to OH stretching [32]. The absorption band at 1382 cm\(^{-1}\) confirms the presence of NO\(_3\)\(^{-}\) group as an anion between the Zn-Al LDH layers [29]. Chitosan–LDH composite (Figure 1c), the absorption spectrum shows the presence of two bands at 3448, 1382 cm\(^{-1}\) characteristic to both chitosan and LDH. A shift occurred in the peak at 1654 cm\(^{-1}\) due to the \( \nu_3 \) vibration of NO\(_3\)\(^{-}\).

The crystal structure of both Chitosan and the Zn-Al LDH along with that of the Chitosan-LDH composite was studied by X-ray powder diffraction. As shown in Figure 2, the diffract gram of Chitosan nanoparticles is composed of two main peaks at approximately 9.4 ° and 19.2° which is similar to previously reported results [33]. The two peaks indicate a relative high degree of crystallinity for the prepared Chitosan nanoparticles [34]. The XRD pattern of Zn-Al LDH shows two intense peaks at 2θ values of 11.7° and 23.2° corresponding to (003) and (006) plane, respectively (ICDD card 48-1022) [35]. The sharp peaks indicate high crystallinity of the prepared LDH nanoparticles. For the chitosan-LDH composite, it is assumed that the peaks for Chitosan has shielded those of LDH causing the noticed broadening in the (003) and (006) peaks. The peaks at 2θ values of approximately 34.7 °, 39.2 °, 46.9 °, 60.4 ° and 61.8 ° correspond to the LDH plane (012), (015), (018), (110) and (113) respectively.

Figure 3a, shows FESEM micrograph of the Zn-Al-NO\(_3\) LDH. Hexagonal like sheets of the LDH phase is clearly observed. In case of the Chitosan-LDH nano-composite (Figure 3b), the layered sheets of LDH are no longer hexagonal [18]. It can be also observed that the Chitosan nanoparticles are distributed over the surface of the LDH phase in the form of aggregates. Figure 4a shows a HRTEM micrograph of the Zn-Al-NO\(_3\) LDH. It can be observed that the layers have a rectangle like shape with approximate dimensions of 430 x 400 nm. Figure 4b shows the homogenous distribution of Chitosan aggregates over the surface of the LDH phase in the Chitosan-LDH composite.

3.2. Metal adsorption study

The synthesized chitosan–LDHs composite was tested for removing Cu\(^{2+}\) and Fe\(^{2+}\) ions from aqueous solutions for water treatment.

3.2.1. Influence of pH on the efficiency of removal metal ions. The pH value is an important factor for the removal of heavy metals and adsorption of metal ions because it affects the degree of ionization of the solution, the surface charge of the adsorbate, the dissociation of the functional groups on the active sites of the adsorbent [36]. Moreover, the active sites can be protonated or deprotonated depending on...
the value of pH. Binding sites are deprotonated at high pH; thereby different functional groups are available for binding of metal ions producing attractive forces responsible for removal heavy metals from waste water [5,37]. At low pH, the sites of the adsorbent are positively charged where repulsion occurs between the metal ions and the adsorbent and consequently decreases the removal capacity. On the other hand, the removal of the metal depends on the direct relationship with the concentration of the metal. In the current study, Chitosan-LDH composite and an initial metal concentration are of 30 ppm. The optimum pH for both metals was found to be pH 5 with a maximum percentage removal of 54.06% and 91.5 for Cu$^{2+}$ and Fe$^{2+}$, respectively. Moreover, increasing the pH above 6 leads to the precipitation of insoluble copper hydroxide and iron hydroxide, causing a decrease in the removal of Fe$^{2+}$ and Cu$^{2+}$ ions. Adsorption equilibrium studies were conducted under optimum conditions. As shown in figure 5, the removal efficiency of Cu$^{2+}$ and Fe$^{3+}$ ions increased with increasing pH solution where the removal efficiency of Fe$^{2+}$ increased from 49% to 91.5% when the pH value was increased from 1 to 5 and the removal efficiency of Cu$^{2+}$ increased from 6.6% to 54.06%.

![Figure 1. FTIR of (a) chitosan (b) LDH (c) chitosan-LDH.](image1)

![Figure 2. XRD patterns of (a) chitosan (b) LDH (c) chitosan-LDH.](image2)

![Figure 3. FESEM micrographs of (a) LDH (b) chitosan-LDH composite.](image3)
Figure 4. HRTEM micrographs of (a) LDH (b) chitosan-LDH composite.

3.2.2. Influence of contact time on the efficiency of removal metal ions. The effect of contact time was studied on removal efficiency of Cu$^{2+}$ and Fe$^{2+}$ ions by the Chitosan-LDH at pH 5, initial metal ion concentration of 30 ppm using 40 mg of adsorbent. The removal efficiency for both ions increased rapidly during the first 30 minutes reaching 32.6% and 55% for Cu$^{2+}$ and Fe$^{2+}$, respectively and then onwards increased slightly as shown in Figure (6). The sharp increase in the first 30 minutes may be attributed to the presence of large number of vacant active sites on the surface of the nanocomposite. Increasing in removal of heavy metals is slight to the degree of equilibrium as shown in Figure 6.

![Figure 5](image5.png)  
**Figure 5.** Influence of pH on the removal efficiency using (40 mg) and (30 ppm) at 20 h.

![Figure 6](image6.png)  
**Figure 6.** Influence of contact time on the removal efficiency using (40 mg) , (30 ppm) and (pH=5).

![Figure 7](image7.png)  
**Figure 7.** Influence of adsorbent amount on the removal efficiency using (30 ppm), (pH=5) at 20 h.
3.2.3. Influence of adsorbent amount on the efficiency of removal metal ions. The current study includes the effect of varying the adsorbent amount on the metal uptake for Fe$^{2+}$ and Cu$^{2+}$ from 0.01 to 0.05 gm, while keeping the other factors constant as pH = 5, concentration (30 ppm), constant time and at room temperature. The removal efficiency increased from 62.82 to 97.19% for Fe$^{2+}$ and from 29.2 to 66.66% for Cu$^{2+}$ ions when the adsorbent dose increased from 10 to 50 mg as shown in Figure 7; this increase can be attributed to the increase of the number of active sites on the surface area of the adsorbent with the increase of adsorbent dose.

3.2.4. Adsorption isotherm. Adsorption isotherms describe the relationship between the amount of the adsorbed substance per unit mass of adsorbent and its concentration in the equilibrium solution at constant temperature. They are used to describe adsorption equilibrium for waste water treatments. The equilibrium adsorption isotherms are essential to determine the adsorption capacity of Cu$^{2+}$ and Fe$^{2+}$ ions and to detect the nature of adsorption onto the Chitosan- LDH catalyst. Langmuir and Freundlich isotherms are the two most reliable models that can provide insights about the characteristics of the adsorbent surface. The Langmuir model assumes that the interaction occurs on the surface forming a mono-layer of adsorbate on the surface of adsorbent. The linear form of Langmuir equation is written as:

$$\frac{c_e}{q_e} = \frac{1}{q_0 k_L} + \frac{1}{q_0} c_e$$

Which $c_e$ (mg/l) is the equilibrium concentration of adsorbate, $q_e$ (mg/g) the amount adsorbed, $q_0$ and $k_L$ are Langmuir constants related capacity and rate of and adsorption, respectively. The values of $C_e/q_e$ was plotted vs. $C_e$ for both Cu$^{2+}$ and Fe$^{2+}$ as shown in Figure 8 and the model constants were estimated and listed in Table 1. Another important factor can be determined by Langmuir model is the separation factor or equilibrium Parameter, $R_L$ can be expressed as:

$$R_L = \frac{1}{1 + K_L C_0}$$

$C_0$ is the initial concentration of the metal ions (mg/g), the value of $R_L$ shows to shape of the isotherm which can be favorable (0<R<1) or (R=1) linear, (R=0) irreversible, (R>1) unfavorable [38]. $R_L$ values for Cu$^{2+}$ and Fe$^{2+}$ adsorption onto chitosan-LDH were calculated to be less than 1 and greater than zero, meaning that the adsorption was favorable. The Freundlich model assumes that the absorption occurs on a heterogeneous surface where the existing adsorption sites are not equivalent. The linear form of Freundlich equation is expressed as:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e$$

Where $k_F$ is the adsorption capacity, N (n) is the isotherm exponent and (n) giving an indication of how favorable the adsorption process. 1/n is the slope of the linear relation and its value ranging from 0 to 1. The surface becomes more heterogeneous as its value gets closer to 0. The Freundlich model parameters were estimated for both Cu$^{2+}$ and Fe$^{2+}$ through plotting $\ln q_e$ vs $\ln C_e$ in Figure 9. However, the higher correlation coefficient of Cu$^{2+}$ obtained from Langmuir model ($R^2 = 0.9759$) compared to Freundlich plot ($R^2 = 0.9206$) suggests the adsorption was taken place on the homogeneous surface of the adsorbent much better than the multilayer adsorption. While, for Fe$^{2+}$, the higher correlation coefficient of Fe$^{2+}$ was from Langmuir model ($R^2=0.7495$) compared to Freundlich plot ($R^2=0.8441$) suggesting the adsorption was occurred on the heterogeneous surface of the adsorbent much better than the monolayer adsorption.

As shown in Table 2, the value of $R^2$ for Cu$^{2+}$ adsorption over the surface of the Chitosan-LDH composite was high for Langmuir model compared to that of Freundlich model showing a better fit of the data. The opposite is true for the case of Fe$^{2+}$ showing the possibility of multilayer adsorption over the surface of the Chitosan-LDH composite.

3.2.5. Adsorption kinetics analysis. Studying the adsorption kinetics, describes the removal rate of the solute, and this rate controls the residence time of removal the adsorbate at the solid solution interface
including the diffusion process. The mechanism of adsorption depends on the physical and chemical characteristics of the adsorbent. The adsorption kinetics explains the solute uptake on the adsorbent and its depended on the contact time that ranged from 30 to 180 minutes for removal of Cu$^{2+}$ and Fe$^{2+}$. Figure 10 displays the kinetics of metal ions adsorption using pseudo first order and pseudo second order. The pseudo-first kinetic order assumes the physical sorption binding according the following equation:

$$\ln(q_e - q_t) = \ln q_e - K_1t$$

(6)

Where, $q_e$ and $q_t$ are the amounts of metal on the adsorbent at equilibrium and at the time. $K_1$ is the pseudo-first adsorption rate constant. $q_e$ and $k_1$ are the slope and intercept on the linear plot by the relation between time (min) and $\ln (q_e - q_t)$. While, the pseudo second order equation is written as:

$$t/q_t = 1/K_2/q_e^2 + t/q_e$$

(7)

Where, $K_2$ is pseudo second adsorption rate constant (min$^{-1}$) and. $K_2$ is calculated from the slope of the linear plot $t/q_t$ versus $t$ (min).

| Table 1. Langmuir and Freundlich isotherm factors for Cu$^{2+}$ and Fe$^{2+}$. |
|---------------------------------------------------------------|
| Isotherm                        | Cu$^{2+}$ | Fe$^{2+}$ |
|--------------------------------|-----------|-----------|
| Langmuir model                  |           |           |
| $q_0$ (mg/g)                    | 23.0733   | 5.0403    |
| $k_L$ (L/mg)                    | 0.2672    | 0.1902    |
| $R_L$                           | 0.0696    | 0.0951    |
| $R^2$                           | 0.9759    | 0.7495    |
| Freundlich model                |           |           |
| $K_f$                           | 9.6234    | 25.0056   |
| $1/n$                           | 0.2249    | 0.2726    |
| $N$                             | 4.4464    | 3.6683    |
| $R^2$                           | 0.9206    | 0.8441    |

| Table 2. Kinetic factors of pseudo first order and pseudo second order for removing Cu$^{2+}$ and Fe$^{2+}$. |
|---------------------------------------------------------------|
| Order model                  | Cu$^{2+}$ | Fe$^{2+}$ |
|--------------------------------|-----------|-----------|
| Pseudo first order            |           |           |
| $q_e$ calc (mg/g)             | 18.137    | 16.0225   |
| $q_e$ exp (mg/g)              | 16.875    | 34.35     |
| $k_1$ (min$^{-1}$)            | -0.021    | -0.125    |
| $R^2$                         | 0.9024    | 0.9914    |
| Pseudo second order           |           |           |
| $q_e$ calc (mg/g)             | 21.231    | 25.57     |
| $q_e$ exp (mg/g)              | 16.875    | 34.35     |
| $k_2$ (min$^{-1}$)            | 1.28*10$^3$ | 2.85*10$^3$ |
| $R^2$                         | 0.9958    | 0.9523    |
According to Table (2), \( q_e, k_1, K_2 \) and \( R_2 \) (the correlation coefficient) from the linear plots, For \( Cu^{2+} \), the pseudo-second-order linear plots revealed higher \( R^2 \) values than the pseudo-first-order while For \( Fe^{2+} \), the pseudo-first order linear plots had higher \( R^2 \) values than the pseudo-second order. The results showed that the adsorption of \( Cu^{2+} \) followed pseudo second order and controlled by chemical adsorption while \( Fe^{2+} \) adsorption was physical and followed first order.

### 3.3 Antimicrobial activity
The antibacterial activity was studied for Chitosan, LDH, and Chitosan-LDH composite against Gram-positive and Gram-negative by agar disc diffusion method. Table (4) displays the measurements of inhibition zone. The result indicated that Chitosan-LDH composite exhibited more antimicrobial activity compared to Chitosan and LDH as parents. The inhibition zone against Lactobacillus sakei LMG was (15mm), Corynebacterium sp (16mm), Staphylococcus aureus (16mm), Escherichia coli AO5 (14mm). The high efficiency of Chitosan-LDH composite was due to the large surface area that provides better contact with microorganisms. Table 5 exhibits the (MIC) values of Chitosan, LDH, and Chitosan-LDH composite, the result showed that the lowest concentration of the tested materials inhibited the growth of Gram–positive bacteria i.e. the tested materials possessed strong antibacterial activity against Gram-positive bacteria The (MIC) value of Chitosan- LDH against Corynebacterium sp was less than 6.25 μg/mL.

---

**Figure 8.** Langmuir adsorption isotherm graphs for removing \( Cu^{2+} \) and \( Fe^{2+} \).

**Figure 9.** Freundlich adsorption isotherm graphs for removing \( Cu^{2+} \) and \( Fe^{2+} \).

**Figure 10.** Kinetic plot (a) pseudo first order (b) pseudo second order for \( Cu^{2+} \) and \( Fe^{2+} \).

### 4. Conclusion
In this study, Chitosan-LDH composite was chosen as an adsorbent for removing metal ions from waste water. It approved satisfactory results for Cu$^{2+}$ and Fe$^{2+}$. The removal efficiency of metal ions was affected by the pH; the adsorption of Cu$^{2+}$ and Fe$^{2+}$ ions increased with the increase of the pH values from 1 to 5. The pH 5 was the optimum pH for maximum adsorption for iron and copper, initial concentration was 30 ppm and 40 mg adsorbent. Kinetic studies suggested that the adsorption of Cu$^{2+}$ by Chitosan-LDH composite was chemical adsorption and followed the second-order kinetics model while Fe$^{2+}$ was physical and followed first order kinetics model. The removal of iron was better than copper; the removal of iron ions achieved to 99.3%, but for copper achieved to 85.6%. Chitosan–LDH composite was tested for its efficiency against some species of microorganisms such as different gram positive (Lactobacillus sakei LMG 2313, Corynebacterium sp, Staphylococcus aureus 5247) and gram negative bacteria (Escherichia coli AO5). Strong antibacterial activity against Gram-positive bacteria achieved; where the lowest concentration of antimicrobial/antifungal agent inhibited the growth of Gram positive bacteria or giving no visible growth.

Table 3. Comparing our results with previous studies on the same metal ions.

| Adsorbent                      | Metal ion | % Removal | pH | Reference |
|--------------------------------|-----------|-----------|----|-----------|
| polyamiline graft chitosan beads | Cu$^{2+}$  | 79.8      | 5  | [2]       |
| Nano chitosan / Soduim ALGINATE/Microcrystalline cellulose | Cu$^{2+}$  | 76        | 6  | [31]      |
| Chitosan-LDH                   | Cu$^{2+}$  | 85.6      | 5  | Current study |
| Chitosan-LDH                   | Fe$^{2+}$  | 99.3      | 5  | Current study |
| Synthesized Chitosan           | Fe$^{2+}$  | 72        | 8  | [39]      |

Table 4: The zone of inhibition was measured by a scale and the measurements in mm are tabulated.

| Gram positive rods | Gram positive cocci | Gram negative rods | Yeast |
|--------------------|---------------------|--------------------|-------|
| Bacillus subtilis  | Lactobacillus sakei LMG 2313 | Corynebacterium sp. | Listeria innocua 2710 | Mycobacterium phlei | Enterococcus faecalis | MRSA | Staphylococcus aureus | Esherichia coli ATCC | Esherichia coli AO5 | Pseudomonas aeruginosa | Proteus vulgaris | Candida albicans |
| -                  | -                   | -                  | -            | -                  | -                  | -   | -                    | -                  | -                  | -                  | -                | -            |
| Chitosan           | -                   | -                  | -            | -                  | -                  | -   | -                    | -                  | -                  | -                  | -                | -            |
| LDH                | -                   | -                  | -            | -                  | -                  | -   | -                    | -                  | -                  | -                  | -                | -            |
| LDH-Chitosan       | 15                  | 16                 | -            | -                  | -                  | 16  | -                    | 14                 | -                  | -                  | -                | -            |

Table 5. the MIC was measured as μ g/ml.

| Gram positive rods | Gram positive cocci | Gram negative rods | Yeast |
|--------------------|---------------------|--------------------|-------|
| -                  | -                   | -                  | -    |
| Chitosan           | -                   | -                  | -    |
| LDH                | -                   | -                  | -    |
| LDH-Chitosan       | -                   | -                  | -    |
Chitosan | Bacillus subtilis | Lactobacillus sakei | Lactococcus lactis | Corynebacterium sp. | Listeria innocua | Mycobacterium phlei | Enterococcus faecalis | MRSA | Staphylococcus aureus | 5247 | Escherichia coli ATCC 8739 | Escherichia coli AO5 | Pseudomonas aeruginosa | Proteus vulgaris | Candida albicans
---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
LDH | >800 | 800 | 400 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800
LDH-Chitosan | >800 | 12.5 | < 6.25 | >800 | >800 | >800 | >800 | >800 | 400 | >800 | 200 | >800 | >800 | >800 | >800 | >800 | >800 | >800 | >800

Also, Chitosan-LDH composite exhibited good antimicrobial potency with MIC value < than 6.25 μg/mL. In a nutshell, Chitosan-LDH composite has evidenced that it is a good adsorbent for removal heavy metals and a good antimicrobial for water treatment process.

References
[1] Alimohammadi V, Sedighi M and Jabbari E 2017 Experimental study on efficient removal of total iron from wastewater using magnetic-modified multi-walled carbon nanotubes Ecol. Eng. 102 90–7
[2] Igberase E, Osifo P and Ofomaja A 2014 The adsorption of copper (II) ions by polyaniline graft chitosan beads from aqueous solution: equilibrium, kinetic and desorption studies J. Environ. Chem. Eng. 2 362–9
[3] Wan M-W, Kan C-C, Rogel B D and Dalida M L P 2010 Adsorption of copper (II) and lead (II) ions from aqueous solution on chitosan-coated sand Carbohydr. Polym. 80 891–9
[4] Ngah W S W, Ab Ghani S and Kamari A 2005 Adsorption behaviour of Fe (II) and Fe (III) ions in aqueous solution on chitosan and cross-linked chitosan beads Bioresour. Technol. 96 443–50
[5] Sivakami M S, Gomathi T, Venkatesan J, Jeong H-S, Kim S-K and Sudha P N 2013 Preparation and characterization of nano chitosan for treatment wastewaters Int. J. Biol. Macromol. 57 204–12
[6] Al-Karawi A J M, Al-Qaisi Z H J, Abdullah H I, Al-Mokaram A M A and Al-Heetimi D T A 2011 Synthesis, characterization of acrylamide grafted chitosan and its use in removal of copper (II) ions from water Carbohydr. Polym. 83 495–500
[7] Ngah W S W, Teong L C and Hanafiah M 2011 Adsorption of dyes and heavy metal ions by chitosan composites: A review Carbohydr. Polym. 83 1446–56
[8] Esmat M, Farghali A A, Khedr M H and El-Sherbiny I M 2017 Alginate-based nanocomposites for efficient removal of heavy metal ions Int. J. Biol. Macromol. 102 272–83
[9] Wang X, Guo Y, Yang L, Han M, Zhao J and Cheng X 2012 Nanomaterials as sorbents to...
remove heavy metal ions in wastewater treatment J. Environ. Anal. Toxicol. 2 1000154

[14] Negm N A, El Sheikh R, El-Farargy A F, Hefni H H H and Bekhit M 2015 Treatment of industrial wastewater containing copper and cobalt ions using modified chitosan J. Ind. Eng. Chem. 21 526–34

[15] Muhammed R, Junise V, Saraswathi P, Krishnan P and Dilip C 2010 Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of tuberculosis Res J Pharm Biol Chem Sci 1 383–90

[16] Ribeiro L N M, Alcântara A C S, Darder M, Aranda P, Araújo-Moreira F M and Ruiz-Hitzky E 2014 Pectin-coated chitosan-LDH bionanocomposite beads as potential systems for colon-targeted drug delivery Int. J. Pharm. 463 1–9

[17] Nejati K, Keypour H, Nezhad P D K, Rezvani Z and Asadpour-Zeynali K 2015 Preparation and characterization of cetirizine intercalated layered double hydroxide and chitosan nanocomposites J. Taiwan Inst. Chem. Eng. 53 168–75

[18] Abdallah S H 2014 Kinetic Controlled Release Study of Salicylate Anion from Synthesized and Characterized of Ni/Al-salicylate-Layered Double Hydroxide Nanohybrid Journals kufa chemical 26–33

[19] Chang Z, Wu C, Song S, Kuang Y, Lei X, Wang L and Sun X 2013 Synthesis mechanism study of layered double hydroxides based on nanoseparation Inorg. Chem. 52 8694–8

[20] Zhao Y, Xiao F and Jiao Q 2011 Hydrothermal synthesis of Ni/Al layered double hydroxide nanorods J. Nanotechnol. 2011

[21] Moaty S A A, Farghali A A, Moussa M and Khaled R 2017 Remediation of waste water by Co–Fe layered double hydroxide and its catalytic activity J. Taiwan Inst. Chem. Eng. 71 441–53

[22] Mahjoubi F Z, Khalidi A, Abdennouri M and Barka N 2017 Zn–Al layered double hydroxides intercalated with carbonate, nitrate, chloride and sulphate ions: Synthesis, characterisation and dye removal properties J. Taibah Univ. Sci. 11 90–100

[23] Moaty S A A, Farghali A A and Khaled R 2016 Preparation, characterization and antimicrobial applications of Zn-Fe LDH against MRSA Mater. Sci. Eng. C 68 184–93

[24] Elgiddawy N, Essam T M, El Rouby W M A, Raslan M and Farghali A A 2017 New approach for enhancing Chlorella vulgaris biomass recovery using ZnAl-layered double hydroxide nanosheets J. Appl. Phycol. 1–9

[25] Mahmoud R, Moaty S A, Mohamed F and Farghali A 2017 Comparative Study of Single and Multiple Pollutants System Using Ti–Fe Chitosan LDH Adsorbent with High Performance in Wastewater Treatment J. Chem. Eng. Data 62 3703–22

[26] Seeley H W, VanDemark P J and Lee J J 1975 Microbes in action : a laboratory manual of microbiology Microbes action. A Lab. Man. Microbiol. 450

[27] Ahmed S H, Amin M A, Saafan A E, El-Gendy A O and ul Islam M 2017 Measuring susceptibility of Candida albicans biofilms towards antifungal agents J. Microbiol. Biotechnol. Res. 3 149–56

[28] El-Gendy A O, Essam T M, Amin M A, Ahmed S H and Nes I F 2013 Clinical screening for bacteriocinogenic Enterococcus faecalis isolated from intensive care unit inpatient in Egypt J. Microb. Biochem. Technol 4 161–7

[29] Sader H S, Flamm R K and Jones R N 2013 Antimicrobial activity of daptomycin tested against Gram-positive pathogens collected in Europe, Latin America, and selected countries in the Asia-Pacific Region (2011) Diagn. Microbiol. Infect. Dis. 75 417–22

[30] Tarek N, Hassan H M, AbdelGhani S M M, Radwan I A, Hammouda O and El-Gendy A O 2014 Comparative chemical and antimicrobial study of nine essential oils obtained from medicinal plants growing in Egypt Beni-Suef Univ. J. Basic Appl. Sci. 3 149–56

[31] Vijayalakshmi K, Gomathi T, Latha S, Hajeeth T and Sudha P N 2016 Removal of copper (II) from aqueous solution using nanochitosan/sodium alginate/microcrystalline cellulose beads Int. J. Biol. Macromol. 82 440–52

[32] Ahmed A A A, Talib Z A, Bin Hussein M Z and Zakaria A 2012 Zn-Al layered double hydroxide prepared at different molar ratios: Preparation, characterization, optical and
dielectric properties. *J. Solid State Chem.* **191** 271–8

[33] Wang Y, Pitt-Barry A, Habtemariam A, Romero-Canelon I, Sadler P J and Barry N P E 2016 Nanoparticles of chitosan conjugated to organo- ruthenium complexes *Inorg. Chem. Front.* **3** 1058–64

[34] Qi L, Xu Z, Jiang X, Hu C and Zou X 2004 Preparation and antibacterial activity of chitosan nanoparticles *Carbohydr. Res.* **339** 2693–700

[35] Qu J, He X, Li X, Ai Z, Li Y, Zhang Q and Liu X 2017 Precursor preparation of Zn-Al layered double hydroxide by ball milling for enhancing adsorption and photocatalytic decoloration of methyl orange *RSC Adv.* **7** 31466–74

[36] Yang F, Sun S, Chen X, Chang Y, Zha F and Lei Z 2016 Mg–Al layered double hydroxides modified clay adsorbents for efficient removal of Pb 2+, Cu 2+ and Ni 2+ from water *Appl. Clay Sci.* **123** 134–40

[37] Lin Y, Hong Y, Song Q, Zhang Z, Gao J and Tao T 2017 Highly efficient removal of copper ions from water using poly (acrylic acid)-grafted chitosan adsorbent *Colloid Polym. Sci.* **295** 627–35

[38] Martins A C, Pezoti O, Cazetta A L, Bedin K C, Yamazaki D A S, Bandoch G F G, Asefa T, Visentainer J V. and Almeida V C 2015 Removal of tetracycline by NaOH-activated carbon produced from macadamia nut shells: Kinetic and equilibrium studies *Chem. Eng. J.* **260** 291–9

[39] Hadi A G 2016 Removal of Fe (II) and Zn (II) ions from aqueous solutions by synthesized chitosan *Int. J. ChemTech Res.* **9** 343–9