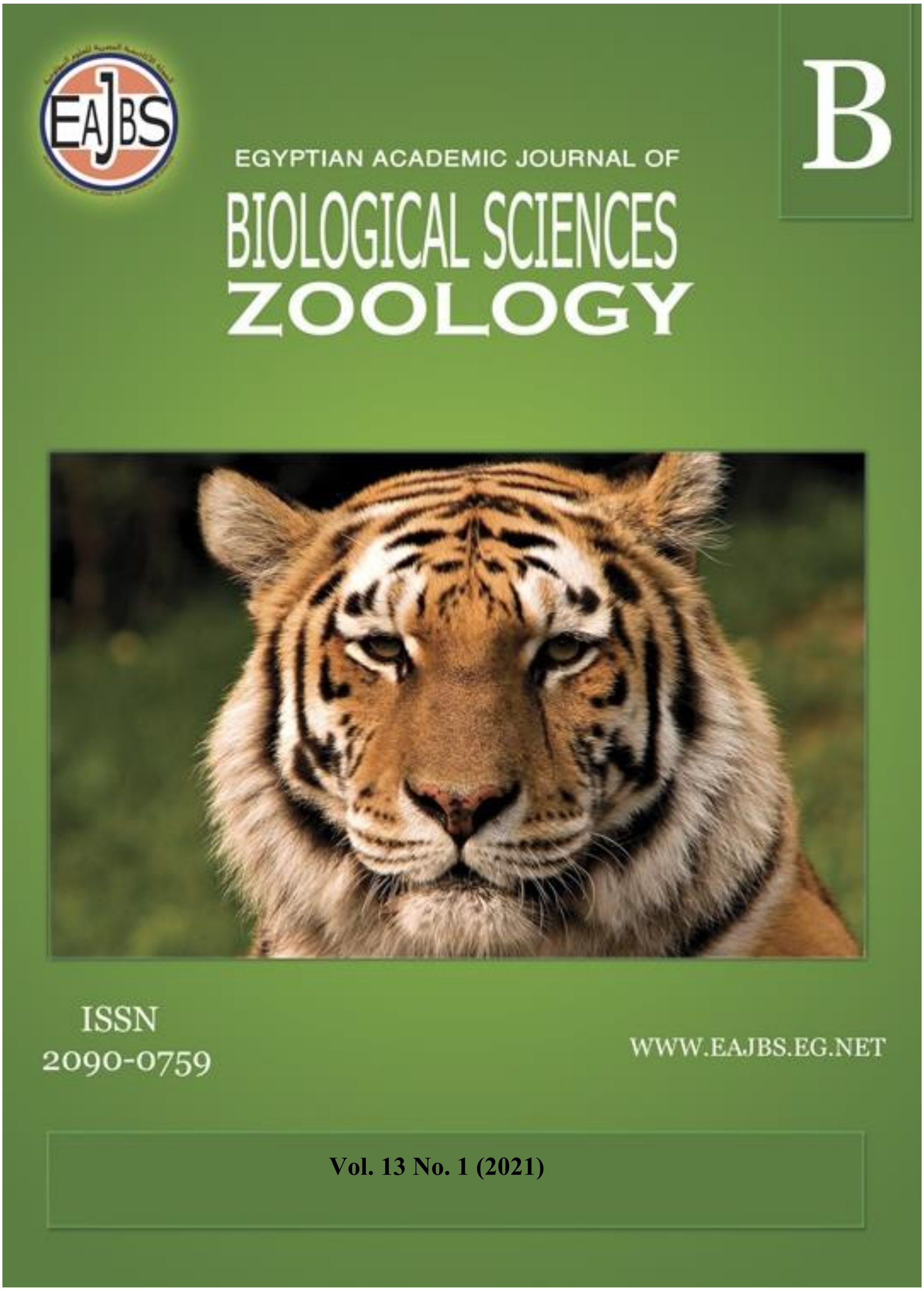

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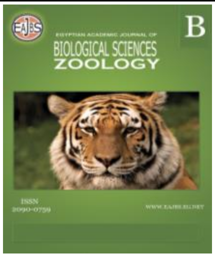

Effect of Using Chitosan as Adsorbing Material for Reducing Heavy Metals Content from Synthetic Polluted Water

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ABSTRACT

The present work aims to investigate the effect of Chitosan (as a natural adsorbent) for removing five heavy metals from synthetic polluted water after different contacting periods to be suitable for aquaculture and other purposes. Three different concentrations: 5, 7.5 and 10 g / l of chitosan were tested for their removal efficiency for 1 ppm of Fe, Zn, Cu, Cd and Pb at different contact periods. The effect of different contacting periods (30 Min, 1, 3, 6, 12, 18 and 24 hours) was tested with the three investigated concentrations of chitosan on the removal efficiency of the five metals. It's revealed that the removal efficiency increases with the increase in the contracting period, where the lowest values of removal efficiency for Fe, Zn, Cu, Cd and Pb were 27.7, 48.4, 68.4, 22.2 and 88.3 %, respectively after 30 Minutes, while the highest ones were 95.2, 91.9, 89.1, 94.1 and 99.9 %, respectively after 24 hours. The equilibrium adsorption behaviour data were fitted well to Langmuir and Freundlich adsorption isotherm models. R^2 for Langmuir values for removing the investigated metals ranged between (0.947 to 1), while for Freundlich R^2 values ranged between (0.931 to 0.992). Isothermal studies proved that chitosan was highly potential for removing all metals from polluted water.

INTRODUCTION

The environmental pollution with heavy metals is one of the major concerns for both governmental and non-governmental (NGOs) bodies that stack holds Egyptian health and welfare improvements. Metals are natural trace components of the aquatic ecosystem, but their levels have been increased due to industrial wastes, geochemical structure, agricultural and mining activities (Al Naggar *et al*., 2018). Heavy metals such as, chromium, cobalt, copper, nickel, zinc, mercury and lead are produced mainly from major industries such, mining operations, tanneries and metal plating. Those industries are the main contributor to heavy metal contamination (Quek *et al*., 1998). Raw untreated discharged wastewaters containing heavy metals constitute a direct great risk for the aquatic ecosystem; while, the direct discharge into the sewerage system may negatively affect the subsequent biological wastewater treatment (Matis *et al*., 2004). Heavy metals are considered a critical concern to

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aquatic ecosystem Contamination due to, their potential toxicity and accumulation in aquatic habitats (Tscheikner-Gratl *et al*., 2019). Pollution of the aquatic environment by inorganic and organic chemicals is a major factor posing a serious threat to the survival of aquatic organisms including fish (Aly *et al*., 2020). Metals that are deposited in the aquatic environment may accumulate in the food chain and cause ecological damage and a threat to human health (Grimanis *et al*. 1978 and Aderinola *et al*., 2009). In general, heavy metals are infiltrated into the water bodies via industrial, agriculture, domestic waste and/ or acidic rain that break down soils and liberating heavy metals into rivers, lakes and streams (Bagul *et al*., 2015). Heavy metals are introduced to the aquatic food chain either directly through the digestive tract through consumption of contaminated water and/or food, or non-dietary routes across permeable membranes such as gills (Burger *et al*. 2002). Fish are widely used as environmental indicators “biomarker or biosensor” in the aquatic ecosystem due to their easy contamination with a wide range of chemicals such as metals, organochlorine and organophosphate pesticides, herbicides, polyaromatic hydrocarbons, polychlorinated biphenyls, dioxins and rapid physiological reaction to chemical toxicity in the environment; those reactions could be changes in feeding habits, opercular rhythm, gills and odd swimming or difficulties (Gemaque *et al*., 2019). The heavy metal could exhibit its detrimental effects by bioaccumulation and biomagnification individually or combined. The first, process in which a chemical pollutant enters into the body of an organism and is not excreted, but rather collected in the organism’s tissues; while the latter is the increase of concentration of a toxic chemical the higher, we go on the food chain (Mann *et al*. 2011). Kim *et al*., 2004 stated that; liver, intestines, gills and kidneys are identified as the main depository of metals in the fish body and are mainly investigated for metal accumulations. Water chemistry, fish species, life stage and temperature of the water are the main variables that affect any given heavy metal toxicity in freshwater fish (McGeer *et al*., 2000). Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals and species (Heath, 1987). Chronic exposure of fish to waterborne Cu, Cd, or Zn had manifested physiological and behavioral changes such as loss of appetite, reduced growth, ion loss (McGeer *et al*., 2000). As well as, damage in organ structure, alteration in enzyme activities hormonal and functions reproductive impairment (Amer and Ahmed, 2019) and mortality. Pb is neurotoxic and could generate damage due to its capacity to join erythrocytes; in addition to, its ability to substitute calcium and zinc in the cellular organelles like the mitochondria (Zuluaga Rodríguez *et al*., 2015). Surplus dissolved iron in the water can cause the formation of flakes that obstruct gills causing respiratory disorders. Moreover, Animals that feed on high levels of iron diets may encounter growth reduction, high feed conversion (FCR), diet rejection, mortality, and histopathological damage in the liver due to iron accumulation (Gemaque *et al*., 2019).

Various methods including chemical precipitation (Hu *et al*., 2010), nanofiltration (Al Rashdi *et al*., 2013), solvent extraction (Cerna, 2014), ion exchange (Vaaramaa and Lehto 2003), reverse osmosis (Zhang *et al*., 2014) and adsorption (Coskun *et al*., 2006) have been extensively studied in the recent decade to decontaminate the polluted waters. Out of all these methods, adsorption is particularly attracting scientific focus mainly because of its high efficiency, low cost, easy handling and high availability of different adsorbents. Scientists have focused their momentum on the search for low cost and easily available biomaterials for wastewater treatment. Chitosan is one of the well-known natural polymers that have received considerable attention for water treatment (Ahmed *et al*., 2014). Chitosan is a versatile polyaminosachride produced by alkaline N-deacetylation of chitin involving deproteination and deacetylation. Owing to many attractive properties such as hydrophilicity, biocompatibility, biodegradability, non-toxicity, presence of very reactive amino ($-NH_2$) and hydroxyl (-OH) groups in its backbone and amino groups on the chain are involved in a special interaction with metals, chitosan shows good complexation (chelating) ability with metals so can be used as an effective material for the removal of heavy metals from wastewaters (Wu *et al*., 2001) and (Rinaudo, 2006).

The present work aims to investigate the effect of Chitosan (as a natural adsorbent) for removing five heavy metals from water at different contact periods to be suitable for aquaculture and other purposes.

MATERIALS AND METHODS

This work take place in Limnology Department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia Governorate, that belonging to the Agricultural Research Center, Cairo, Egypt, to investigate the effect of Chitosan particles on removing five heavy metals from the water with pH relevance in fish aquaculture (7.3). Water temperature was measured by using oxygen meter (WPA 20 Scientific Instrument). pH was measured by using a glass electrode pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA) according to APHA (1992).

Heavy Metals Preparation and Determination:

The experimental synthetic polluted water was prepared by using 1000 ppm stander solutions (Banco Co.) of each metal (Fe, Zn, Cu, Cd and Pb) and then 1 ml of each solution completed to 1 liter by redistilled water (Parker, 1972). The residues of the investigated metals in different samples after treating the prepared aqueous solution with chitosan were determined at different contacting periods.

Chitosan Preparation Method:

The chitosan was extracted according to the method reported by (Kurita 2001) and modified by (Ahmed *et al.*, 2020).

Effect of Chitosan Dosage and Contact Period:

Chitosan doses of (5, 7.5 and 10 g) were added separately into 1-liter Beaker includes 1 ppm of each investigated metal (Fe, Zn, Cu, Cd and Pb) to determine the optimum dose of Chitosan suitable for removing the five heavy metals from water. The tested solutions were stirred vigorously by a magnetic stirrer at room temperature for the whole period. Samples were taken at different contact periods (30 min, 1 hour, 3 hours, 6 hours, 12 hours, 18 hours and 24 hours), respectively. All samples were represented in two replicates. Samples were filtered and the residues in each sample were determined and then the removal efficiency of different investigated chitosan dosages at different tested contact periods was calculated.

Heavy Metals Residues Determination:

Water samples were digested by Nitric acid digestion method according to APHA (**2000**), and then filtered and the investigated heavy metals residues: iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), and Lead (Pb) were determined by using Flame Atomic Absorption Spectrophotometer (Thermo Electron Corporation S Series AA Spectrometer) for as mg / l.

Isothermal Studies:

The experimental data for the removal of the five metal ions by chitosan over the studied concentration range were processed in accordance with the most widely used adsorption isotherms (Langmuir and Freundlich) in order to calculate adsorption maximum and binding energies for using chitosan for the removal of all metal ions from contaminated water.

The Langmuir isotherm equation (Rao, *et al*., 2009) can be written in linear form as:

$$C_e / q_e = C_e / Q_m + 1 / Q_m b$$

Where: q_e and C_e are the metal equilibrium concentration is adsorbed and liquid phase in mmol/g and mmol/l, respectively. Those constants were calculated from the intercept and slope of the linear plot of C_e/q_e vs C_e.
The Fruendlich isotherm equation (Rao, *et al*., 2009) can be written in the linear form as:
$\log q = \log k + 1/n \log C$

Where k and n are indicators of sorption capacity (in mg/g) and intensity respectively. These Fruendlich constants were calculated from the slope and intercept of the linear plot, with log q vs log C.

Statistical analysis

The statistical analysis was applied according to Steel and Torrie (1980) data of the frequent samples were analyzed with the repeated statement (SAS, 2009). For detecting the significant differences among means, Duncan Multiple range tests (Duncan's, 1955) were used.

$$Y_{ijk} = \mu + Con_i + Rep_{ij}(Con)_{ij} + Time_k + (Time*Con)_{kj} + e_{ijk}$$

Where:
Y_{ijk} = observations;
μ = is the overall mean;
Con_i = is the effect of concentrations;
$Rep_{ij}(Con)_{ij}$ = is the effect of the concentrations within the replicate.
$Time_k$ = is the effect of the time;
$(Time*Con)_{kj}$ = is the effect of the time within Concentrations;
e_{ijk} = random error.

RESULTS AND DISCUSSION

Table (1) showing that there is an evident trend, where the initial concentration of the investigated five heavy metals in the water decreases due to, the effect of the three investigated dosages of chitosan as well as the contact period from the beginning till the end of the experimental.

Residues of Fe, Zn, Cu, Cd and Pb were decreased from their initial concentration (1 mg/l) to 0.37, 0.21, 0.11, 0.15 and 0.03 mg / l, respectively after treating with 5 g Chitosan /l. These residues after treating with 7.5 g Chitosan / l became 0.23, 0.1, 0.13, 0.11 and 0.01 mg / l while these residues became 0.05, 0.08, 0.11, 0.06 and 0.001 mg / l, respectively after treating with 10 g Chitosan / l. The current study indicated that the use of chitosan extracted from shrimp wastes as a chelating biomaterial led to removing heavy metals from contaminated water because it has many positive charges that aid in mineral precipitation (Oliveira Franco *et al*., 2004). (Pradhan *et al*., 2005) showed that, the mechanism of the action of chitosan as a heavy metal removal agent was through its ability to condense heavy metal ions, as the amine groups in chitosan act as electron donors selectively with the metal ions.

The Removal Efficiency of Heavy Metals by Chitosan:

The Removal Efficiency of Fe:

From the obtained results (Fig. 1), it can conclude that the lowest removal efficiencies of the three examined chitosan dosages (5, 7.5 and 10 g/l were 27.7, 43.7, and 69.7 %, respectively after 30 Min of contacting period, while the highest removal efficiencies were 64.2, 77.3 and 94.07 % respectively after 24 hours of contacting period. (Rana *et al*., 2009) showed that, the removal efficiency was 58 % of Fe with 5 g chitosan for 10 mg Fe / l.

The Removal Efficiency of Zn:

Zn had the same trend of Fe where removing efficiency increased with the increase of chitosan dosage as well as contacting period. The lowest removing efficiency (78.9 %) was obtained after treating with 5 g chitosan / l while the highest one (91.9 %) was obtained after mixing with 10 g chitosan / l (Fig. 2). Jain (2013) showed that a similar removal efficiency (91.4 %) of 3 mg Zn / l was obtained as a result of 50 mg chitosan/l. Increases in metal removal with increased pH above 5 can be explained on the basis of the decrease in competition between proton and metal cations for the same functional groups and by a decrease in positive surface charge, which results in a lower electrostatic repulsion between surface and metal ions (Meena *et al*., 2005.

The Removal Efficiency of Cu:

At the end of the contacting period, removal efficiency for chitosan towards Cu was (89.1 %) which was recorded in both 5 g and 10 g chitosan / l concentrations, while 7.5 g chitosan / l concentration was recorded (86.8 %) (Fig.3). Jain (2013) indicated that the highest removal efficiency (88.1 %) of 3 mg Cu / l with / l was obtained after treating with 200 mg chitosan / l for 6 hours.

The Removal Efficiency of Cd:

The obtained results illustrated in (Fig. 4) indicated that the removal efficiency increases with the increasing contracting period for chitosan towards cadmium. The percentages of Cd removal efficiency for the three investigated chitosan dosages (5, 7.5 and 10 mg / l) ranged between (22.2 to 84.5 %) (60 to 89 %) and (76.4 to 94.1 %) respectively. Rana *et al*., (2009) revealed that the removal efficiency of 5 g chitosan / l for 10 mg Cd / l was 75.6 %.

Table. 1: Effect of chitosan on heavy metals residues alongside the experiment

Concentrations	Times	Heavy Metals residues (mg/l)				
		Fe	Zn	Cu	Cd	Pb
	0	1.03^{a}	0.99^{a}	0.99^{a}	0.99^{a}	1.04^{a}
5 g Chitosan / L	30 Min	0.75^{b}	0.51^{b}	0.31^{b}	0.77^{b}	0.12^{b}
	1 Hour	0.61^{c}	0.48^{bc}	0.26^{bc}	0.64^{c}	0.09^{c}
	3 Hour	0.54^{d}	0.41^{c}	0.25^{bc}	0.44^{d}	0.08^{c}
	6 Hour	0.5^{d}	0.38^{cd}	0.17^{c}	0.33^{e}	0.06^{cd}
	12 Hour	0.44^{e}	0.36^{cd}	0.16^{c}	0.25^{ef}	0.05^{d}
	18 Hour	0.37^{f}	0.33^{d}	0.12^{d}	0.18^{f}	0.03^{e}
	24 Hour	0.37^{f}	0.21^{e}	0.11^{d}	0.15^{fg}	0.03^{e}
7.5 g Chitosan / L	30 Min	0.58^{d}	0.34^{d}	0.29^{b}	0.39^{e}	0.1^{b}
	1 Hour	0.48^{e}	0.31^{d}	0.24^{bc}	0.27^{ef}	0.09^{c}
	3 Hour	0.47^{e}	0.28^{de}	0.23^{bc}	0.24^{ef}	0.08^{c}
	6 Hour	0.37^{f}	0.22^{e}	0.2^{bc}	0.2^{f}	0.06^{cd}
	12 Hour	0.3^{fg}	0.18^{ef}	0.18^{c}	0.14^{fg}	0.03^{e}
	18 Hour	0.29^{fg}	0.14^{f}	0.17^{c}	0.11^{g}	0.02^{e}
	24 Hour	0.23^{g}	0.1^{g}	0.13^{d}	0.11^{g}	0.01^{e}
10 g Chitosan / L	30 Min	0.31^{fg}	0.28^{de}	0.21^{bc}	0.23^{ef}	0.1^{b}
	1 Hour	0.15^{h}	0.23^{e}	0.2^{bc}	0.19^{f}	0.07^{c}
	3 Hour	0.1^{i}	0.18^{ef}	0.19^{c}	0.18^{f}	0.04^{e}
	6 Hour	0.09^{i}	0.14^{f}	0.16^{c}	0.17^{f}	0.01^{e}
	12 Hour	0.08^{ij}	0.1^{g}	0.15^{c}	0.14^{fg}	0.005^{f}
	18 Hour	0.06^{j}	0.1^{g}	0.14^{d}	0.13^{fg}	0.003^{fg}
	24 Hour	0.05^{j}	0.08^{h}	0.11^{d}	0.06^{h}	0.001^{g}
± SE		0.04	0.03	0.008	0.018	0.01

Means in the same column having the same superscript letters are not significantly different ($P < 0.05$)

The Removal Efficiency of Pb:

Pb had the highest removal efficiency among the investigated heavy metals. These values were 99.9 %, 99.1 % and 96.7 % after treating with 10 g, 7.5 g and 5 g chitosan/l, respectively (Fig. 5). These results were in agreement with Akinyeye *et al*., (2016) who stated that minimum percentage removal of 0.03 mg Pb^{2+} ion / l was 99.81 % after treating with 0.25 g chitosan / l while the maximum value was 99.93 % after treating with 0.5 g chitosan / l for the same concentration of Pb^{2+}, therefore, it could be noted that removal efficiency of Pb^{2+} ions improved with increasing dose due to availability of more binding sites in the surface of the adsorbent as the dosage increases till a certain border (Asubiojo *et al*., 2009, Choi *et al*., 2009, Rathinam *et al*., 2010, Wan *et al*., 2012).

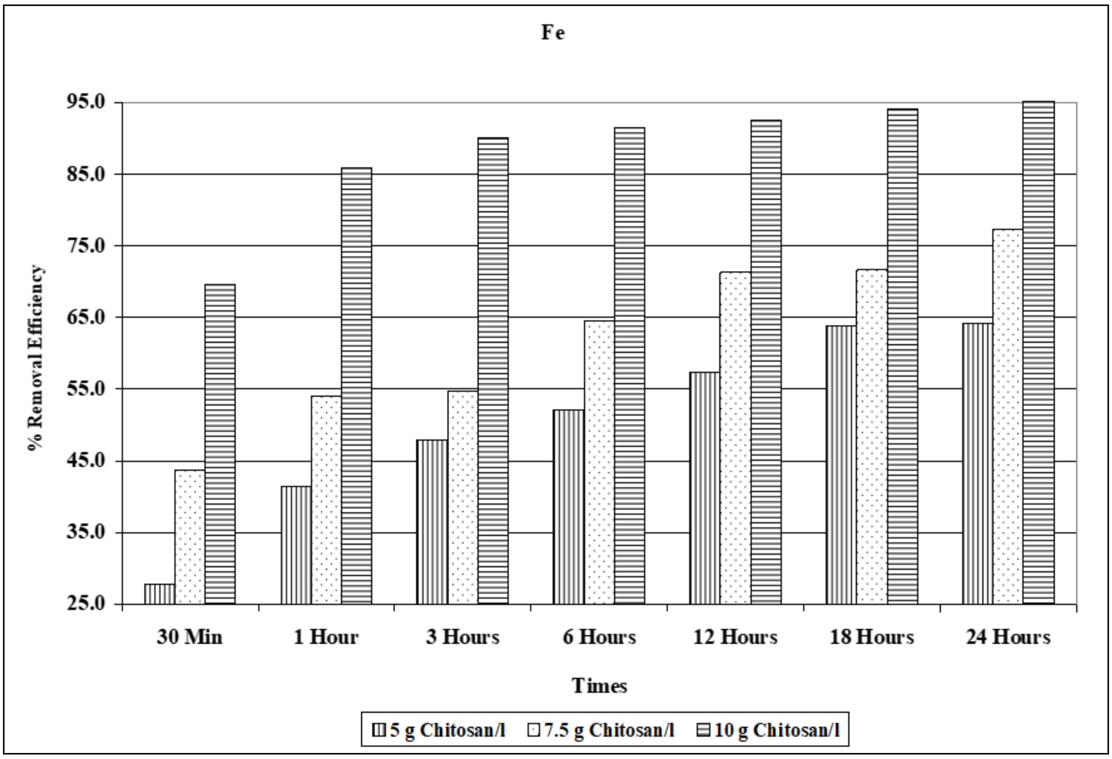

Fig. 1: Removal efficiency of chitosan against Fe in water

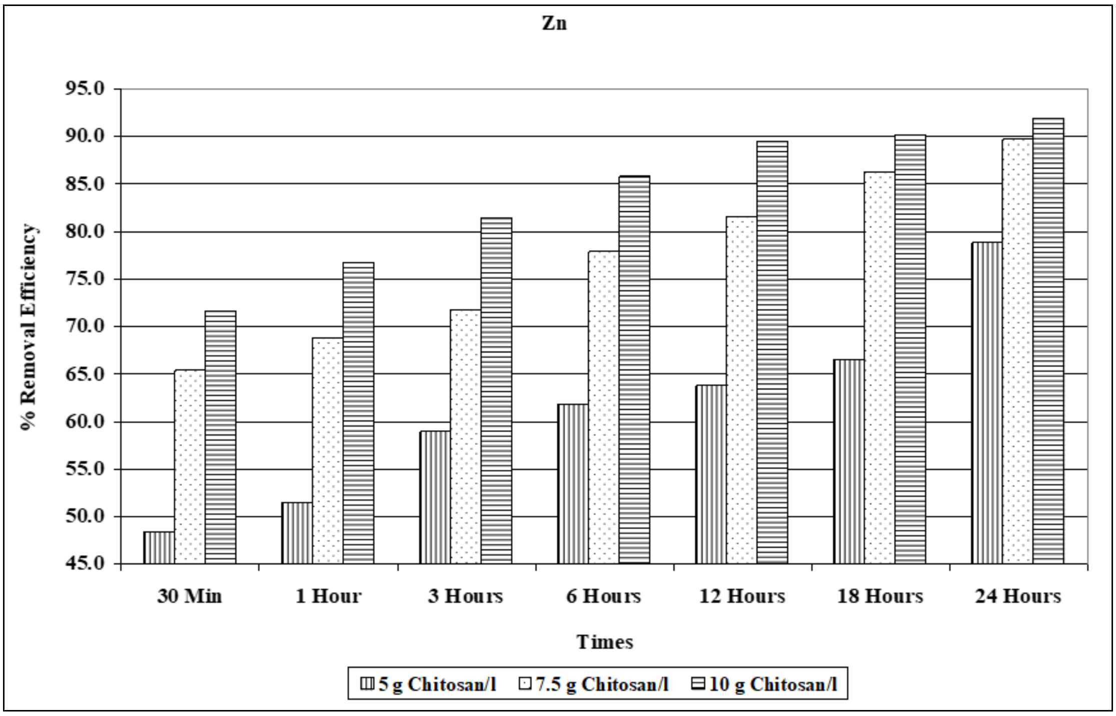

Fig. 2: Effect of chitosan in remove Zn from water in the different experimental times

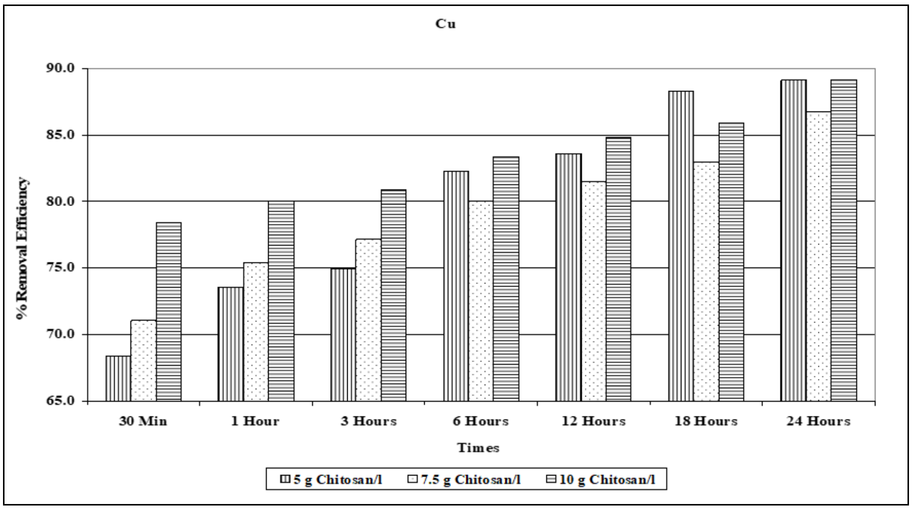

Fig. 3: Cu removing efficiency by chitosan during the experimental period

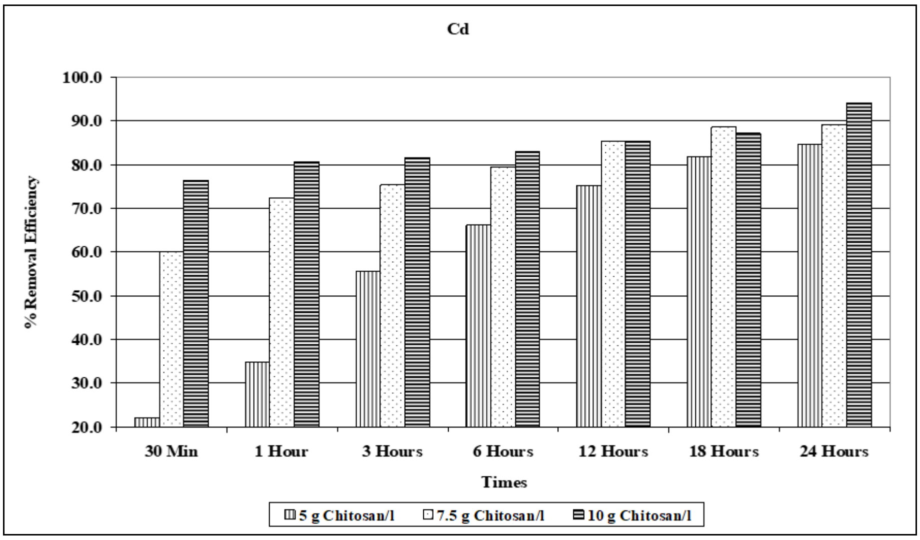

Fig. 4: Efficacy of chitosan for removing Cd from the experimental water

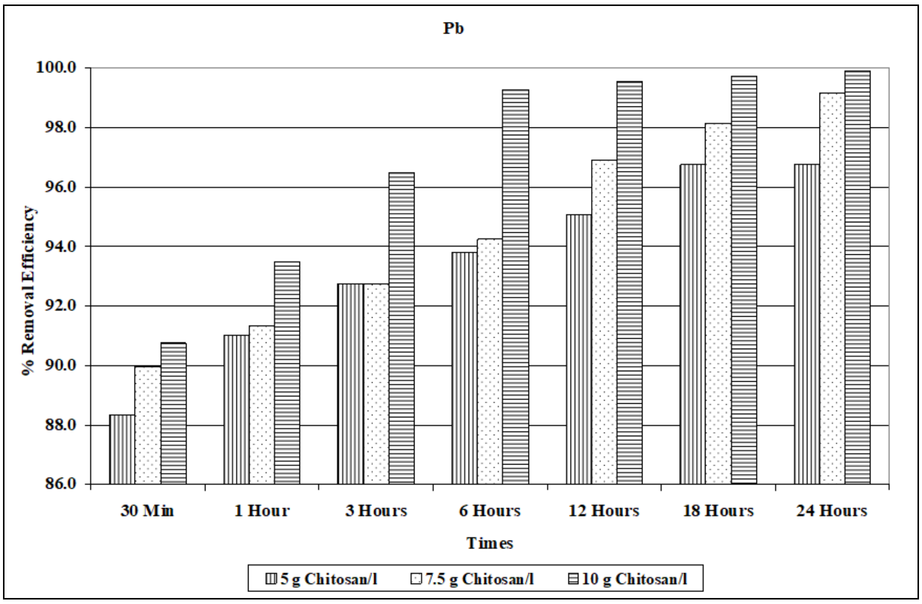

Fig 5: Effect of chitosan in removing Pb during the different experimental periods

The Langmuir Isotherm Equation:

As indicated in Table 2 and Figures 6 – 10, the most fitted Langmuir equation was for lead (1), on the other hand, R^2 values were ranged between 0.947 to 0.998 for Fe to Cd.

The Fruendlich Isotherm Equation:

Chitosan for all five heavy metals was fitted to Fruendlich model, where R^2 values for adsorbing Fe, Zn, Cu, Cd, and Pb by chitosan were 0.931, 0.999, 0.98, 0.986, and 0.992, respectively Table 2 and Figures 11 – 15.

Table 2: Langmuir and Fruendlich parameters for the adsorption of all metals on chitosan.

	Fe			Zn			Cu			Cd			Pb		
C (Metal conc. mg / l)	35.8	22.7	4.8	21.1	10.3	8	10.9	13.2	10.9	15.5	10.9	5.9	3.2	0.8	0.09
Metal removed (mg / l)	64.2	77.3	95.2	78.9	89.7	92	89.1	86.8	89.1	84.5	89.1	94.1	96.8	99.2	99.01
Chitosan dose (g / l)	5	7.5	10	5	7.5	10	5	7.5	10	5	7.5	10	5	7.5	10
Q (Metal removed / g of adsorbent (mg / g)	12.8	10.3	9.5	15.8	12	9.2	17.8	11.6	8.9	16.9	11.9	9.4	19.4	13.2	10
1/C	0.03	0.04	0.21	0.05	0.10	0.13	0.09	0.08	0.09	0.06	0.09	0.17	0.31	1.25	11.11
1/Q	0.08	0.10	0.11	0.06	0.08	0.11	0.06	0.09	0.11	0.06	0.08	0.11	0.05	0.08	0.1
Log C	1.55	1.36	0.68	1.32	1.01	0.9	1.04	1.12	1.04	1.19	1.04	0.77	0.51	-0.1	-1.05
Log Q	1.11	1.01	0.98	1.2	1.08	0.96	1.25	1.06	0.95	1.23	1.07	0.97	1.29	1.12	1

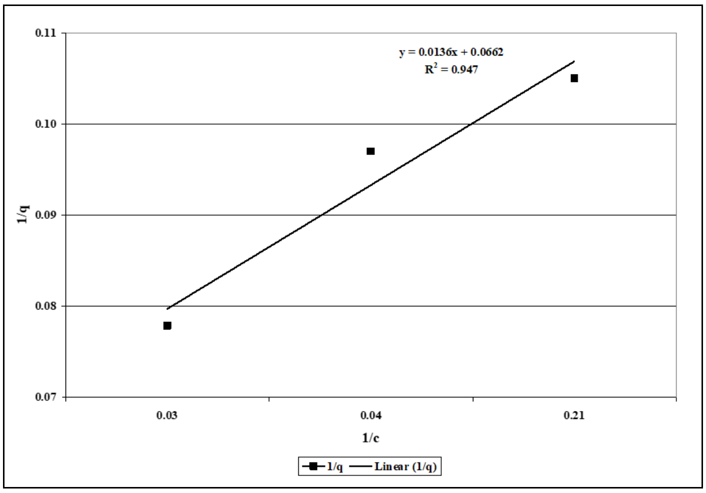

Fig. 6: Langmuir adsorption isotherm of Fe by chitosan.

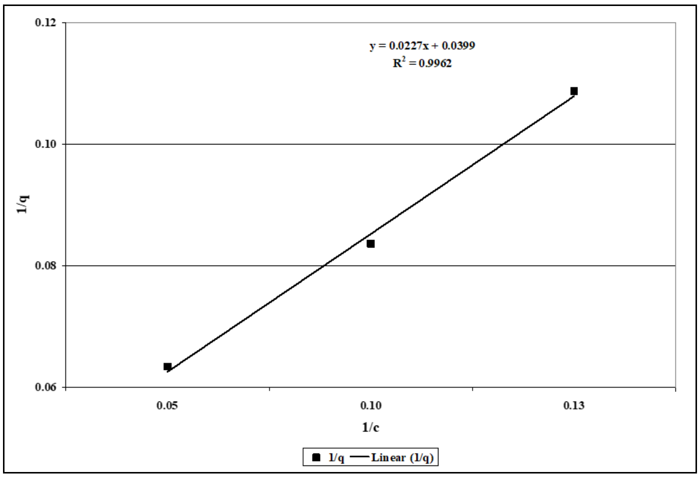

Fig. 7: Langmuir adsorption isotherm of Zn by chitosan.

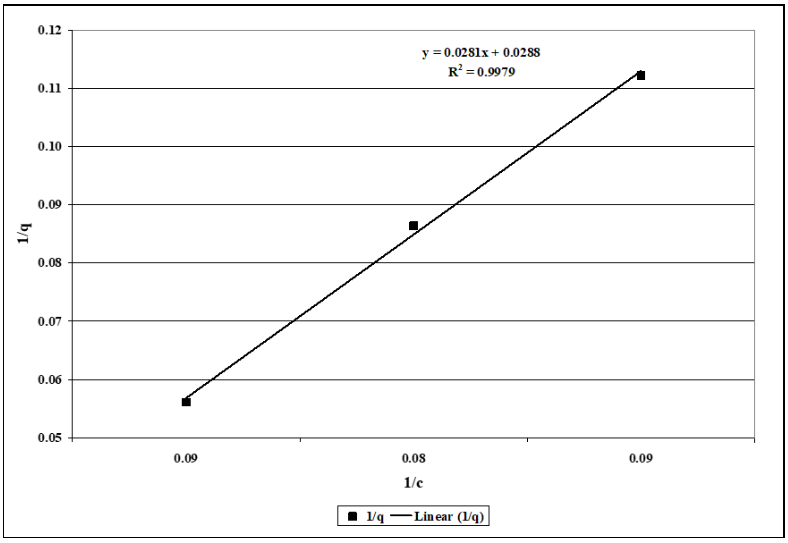

Fig. 8: Langmuir adsorption isotherm of Cu by chitosan.

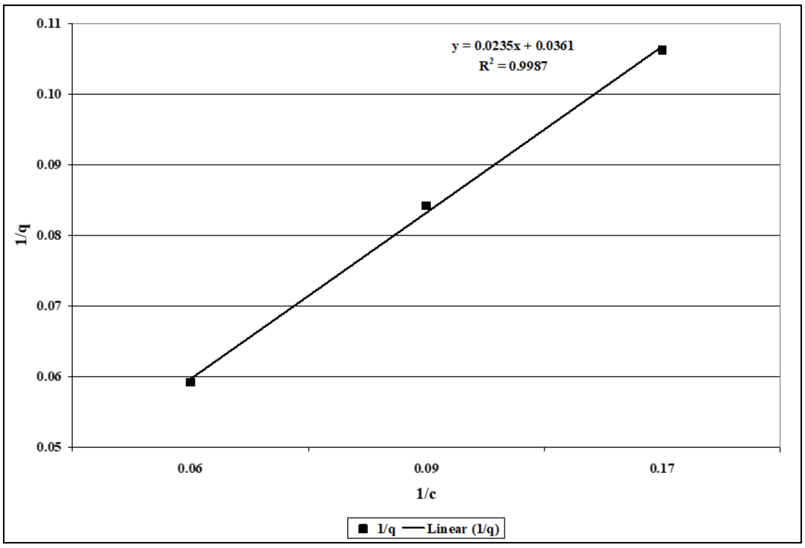

Fig. 9: Langmuir adsorption isotherm of Cd by chitosan.

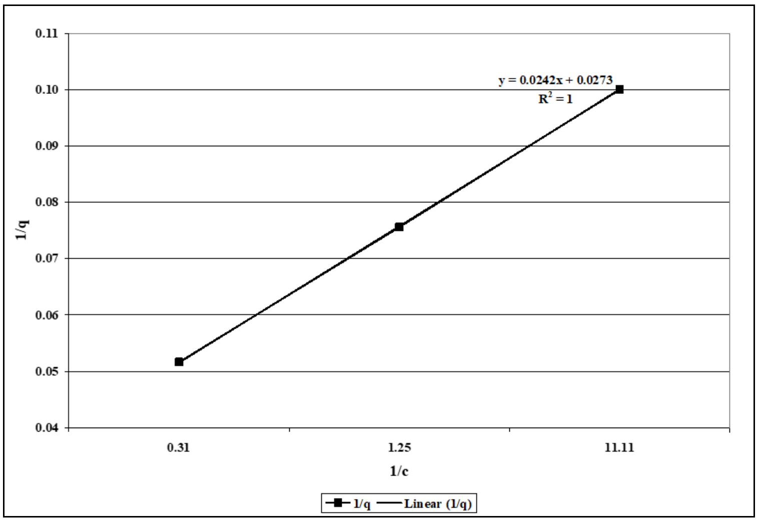

Fig. 10: Langmuir adsorption isotherm of Pb by chitosan.

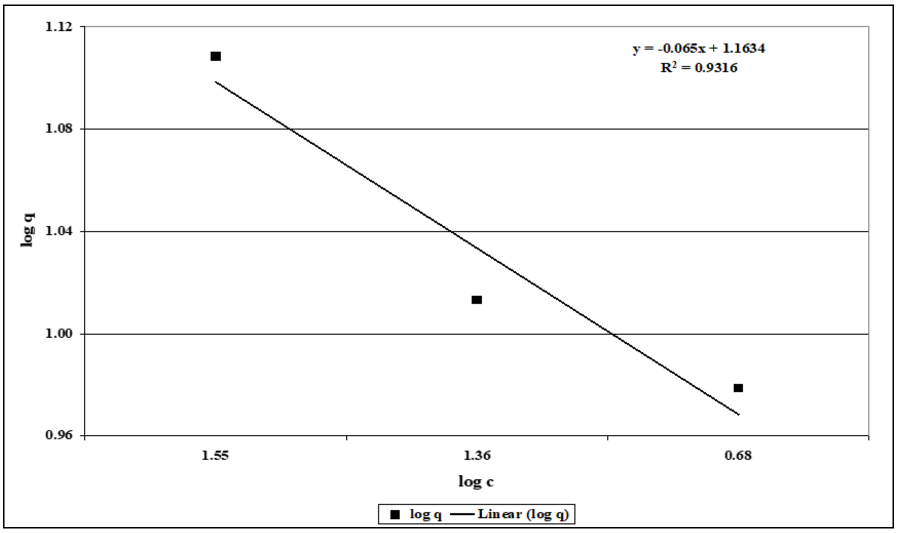

Fig. 11: Fruendlich adsorption isotherm of Fe by chitosan.

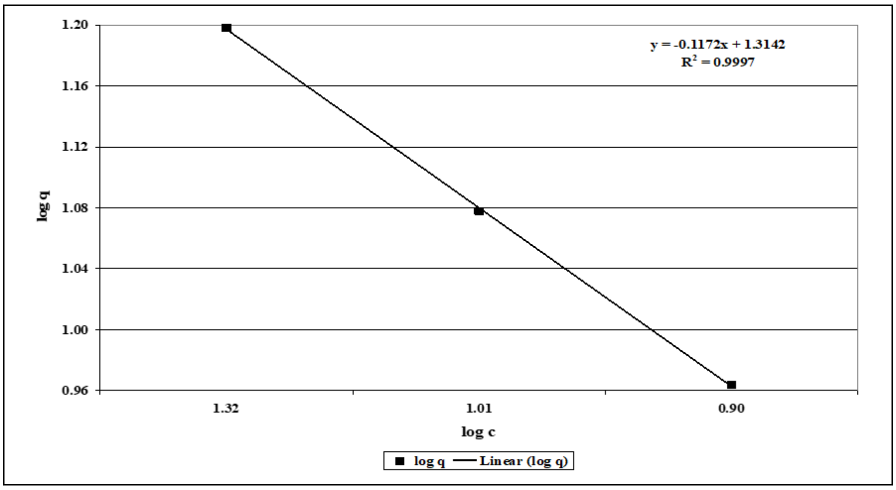

Fig. 12: Fruendlich adsorption isotherm of Zn by chitosan

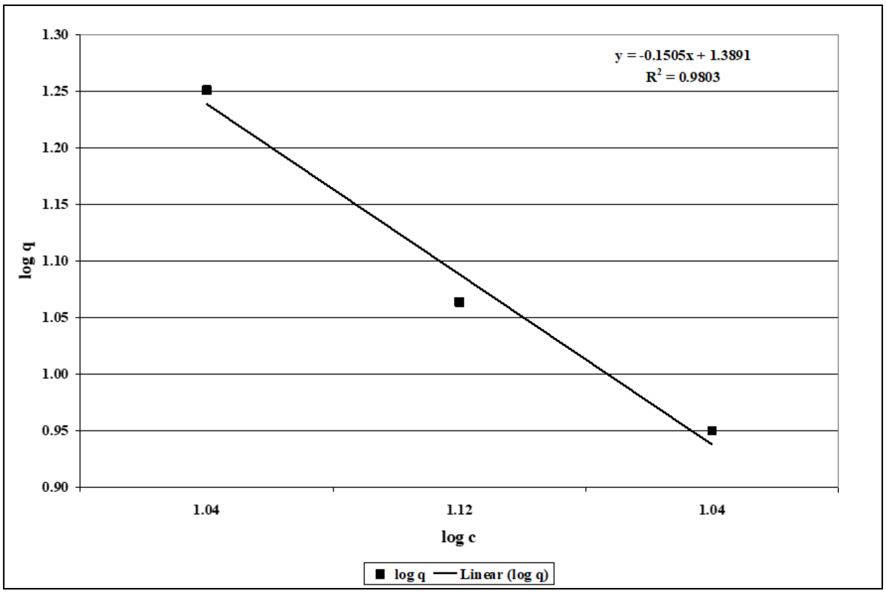

Fig. 13: Fruendlich adsorption isotherm of Cu by chitosan.

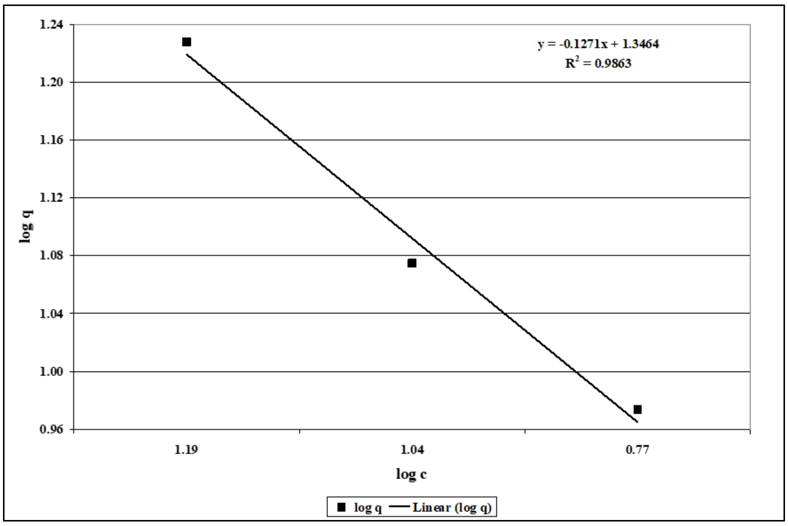

Fig. 14: Fruendlich adsorption isotherm of Cd by chitosan.

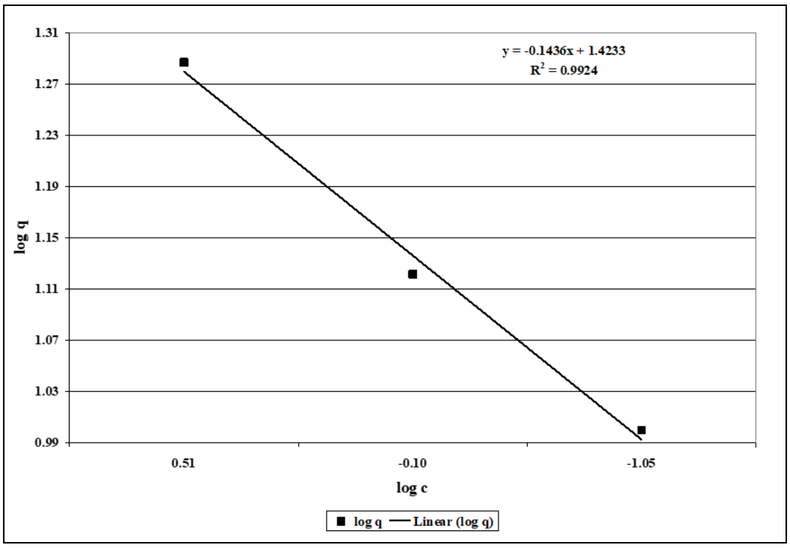

Fig. 15: Fruendlich adsorption isotherm of Pb by chitosan.

Conclusion

The current work indicated that, chitosan extracted from shrimp wastes give a high efficiency as a removal adsorbent for iron, zinc, copper, cadmium and lead ions from contaminated water which pollute different water sources such as river, lakes and seas; which considered of a potential threat for fish and accordingly to man, therefore chitosan representing an effective and environmentally clean material for removing those wastes from an aquatic environment.

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ARABIC SUMMARY

أثر استخدام الشيتوزان كمادة ممتزه لتقليل محتوى المياه الملوثه صناعيا من المعادن الثقيلة

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تهدف الدراسة الحاليه إلى إختبار تأثير الشيتوزان (كممتز طبيعي) مع درجة حموضة الماء ودرجة الحراره المناسبه لتربية الأسماك لإختبار نسب الإزاله لخمسة معادن ثقيله من المياه الملوثه المحضره معمليا حتي يمكن استخدامها في تربية الأحياء المائيه وأغراض أخرى. تم اختبار ثلاث تركيزات مختلفه: 5 و7.5 و 10 جم / لتر من الشيتوزان لكفاءة الإزاله لتركيز 1 جزء في المليون من كل من الحديد، الزنك، النحاس، الكادميوم والرصاص لفترات خلط مختلفه (30 دقيقة، 1، 3، 6، 12، 18 و 24 ساعة). أظهر تأثير فترات الخلط المختلفه مع تركيزات الشيتوزان الثلاثة المدروسه على كفاءة إزالة المعادن الخمسه، أن كفاءة الإزاله تزداد مع زيادة وقت الخلط. حيث كانت أقل قيم كفاءه الإزاله للحديد، الزنك، النحاس، الكادميوم والرصاص (27.7، 48.4، 68.4، 22.2 و88.3 ٪) علي التوالي عند 30 دقيقة، بينما أعلاها كانت (95.2، 91.9، 89.1، 94.1 و 99.9 ٪) على التوالي. تمت ملائمة بيانات سلوك امتصاص التوازن جيدًا لنماذج متساوية الامتزاز لانجموير وفريوندليش. تراوحت قيم R^2 الخاصه بإزالة المعادن التي تم فحصها بين (0.947 إلى 1)، بينما تراوحت قيم R^2 لـ Freundlich بين (0.931 إلى 0.992). أثبتت العديد من الدراسات أن الشيتوزان له قدره عاليه على إزالة جميع المعادن من المياه الملوثه.

الكلمات المفتاحيه: الشيتوزان، المعادن الثقيله، المياه الملوثه، الحديد، الزنك، النحاس، الكادميوم والرصاص.