Effect of different molecular weights of chitosan on the removal efficiencies of heavy metals from contaminated water

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ABSTRACT

In the present study, chitin isolated from locally available shrimp shells was pulverized to obtain three different particle sizes and then deacetylated to obtain various deacetylation degrees and molecular weights of chitosan. In the batch mode experiment, the removal efficiency of heavy metals [Zn(II), Cd(II), Pb(II), and Cu(II)] from synthetic wastewater solutions with different concentrations ranging from 10-30 mg/l using three molecular weights chitosan was evaluated. Cadmium was the best metal chelated by all three types of chitosan and the extent of adsorption was considerably higher in the type 1 of chitosan sample of small particle size with lower of molecular weight and higher of degree of deacetylation compared with types 2 and 3 chitosans. In addition, the maximum percent removal of heavy metal ions was at concentration 10 and 20 mg/l in order of Cd (II > Pb (II) > Cu (II))> Zn (II), while at concentration 30 mg/l the removal efficiency of chitosan was in order of Cd (II) > Cu (II) > Pb (II) > Zn (II). The influence of pH is presented and the maximum uptake of metal ions was obtained at pH 4.0 at room temperature.

INTRODUCTION

Natural biopolymers are industrially attractive because of their capability of lowering transition metal-ion concentration to parts per billion concentrations. Natural materials that are available in large quantities or certain waste from agricultural operations may have potential to be used as environmentally friendly as well as low cost adsorbent materials (Muzzarelli, 1977).

Chitin (poly[β -2-acetoamido-2-deoxy-D-glucopyranose]) is the most abundant natural amino polysaccharide polymer, especially in the exoskeletons of invertebrates (Lerivrey *et al*., 1986). After its deacetylation, it produces chitosan (poly [β-(1-4)-2-amino-2-deoxy- D-glucopyranose]) that recognized as an excellent metal chelating agent, forming stable complexes with different metal ions (Kurita *et al*., 1979; Brine and Austin, 1981; Knorr, 1984; Jha *et al*., 1988; Khan *et al*., 2002), especially with transition metals. Reactive amino groups in chitosan are the responsible groups for complex formation between metal ions and the polymer chain. Chitosan is a heteropolymer consist of D-glucosamine and a small fraction of N-acetyl-D-glucosamine residues (Li *et al*., 1997).

Chitin and its deacetylated derivative, chitosan, have a wide range of uses, not only in the food industry but also in the pharmaceutical, textile, cosmetic industries, and in agriculture (Muzzarelli, 1973; Micera *et al*., 1986).

The major free source of the chitin in the market and local seafood processing industries are fishery wastes such as shrimp, lobster, and crab shells, which considered as one of the promising options to produce chitosan in the laboratory.

Environmental pollutants especially heavy metals is a great environmental problem and had been increased during the last years (Shreadah *et al.*, 2006; Said *et al.* 2006; Younis & Nafea 2012; Younis *et al.*, 2014; El Zokm *et al.*, 2015; Soliman *et al.*, 2018; Younis, 2018; Amin *et al.*, 2018; Soliman *et al.*, 2019; El-Naggar *et al.*, 2019). Chitosan has received considerable interest for heavy metals removal due to its excellent metal-binding capacities and low cost.

Metal oxides can quantitatively adsorb heavy metals from their aqueous solutions and are thus employed in metal pre-concentration (Carbonell *et al.*, 1992; Pannain and Santelli, 1995; Marqués *et al.*, 2001) and metal removal (Cooper *et al.*, 2002).

The removal efficiency of toxic metals from contaminated water is an important target of many researchers worldwide and being studied by many of them using different methodologies and techniques over the years (El-Kassas *et al.*, 2016; Younis *et al.*; 2014; Hwang and Jho, 2018; Meepho *et al.*, 2018; Kiran *et al.*, 2018; Saleh *et al.*,2019). One of the most promising methodologies in this process is the biosorption since using of bioadsorbent material considered as a good alternative to traditional adsorbent materials.

Previous studies have demonstrated the ability of chitosan to uptake metal ions from aqueous solutions (Schmuhl *et al.*, 2001). Therefore, the aim of this study is to carry out the production of different molecular weight of chitosan biopolymer from natural resources as well as studying the influence of chitosan molecular weight and initial concentration of metals on the removal efficiencies of some metal ions [Zn(II), Cd (II), Pb (II), and Cu (II)] from contaminated water.

MATERIALS AND METHODS

The shrimp shells that used for chitosan production was collected from local seafood markets. Shells were collected, and then washed under running warm water to remove soluble organics, adherent proteins and other impurities.

The raw samples of crab shell were pulverized using Macinagosatore SM 90-95 and sieved into three sized 0.5, 1.5, 2.5 mm after dried. The samples were soaked in 4 % HCl at room temperature in the ratio of 1:14 (w/v) for 36 h to demineralization of shells. After that, they were washed with tap water to remove acid and then filtered under vacuum. The demineralized shells were washed for 30 min with double distilled water and then oven-dried.

After that, the demineralized shells were subjected to deproteinization process. In this process, demineralized shells were added to 3% NaOH solution with a ratio 1:10 (w/v). the mixture was stirred with constant stirring rate for 1 hr at 60°C (No *et al.*, 1989). The boiled sample was removed and allowed to cool at room temperature for 30 minutes, filtered under vacuum, and then washed with double distilled water for 30 minutes and oven-dried.

Pigments can be removed using acetone for 10 min and dried for 2 hr at ambient temperature, followed by bleaching with 0.315 % sodium hypochloride (NaOCl) solution with a solid to solvent ratio of 1:10 (w/v) for 5 minutes at ambient temperature (No *et al.*, 1989). The final product obtained after this step was Chitin.

Chitosan was prepared from the previous isolated chitin by its treatment with 50% sodium hydroxide for 90 minute. The ratio of solid to alkaline solution was 1:10

(w/v). The purified samples were thoroughly washed with double distilled water until neutral pH. Later the samples were dried at room temperature. Acidic reagents without hydrolysis of the polysaccharide cannot remove the N-acetyl groups, thus, alkaline methods must be employed for N-deacetylation (Muzzarelli, 1977).

Moisture content (%) was determined gravimetrically according to the following equation that described by Black (1965):

$$\text{Moisture content}, \% = \frac{(wet\ weight,\ g - dry\ weight,\ g)}{wet\ weight,\ g} * 100 \quad \text{(1)}$$

Solubility was determined according to method described by Nessa *et al.* (2010). Ash content of chitin and chitosan were determined according to standard method described by (AOAC, 1990). Nitrogen content (The total nitrogen was determined using Kjeldahl procedure (Bremner, 1960). For the determination of viscosity- average molecular weight (Dalton), the chitosan was dissolved in a mixture of 0.1 M acetic acid with 0.2 M NaCl, then the automated solution viscometer was used to was calculated using the equation

$$\text{Molecular weight} = anti\ log\ \frac{Log\ (\eta) + 3.8601}{0.850} \quad \text{(2)}$$

Batch adsorption experiments

To study the effect of molecular weight of chitosans on metal ions sorption, 25mg of different molecular weight of chitosan (68, 112, 172 KDa) were added to 25 mL solutions containing 10, 20 and 30 mg/L of each [Cu(II), Cd(II), Pb(II), and Zn(II)] for estimate the sorption capacity and the mixtures were shaken for 3 h at $20\pm1°C$. After equilibrium was reached, the final concentrations (mg/L) of metal ions in the filtered samples were measured by atomic absorption spectrometer (Model: AA-6800 Shimadzu). The results are based on three replicate experiments.

The removal efficiency (R, %) as well as sorption capacity (q, mg/g) were calculated according to the following equations:

$$R\ (\%) = \frac{(Co - Cf)}{Co} * 100 \quad \text{(3)}$$

$$q\ (mg/g) = \frac{(Co - Cf)}{m} * V \quad \text{(4)}$$

where C_0 and C_f are the initial and final metal concentrations (mg/L) in the aqueous solution, respectively, V is the volume of metal ion solution (L), and m is the weight of sorbent material (g).

RESULTS AND DISCUSSION

According to particle size, three types of chitosan were obtained (types 1-3). Fig. 1 and Table 1 showed that, the moisture content of chitin sample was (5.22 %) and for chitosans were 5.4 %, 5.8% and 5.8% for type 1, 2 and 3 respectively. Due to the hygroscopic nature of Chitosan (Khan *et al.*, 2002), it had relatively higher moisture content than chitin during storage (Nessa *et al.*, 2010). According to Li *et al.* (1992), commercial chitosan products contain less than 10% moisture content. Chitin ash content was (0.2 %), while chitosan ash content varied from 0.22% to 0.27%. Ash content is considered as an indicator for the effectiveness of demineralization process (DM). The solubility of chitosan is highly affected by residual ash content, which in turn contributing to lowering its viscosity .According to No and Lee (1995), a high

quality grad of chitosan should have less than 1% of ash content. The degree of deacetylation (DD) of chitosan samples in the present study ranged from 77% to 82%. Our result was agreed with that obtained by No *et al*., (2003), who found that DD of chitosan ranges from 56% to 99%.
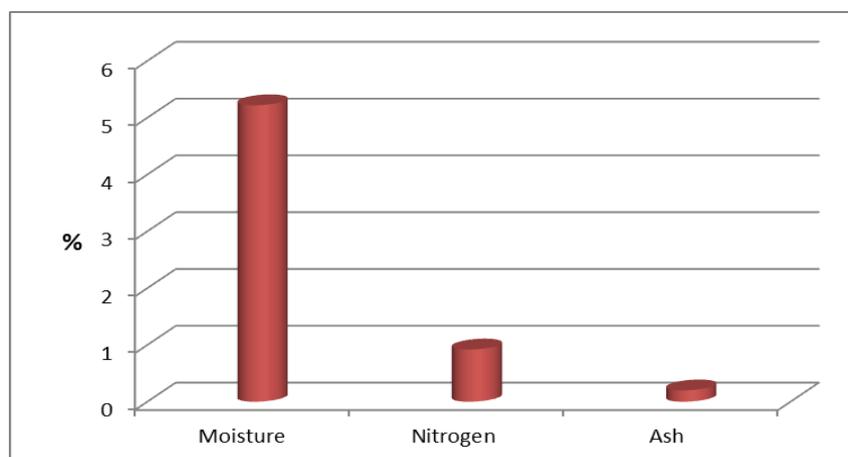

Fig. 1: The chemical composition of chitin prepared from crab shell

As can be observed in Table 1, type 1 of chitosan which had lower of particle size (0.5 mm) was the largest degree of deacetylation (DD), while type 3 which had higher of particle size (2.5 mm) was the lowest degree of deacetylation.

The high density of positive charge is highly coincided with the highest degree of deacetylation (77– 82%) and this makes crab chitosans unique for industrial applications. The degree of deacetylation and the molecular weight of chitosan were found to be key factors that affect the properties of produced chitosan (Aranaz *et al*., 2009).

The chitosan with various deacetylation degrees exhibit different removal efficiencies of the four studied heavy metal ions. The percent removal of Zn(II), Cd (II), Pb (II) and Cu (II) positively increased with the degree of chitosan deacetylation (Fig. 2). These can be attributed to the increasing of active sites such as amino and hydroxyl groups, which has a great potential as an adsorbent for removal of heavy metal ions.

Table 1 Characteristics of three different types of chitosan (Types 1–3) prepared from crab shell.

Properties	Type1	Type2	Type3
Practical size(mm)	0.5	1.5	2.5
color	Cream white	Cream white	Cream white
Moisture (%)	5.4	5.8	5.8
Nitrogen (%)	0.87	0.91	0.92
Ash (%)	0.27	0.24	0.22
Solubility in acid %	94.5	92.7	91.8
MM (KDa)	68	112	172
DD(%)	82	78,3	77
viscosity, $[\eta]$,mm^2/s	1.7	2.8	3.9
Density, kg/l	0.297	0.238	0.2

It has been reported that chitosan with the highest degree of deacetylation showed the highest removal efficiency (Saha *et al*., 2005) and this may be due to a growing number of amino and hydroxyl groups (Wu *et al*., 2001).

Molecular weight and viscosity of chitosan were (68, 112, 172 KDa) and (1.7, 2.8 and 3.9 mm^2/s) for the chitosan types 1, 2 and 3, respectively. When molecular weight is lower, viscosity of chitosan also tends to decrease (No and Meyers, 1995)

and consequence decreasing its ability to chelate metal ions. According to Tariq *et al*., (2014), the chelation ability increases as the degree of acetylation increases.

Viscosity of chitosan is controlled by some factors such as degree of deacetylation, molecular weight, concentration, pH and temperature, etc. Solubility of chitosan samples ranged from 91.8 to 94.5. Brine and Austin (1981) noted that, incomplete removal of protein may be the key factor that lowers solubility values. Micera *et al.* (1986) reported that, the density of chitosan from craw fish shell dependents on the particle size and porosity of the material.

The relationship between molecular weight of chitosan and heavy metal content at different concentrations were shown in (Figures 2, 3 and 4). It was obvious that the removal efficiency of Zn (II), Cd (II), Pb (II) and Cu (II) increased with the decreased molecular weight of chitosan. One exception was Cd (II), which showed no difference in the removal efficiency at concentration of 10 and 20 mg/l.

The percent removal of Cd (II) was the largest while that of Zn (II) was the lowest and the maximum percent removal of heavy metal ions followed Cd (II) > Pb (II) > Cu (II) > Zn (II) for metals concentration 10 and 20 mg/l, whereas percent removal of heavy metal ions followed Cd (II)> Cu (II) > Zn (II) > Pb (II) for metals concentration 30 mg/l.

Chitosan with lower molecular weight (68 KDa) exhibits the highest affinity for metal ions studied, despite having almost the same surface charge as chitosan with medium molecular weight (112 KDa) and chitosan with higher molecular weight (172 KDa) . The affinity of metal ions towards different molecular weight of chitosan was: 68 KDa > 112 KDa> 172 KDa. This due to the higher distribution of amino groups at the surface of chitosan with lower molecular weight, the consequent increase an electrostatic attraction between the sorbent and metal ions. A comprehensive knowledge of the Mw and DDA of chitosan is essential for its applications (Yaghobi, 2012).
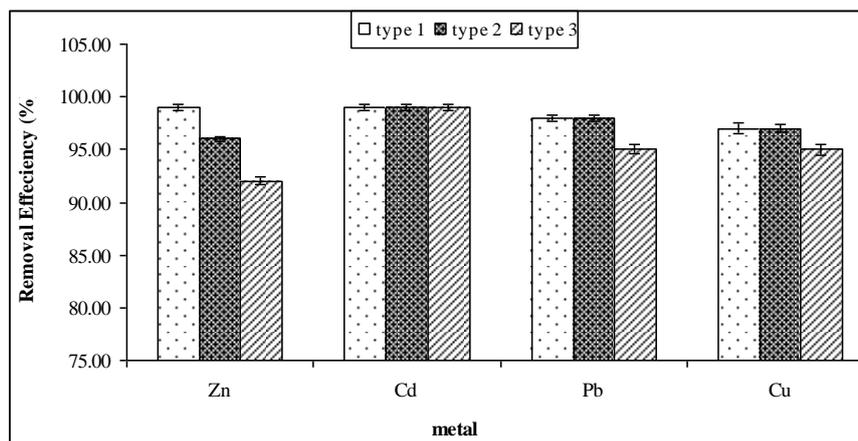

Fig. 2: Effects of chitosan molecular weight on the removal efficiency of metal ions at concentration 10 mg/l
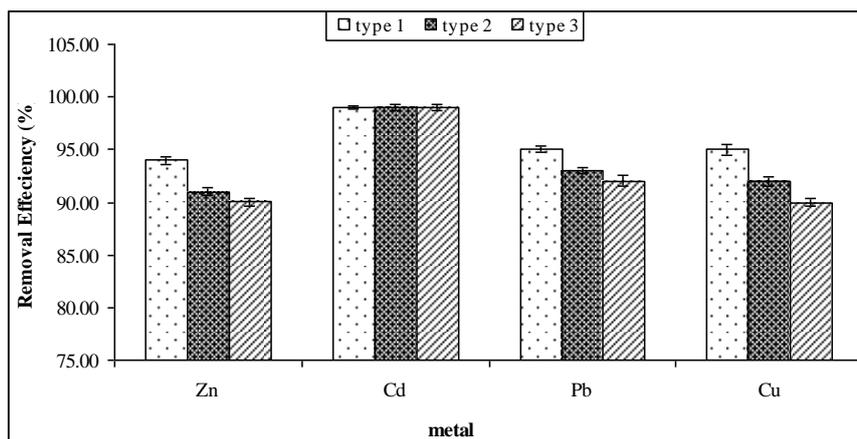
Fig. 3: Effects of chitosan molecular weight on the removal efficiency of metal ions at concentration 20 mg/l
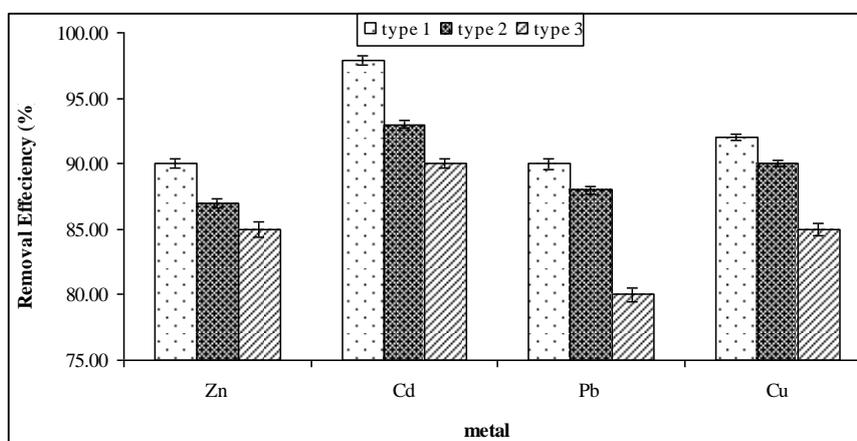

Fig. 4: Effects of chitosan molecular weight on the removal efficiency of metal ions at concentration 30 mg/l

The sorption capacity

The adsorption capacity of metal removal by chitosan is the amount of adsorbate that can be taken up from the solution by adsorbent per unit mass of the adsorbent.

Table 2 shows the sorption capacity of metals onto chitosan at pH 4. The equilibrium sorption uptake were obtained at an initial metal concentration range of 10–30 mg/L. Cadmium ions are more favorably retained onto lower molecular weight of chitosan and its sorption capacity was 29.37 mg/g at an initial concentration of 30 mg/L, while lead has low affinity towards the higher molecular weight of chitosan at the same initial concentration (30 mg/l); the sorption capacity was 24 mg/g. In general, the adsorption capacities are in the order $Cd^{2+} > Pb^{2+} > Cu^{2+} > Zn^{2+}$. This can be explained by the fact that, nature of the cation is very important in the mechanism of interaction (Rhazi *et al*. 2002). For the set of metallic ions, El-Gendy *et al*. (2013) stated that charge, ionic size, softness and hardness of each ion are the key factors that control the selectivity of metal ions by a biosorbent.

From the results of the present study we concluded that, type 1 of chitosan exhibits the highest removal efficiency and forming stable complexes with investigated metal ions compared with types 2 and 3. Sorption capacity of chitosan increases with the decreasing of particle size. The sample of the particle size 0.5 mm

exhibits maximum adsorption capacities and this may be due to the larger surface area of the adsorbent material. The extent of adsorption is considerably higher in the case of chitosan sample of small particle size with lower of molecular weight and higher of DD because chitosan with the highest DD containing the biggest amount of free $-NH_2$ groups.

Table 2. Sorption capacity of metals onto chitosan sorbents. Experimental conditions: $C_0 = 10$, 20 and 30 mg/L; sorbent dose = 25mg/25 mL.

Type of Chitosan	Sorption capacity (mg/g)			
	Zn	Cd	Pb	Cu
$C_0 = 10$ mg/L				
type 1	9.9±0.27	9.9±0.25	9.8±0.20	9.7±0.25
type 2	9.6±0.47	9.9±0.14	9.8±0.36	9.7±0.13
type 3	9.2±0.19	9.9±0.16	9.5±0.14	9.5±0.20
$C_0 = 20$ mg/L				
type 1	18.8±0.20	19.8±0.20	19±0.26	19±0.38
type 2	18.2±0.19	19.8±0.20	18.6±0.18	18.4±0.23
type 3	18±0.20	19.8±0.23	18.4±0.13	18±0.23
$C_0 = 30$ mg/L				
type 1	27±0.23	29.37±0.20	27±0.22	27.6±0.13
type 2	26.1±0.20	27.9±0.20	26.4±0.25	27±0.23
type 3	25.5±0.23	27±0.30	24±0.23	25.5±0.38

The metal ion sorption capacity by different molecular weight of chitosan is directly related to their specific surface area and the functional groups which induce negative charge on the chitosan surface (Eric Guibal *et al*. 2014).

In general, chitosan is known to be an excellent metals ligand, forming stable complexes with many metal ions by coordination (No *et al*., 1989; Muzzarelli and Tanfani, 1982), this because of the regular distribution of amino groups which has pKa 6.3 (Udaybhaskar *et al*., 1990) in the polymer chain (No *et al*., 1989), which are considered the major effective binding sites for metal ions. The nitrogen electrons present in the amino groups can establish dative bonds with transition metal ions and some hydroxyl groups in these biopolymers may function as second donors; hence deprotonated hydroxyl group can be involved in the coordination in the metal ions (No and Lee, 1995, Younis *et al*., 2014).

REFERENCES

Amin, H. F.; Ahmed, O. M.; Rasmey, A. M.; Younis, A. M. and Bekhit, A. A. (2018). Effect of technological processing on the safety of Indian mackerel (*Rastrelliger kangurata*) from Suez, Egypt. Egyptian Journal of Aquatic Biology & Fisheries, 22(5): 273- 284.

AOAC, (1990). Official Methods of Analysis, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.

Aranaz, I.; Mengíbar, M.; Harris, R.; Paños, I.; Miralles, B.; Acosta, N.; Galed, G. and Heras, Á. (2009). Functional characterization of chitin and chitosan. Curr. Chem. Biol., 3:203–230.

Brine, C. J. and Austin P. R. (1981). Chitin variability with species and method of preparation. J. Comp. Biochem. Physiol. - Part B: Biochemistry & Molecular Biology, 69 (2): 283-286.

Carbonell, V.; Salvador, A. and Guardia, M. (1992). Literature survey of the on-line preconcentration in flow-injection atomic spectrometric analysis. Fresenius J. Anal Chem., 342(7): 529-537, DOI: 10.1007/BF00321909.

Cooper, C.; Jiang, J. Q. and Ouki, S. (2002). Preliminary evaluation of polymeric Fe- and Al-modified clays as adsorbents for heavy metal removal in water treatment. J. Chem Technol Biotechnol., 77 (5):546-551. DOI: 10.1002/jctb.614

El Zokm, G.M.; Okbah, M.A. and Younis, A.M. (2015). Assessment of Heavy Metals Pollution Using AVS-SEM and Fractionation Techniques in Edku Lagoon Sediments, Mediterranean Sea, Egypt. Journal of Environmental Science and Health, Part A., 50: 1-14.

El-Gendy, A. A.; Mohamed, S. H. and Abd-Elkader, A. H. (2013). Ion exchanger from chemically modified banana leaves. Carbohydr Polym, 96:481–486. https://doi.org/10.1016/j.carbpol.2013.04.031

El-Kassas, H. Y.; Aly-Eldeen, M. A. and Gharib, S. M. (2016). Green synthesis of iron oxide (Fe_3O_4) nanoparticles using two selected brown seaweeds: Characterization and application for lead bioremediation. Acta Oceanol. Sin., 2016.

El-Naggar, M.; Younis, A. M.; Amin, H. F.; Ahmed, O. M. and Mosleh, Y. Y. (2019). Assessment of Polycyclic Aromatic Hydrocarbons and Heavy Metals Contamination in the Egyptian Smoked Herring (Clupeaharengus), Polycyclic Aromatic Compounds, DOI: 10.1080/10406638.2018.1555173.

Eric, G.; Thierry, V. and Ricardo, N. (2014). Metal ion biosorption on chitosan for the synthesis of advanced materials. J Mater Sci., 49:5505–5518.

Jha, I. N.; Iyengar, L. and Rao, A. V. (1988). Removal of Cadmium Using Chitosan. J. Environmental Engineering, 114(4): 962-974. DOI: 10.1061/(ASCE)0733-9372(1988)114:4(962).

Khan, T.; Peh, K. and Chang, H. S. (2002). Reporting Degree of Deacetylation Values of Chitosan: The Influence of Analytical Methods. J. pharmacy and pharmaceutical Sciences, 5(3): 205-212.

Kiran, M. G.; Pakshirajan and K., Das, G. (2018). Heavy metal removal from aqueous solution using sodium alginate immobilized sulfate reducing bacteria: Mechanism and process optimization. Journal of Environmental Management, 218: 486–496.

Knorr, D. (1984). Use of chitinous polymers in food – A challenge for food research & development. J. Food Technology, 38: 85-97.

Kurita, K.; Sannan, T. and Iwakura, Y. (1979). Studies on chitin. VI. Binding of metal cations. J. Applied Polymer Science, 23 (2): 511-515. DOI: 10.1002/app.1979.070230221.

Lerivrey, J.; Dubois, B. and Decock, P. *et al*. (1986). Formation of D-glucosamine complexes with Cu(II), Ni(II) and Co(II) ions. Inorganica chimica acta, 125 (4): 187-190. DOI:10.1016/S0020-1693(00)81209-8.

Li, Q.; Dunn, E. T.; Grandmaison, E. W. and Goosen, M. F. A. (1992). Applications and Properties of Chitosan. J. of Bioactive Compatible Polymers, 7 (4): 370-397. DOI: 10.1177/088391159200700406.

Li, Q.; Dunn, E. T.; Grandmaison, E. W. and Goosen, M. F. A. (1997). Applications and properties of chitosan. In: Applications of Chitin and Chitosan. Technomic Publishing Co. Inc.

Marqués, M. J.; Morales-Rubio, A.; Salvador A. and de la Guardia M. (2001). Chromium speciation using activated alumina microcolumns and sequential injection analysis-flame atomic absorption spectrometry. Talanta, 53 (6): 1229-1239. DOI:10.1016/S0039-9140(00)00616-0.

Meepho, M.; Sirimongkol, W. and Ayawanna J. (2018). Samaria-doped ceria nanopowders for heavy metal removal from aqueous solution. Materials Chemistry and Physics, 214: 56–65.

Micera, G.; Deiana, S. and Dessi, A. (1986). Chitin in nature and technology, New York, NY: Plenum Press., 565 p.

Muzzarelli, R. A. A. (1973). Natural chelating polymers, New York. NY Pergamon Press.

Muzzarelli, R. A. A. (1977). Chitin, New York, NY: Pergamon Press, 309p.

Nessa, F.; Masum, S.h.; Asaduzzaman, M.; Roy, S. K.; Hossain, M. M. and Jahan, M. S., (2010). A Process for the Preparation of Chitin and Chitosan from Prawn Shell Waste. Bangladesh J. Sci. Ind. Res., 45(4), 323-330.

No, H. K. and Lee, M. Y.(1995). Isolation of Chitin from Crab Shell Waste. J. Korean Society of Food Science and Nutrition, 24 (1): 105-113.

No, H. K. and Meyers, S. P. (1995). Preparation and Characterization of Chitin and Chitosan – A Review. J. of Aquatic Food Product Technology, 4(2), 27-52. DOI: 10.1300/J030v04n02_03.

No, H. K.; Lee, S. H.; Park, N. Y. and Meyers, S. P. (2003). Comparison of Physicochemical, Binding, and Antibacterial Properties of Chitosans Prepared without and with Deproteinization Process. J. Agr. Food Chem., 51 (26): 7659-7663. DOI: 10.1021/jf030226w.

No, H. K.; Meyers, S. P. and Lee, K. S. (1989). Isolation and characterization of chitin from crawfish shell waste. J. Agricultural and Food Chemistry, 37(3): 575-579. DOI: 10.1021/jf00087a001.

Pannain, M. C. and Santelli, R.E. (1995). On the spectrophotometric flow injection determination of chromium (VI) in natural waters after on-line preconcentration on activated alumina. Talanta, 42 (11): 1609-1617. DOI:10.1016/0039-9140(95)01613-9.

Rhazi, M.; Desbrieres, J.; Tolaimate, A.; Rinaudo, M.; Vottero, P. and Alagui, A. (2002). Influence of the nature of the metal ions on the complexation with chitosan. Application to the treatment of liquide waste. Eur Polym J., 38:1523–30.

Saha, T.K.; Karmaker, S.; Ichikawa, H. and Fukumori, Y. (2005). Mechanisms and kinetics of trisodium 2-hydroxy-1,1-azonaphthalene-3,4,6-trisulfonate adsorption onto chitosan. J Colloid Interf Sci., 286: 433-9.

Said, T.O.; Farag, R.S.; Younis, A.M. and Shreadah, M.A. (2006). Organotin Species in Fish and Bivalves Samples Collected from the Egyptian Mediterranean Coast of Alexandria, Egypt. Bulletin of Environmental Contamination and Toxicology, 77: 451-458.

Schmuhl, R.; Krieg, H. M. and Keizer, K. (2001). Adsorption of Cu(II) and Cr(VI) ions by chitosan: Kinetics and equilibrium studies. Water SA, 27 (1):1–7.

Shreadah, M.A.; Said, T.O., Younis, A.M. and Farag, R.S. (2006). Speciation of Organotin Compounds in Sediments of Semi-Closed Areas along the Mediterranean Coast of Alexandria," Chemistry and Ecology, 22 (5): 395-404.

Sobahi, T. R.A.; Abdelaa, M. Y. l. and Makki, M. S.I. (2014). Chemical modification of Chitosan for metal ion removal. Arabian Journal of Chemistry, 7: 741–746.

Soliman, N.F.; Younis, A.M. and Elkady, E.M. (2019). An insight into fractionation, toxicity, mobility and source apportionment of metals in sediments from El Temsah Lake, Suez Canal. Chemosphere 222: 165-174.

Soliman, N. F.; Younis, A. M.; Elkady, E. M. and Mohamedein, L.I. (2018). Geochemical associations, risk assessment, and source identification of selected

metals in sediments from the Suez Gulf, Egypt. Hum Ecol Risk Assess, 1-17. DOI: 10.1080/10807039.2018.1451301

Hwang, S. K. and Jho, E. H. (2018). Heavy metal and sulfate removal from sulfate-rich synthetic mine drainages using sulfate reducing bacteria. Science of the Total Environment, 635:1308–1316.

Udaybhaskar, P.; Iyengar, L. and Prabhakara Rao, A. V. S. (1990). Hexavalent chromium interaction with chitosan. J. Applied Polymer Science, 39 (3): 739-747. DOI: 10.1002/app.1990.070390322.

Wu, F. C.; Tseng, R. L.; and Juang, R. S. (2001). Enhanced abilities of highly swollen chitosan beads for color removal and tyrosinase immobilization. J. Hazardous Materials, 81 (1-2): 167-177. DOI: 10.1016/S0304-3894(00)00340-X.

Yaghobi, N. (2012). Controlling chitosan's molecular weight via multistage deacetylation. Biotechnol. Bioprocess Eng. 17: 812–817

Younis, A.M.; El-Zokm, G.M. and Okbah, M.A. (2014). Spatial Variation of Acid-Volatile Sulfide and Simultaneously Extracted Metals in Egyptian Mediterranean Sea Lagoon Sediments. Environmental Monitoring and Assessment, 186: 3567-3579. http://dx.doi.org/10.1007/s10661-014-3639-3

Younis, A.M.; Kolesnikov, A.V. and Desyatov, A.V. (2014). Efficient Removal of La(III) and Nd(III) from Aqueous Solutions Using Carbon Nanoparticles. American J. Anal. Chem., 5: 1273-1284. http://dx.doi.org/10.4236/ajac.2014.517133.

Younis, A. M. (2018). Environmental Impacts on Egyptian Delta Lakes' Biodiversity: A Case Study on Lake Burullus. A.M. Negm *et al*. (eds.), Egyptian Coastal Lakes and Wetlands: Part II – Climate Change and Biodiversity, Hdb Env Chem, DOI 10.1007/698_2017_120.

Younis, A.M. and Nafea, S. M. (2012). Impact of Environmental Conditions on the Biodiversity of Mediterranean Sea Lagoon, Burullus Protected Area, Egypt. World Applied Sciences Journal, 19: 1423-1430.

Younis, A.M.; Soliman Y. A.; Elkady E. M. and El-Naggar M. H. (2018). Assessment of polycyclic aromatic hydrocarbons in surface sediments and some fish species from the Gulf of Suez, Egypt. Egyptian Journal of Aquatic Biology & Fisheries, 22(4): 49- 59.