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Removal of Heavy Metal from Contaminated Water by Biopolymer Crab Shell Chitosan

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Abstract: The study focuses on potential of using crab shell chitosan as a low-cost biosorbent, for heavy metals removal from aqueous solutions in an adsorption filtration system. Chitosan was synthesized from chitin by the treatment of strong alkali solution under reflux condition and chitin was extracted from crab shell followed by decalcification and deproteinization treatment. Spectrometric (AA and UV) method was employed to detect the heavy metals concentration. Prepared 10 mg L^{-1} solutions of zinc, lead, cadmium, cobalt, nickel, chromium, manganese and iron were passed through the 5 g of chitosan separately and it was found that chitosan was excellent adsorbent in removing mentioned heavy metals. The removal efficiency of chitosan was in the following order Mn>Cd>Zn>Co>Ni>Fe>Pb>Cr. The result also showed that the time required for 100% breakthrough of prepared chitosan for Mn and Zn was approximately 27 and 23 h whereas it was only 16 h for both Cr and Pb, respectively. The research revealed that prepared chitosan showed better removal performance for Mn, Cd, Zn whereas the removal efficiency was satisfactory for Co, Ni and Fe but it exhibited relatively least performance for Pb and Cr.

Key words: Bioremediation, environmental pollution, metal chelating agent, toxic materials, sorption mechanism

INTRODUCTION

Contamination of diverse water resources by heavy metal is a great concern because of their detrimental effect on human, animal and plants (Li and Bai, 2006; Sharma et al., 2008). Heavy metals are very harmful because of their non-biodegradable nature, long biological half-lives and their potentiality to be accumulated in different body parts (Manaham, 2005; Wilson and Pyatt, 2007). It is well known that some heavy metals which are essential component in metabolism are toxic when present in high concentration (Gazso, 2001). Trace metal like iron, copper, zinc, cobalt and manganese are essential metals since they play important role in biological systems (Mashi et al., 2004; Garcia et al., 2005). However, some research also confirmed that heavy metals such as Cd, Pb, Cu, Zn and Ni have carcinogenic or toxic effects on human beings and environment (Trichopoulos, 2001; Turkdogan et al., 2003; Kocasoy and Sahin, 2007). Heavy metals enter in the environment through a wide variety of sources, including combustion, wastewater discharges and manufacturing facilities (Jarup, 2003;

Singh et al., 2004). Mining and processing of metal ore can be a significant source of heavy metal contamination of the environment (Dudka and Adriano, 1997; Navarro et al., 2008). One of the major routes of the excessive accumulation of heavy metals in agricultural soil is wastewater irrigation that adulterates the soil and later affects the food quality and safety (Muchuweti et al., 2006). Other potential sources of entering the heavy metals to the human body are inhalation and ingestion through the plants produced in contaminated areas (Tripathi et al., 1999; Mendil, 2006). It is observed that serious systemic health problems can develop as a result of excessive dietary accumulation of heavy metals such as Cd and Pb in human body (Oliver, 1997).

In recent years, biosorption by biologically originated materials in removing heavy metals has drawn more and more attention, largely due to the unique properties of these biomaterials being environmentally benign, low cost, effective at low metal concentrations and easily reusable. Chitosan attracted particular consideration because of its capability to adsorb various heavy metal ions chemically or physically (De Castro Dantas et al.,

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2001;Vincent and Guibal, 2001; Rhazi *et al.*, 2002). The nitrogen in the amino group of the chitosan molecule acts as an electron donor and is presumably responsible for selective chelation with metal ions (Bassi *et al.*, 1999; Pradhan *et al.*, 2005). Chitosan can be produced easily from Chitin, poly-β-(1, 4)-*N*-acetyl-D-glucosamine and it is the most common polysaccharide after cellulose found in the nature extracted from crustacean shells, such as prawns, crabs, insects and shrimps (Xu *et al.*, 2008; Xu-fen *et al.*, 2007; Rinaudo, 2006).

Several processes have been developed and used for removing metals dissolved in industrial wastewater over the years. The techniques are: chemical precipitation, ion exchange, membrane filtration, solvent extraction, or adsorption (Chiron *et al.*, 2003; Gupta and Ali, 2004; Molinari *et al.*, 2004). Conventionally, precipitation is the most common technique for removal of metals, but this method leads to the production of toxic sludge. On the other hand, solvent extraction technique is not suitable for effluents containing less than 1 g L^{-1} of targeted heavy metals and ion exchange processes are too expensive due to the high cost of synthetic resins (Selatnia *et al.*, 2004). In consequence, new separation methods are required to reduce heavy metal concentrations to environmentally acceptable levels at affordable cost (Klimmek *et al.*, 2001; Han *et al.*, 2006). Adsorption filtration method is preferred for heavy metals removal in small-scale treatment system because of its simplicity, ease of operation and handling, regeneration capacity and sludge free operation (Thirunavukkarasu *et al.*, 2003; Nurul *et al.*, 2006).

Although, a number of researchers are available on removal of heavy metals by chitosan from other sources but very few researches have been reported on crab shell chitosan. Therefore, the current study examines the capability of chitosan obtained from crab shell for removing toxic heavy metals by adsorption filtration method.

MATERIALS AND METHODS

Preparation of chitosan from crab shell: The research project was conducted from February, 2006 to November, 2007, in the Department of Chemistry, Jahangirnagar University, Dhaka, Bangladesh. The raw materials i.e., crab shell was collected from Bio-Chemical and Seafood Export Co., Dhaka, Bangladesh. It was then dried, crushed and decalcified by treatment with 10% HCl, the acid being changed everyday. The dorsal cover of the shell was peeled off on the third day. Its inner layer was removed by rubbing it away with the fingers. Thereafter, the rest of bone was broken into smaller bits. Decalcification was

completed in about four days which can be represented in the following reaction.

$$2HCl + CaCO_3 = CO_2 + Ca^{2+} + 2Cl^- + H_2O$$
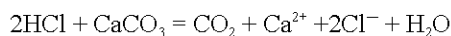
The resulting chitin protein complex of the dorsal cover was deproteinized by the treatment of 10% (w /v) NaOH at 103 to 105°C in an autoclave. The septa region was deproteinized in 6 h treatment under similar conditions.

Several studies confirmed that chitosan, a poly-*N*-acetylglucosamine, is obtained by the deacetylation of chitin (Crini and Badot, 2008; Orrego and Valencia, 2009; Dambies *et al.*, 2000). To follow this process chitin shells collected from crab shell were dried and again crushed and boiled successively with 5% HCl for several hours then it was filtered in a Buckner funnel. The solid was added to sodium hydroxide (20% aqueous solution) and refluxed for 4 h and was filtered. The reaction mass was finally added to the deionized water and was again boiled for several hours. After cooling and washing, it was finally dried at 105°C. The powder thus obtained was boiled with 3 N acetic acid and the solution was cooled and filtered. The resulting slurry was neutralized with 15% KOH solution in a large beaker and the precipitate was then filtrated and thoroughly washed with hot water and deionized water. This resulting chitosan precipitates was dried at 105°C and ground to pass through a US standard 40-mesh screen. The individual particle size of this material ranges between 0.32 and 2.0 mm. The IR spectrum of prepared chitosan was taken in KBr disc.

Chemicals used for metal solution: Different metal solutions were prepared for passing through the column packed with crab shell chitosan and mother solution of 100 mg L^{-1} of zinc, nickel, iron, lead, cadmium, manganese and 200 mg L^{-1} chromium were prepared from zinc chloride, nickel chloride, iron (III) chloride, lead chloride, cadmium chloride, manganese (II) sulphate and chromium (III) sulphate, respectively. All chemicals used were Mark analytical grade including standard stock solution of known concentrations of different heavy metals.

Column preparation and calculation of adsorbed amount: Eight columns were made with 5 g uniformly grained prepared Chitosan and the prepared 10 mg L^{-1} each heavy metal solution was passed through the columns until the break through volume as well as saturation volume of the materials was reached. Water samples that passed through the columns were collected in the sample bottles after several times interval. The flow rate of the columns

Table 1: Analytical condition for measurement of various elements in water using AAS

Elements	Wave length (nm)	Slit (nm)	Lamp current (mA)	Calibration range ($\mu g\ L^{-1}$)	Detection limited ($\mu g\ L^{-1}$)
Fe	248.3	0.2	12	0.0-3.0	0.050
Zn	213.9	0.2	8	0.0-1.6	0.005
Cd	228.8	0.5	8	0.0-1.2	0.016
Pb	283.3	0.5	10	0.0-3.0	0.060
Cr	357.9	0.5	10	0.0-2.0	0.010
Ni	232.0	0.2	12	0.0-3.0	0.015
Co	240.7	0.5	10	0.0-1.0	0.030

were measured 3.5 mL min^{-1}. The amount of metal adsorbed in each volume interval was calculated from the following equation.

$$\text{Total amount adsorbed} = X_p + (10\text{-}C) \times V \times 10^{-3}$$

where, X_p is the previous absorbed amount, C is the concentration in volume (V) and V is the volume interval that passed through the column.

Heavy metal analysis: Heavy metals (Fe, Zn, Cd, Pb, Cr, Ni and Co) analyses were carried out using Atomic Absorption Spectrophotometer (SHIMADZU AA-6800). The AAS was calibrated for all the metals by running different concentrations of standard solutions. Average values of three replicates were taken for each determination. Quantitative estimation of Mn in the digested samples was carried out using UV Spectrophotometer (Sequoia-Tuner, Model-390) following permanganate oxidation method at 522 nm (Charlot, 1964). Because of its distinctive color and stability, permanganate ion is preferably used as the determination form for manganese. For the measurement of all metals in water samples, analytical conditions are very essential to get accurate result and they are summarized in Table 1.

RESULTS AND DISCUSSION

Infrared Spectra (IR) was taken for both chitin and chitosan. In the IR of chitin and chitosan all the stretching frequencies for the different functional groups were almost same. But the stretching frequency of C = O was missing in the spectrum of the prepared chitosan. Secondary amide in the solid state usually showed the C = O absorption near 1640 cm^{-1} but in the spectrum of prepared chitin two strongly perpendicular bands were observed near the frequency at 1652 cm^{-1}.

The findings of the present research revealed that chitosan can successfully remove manganese from contaminated water. 10934.5 mg kg^{-1} of manganese was passed through the prepared chitosan composite and it was found that the amount of manganese which absorbed by chitosan was 8350.8 mg kg^{-1} and manganese removal

efficiency of chitosan was 76.3% (Table 2). Similar performance was also observed with cadmium and zinc. For cadmium and zinc, 9183.9 and 9625.4 mg kg^{-1} were passed through the chitosan and the amount adsorbed by them was 6949.0 and 6301.6 mg kg^{-1} which indicates that the removal efficiency of chitosan for Cd and Zn was 75.6 and 65.4%, respectively (Table 2). The removal efficiency of chitosan for nickel, iron and cobalt was found nearly 58%. Again for passing of Ni 8164.4 mg kg^{-1}, Fe 8144.3 mg kg^{-1} and Co 8032.0 mg kg^{-1} through the chitosan, the adsorption amount of metals was 4710.4, 4683.7 and 4712.4 mg kg^{-1}, respectively (Table 2). Furthermore, it is found that the removal performance of Pb and Cr was relatively lower than all the other heavy metals, which were about 43.6 and 36.5% respectively. After passing 6183.3 and 6668.6 mg kg^{-1} of Pb and Cr through crab shell chitosan the study showed that the amount of Pb and Cr absorbed by chitosan was 2699.3 and 2436.8 mg kg^{-1}, respectively (Table 2).

The effect of flow rate was also a significant factor on prepared chitosan. The influent (Co) and effluent (Ce) concentrations were calculated for each heavy metal (Chen and Chung, 2006). For calculating the 100% breakthrough time of chitosan for each heavy metal, the Ce/Co was plotting against different time interval in Fig. 1. It is observed in Fig. 1 that crab shell chitosan can remove 100% of Mn and Cd up to about 14 and 11 h, respectively. After 10 h the influent and effluent ratio of Mn, Cd, Zn, Co, Ni, Fe, Pb and Cr was 0.001, 0.020, 0.132, 0.322, 0.467, 0.354, 0.594 and 0.612, respectively (Fig. 1). Approximately 27 h was required to reach 100% breakthrough of prepared chitosan for Mn whereas 16 h was required for both Cr and Pb (Fig. 1). Furthermore, the complete breakthrough time for Fe, Ni and Co was nearly 20 h whereas for Zn and Cd it was 23 and 22 h, respectively (Fig. 1a-h).

The IR spectra of chitosan were compared with the IR spectra of chitin. The Infrared spectra clearly show a marked change due to the removal of acetyl groups from the amino nitrogen. The C = O stretching frequency at 1640 cm^{-1} was missing in the spectrum of chitosan which was obtained from deacetylation of the chitin but two bands occurred at 1652 cm^{-1} in chitin that is supported by the observation of Shin *et al.* (2001). This band is due to the antisymmetric deformational vibration due to the formation –NH$_3^+$ because chitin is dissolved in HCl during the reaction. The band appearing at 1521 cm^{-1} may be assigned to the symmetric NH$_3^+$ deformational mode. In addition, the IR spectrum of the prepared Chitosan was confirmed from pure Chitosan. The physical and chemical property of the prepared chitosan was compared with the original (97% pure) chitosan and it was observed that this

Table 2: Heavy metal removing performance of biopolymer chitosan

Absorbent (mg)	Initial conc. (mg L^{-1})	Volume passed	Mn Conc. (mg L^{-1})	Mn Absorbed (mg)	Cd Conc. (mg L^{-1})	Cd Absorbed (mg)	Zn Conc. (mg L^{-1})	Zn Absorbed (mg)	Ni Conc. (mg L^{-1})	Ni Absorbed (mg)
5	10	50	0.00	0.50	0.1087	0.4940	0.3354	0.4832	1.0341	0.4480
		100	0.00	1.00	0.1095	0.9880	0.5296	0.9567	1.1128	0.8960
		350	0.00	3.50	0.1288	3.4550	0.6256	3.3003	1.5237	3.0150
		600	0.00	5.9980	0.1381	5.9890	0.6566	5.6353	1.5813	5.1190
		850	0.0070	8.4950	0.1421	8.4530	0.7561	7.9453	2.0599	7.1040
		1100	0.0090	10.9920	0.1419	10.9170	0.9163	10.2153	2.0900	9.0810
		1350	0.0100	13.4880	0.1613	13.3760	1.0342	12.4563	2.4373	11.0610
		1600	0.0130	15.9840	0.1851	15.8290	1.1268	14.6743	2.5372	13.0260
		1850	0.0150	18.4790	0.1969	18.2790	1.3516	16.8363	3.0331	14.7670
		2100	0.0180	20.9740	0.2070	20.7270	1.3240	19.0053	3.1299	16.4840
		2350	0.0200	23.4690	0.2108	23.1740	1.4609	21.1393	3.6892	18.2390
		2600	0.0532	25.9557	0.7321	25.4910	1.9612	23.1490	4.6715	19.5711
		2850	0.0859	28.4342	1.0952	27.7172	2.8378	24.8585	5.3649	20.7299
		3100	1.1956	30.6353	2.4356	29.6083	3.8196	26.4036	6.1593	21.6901
		3350	1.9867	32.6387	3.3216	31.2779	4.9032	27.6778	6.9876	22.4432
		3600	2.5760	34.4947	4.5638	32.6369	5.0798	28.9078	7.8232	22.9874
		3850	3.3218	36.1643	5.8291	33.6796	5.9632	29.9170	8.7837	23.5415
		4100	4.1295	37.6319	7.1644	34.3885	6.8632	30.7012	9.9567	23.5523
		4350	5.0325	38.8738	8.5912	34.7407	7.9501	31.2164	-	-
		4600	5.9862	39.8772	9.9825	34.7451	8.9100	31.4889	-	-
		4850	6.6788	40.7075	-	-	9.9231	31.5081	-	-
		5100	7.5289	41.3253	-	-	-	-	-	-
		5350	8.5216	41.6949	-	-	-	-	-	-
		5600	9.7630	41.7542	-	-	-	-	-	-

Absorbent (mg)	Initial conc. (mg L^{-1})	Volume passed	Fe Conc. (mg L^{-1})	Fe Absorbed (mg)	Pb Conc. (mg L^{-1})	Pb Absorbed (mg)	Cr Conc. (mg L^{-1})	Cr Absorbed (mg)	Co Conc. (mg L^{-1})	Co Absorbed (mg)
5	10	50	1.4083	0.4330	1.8375	0.4081	4.0632	0.2960	0.7270	0.4630
		100	1.3382	2.4760	1.8955	0.8133	4.5019	0.5700	1.2200	0.9020
		350	1.8243	4.4640	2.4176	2.7089	5.1846	1.7730	1.4092	3.0490
		600	2.0448	6.4030	3.5809	4.3137	5.3055	2.9460	1.8573	5.0840
		850	2.2403	8.2560	3.5355	5.9299	5.3833	4.1000	1.8598	7.1190
		1100	2.5861	9.9770	4.2563	7.3658	5.4956	5.2260	2.1063	9.0920
		1350	3.1124	11.6950	4.7557	8.6769	5.4506	6.3630	2.3229	11.0110
		1600	3.1274	13.3830	4.7670	9.9851	5.5389	7.4780	2.6366	12.8510
		1850	3.2477	14.9970	5.1075	11.2082	6.0452	8.4660	3.0325	14.5920
		2100	3.5434	16.6550	5.9418	12.2227	6.1221	9.4350	3.2242	16.2850
		2350	3.8631	18.1892	7.2186	12.9180	6.4850	10.3130	3.4333	17.9260
		2600	4.3858	19.5928	8.4964	13.2939	6.9479	11.0760	3.9893	19.4287
		2850	5.2106	20.7901	9.2156	13.4900	7.4852	11.7047	4.6415	20.7683
		3100	5.9861	21.7936	9.9732	13.4967	8.1290	12.1725	5.7493	21.8310
		3350	6.8632	22.5778	-	-	9.9532	12.1842	6.8003	22.6310
		3600	7.8912	23.1050	-	-	-	-	7.6942	23.2074
		3850	8.8132	23.4017	-	-	-	-	8.7852	23.5111
		4100	9.9321	23.4187	-	-	-	-	9.7952	23.5623
		4350	-	-	-	-	-	-	-	-
		4600	-	-	-	-	-	-	-	-
		4850	-	-	-	-	-	-	-	-
		5100	-	-	-	-	-	-	-	-
		5350	-	-	-	-	-	-	-	-
		5600	-	-	-	-	-	-	-	-

pure chitosan is inactive in both acid and base but the prepared chitosan is slightly soluble in acid and it is basic impurity as $CaCO_3$ was present in a large scale.

From the present research, it is observed that the sorption capacity of crab shell chitosan was decreased in the following order Mn>Cd>Zn>Co>Ni>Fe>Pb>Cr. There are very few studies available on the removal of heavy metal by crab shell chitosan however the chitosan produced from Fly Larva Shells (FLS) revealed that the sorption ability was decreased in order of Cu>Mn>Ni>Zn>Pb>Fe (Gyliene *et al.*, 2002). This study also noted that chitin and chitosan produced from FLS act as sorbents for free metal ions up to 600-800 mmol kg^{-1}. Previous research by An *et al.* (2001) found that in case of Cd the removal capacity of crab shell of *Chinoneceetes opilio* was considerably higher than the other sorbents such as cation exchange resin, zeolite, granular activated carbon, powdered activated carbon. Another study concluded that the chitosan prepared for crab shell has a cadmium uptake capacity (on a per weight basis) greater
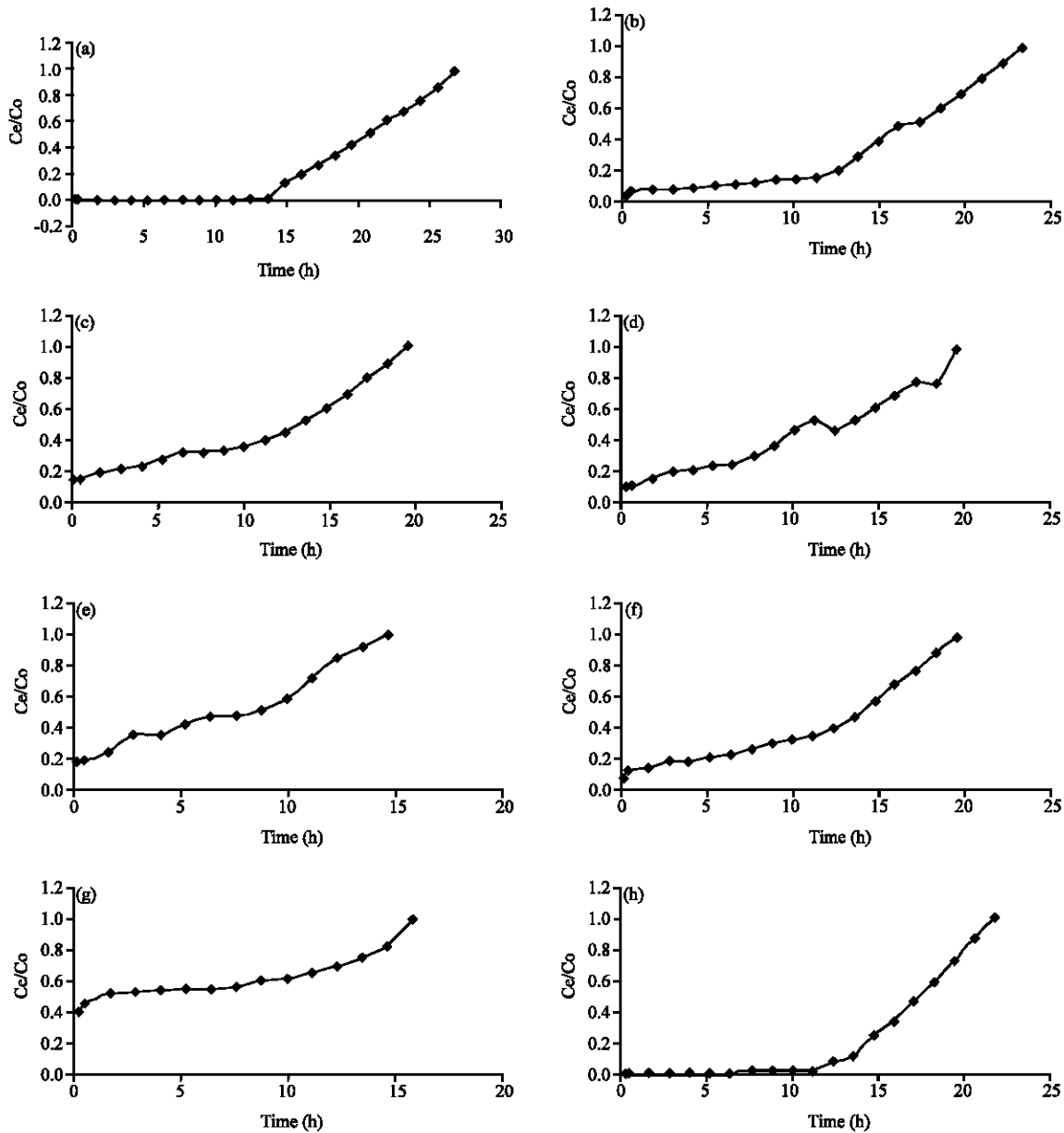
Fig. 1: Effect of flow rates on each metal adsorption by crab shell chitosan packed column, (a) Mn, (b) Zn, (c) Fe, (d) Ni, (e) Pb, (f) Co, (g) Cr and (h) Cd

than porous alumina, hydrous ferric oxide and nonporous chitosan (Evans *et al.*, 2002). For an equilibrium aqueous concentration of 10 mg kg^{-1} cadmium, cadmium uptake by porous alumina ranges from 20000 to 50000 mg kg^{-1} (Papelis *et al.*, 1995) and 8000 mg kg^{-1} by hydrous ferric oxide (Dzombak and Morel, 1990).

Lower removal capability of chitosan for chromium and lead might be due to relative weak chelating ability of chitosan on chromium and lead which is in good agreement with results reported earlier by Kartal and

Imamura (2005). However, chitosan coated AOPSC (acid treated oil palm shell charcoal) exhibited better performance on removing chromium than uncoated crab shell chitosan and it is shown that the adsorption capacity of chitosan coated AOPSC for chromium was 15400 mg kg^{-1} (Nomanbhay and Palanisamy, 2005). Similar trend was observed for chromium for other adsorbent (5000-12000 mg kg^{-1}) (Schmuhl *et al.*, 2001).

To estimate the each adsorption amount of heavy metals on chitosan materials, influent (Co) and effluent

(Ce) concentration were calculated and it was observed that initially the adsorption amount of metals on the prepared chitosan was expectedly high and it was gradually decreased over time in Fig. 1. However, the adsorption amount was not uniformly decreased with time and a probable reason was that the passing rate of the prepared heavy metal solution through the column was not same. Surface area and contact time also played a vital role for the adsorption of heavy metals in prepared chitosan. The shape of the uptake isotherms (Fig. 1) showed that the uptake process is a complex one. Simple ion adsorption on the chitosan surface does not appear to be the dominant mechanism as is inferred from literature. It is concluded from the previous work where under moderate magnification, metal containing aggregates were observed on the polymer (Rana *et al.*, 2009). So a possible uptake mechanism is the formation of metal-containing nodules on the polymer surface.

Furthermore, absorption presumably by diffusion of metal ions into the polymer is also playing a part in the total process. Further chitosan embedded or laced with calcium carbonate in the composite materials may create some cryptand sites of right size so that some metals are simply caged at those sites. Thus, a combination of nodular formation, cryptand site formation, ion adsorption and ion absorption account for the total uptake. The greater uptake is attributed due to the free amino group of chitosan having its lone pair electrons can act as a specific chemical bonding sites for metal ions capable of forming complex ions. Research by Guibal (2004) also confirmed that metal cations can be adsorbed by chelation on amine groups of chitosan in near neutral solutions. The present research is also strongly support the study of Pradhan *et al.* (2005) that the high adsorption capacity could be ascribed by the following factors: (1) the high hydrophilicity of chitosan with a large number of hydroxyl groups; (2) the large number of primary amino groups with high activity and (3) the flexible structure of the polymer chains of chitosan, which enables a suitable configuration for complexation with metal ions.

CONCLUSION

The study showed the first details study of crab shell chitosan, an abundant low-cost biopolymer, for removing hazardous trace elements. The removal performance of chitosan was not found same for all the studied heavy metals, it was better for manganese, zinc and cadmium whereas satisfactory level was not observed for lead and chromium. In addition, the study concluded that the use crab shell chitosan for heavy metals removal appears to be technically feasible, eco-friendly and with high efficacy. However, further studies are needed to unveil the complete removal mechanism of chitosan for heavy metals.

REFERENCES

An, H.K., B.Y. Park and D.S. Kim, 2001. Crab shell for the removal of the heavy metals from aqueous solution. Water Res., 35: 3551-3556.

Bassi, R., S.O. Prasher and B.K. Simpson, 1999. Effects of organic acids on the adsorption of heavy metal ions by chitosan flakes. J. Environ. Sci. Health A, 34: 289-294.

Charlot, G., 1964. Colorimetric Determination of Elements (Principles and Method). 1st Edn., American Elsevier Publishing Company, Inc., USA., ISBN: 0444401040.

Chen, C.C. and Y.C. Chung, 2006. Arsenic removal using a biopolymer chitosan sorbent. J. Environ. Sci. Health, 41: 645-658.

Chiron, C., R. Guilet and E. Deydier, 2003. Adsorption of Cu (II) and Pb (II) onto a grafted silica: Isotherms and kinetic models. Water Res., 37: 3079-3086.

Crini, G. and P.M. Badot, 2008. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. Progress Polym. Sci., 33: 399-447.

Dambies, L., E. Guibal and A. Roze, 2000. Arsenic (V) sorption on molybdate-impregnated chitosan beads. Colloids Surfaces A Physicochem. Eng. Aspects, 170: 19-31.

De Castro Dantas, T.N., A.A. Dantas Neto, A. De, M.C.P. Moura, E.L. Barros Neto and E. de Paiva Telemaco, 2001. Chromium adsorption by chitosan impregnated with microemulsion. Langmuir, 17: 4256-4260.

Dudka, S. and D.C. Adriano, 1997. Environmental impacts of metal ore mining and processing: A review. J. Environ. Qual., 26: 590-602.

Dzombak, D.A. and F.M.M. Morel, 1990. Surface Complexation Modeling: Hydrous Ferric Oxide. Wiley, New York, ISBN: 978-0-471-63731-8.

Evans, J.R., W.G. Davids, J.D. MacRae and A. Amirbahman, 2002. Kinetics of cadmium uptake by chitosan-based crab shells. Water Res., 36: 3219-3226.

Garcia, J.C.R., J.B. García, C.H. Latorre, S.G. Martin and R.M.P. Crecente, 2005. Direct and combined methods for the determination of chromium, copper and nickel in honey by electrothermal atomic absorption spectroscopy. J. Agric. Food Chem., 53: 6616-6623.

Gazso, L.G., 2001. The key microbial processes in the removal of toxic metals and radionuclides from the environment. Central Eur. J. Occup. Environ. Med., 7: 178-185.

Guibal, E., 2004. Interactions of metal ions with chitosan-based sorbents: A review. Separation Purification Technol., 38: 43-74.

Gupta, V.K. and I. Ali, 2004. Removal of lead and chromium from wastewater using bagasse fly ash-a sugar industry waste. J. Colloid Interface Sci., 271: 321-328.

Gyliene, O., R. Rekertas and M. Salkauskas, 2002. Removal of free and complexed heavy-metal ions by sorbents produced from fly (*Musca domestica*) larva shells. Water Res., 36: 4128-4136.

Han R., W. Zou, Z. Zhang, J. Shi and J. Yang, 2006. Removal of copper (II) and lead (II) from aqueous solution by manganese oxide coated sand. I. Characterization and kinetic study. J. Hazardous Mater., 137: 384-395.

Jarup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull., 68: 167-182.

Kartal, S.N. and Y. Imamura, 2005. Removal of copper, chromium and arsenic from CCA-treated wood onto chitin and chitosan. Bioresou. Technol., 96: 389-392.

Klimmek, S., H.J. Stan, A. Wilke, G. Gunke and R. Buchholz, 2001. Comparative analysis of the biosorption of cadmium, lead, nickel and zinc by alga. Environ. Sci. Technol., 35: 4283-4288.

Kocasoy, G. and V. Sahin, 2007. Heavy metal removal from industrial wastewater by clinoptilolite. J. Environ. Sci. Health Part A, 42: 2139-2146.

Li, N. and R. Bai, 2006. Development of chitosan-based granular adsorbents for enhanced and selective adsorption performance in heavy metal removal. Water Sci. Technol., 54: 103-113.

Manaham, S.E., 2005. Environmental Chemistry. 8th Edn., Lewis Publisher, Boca Raton, Florida, ISBN 1566706335.

Mashi, S.A., S.A. Yaro and A.S. Haiba, 2004. Cu, Mn, Fe and Zn levels in soils of shika area Nigeria. Biomed. Environ. Sci., 17: 426-431.

Mendil, D., 2006. Mineral and trace metal levels in some cheese collected from Turkey. Food Chem., 96: 532-537.

Molinari, R., T. Poerio, R. Cassano, N. Picci and P. Argurio, 2004. Copper (II) removal from wastewaters by a new synthesized selective extractant and SLM viability. Ind. Eng. Chem. Res., 43: 623-628.

Muchuweti, M., J.W. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J.N. Lester, 2006. Heavy metal content of vegetables irrigated with mixture of wastewater and sewage sludge in Zimbabwe: Implications for human health. Agric. Ecosyst. Environ., 112: 41-48.

Navarro, M.C., C. Perez-Sirvent, M.J. Martinez-Sanchez, J. Vidal, P.J. Tovar and J. Bech, 2008. Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone. J. Geochem. Explorat., 96: 183-193.

Nomanbhay, S.M. and K. Palanisamy 2005. Removal of heavy metal from industrial wastewater using chitosan coated oil palm shell charcoal. Elect. J. Biotechnol., 8: 43-53.

Nurul, A.M., K. Satoshi, K. Taichi, B. Aleya and K. Hideyuki *et al.*, 2006. Removal of arsenic in aqueous solutions by adsorption onto waste rice husk. Ind. Eng. Chem. Res., 45: 8105-8110.

Oliver, M.A., 1997. Soil and human health: A review. Eur. J. Soil Sci., 48: 573-592.

Orrego, C.E. and J.S. Valencia, 2009. Preparation and characterization of chitosan membranes by using a combined freeze gelation and mild crosslinking method. Bioprocess Biosyst. Eng., 32: 197-206.

Papelis, C., P.V. Roberts and J.O. Leckie, 1995. Modeling the rate of cadmium and selenite adsorption on micro-and mesoporous transition aluminas. Environ. Sci. Technol., 29: 1099-1108.

Pradhan, S., S.S. Shukla and K.L. Dorris, 2005. Removal of nickel from aqueous solutions using crab shells. J. Hazardous Mater. B, 125: 201-204.

Rana, M.S., M.A. Halim, S.A.M. Waliul Hoque, K. Hasan and M.K. Hossain, 2009. Bioadsorption of arsenic by prepared and commercial crab shell chitosan. Biotechnology, 8: 160-165.

Rhazi, M., J. Desbrieres, A. Tolaimate, M. Rinaudo, P. Vottero, A.Alagui and M.E. Meray, 2002. Influence of the nature of the metal ions on the complexation with chitosan-Application to the treatment of liquid waste. Eur. Polymer J., 38: 1523-1530.

Rinaudo, M., 2006. Chitin and chitosan: Properties and applications. Progress Polym. Sci., 31: 603-632.

Schmuhl, R., H.M. Krieg and K. Keizer, 2001. Adsorption of Cu (II) and Cr (VI) ions by chitosan: Kinetics and equilibrium studies. Water SA, 27: 1-8.

Selatnia, A., A. Boukazoula, N. Kechid, M.Z. Bakhti, A. Chergui and Y. Kerchich, 2004. Biosorption of lead (II) from aqueous solution by a bacterial dead Streptomyces rimosus biomass. Biochem. Eng. J., 19: 127-135.

Sharma, R.K., M. Agrawal and F.M. Marshall, 2008. Heavy metal (Cu, Zn, Cd and Pb) contamination of vegetables in urban India: A case study in Varanasi. Environ. Pollut., 154: 254-263.

Shin, Y., D.I. Yoo and J. Jang, 2001. Molecular weight effect on antimicrobial activity of chitosan treated cotton fabrics. J. Applied Polym. Sci., 80: 2495-2501.

Singh, K.P., D. Mohon, S. Sinha and R. Dalwani, 2004. Impact assessment of treated/untreated waste water toxicants discharge by sewage treatment plants on health, agricultural and environmental quality in waste water disposal area. Chemosphere, 55: 227-255.

Thirunavukkarasu, O.S., T. Viraraghavan and K.S. Subramanian, 2003. Arsenic removal from drinking water using iron oxide-coated sand. Water Air Soil Pollut., 42: 95-111.

Trichopoulos, D., 2001. Epidemiology of Cancer. In: Cancer: Principles and Practice of Oncology, DeVita, V.T. (Ed.). Lippincott Company, Philadelphia, ISBN: 0-781-72229-2, pp: 231-258.

Tripathi, R.M., R. Raghunath, V.N. Sastry and T.M. Krishnamoorthy, 1999. Daily intake of heavy metals by infants through milk and milk products. Sci. Total Environ., 227: 229-235.

Turkdogan, M.K., F. Kilicel, K. Kara and I. Tuncer, 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey Environ. Toxicol. Pharmacol., 13: 175-179.

Vincent, T. and E. Guibal, 2001. Cr (VI) extraction using aliquat 336 in a hollow fiber module made of chitosan. Ind. Eng. Chem. Res., 40: 1406-1411.

Wilson, B. and F.B. Pyatt, 2007. Heavy metal dispersion, persistence andbioaccumulation around an ancient copper mine situated in Anglesey, UK. Ecotoxicol. Environ. Safety, 66: 224-231.

Xu, Y., C. Gallert and J. Winter, 2008. Chitin purification from shrimp wastes by microbial deproteination and decalcification. Applied Microbiol. Biotechnol., 79: 687-697.

Xu-Fen, Z., Z. Ying and F. Jun-Li, 2007. Analysis of both chitinase and chitosanase produced by *Sphingomonas* sp. CJ-5. J. Zhejiang Univ. Sci. B, 8: 831-838.