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An Experimental and Modelling Study of Selected Heavy Metals Removal from Aqueous Solution Using *Scylla serrata* as Biosorbent

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ABSTRACT

This study was conducted using crab shells as a biosorbent to remove Cu and Cd with different initial concentrations of 1, 5, 10, 15, and 20 mg/L in a biosorption treatment process. Crab shells were selected as biosorbents due to their abundance in the environment and ready availability as waste products from the market place. This study aimed to determine the ability of *Scylla Serrata* shells to remove Cu and Cd in an aqueous solution, as well as to provide a comparison of the removal rate between the two metals. The data were incorporated into hydrochemical software, PHREEQC, to investigate the chemical speciation distribution of each heavy metal. The shells of *S. serrata* were found to have a significant ($p < 0.05$) ability to remove Cu and Cd following the treatment. After six hours of treatment, the crab shells had removed 60 to 80% of both metals. However, the highest removal percentage was achieved for Cu with up to 94.7% removal rate in 5 mg/L initial Cu concentration, while 85.1% of Cd was removed in 1 mg/L initial solution, respectively. It can be concluded that the shells of *S. serrata* could remove Cu and Cd better with significant results ($p < 0.05$) in 1 and 5 mg/L initial concentrations, respectively.

Keywords: Biosorption, Cd, Cu, crab shell, PHREEQC

INTRODUCTION

Biosorbents can be divided into living or dead materials, which are capable of carrying biosorption mechanisms (Shareef, 2009; Yadanaparthi *et al.,* 2009). The benefits of using living biosorbents are that they are self-renewing, and therefore, the active transport may take higher metal concentrations from

the media of aqueous solution. Some examples of these biosorbent activities include bacteria (Adeogun *et al.*, 2012; Castillo-Zacarías *et al.*, 2011), fungi (Yazdani *et al*., 2009), algae (Leborans & Novillo, 1996), and living plants (Park *et al.,* 2008). These biosorbents are only different in their uptake mechanisms. In contrast, dead biosorbents are chosen because of their ability to avoid the toxicity problems towards the sample and the environment. They do not cause secondary pollution or have any side effect prior to the treatment. In addition, defunct biosorbents often require shorter time, are easier to apply, and most importantly, provide a non-destructive recovery of bound metals, which allow regeneration of the biosorbent materials. Thus, the majority of biosorption studies preferred using defunct biosorbents, which offer more advantages compared to living organisms (Schiewer & Volesky, 2000). Biosorption of heavy metals is an alternative technology in wastewater treatment, instead of using additional chemical and high cost technology. Natural biological materials from different kinds of waste have proven the same efficiency rate with less harm to the environment and human health (Demirbas, 2008).

Crab shell is one of the most applied and efficient natural products used as a biosorbent in treating metal-polluted water (Evan *et al*., 2008; Kim, 2003) and toxic materials (Alkan *et al*., 2004). Generally, crab shells consist of calcium carbonate and protein (29.19%), ash (40.60%), lipid (1.35%) and chitin (26.65%) on a dry weight basis (Tokura & Nishi, 1995). Calcium carbonate and chitin in the crab shells are found to be the most effective materials in removing heavy metals, especially copper and lead. This is because calcium carbonate forms strong copper-carbonate and lead-carbonate bonds when reacting with copper and lead, while chitin acts as an adsorbent for precipitation in the presence of those heavy metals (Lee *et al*., 1998). Vijayaraghavan *et al*. (2006) utilized crab shells for the biosorption of copper (II) and cobalt (II) in their study. Based on their study, crab shells have been proven to be the best adsorbent with the highest efficient removal of heavy metal. The biosorbent, which is around 0.767 mm in size with a dosage of 5g/L and an initial pH solution of 6, adsorbed heavy metals up to 90% from solution, i.e. 30% higher than other adsorbents. In addition, the biosorption capacity depends on the pH solution, for which the maximum copper (II) and cobalt (II) uptakes were 243.9 mg/g and 322.6 mg/g, respectively, and the results were observed at pH6. Another study by An *et al*. (2001) revealed that crab shells had the highest removal efficiency among other adsorbents such as zeolite, powdered activated carbon and granular activated carbon. This makes crab shells the most effective and applicable adsorbents for metal removing treatment, which combined with their abundance in the environment as waste and its cost effectiveness, making them a reasonable adsorbent in larger treatments such as industrial treatment plants.

Heavy metals exist naturally in the environment in different orders such as sedimentary, magmatic, metamorphic rocks, weathering and soil structure. Anthropogenic activities, mainly from the human activities also contribute to their existence, which usually originate from industrial and agricultural sources (Bradl, 2005). Copper and its compounds, which are very complex and involved in many metabolic processes in living organisms, are ubiquitously distributed in the environment and consist of the redox potential of Cu(I) or Cu(II) (Scheinberg, 1991). Copper is generally known as a non-toxic element in lower concentrations, but it may have an adverse effect on health (Bisone *et al.*, 2012) such as nausea (Ali & Aboul-Enein, 2006) when in higher concentrations.

Cadmium, which is a volatile element, is not essential for plants, animals or human beings. High doses of cadmium can have toxic effects on plants, animals and human beings. The target of cadmium concentration in the human organs is the kidney. In more specific, cadmium is considered toxic when its concentration reaches15 mg/L or higher (Stoeppler, 1991).

The shell of *S. serrata*, a waste material disposed of by the fishing industry, could be a viable and cost effective adsorbent for the removal of heavy metals. It is cheap and abundantly available in the environment, making it the most suitable material to remove copper and cadmium from an aqueous solution. The present study was conducted to determine the *S. serrata* shells' ability to remove copper (Cu) and cadmium (Cd) from an aqueous solution based on the removal rate of the heavy metal. The efficiency of removal was also calculated to determine the most suitable application for the removal of heavy metal using *S. serrata* shells as the biosorbent. Lastly, the chemical speciation of each heavy metal was modelled using hydrochemical software known as PHREEQC.

MATERIALS AND METHODS

Sorbent Collection and Preparation

The sorbent used throughout this study was crab (*S. serrata*) shells obtained from the local wet market. The crab *S. serrata* belongs to the Phylum Arthropode, Class Malacostrace and Family Portunidae. *S. serrata* (mud crab) is easily and abundantly found in the estuaries or mangrove areas in Malaysia and other developing countries in Asia such as Indonesia, Thailand, Vietnam and Sri Lanka (Overton á *et al.*, 1997). The smallest *S. serrata* is found to have a shell width of 11.0 cm, and it can reach up to 24.0 cm in size (Jirapunpipat *et al.,* 2008; Overton á *et al.*, 1997), depending on the surrounding conditions. For this study, mature *S. serrata* (in the range size of 8.6-9.2 cm) (Ikhwanuddin *et al.,* 2011) of similar shapes were selected and transferred to the laboratory for further analysis. The shells were steamed at 120˚C to separate the crabmeat and then thoroughly rinsed in tap water, followed by deionized water (Milli Q; 18 MΩ c/m). Subsequently, the shells were dried in the oven overnight at 30°C to make sure they were totally dry. There was no special treatment applied at this step of the experiment. After they had been totally dried and no weight changes were observed, the shells of *S. serrata* were pulverized (Juang *et al.*, 1999) into fragment in the sizes between 2.00-2.50 mm and stored at room temperature (Evan *et al.*, 2008) until further analysis.

Sorption Experiment

All the sorption experiments were carried out in batch experiments with different initial concentrations of Cu and Cd solutions (1, 5, 10, 15 and 20 mg/L), representing the low range (<100 mg/L) of heavy metal concentrations (Liu *et al.*, 2009). All the solutions used in this experiment were analytical grade (Merck, India) reagent cadmium standard solution and copper standard solution (for Flame Atomic Absorption Spectrometry). The batch experiments were done in triplicate by using an Erlenmeyer flask containing 100ml solution for each metal. The laboratory apparatus used in this experiment were acid-washed by soaking them overnight in 5% (v/v) nitric acid (HNO_3) before rinsing thoroughly with distilled water. This procedure was

done to ensure that any available contaminants, ions and trace elements of cleaning reagent were removed before the experiments (APHA, 2005). With 100ml heavy metal in each flask, the pH of the solutions was adjusted to pH6 (±0.1) by adding a significant amount of 1.0M NaOH or 1.0M HCl. This pH value was chosen as the optimal pH solution because the uptake of heavy metals could be increased with the increase in pH, in which the maximum pH needed for biosorption ranged from 4.5 to 6.0 (±0.1) (Vijayaraghavan *et al*., 2006). Each flask was added 0.1g of crushed crab shells before sealing with a piece of parafilm to avoid any external contamination. Immediately afterwards, all the flasks were placed in an incubator rotary shaker and the temperature was set at 30°C, with a shaking speed of 150rpm. Samples of the heavy metal solution (3ml) were drawn out at every 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, and 1.75 h to obtain a precise breakthrough curve, which was followed by an interval time of 2 h up to 6 h of contact time. Different flasks indicate a different interval time. The use of the incubator rotary shaker was to ensure the crushed crab shells could mix well with the heavy metal solutions in the flasks. The samples were immediately centrifuged for 10 minutes in a centrifuge at 3000 rpm. After 10 minutes, the supernatant sample was pipetted out into another centrifuge tube and preserved until heavy metal analysis. Preservation was undertaken according to APHA (2005), in which the samples were kept in the refrigerator at a temperature of 4°C. The selected heavy metals were analyzed using Flame Atomic Absorption Spectrometry (FAAS) (Perkin Elmer AAS 3300). The accuracy of the FAAS performance was assessed based on the external standards, which had been prepared by diluting the specific stock standards of Cd and Cu (Merck, Germany) into a series of concentrations with the same mixture used for the sample pre-treatment. The recovery rates calculated for Cd and Cu ranged from 100.07% to 101.88% (±0.5%), depending on the elements analyzed (Table 1).

TABLE 1
Determination of the recovery percentage of metals

Element	Concentration (mg/)	Recovery (%)	Working Curve (r)	Detection Limit Ranges
Cd	1	100.29	1.0000	
	5	101.14	1.0000	
	10	100.76	1.0000	1.0-10.0
	15	100.27	0.9998	
	20	101.38	0.9999	
Cu	1	100.07	1.0000	
	5	100.15	1.0000	
	10	101.88	0.9997	1.0-10.0
	15	101.03	1.0000	
	20	101.67	0.9999	

Modelling Using PHREEQC

Data from the removal experiments were calculated using the hydrochemical model PHREEQC. PHREEQC is a useful tool for simulating a variety of reactions and processes in natural waters

or laboratory experiments (Parkhurst & Appelo, 2005). In this study, PHREEQC was applied to obtain the chemical speciation of Cu and Cd during the experiments (Parkhurst & Appelo, 2005). PHREEQC performs speciation calculations based on the law of mass action, which states that for a reaction of the generalized type:

$$aA + bB \leftrightarrow cC + dD \qquad \text{(Eq. 1)}$$

Whereas, the distribution at thermodynamic equilibrium of the species at the left and right side of the reaction is given by:

$$K = \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b} \qquad \text{(Eq. 2)}$$

Where, K = equilibrium constant and the bracketed quantities denote activities or effective concentrations. In more specific, a, b, c and d = the number of moles of the reactants A, B, and the end products C, and D, respectively, for the given reaction of the mass-action law. In particular, the term K is defined as the solubility product of dissolution/ precipitation of minerals.

Data Analysis

The raw data obtained from the experiments were analyzed for the statistical analysis using the Statistical Package for Social Science (SPSS version 17) software. The statistical analysis was done for the descriptive statistics, one-way ANOVA and correlation analyses based on the heavy metals and time consumption of the treatment. The variances of the treated heavy metal solutions can be described using this statistical analysis in terms of variability.

The removal rate of heavy metals, R (%) in each interval time was calculated based on the following equation:

$$R = \left[\frac{C_\beta - C_\alpha}{t} \right] \qquad \text{(Eq. 3)}$$

where C_β indicates the concentration before that interval time (mg/L) and C_α indicates the concentration at the time interval of contact time (mg/L). This calculation was performed as a percentage to show the removal amount of heavy metal in respective time. The higher it is, the better the performance of heavy metal removal will be within the time.

In order to obtain the efficiency of heavy metal removal, E (%) was calculated based on the percentage of efficient removal after the treatment, as shown in the equation below:

$$E = \left[\frac{C_o C_f}{C_o} \times 100 \right] \qquad \text{(Eq. 4)}$$

C_o refers to the initial heavy metal concentrations (mg/L) and the C_f refers to the final heavy metal concentrations (mg/L) after the treatment.

RESULTS AND DISCUSSION

Sorption Experiment

The results for the Cu removal are shown in Figure 1, with a rapid increase of cadmium removal at the initial stages of the 2-h treatment. Specifically, the Cu removal within 2 h of adsorption was between 38.9 and 90.23% (1-20 mg/L) before it slowed down after a few hours, depending on the initial Cu concentration used. From the graph of the remaining concentration of Cu, the treatment using 1 mg/L Cu at the beginning of experiments clearly resulted in the best Cu removal of 0.416 ± 0.010 mg/L within 0.25 h or 15 minutes of the treatment. In comparison, other concentrations only achieved between 10.2% and 30.9% of Cu removal, while Cu in 1 mg/L had already achieved 58.4% of Cu removal. However, in the middle of the treatment (3h), the removal trend shifted to the 5 mg/L initial concentration, which had previously been dominated by 1 mg/L. This trend continued until the end of the six hour treatment, whereby 5 mg/L gave the highest Cu removal of 94.7% (Table 2). Finally, the remaining concentration of Cu in the solution was 0.266 ± 0.024 mg/L, with a total concentration of 4.774 ± 0.024 mg/L being successfully removed by using the crushed crab shells.

In the 10 mg/L Cu initial concentration, only 6.35 ± 0.072 mg/L of Cu metal was removed after four hours of treatment using the crushed crab shells. However, by the end of the experiment (6 hours), the remaining concentration was slightly increased to 3.93 ± 0.067 mg/L (see Figure 1). This indicates that the desorption mechanism of Cu occurred using the crushed crab shells. As for the 15 and 20 mg/L Cu treatments, 10.61 ± 0.053 and 16.38 ± 0.059 mg/L were removed, respectively, by the use of the crab shells by the end of the experiment (6 hours).
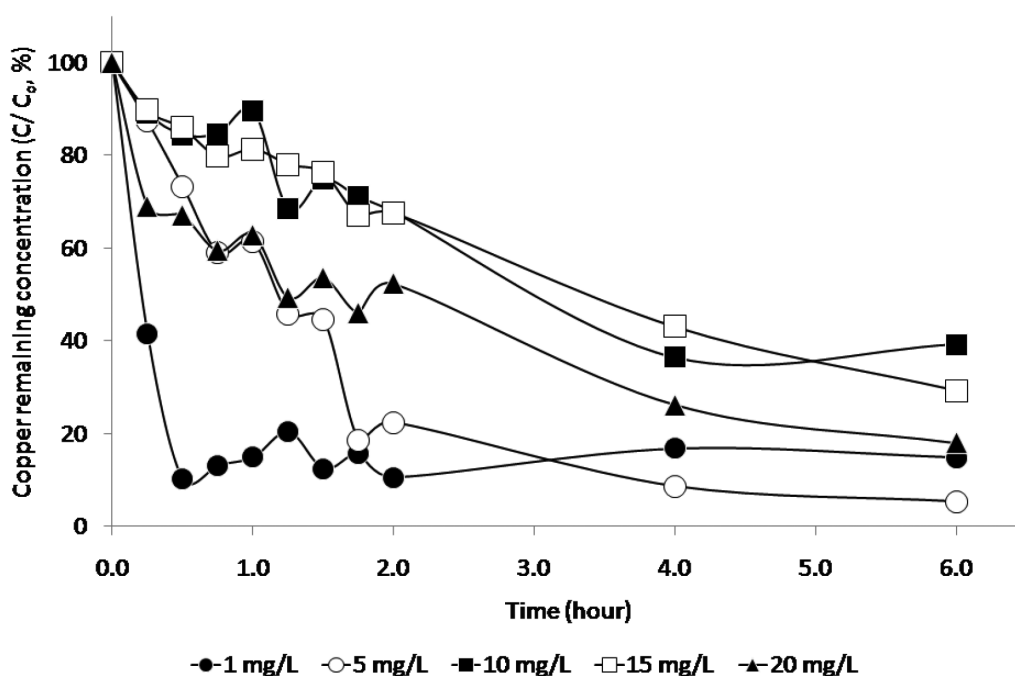

Fig.1. Copper remaining concentration (C/ C_o, %) after six hours of treatment using crab shells

Both the Cu solutions, in the concentrations of 1 mg/L and 5 mg/L, were found to be in the equilibrium state after 0.5 and 2 hours, respectively. For the Cu solutions in the concentrations of 10, 15 and 20 mg/L, however, no equilibrium state was observed, even after the experiments had been carried out for 6 hours. Similarly, it is suggested that the time for the attainment of equilibrium increased with the concentration of metals. These findings are parallel with the study conducted by Vijayaraghavan *et al*. (2006), who found a significant change in the initial Cu concentration ranging from 50 to 200 mg/L. Eventually, the metal amount being adsorbed was increased from 75.4 to 197.7 mg/L. Therefore, the equilibrium state for the higher concentrations of Cu (10, 15 and 20 mg/L) requires a longer time to be achieved.

The maximum sorption capacity was recorded for Cu removal with an initial concentration of 5 mg/L, with 94.7% of Cu being removed after 6 h of treatment. The 6-h treatment might seem inadequate for a complete (100%) removal, however, it showed that it could still achieve >90% removal within that time. Having shown its ability to remove heavy metals, the next factor to consider is the time required. The faster it can remove heavy metals, the greater chance it has of being an efficient sorbent.

The results of the Cd removal experiments are shown in Figure 2. In the Cd removal treatment, the variations of Cd concentration were also obtained. The initial cadmium removal is rapid throughout the treatment but it became slow after a few hours, depending on the initial Cd concentration being used. The slow removal rate is likely to be due to the saturation of Cd concentration into the biosorbent. Since it attached more and more Cd to its surface, it became saturated and therefore, the remaining Cd can only be adsorbed in minimal amounts. Similarly, Evans *et al*. (2002) found that the longer time for Cd uptake resulted in a slower Cd uptake rate in respect of crab shells as a biosorbent.

In the first 2 hours, all the treatments showed that they had achieved the equilibrium state, while the removal rate after that time interval showed a similar trend. When identical trend was repeated for Cu removal, the 1 mg/L initial metal concentration always gave the best metal removal in the first 15 minutes of the treatment. This phenomenon continued until the end of the treatment through which 1 mg/L initial Cd concentration achieved the highest Cd removal rate of 85.1% or a total of 0.851 ± 0.004 mg/L of Cd was removed by the end of experiment.

From the experiment, 5 mg/L of Cd concentration showed the highest desorption (0.69 ± 0.011 mg/L) from 0.25 h to 1.25 h of the treatment. This was repeated for the next 15 minutes but in a lower amount (0.22 ± 0.103 mg/L). Higher initial Cd concentrations (10, 15 and 20 mg/L) were only able to remove Cd in amounts ranging from 49.3 to 64.7%, while the lowest percentage was detected for the 20 mg/L initial concentration. This might be due to the saturation of the solution, which required a longer time to gradually remove Cd (Evans *et al*., 2002).

In this study, the ability of all the initial Cd concentrations and their removal rates after the treatment using the crushed crab shells to remove Cd were assessed. Figure 2 shows the ability of these initial Cd concentrations since the decreasing amount of Cd showed a downward trend. To support this result, the results obtained from the statistical analysis using the one-way ANOVA showed a significant difference ($p< 0.05$) after the use of crushed crab shells. The efficiency of the crushed crab shells to remove Cd in aqueous solution is shown in Table 2.

TABLE 2
Results of Cu and Cd removal efficiency from different initial concentrations (mg/L)

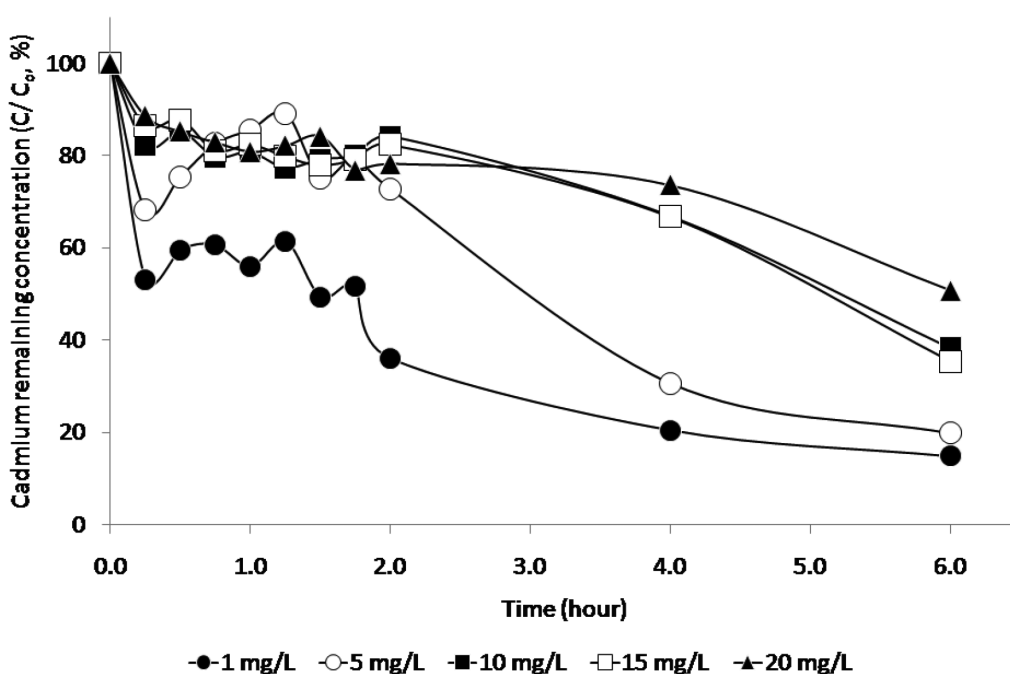| Initial Heavy Metal Concentrations (%)	Removal Efficiency (%)	
	Cu	Cd
1	85.1	85.1
5	94.7	80.2
10	60.7	61.7
15	70.7	64.7
20	81.9	49.3

Fig.2. Cadmium remaining concentration (C/ C$_o$, %) after six hours of treatment using crab shells

Additionally, this study also showed that with increasing initial concentration, the removal efficiency by the crushed crab shells also decreased (see Figure 3). A strong correlation (r= -0.950, p< 0.05) between the initial concentration solution and the removal efficiency for Cd is shown in Figure 3. In regards to the variation in the initial Cd concentration, the more concentrated the solution, the lower the tendency of the Cd metal to bind to the surface of the crushed crab shells. This might be due to the equilibrium state of the surface environment in which the mechanism happened (Ismail & Aris, 2012; Reddad *et al*., 2002). Therefore, it is suggested that future experiments use the lowest initial concentration (1 mg/L) of Cd because of its cost effectiveness and ability to produce the most efficient result. Thus, it is suggested that the trend for total Cd adsorbed by the crushed crab shells follow a decreasing trend of 1 mg/L > 5 mg/L > 15 mg/L > 10 mg/L > 20 mg/L (r= -0.950, p< 0.05).
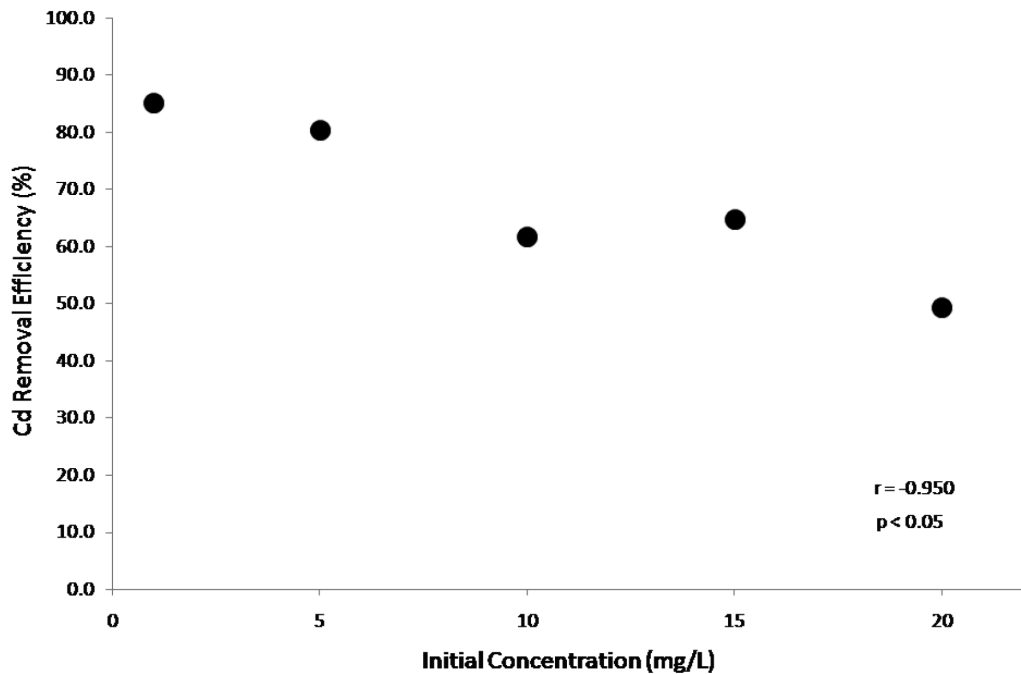
Fig.3. The relationship between total Cd removed with the initial concentration for the treatment

However, the removal experiments for Cu showed a weak correlation ($r = -0.363$, $p < 0.05$) between the removal efficiency and the initial metal concentrations (Figure 4). Even though the highest amount of Cu was removed at the end of the experiments using 20 mg/L Cu (16.38 $\pm$ 0.159 mg/L out of 20 mg/L), it was not as efficient as using 1 mg/L (0.851 $\pm$ 1.021 mg/L out of 1 mg/L) of Cu in the initial stage. Based on the experiments, it is suggested to choose 5 mg/L of initial Cu concentration in the future when attempting to treat Cu from industrial wastewater effluent. This is particularly due to the effective and efficient removal rate of 94.7% attained when using the suggested initial Cu concentration (Table 1). Despite the concentration amounts being used, another crucial aim is to remove as much Cu as possible in a short time period. This has been proven by the statistical analysis, which showed no significant difference ($p = 0.391$) between the 1 and 5 mg/L solutions.

Comparing the removal efficiency rates of Cu and Cd, Figure 5 illustrates that the fastest removal rate achieved for Cu was within two hours of treatment, with almost 92.4% of Cu being removed, and only 0.076 $\pm$ 0.002 mg/L Cu remained in the solution. The removal rate of Cu was more rapid than Cd ($p < 0.05$). An experiment using 1 mg/L initial metal concentration was chosen as a comparison since this experiment yielded the same removal efficiency of 85.1% by the end of the experiment. As a more abundant element in the environment compared to Cd (Ellingsen *et al*., 2007; Nordberg *et al*., 2007), Cu seems to be adsorbed faster. In the Cu experiments, however, the negative amounts indicated that desorption most likely occurred because the surface area of the crab shells had reached their capacity for adsorption.
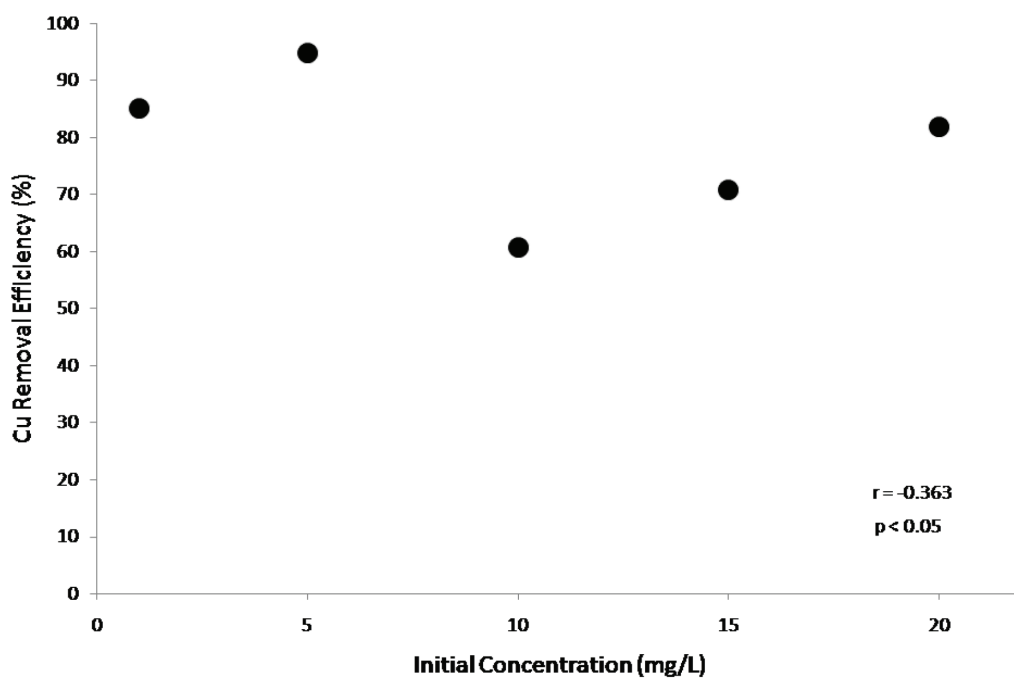
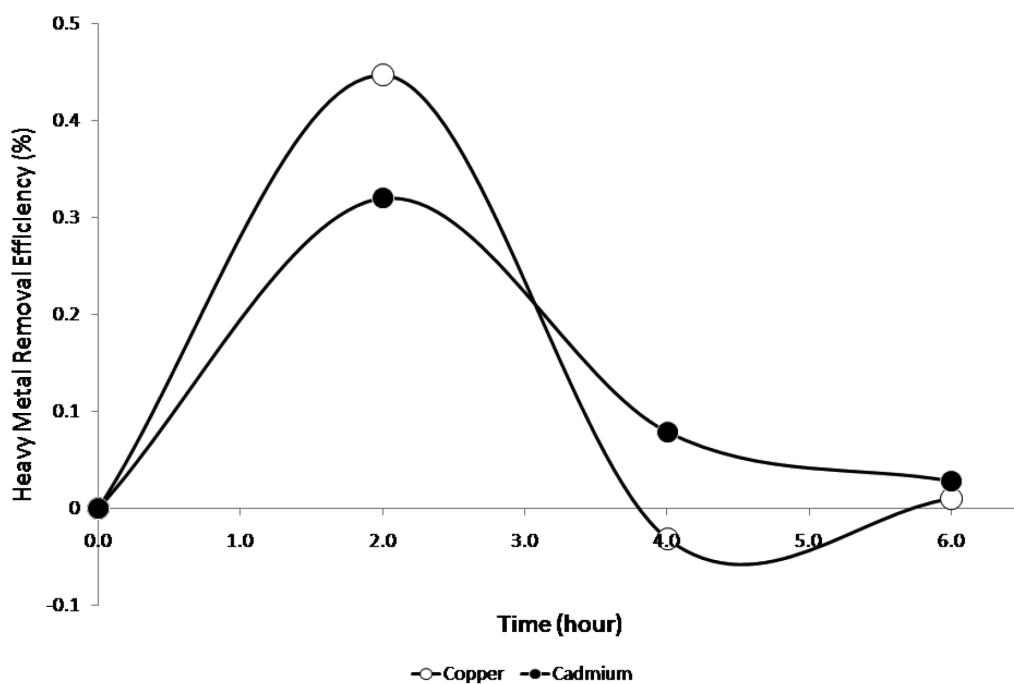Fig.4. The relationship between total Cu removed and the initial concentration for the treatment

Fig.5. Variation of the heavy metals removal efficiency rates from beginning until the end of the treatment using the initial 1 mg/L metals solution

PHREEQC Modelling

The data obtained were also modelled using hydrochemical software with PHREEQC (Parkhurst & Appelo, 2005). Basically, PHREEQC was used to identify the dominant chemical speciation of heavy metals during the experiment, based on the results obtained. This software determined the most abundant species during the treatment, and came out with the amount of species prediction. However, to obtain the exact amount of speciation, further experiments must be done. In this experiment, PHREEQC was used to calculate the dominant metal species in the solution during the experiment. The Cu experiments (see Figure 6) suggest that divalent Cu (Cu^{2+}) seems to be the most abundant species in the aqueous solution. In fact, this metal species is the most stable form that exists in normal atmospheric and pressure conditions (Appelo & Postma, 2005). A similar observation was encountered for the Cd experiment (Figure 7), in which the divalent Cd (Cd^{2+}) also tended to be the most abundant in that solution. Following that, cadmium hydroxide ($Cd(OH)^{+}$) was the second most abundant, with approximately 44.01% of $Cd(OH)^{+}$.
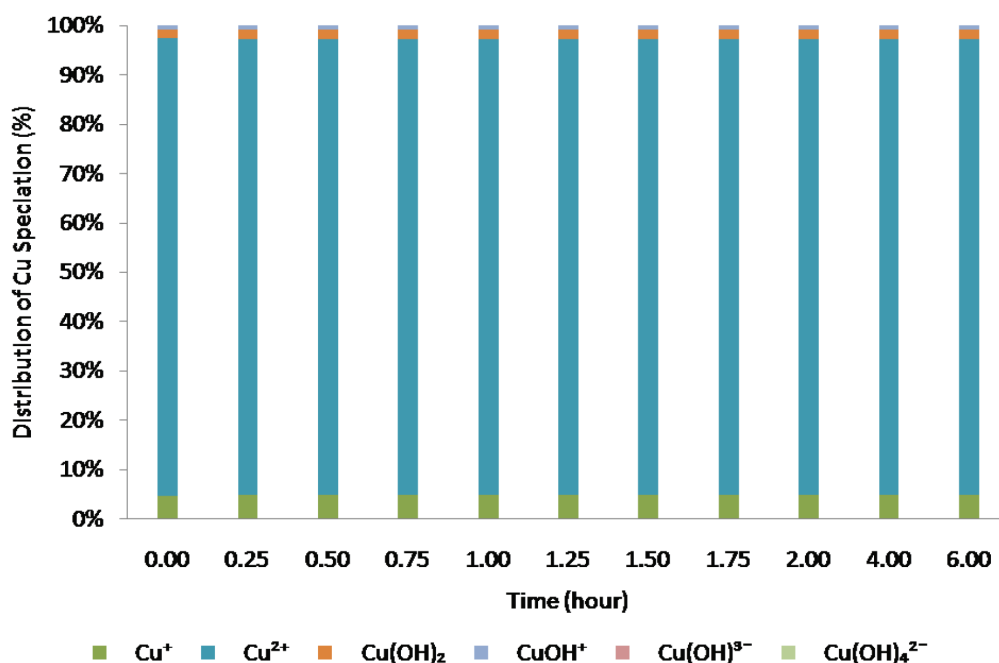

Fig.6. Distribution of Cu speciation throughout the treatment of six hours using initial mg/L Cu

CONCLUSION

In summary, the crushed shells of *S. serrata* have a high sorption capacity for Cu and Cd. Since this study mainly focused on the ability of crushed crab shells to remove Cu and Cd, it was proven that this biosorbent could remove up to 94.7 and 85.1% Cu and Cd, respectively. From the calculated removal efficiencies, *S. serrata* was found to be a more effective adsorbent in removing Cu than Cd for both 5 and 1 mg/L. The application of *S. serrata* in low amounts
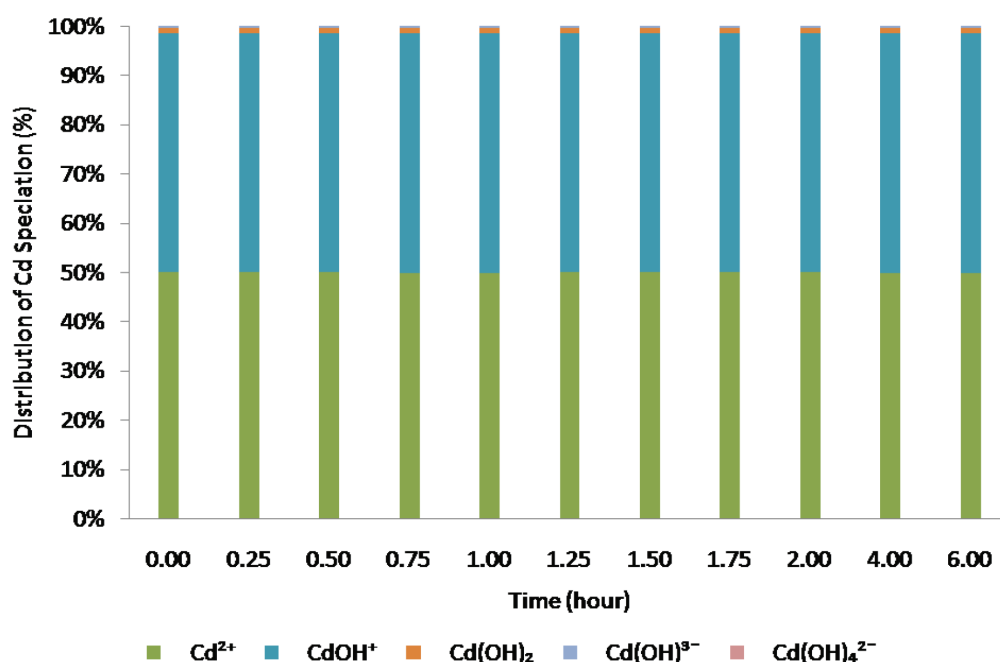
Fig.7. Distribution of Cd speciation throughout the treatment of six hours using initial 1 mg/L Cd

that eventually achieved high removal of heavy metals for Cu and Cd suggests that *S. serrata* could be a very effective and efficient biosorbent. With the constant pH of 6 and the temperature maintained at 25°C, this experiment perfectly suited the wastewater effluent characteristics from mining or metal refining industries. Coupled with its low cost, easy availability (since it is a waste product from the fishing industry), as well as its abundance in the environment, crab shells are undeniably suitable adsorbents for heavy metals from industrial wastewater.

REFERENCES

Adeogun, A. L., Kareem, S. O., Durosanya, J. B., & Balogun E. S. (2012). Kinetics and equilibrium parameters of biosorption and bioaccumulation of lead ions from aqueous solutions by *Trichoderma Longibrachiatum*. *Journal of Microbiology, Biotechnology and Food Sciences, 1*(5), 1221-1234.

Ali, I., & Aboul-Enein, H. Y. (2006). *Instrumental Methods in Metal Ion Speciation*. London: Taylor & Francis, pp. 376.

Alkan, M., Demirbaş, **Ö.,** & Doğan, M. (2004). Removal of Acid Yellow 49 from Aqueous Solution by Adsorption.*Fresenius Environmental Bulletin, 1311*(a), 1112-1121.

An, H. K., Park, B. Y., & Kim D. S. (2001). Crab shell for the removal of heavy metals from aqueous solution. *Water Research, 35*(15), 3551-3556.

APHA. (2005). *Standard Methods for the Examination of Water and Wastewater* (21st Edn.). Washinton: American Water Works Association, Water Environment Federation, pp. 1368.

Appelo C. A. J., & Postma D. (2005). *Geochemistry, Groundwater and Pollution* (2nd Edn,). Balkema, Rotterdam, pp. 649.

Bisone, S., Blais, J. F., Drogui, P., & Mercier, G. (2012). Toxic metal removal from polluted soil by acid extraction. *Water Air Soil Pollution, 223*, 3739-3755.

Bradl, H. B. (2005). *Heavy Metal in the Environment: Origin, Interaction and Remediation*. London: Elsevier Academic Press, pp. 268.

Castillo-Zacarías, C. J., Suárez-Herrera, M. A., Garza-González, M. T., Sánchez-González, M. N., & López-Chuken, U. J. (2011). Biosorption of metals by phenol-resistant bacteria isolated from contaminated industrial effluents. *Journal of Microbiology Research, 5*(18), 2627-2631.

Demirbas, A. (2008). Heavy metal adsorption onto agro-based waste materials: A review. *Hazardous Materials, 157*(2-3), 220-229.

Ellingsen, D. G., Hom, N., & Aaseth J. (2007). Copper. In *Handbook of Toxicology of Metals* (p. 975). New York: VCH Publishers.

Evans, J. R., Davids, W. G., MacRae, J. D., & Amirbahman, A. (2002). Kinetics of Cadmium uptake by chitosan-based crab shells. *Water Research, 36*(13), 3219-3226.

Ikhwanuddin, M., Azmie G., Juariah H. M., Zakaria M. Z., & Ambak M. A. (2011). Biological information and population features of mud crab, genus *Scylla* from mangrove areas of Sarawak, Malaysia. *Fisheries Research*, *108*(2-3), 299-306.

Ismail, F. A., & Aris, A. Z. (2013). Experimental determination of Cd2+ adsorption mechanism on low-cost biological waste. *Frontiers of Environmental Science and Engineering.* DOI 10.1007/s11783-013-0488-1.

Jirapunpipat, K., Aungtonya, C., & Watanabe, S. (2008). Morphological study and application of multivariate analysis for the mud crab genus *Scylla* Klong Ngao Mangrove, Ranong Province, Thailand. *Phuket mar. Boil. Cent. Res. Bull., 69*, 7-24.

Juang, R. S., Wu, F. C., & Tseng, R. L. (1999). Adsorption removal of Copper(II) using chitosan from simulated rinse solutions containing chelating agents. *Wat. Res., 33*(10), 2403-2409.

Kim, D. S. (2003). The removal by crab shell of mixed heavy metal ions in aqueous solution. *Bioresource Technology*, *87*(3), 355-357**.**

Leborans, G. F., & Novillo, A. (1996). Toxicity and bioaccumulation of Cadmium in *Olisthodiscus Luteus* (Raphidophyceae). *Wat. Res., 30*(1), 57-62.

Lee, M. Y., Shin, H. J., Lee, S. H., Park, J. M., & Yang J. W. (1998). Removal of Lead in a Fixed-Bed Column Packed with Active Carbon and Crab Shell. *Separation Science and Technology, 33*(7), 1043-1056.

Nordberg, G. F., Nogawa K., Nordberg, M., & Friberg, A. L. T (2007). Cadmium. In *Handbook of Toxicology of Metals* (p. 975). New York: VCH Publishers.

Overton, J. L., Macintosh, D. J., & Thorpe, R. S. (1997). Multivariete analysis of the mud crab Scylla serrata (Brachyura: Portunidae) from four locations in Southeast Asia. *Marine Biology*, *128*, 55-62.

Park, D. P., Lim, S. R., Yun, Y. S., & Park, J. M. (2008). Deveopment of a new Cr(VI)-biosorbent from agricultural biowaste. *Bioresource Technology*, *99*, 8810-8818.

Parkhurst, D. L., & Appelo, C. A. J. (2005). User's Guide to PHREEQC Version 2-A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations. *U.S. Geological Survey, Water Resource Investigation Report.* pp. 99-4259.

Reddad, Z., Gerente, C., Andres, Y., & Cloires, P. L. (2002). Adsorption of several metal ions onto a low-cost biosorbent: kinetic and equilibrium studies. *Environmental Science Technology, 36*(9), 2067-2073.

Scheinberg, H. (1991). Copper. In *Metals and Their Compounds in the Environment, Occurrence, Analysis, and Biological Relevance* (pp. 891-904). New York: VCH Publishers.

Schiewer, S., & Volesky, B. (2000). Biosorption process for Heavy metal removal. In *Environmental Microbe-Metal Interactions* (pp. 329-362). Washington, D.C.: ASM Press, Washington.

Shareef, K. M. (2009). Sorbents for contaminants uptake from aqueous solutions. Part I: Heavy metals. *World Journal of Agricultural Sciences, 5*(S), 819-831.

Stoeppler, M. (1991). Cadmium. In *Metals and Their Compounds in the Environment, Occurrence, Analysis, and Biological Relevance* (pp. 803-835). New York: VCH Publishers.

Tokura, S., & Nishi, N. (1995). Specification and Characterizationof Chitin and Chitosan. In *Chitin and Chitosan the Versatile Environmentally Friendly Modern Materials* (pp. 67-86). Bangi: Penerbit Universiti Kebangsaan Malaysia.

Vijayaraghavan, K., Palanivelu, K., & Velan, M. (2006). Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresource Technology, 97*(12), 1411-1499.

Yadanaparthi, S. K. R., Graybill, D., & Wandruszka, R. V. (2009). Review: Adsorbents for the removal of arsenic, cadmium, and lead from contaminated waters. *Journal of Hazardous Materials*, *171*, 1-15.

Yazdani, M., Yap, C. K., Abdullah, F., & Tan, S. G. (2009). Trichoderma atroviride as a bioremediator of Cu pollution: An in vitro study. *Toxicological and Environmental Chemistry*, *91*(7), 1305-1314.