

## Analysis of copper removal using different seafood wastes by differential pulse voltammetry (DPV)

Joel Ashervin Zachariah<sup>a</sup>, Mohd Rawa Ispal<sup>b</sup>, Michelle Soo Oi Yoon<sup>a\*</sup>

<sup>a</sup>Aquatic Science Programme, Faculty of Applied Sciences, UCSI University KL, No. 1, Jalan Menara Gading, Taman Connaught, 56000 Cheras, Kuala Lumpur, Malaysia

<sup>b</sup>BioGenes Technologies Sdn. Bhd., Technology Incubation Centre, Infrastructure University Kuala Lumpur (IUKL), UniPark Suria, Jalan Ikeram-Uniten, 43000 Kajang, Selangor Darul Ehsan, Malaysia

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**Abstract.** Current methods of heavy metal removal are shown to have several drawbacks: cost of operation, generation of toxic sludge and complicated procedures in the treatment. In this study, the ability of seafood waste as a sustainable biosorbent was investigated. Three commonly consumed seafood wastes in Malaysia were chosen for this study: crab shells, prawn shells and cockle shells. This study aimed to determine the differences in copper removal between crab, prawn and cockle shells at fixed concentrations and weights of shells. Differential pulse voltammetry using a modified screen-printed electrode was used to determine the concentration of copper for each waste sample. Removal of copper was first observed at different shell weights between 20 and 100 mg and at a fixed copper concentration of 20 ppm. Shell weights with the highest removal, 20 mg and 40 mg, were then used for further observation at concentrations between 1-20 ppm. At a weight of 20 mg, prawn shells showed the highest removal percentage of copper at 34.40% while crab shells and cockle shells showed the highest removal percentage of 31.22% and 12.78%, respectively. This was likely due to the presence of calcium carbonate and chitin as the main component in crab and prawn shells. Cockle shells showed low removal efficiency under these conditions. In conclusion, untreated crab shells and prawn shells were shown to successfully remove copper in water.

**Keywords:** copper, seafood waste, Malaysia, sustainability, biosorbent, screen-printed electrode (SPE)

## INTRODUCTION

Copper is a reddish metal that occurs naturally in water, rocks and sediments. Copper enters the environment through anthropogenic sources such as mining and from factories that make or use copper metal or copper compounds. The according The WHO Guidelines for Drinking-water Quality (2004) states that the acute lethal dose for adults lies between 4 and 400 mg of

copper (II) ion per kg of body weight. Metal finishing processes such as electroplating, etching, and preparation of metal components for various industries, have been identified as a major source of wastes containing high concentrations of copper in several states in Peninsular Malaysia (Shazili *et al.*, 2006). Chemical precipitation, membrane separation, electrochemical

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\*Author for correspondence: Michelle Soo Oi Yoon, Faculty of Applied Sciences, UCSI University KL, No. 1, Jalan Menara Gading, Taman Connaught, 56000 Cheras, Kuala Lumpur, Malaysia. Email – michellesoo@ucsiuniversity.edu.my

deposition, ion exchange and reverse osmosis are regular processes for removing heavy metals from. However, these processes are fairly expensive and inefficient when heavy metal concentrations are less than 100 mg/L (Pugazhenthiran *et al.*, 2015).

Seafood such as prawn, crabs and cockles are a well-loved delicacy in Malaysia. After meals, the leftover consisting of shells are often thrown away with garbage, ending up in landfills. This results in accumulation of seafood waste over time which results in odor and microbe pollution that eventually pollutes coastal environments thereby affecting marine life and the people who live in the vicinity. Various studies have shown that waste materials such as banana pith, corn cob, saw dust, date piths, rice husk ash, spent tea leaves, lemon peel, apple pomace and fruit peel are useful low-cost adsorbents (Sudha *et al.*, 2014). Previous studies have shown that the adsorption properties of chitin have proven to be effective (Peiselt da Silva *et al.*, 2004). Chitin's deacetylated form, chitosan has adsorptive properties that have been proven to be more effective than chitin.

Seafood waste could be utilized as a cheaper, sustainable alternative to treating wastewater containing heavy metals such as copper. The shells of crustaceans and bivalves have shown biosorption properties. Termed as adsorption, this is a physiochemical process that occurs in different types of biomasses, allowing it to passively concentrate and adhere contaminants onto its cellular structure (Kim *et al.*, 2006). This method has been studied in a number of cases as a technique for heavy metal removal from water (Nascimento *et al.*, 2019). The process does not depend on metabolism, since the metal ions accumulate on the surface of the biosorbents such as the seafood wastes. Therefore, with these in mind, we chose to study the biosorption efficiency of copper using three types of untreated seafood wastes: crab, prawn and cockle shells. In this study, screen printed carbon electrodes (SPCE) were used to determine the amount of copper in the solutions. The objectives of this study are to determine the differences in copper removal between crab, prawn and cockle shells at fixed concentrations and weights of shells.

## MATERIALS AND METHODS

### *Sorbent preparation*

The crab shells of *Scylla* spp., prawn shells of *Penaeus* spp. and cockle shells of *Anadara* spp., were collected from a seafood restaurant in Sungai Long, Cheras, Malaysia. The shells of the mentioned crustaceans were identified based on identification characteristics based on previous literature (Budín *et al.*, 2014). The shells were boiled separately in distilled water in a 500 mL beaker for approximately two hours under a fume hood to remove the excess meat and seasonings on the shells as these shells were cooked prior to experimentation. No chemicals were used to pretreat the shells. The shells were placed on sheets of aluminum foil and dried in an oven at 60°C for an hour to remove moisture. The shells were then crushed using a pestle and mortar, and then sieved through a 0.5 mm pore size sieve to ensure the shell sizes were standardised (Figure 1) (Vijayaraghavan *et al.*, 2011).

### *Preparation of screen-printed carbon electrodes (SPCE)*

The SPCE's were purchased from BioGenes Technologies Sdn Bhd. The working electrode of the SPCE's were then rinsed with 10 mL of distilled water using a 10 ml micropipette. The SPCE's were then left to dry in a plastic container. An amount of chitosan was mixed with an amount of acetic acid and was placed on a magnetic stirrer for two hours. The exact amount of chitosan used cannot be specified at the moment due to a confidentiality agreement with Biogenes Technologies Sdn Bhd. (Tang, 2019, pers. comm.). 10 µl of the chitosan solution was then carefully drop-coated on the working electrode of the SPCEs. Once the SPCE's had been drop-coated, they were left in the plastic container to dry for 24 hours.

### *Sorption experiments*

An initial test was done to determine the detection limit of copper on the screen printed carbon electrode with different copper concentrations (20, 40, 60, 80 and 100 ppm). A pre-test was first conducted with a copper concentration of 20 ppm with different weights of the shells to determine the shell weight with the highest sorption capacity.

Initial readings of the copper solution were first taken by differential pulse voltammetry (DPV) using the SPE which was connected to a galvanostat (Metrohm Autolab PGSTAT101). The first batch of experiments were carried out in 100 mL conical flasks with different shell weights (20, 40, 60, 80 and 100 mg). The prepared flasks were then sealed using parafilm to prevent evaporation. The conical flasks were then placed in a rotary incubator set at 30°C and 150 rpm for 24 hours. The solution was then filtered through separate filter papers with pore sizes of 5-13 µm to remove the shells and the filtered solution was

analyzed to obtain the final readings. The following experiment was then repeated using the weight of shells obtained from the pre-test, which was then tested with different copper concentrations (1, 5, 10, 15 and 20 ppm). The removal efficiency was calculated (formula given below) where  $C_0$  is the initial peak reading and  $C_1$  is the final peak reading. A multifactorial ANOVA was conducted for both sets of experiments.

$$\% = \left( \frac{C_0 - C_1}{C_0} \right) \times 100$$



**Figure 1.** Crushed and sieved crab shells using a 0.5 mm pore size sieve. Prawn and cockle shells were also crushed and sieved to a size of 0.5mm or less.

## RESULTS AND DISCUSSION

The results showed that the readings beyond 20 ppm had shifted to the right beyond the range of copper which is usually between -0.4V and -0.2V, indicating a detection limit (Figure 2). Thus the optimum concentration to be used in the pre-test was 20 ppm. The following phase was to narrow

down and determine the shell weight, (20, 40, 60, 80 and 100 mg) with the highest sorption capacity. The highest removal percentage was observed at 20 mg and 40 mg (Table 1).

### ***Removal of 20 ppm of copper between shell weights 20, 40, 60, 80, 100 mg***

For crab shells, the highest percentage of copper removal was observed at 20 mg and the lowest at

80 mg of crab shells with removal percentages of 8.367% and 0.372% (Table 1), respectively. This may be due to that fact that as the weight of the adsorbent increased the total adsorption sites of copper reduces as a result of overlapping and aggregation of adsorption sites (Ndi Nsami *et al.*, 2019). We assume that the aggregation of binding sites means that the binding sites had become tight clusters.

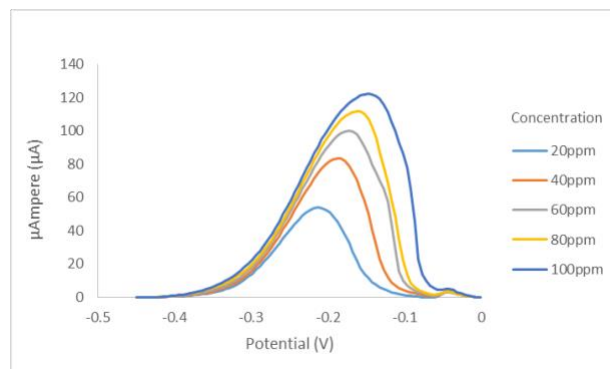
The highest removal percentage for prawn shells was seen at 20 mg with a removal percentage of 20.49% while the lowest removal efficiency was seen at 100 mg for prawn shells with a removal percentage of 10.65% (Table 1). An increase in biosorbent concentration increases the biosorbed metal ions because of an increase in surface area of the biosorbent (Vijayaraghavan *et al.*, 2006).

As for cockle shells, the removal percentage was lower compared to the other shells. The highest removal percentage was at 20 mg of cockle shells with a removal percentage of 2.628% and the lowest was observed at 100 mg of cockle shells with a removal percentage of -11.02% (Table 1). The negative removal percentage for 100 mg shell weight could be an anomaly and we attribute this to the disassociation of calcium carbonate (due to the presence of hydrochloric acid in the buffer) to calcium and carbonate in which the release of copper ions could have interfered with results obtained from the use of anodic stripping voltametry.

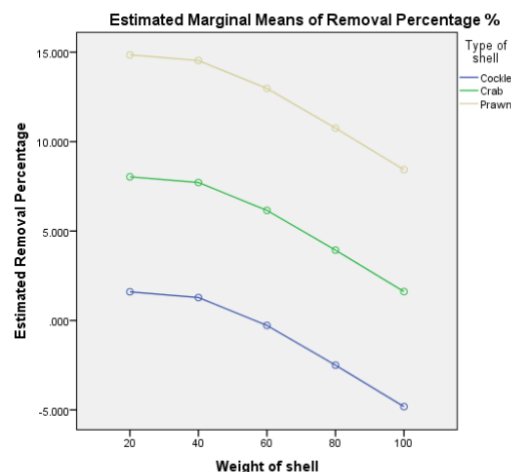
The multifactorial ANOVA showed a significance between the removal percentage of copper and the type of shell used (Table 2). However there was no significance between the removal percentage and weights of shells. A graph was plotted to determine the estimated marginal means of the removal percentage which suggested that the removal percentage decreased as the weight of shells increased (Figure 3). Thus the following part of the experiment was conducted with shell weights of 20 and 40 mg.

Removal efficiency improves as the amount of adsorbent is increased. However, in some cases, the opposite is seen because metal ions are not able to bind with all of the exchangeable sites

of the adsorbent or due to complex ion interactions, which results in a low metal uptake (Morris and Sneddon, 2011). Alwi *et al.* (2015) also showed that the adsorption capacity of shrimp shells increased between 10 mg and 50 mg but then decreased as the shell weights was increased up to 100 mg.



**Figure 2.** Differential pulse voltammograms (DPV) of copper concentration 20 ppm to 100 ppm, measured in a working buffer of HCL/KCL (pH 4.5) with a scanning rate of 50 mV/s. The graph begins to shift to the right beginning at 40ppm indicating a detection limit.



**Figure 3.** Estimated marginal means between removal percentage of copper and multiple weight of shells (20, 40, 60, 80, 100 mg). The estimated means shows a large variability between the total means of 5.619 and the column means (Type of shell).

**Table 1.** Removal percentage of 20 ppm of copper by different shell weights (20, 40, 60, 80 and 100 mg).

Weight (mg)	Copper Removal Percentage (%)		
	Crab Shell	Prawn Shell	Cockle Shell
20	8.367	13.488	2.628
40	8.134	13.148	2.247
60	4.970	13.119	0.763
80	0.372	11.145	0.670
100	5.603	10.647	-11.023*
	5.49 ± 3.23	12.31 ± 1.31	-0.94 ± 5.7

\*Negative results indicate that the adsorbent contaminated the samples.

**Table 2.** Multifactorial ANOVA for test between different weight of shells and type of shells.

Dependent Variable: Removal Percentage %

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	527.096 <sup>a</sup>	6	87.849	7.744	0.005
Intercept	473.519	1	473.519	41.741	0.000
Shell	439.191	2	219.595	19.358	0.001
Weight	87.906	4	21.976	1.937	0.198
Error	90.753	8	11.344		
Total	1091.368	15			
Corrected Total	617.850	14			

<sup>a</sup>R Squared = 0.853 (Adjusted R Squared = 0.743)

**Table 3.** Removal percentage of copper by shells weighing 20 and 40 mg.

Concentration (mg/L)	Copper Removal Percentage (%)					
	20 mg			40 mg		
	Crab Shell	Prawn Shell	Cockle Shell	Crab Shell	Prawn Shell	Cockle Shell
1	12.267	8.517	-48.795*	3.660	6.803	-110.465*
5	4.605	18.927	-31.874*	0.951	5.293	-35.223*
10	12.243	34.399	-6.570*	9.739	6.495	-11.550*
15	31.220	31.263	6.753	21.169	18.282	-13.436*
20	30.469	31.587	12.781	16.264	17.657	-5.555*
	18.16 ± 11.99	24.94 ± 10.95	-13.54 ± 26.14	10.36 ± 8.45	10.91 ± 6.48	-35.25 ± 43.52

\*Negative results indicate that the adsorbent contaminated the samples

### Removal of copper concentrations 1, 5, 10, 15, 20 ppm with 20 & 40 mg shells

The crab shells at 20 mg showed the highest removal percentage was 31.22% at 15 ppm of copper, which then decreased to 30.469% at 20 ppm of copper (Table 3). This shows that the percentage of copper removal increases from a heavy metal concentration of 1 ppm to 15 ppm however the percentage decreases at higher heavy metal concentration levels. The same can be said for the crab shells weighing 40 mg as the removal percentage decreased at 15 ppm of copper.

The removal percentage of the prawn shells increased with the initial metal concentration but

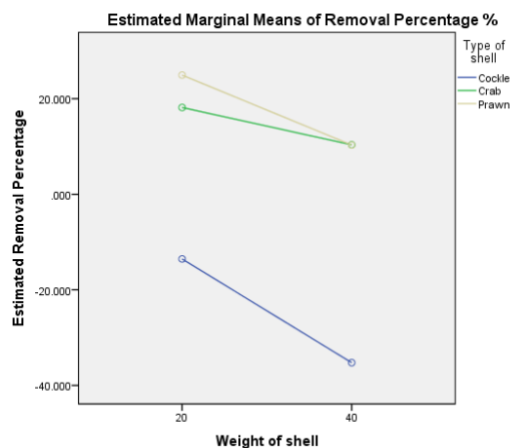
decreased as the concentration of copper increased (Table 3). With more copper content in a solution, larger fractions of active sites are involved in the adsorption process, hence the increase in removal percentage decreases (Vijayaraghavan *et al.*, 2006). Compared to both the crab and cockle shells, the prawn shells showed the lowest standard deviation, 10.95 and 6.48 at both weights, 20 and 40 mg (Table 3).

As for the cockle shells, although the removal percentage begins with a negative value of -48.795, it is seen to increase as the copper concentration increases. Similar to the first test, it is likely that the disassociation of calcium

carbonate led to the interference of copper ions within the shells. Cockle tissue is known to contain different types of heavy metals which could have led to these results (Townsend, 2010). The multifactorial ANOVA indicated a significance of 0.0001 between the types of shells and the removal percentage and this is probably due to the large difference between each shell's adsorption rate. However there is no significance when the weights 20 mg and 40 mg were compared (Table 4). A graph was also plotted to determine the estimated marginal means of the removal percentage which suggests that the removal percentage would be lower with 40 mg shells (Figure 4). Additionally, traces of copper in the cockle's habitat could have possibly been incorporated with the calcium carbonate in the cockle's shell as the cockles grew in size.

The current study is a preliminary one with uncertainties such as negative readings for the cockle shell removal rate. None of the shells were pre-treated by demineralization or deproteinisation. Thus the logical explanation for the higher absorbance by prawn shells is because prawn shells are less dense compared to the crab shells because crab shells mainly constitute of calcium carbonate (Chen *et al.*, 2008; Aklog *et al.*, 2016). Chui *et al.* (1996) showed that with

demineralization and deproteinisation, shrimp chitin showed removal efficiencies up to 99.6% with a copper concentration of 100 ppm. It also could be assumed that the maximum removal percentage could have been reached in less than 24 hours.



Covariates appearing in the model are evaluated at the following values: Copper Concentration = 10.20

**Figure 4.** Estimated marginal means of removal percentage of copper and two weights of shell (20 & 40 mg). The estimated means shows a large variability between the total means of 2.62 and the column means (Weight of shell) with no significant interaction between the two variables.

**Table 4.** Multifactorial ANOVA for test between different 20 & 40 mg shells with different concentrations of copper.

Dependent Variable: Removal Percentage %

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	18497.903 <sup>a</sup>	6	3082.984	11.721	0.000
Intercept	3071.917	1	3071.917	11.679	0.002
Concentration	5730.552	1	5730.552	21.787	0.000
Weight	1623.734	1	1623.734	6.173	0.021
Shell	10902.059	2	5451.029	20.724	0.000
Weight * Shell	241.558	2	120.779	0.459	0.637
Error	6049.588	23	263.026		
Total	24734.372	30			
Corrected Total	24547.491	29			

<sup>a</sup>R Squared = .754 (Adjusted R Squared = 0.689)

### CONCLUSION

The current study has shown that seafood waste can be utilized to adsorb heavy metals, in this case, copper. However among the three shells, cockle

shells may not be the most efficient copper biosorbent as some of the final readings had shown a higher concentration of copper which could be due to cockles being filter feeders thus whatever they consume also becomes part of their shell composition.

More in-depth studies are needed to further prove the copper removal efficiencies of seafood waste incorporating contact time of the shells with the heavy metal solution. Comparative studies could be done to compare the removal percentage of untreated shells and treated shells as the prawn shells and crab shells contain calcium carbonate, chitin and proteins. Thus, although prawn shells and crab shells did show high removal percentages, this study does not determine if the metals were being adsorped to the proteins, chitin or the calcium carbonate from these two shells.

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