Biosorption of lead and chromium in surface water using Philippine mud crab (*Scylla serrata*) and tahong (*Perna viridis*) shells

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Abstract. This study aimed to prove the feasibility of Philippine mud crab (*Scylla serrata*) and tahong (*Perna viridis*) shells in treating heavy metal content of surface water through the removal of lead (Pb) and chromium (Cr). Surface water samples were collected at Pasig River (at the deepest point at Guadalupe, Makati City, Philippines). The surface water samples were then treated with varying biosorbent dosages and contact time. Based on statistical tests, there was a significant difference in the Pb and Cr concentrations between treated and untreated surface water, this means that both *S. serrata* and *P. viridis* shells were effective in absorbing Pb and Cr. However, *S. serrata* shells were more effective in absorbing Pb while *P. viridis* shells proved to absorb more Cr content in surface water.

1. Introduction

Discharge of heavy metals with high solubility in the environment poses risks, such as harmful health effects in the human body once these metals have accumulated [1], when absorbed by living organisms [2]. With this, a high concentration of heavy metals, though some of these metals are essential for life, can prove to become detrimental to the environment due to the formation of complex compounds within the cell [3]. To prevent high dosage disposal of these heavy metals, certain techniques that meet the standards of the Maximum Contaminant Level (MCL) established by the USEPA are executed to treat inorganic effluent in waste water [1]. Limits on the types and concentration of heavy metals was regulated before the discharged of wastewater [1]. This study focuses on lead (Pb) and chromium (Cr) wherein the allowable concentrations present in discharged water are 0.006 mg/L and 0.05 mg/L, respectively. Excess heavy metal can already cause harm in the human body, causing serious health problems or even death. Application of pollution control such as bioavailability, mobility, and toxicity of metals are examples of methods used for the mitigation of harmful environmental effects of said heavy. In the 2012 census conducted by the Philippine Statistics Authority (PSA) regarding the Philippine water supply including sewerage, waste management, and remediation activities for all of the establishments, there are 828 establishments which actively engaged in water supply management [4]. This proved that the awareness about the wastewater

treatment process is already acknowledged by both public and private institutions. Thus, experimentations in laboratories were conducted to search for new, convenient, and cost-effective processes to remove heavy metals from bodies of water. Physicochemical treatments for removal of heavy metals from industrial water were widely used. Industries are using conventional process in removing heavy metals such as chemical precipitation, flotation, ion exchange, electrochemical deposition and adsorption [2].

Chemical precipitation happens when materials was changed when dissolve in water into solid particles. Addition of counter ions to reduce the solubility removes the ionic constituents in the water [5]. However, complete removal needed a certain amount of over dosage. Moreover, silt is the by-product of the process and was regarded as dangerous waste which can potentially harm the environment [6]. On the other hand, flotation was used for the treatment of aqueous and oily effluents which is known to cause serious environmental issues. Advanced equipment, low sludge generation, and high efficiency separation schemes are already available, this shows a great potential in removing oily effluents in wastewater [7]. Ion exchange happens when one or more undesirable ionic contaminants are removed and exchanged by the non-objectionable ionic substance. The contaminant and the exchanged substance must be dissolved and possess the same type of electric charge [8]. It is one of the most appropriate technologies to effectively remove dissolved inorganic ions. However, it does not effectively remove bacteria and operation can be costly over time. Existing researches use electrodeposition in fluidized bed electrode which was found out to be efficient in removal of metal ions in the solution [9]. The said conventional techniques were effective but researchers continued to find and study low-cost wastewater treatment processes, giving birth to the use of flocculants and biosorbents for removal of heavy metals on contaminated water systems.

Biosorbents are materials, which are living or dead, capable of carrying biosorption mechanism [10-12]. In their pursuit of maintaining clean bodies of water, Philippine Local Government Units (LGU's) initiate projects such as clean-up of rivers and coastal areas but the usage of flocculants and biosorbents are not evident in such projects. The activity merely involves gathering accumulated trash in nearby bodies of water or the body of water itself; this completely neglects the heavy metal pollution in the mentioned bodies of water. Recent studies found out that chitin found in shells that are used as biosorbent helps in the adsorption of heavy metals. Chitin is one constituent of the crustacean shells. It allows metal removal from solutions. Shift to crustacean shells as biosorbent is brought about by its non-toxic properties. Chitin can be acetylated to form chitosan which has the ability to form metal complexes [13]. Chitin and chitosan both are organic matters.

This study aimed to give preliminary data on the feasibility of Philippines mud crab (*Scylla serrata*) and tahong (*Perna viridis)* shells as a biosorbents. A thorough investigation regarding the effectivity of *S. serrata* and *P. viridis* shells as biosorbents in terms of varying biosorbent dosage and contact time on the removal of Pb and Cr in surface water was also carried out.

2. Materials and methods

2.1. Preparation of biosorbents
The Philippine mud crab (*Scylla serrata*) and tahong (*Perna viridis*) shells used in this work were collected from a local seafood restaurant located at Angat, Bulacan, Philippines. *S. serrata* shells are composed of 40% ash, 29% calcium carbonate and protein, 26% chitin, and 1.35% lipid [12]. Due to the aforementioned composition of *S. serrata* shell, it is recommended for the purpose of surface water treatment; specifically, chitin and calcium carbonate prove to be the most useful component of *S. serrata* shells when used for surface water treatment. Aris *et al* [12] defined chitin as an adsorbent for precipitation in the presence of heavy metals and copper-carbonate and lead-carbonate bonds are formed when lead and copper react with calcium carbonate. *P. viridis* shells proved to have calcium carbonate, which is one of the components needed in adsorbtion of heavy metals [14]. The shells were washed thoroughly using distilled water, and then dried without further chemical treatment. After drying, the shells were crushed using a mortar and pestle and sieved using a 30-mesh sieve for particle

size homogeneity. The pulverized shells were stored in desiccators prior to batch adsorption experiment.

2.2. Collection, preservation, and preparation of surface water samples

The surface water was collected from the deepest area of Pasig River which is located in Guadalupe, Makati City, Philippines. After sampling, a drop of nitric acid (HNO_3) was immediately added to preserve the sample. The collected surface water samples were filtered using No. 42 Whatman filter paper. The samples were then refrigerated at 4°C, following APHA guidelines [12], for later analysis.

2.3. Batch adsorption

This study used batch adsorption experiment in order to investigate the effects of varying biosorbent dosage and contact time to the uptake of heavy metals (Pb and Cr) in *S. serrata* and *P. viridis* shells.

The pH of the samples was measured using a digital pH meter – CyberScan pH 1500 (Eutech Instruments). According to Vijayaraghavan *et al* [15], in biosorption, the pH range of 4.5 to 6.0 (±0.1) is required in order to maximize the uptake of heavy metals. For pH adjustments, both 1.0 M NaOH and 1.0 M HCl were used. For each sample, varying dosages of *S. serrata* and *P. viridis* shells were added – 0.2 g, 2.0 g, and 5.0 g. The samples were then placed in an incubator shaker set at 37°C and with a speed of 150 revolutions per minute (rpm) at pre-determined time intervals. The treated surface water samples were filtered using No. 42 Whatman filter paper and digested right after.

A standard stock solution of 100 mg/L of Cr and Pb was prepared by dissolving $Pb(NO_3)_2$ and $Cr(NO_3)_3$ in distilled water. Five series standard solutions of $Pb(NO_3)_2$ and $Cr(NO_3)_3$ in the range of 2-10 mg/L was prepared to determine the concentration of the heavy metals in the treated water samples. This range was chosen to provide a well distributed concentration based from the initial mean concentration of Cr (5.9873 mg/L) on the untreated water sample. Then, a blank solution was prepared. The pH of each standard solutions where adjusted to match the pH of water samples. Parallel experiments (controls) were conducted without the addition of *S. serrata* and *P. viridis* shells biosorbents. In order to determine the concentration of Pb and Cr present in the water samples, these were subjected to the atomic absorption spectrometer (AAS). All experimental runs were carried out in triplicates and the average values were reported.

2.4. Statistical treatments

One-way ANOVA was used to determine if a mean difference is present between the heavy metal concentrations of the control group and the test group; the test group being the wastewater samples treated with *S. serrata* and *P. viridis* shells at varying amounts – 0.2 g, 2.0 g, and 5.0 g. In the event where a significant difference exists, based on the analysis, a Tukey post-hoc test was being performed to specify which biosorbent dosage in the test group differs.

To determine the mean difference of the biosorbent type of treated surface water samples, two-way ANOVA was performed where contact time and biosorbent type were set as independent variables to see the difference in the concentration of the two groups. If there is at least one mean concentration of Pb and Cr that is different from each other, based on the analysis, a Bonferroni post-hoc analysis was used to further explain the difference in mean of the given sample.

The use of correlation analysis aimed to determine the strength of association between contact time and biosorbent type towards the concentration of Pb and Cr. Moreover, the statistical analysis determined whether the relationship between contact time and biosorbent type would show if they are negatively or positively correlated. The positive relationship means that as the time increase the concentration at varying amounts of *S. serrata* and *P. viridis* increases. Whereas a negative relationship indicates that, the concentration of *S. serrata* and *P. viridis* both decreases across time.

3. Results and discussion

The results obtained from the batch adsorption experiment is graphically summarizes as shown in figure 1. At varying biosorbent dosages of Philippine mud crab (*Scylla serrata*) and tahong (*Perna*

viridis) shells, the lead (Pb) concentration decreases, conversely, concentration of Chromium (Cr) increases across time. However, when compared to the initial mean concentration of Pb and Cr in surface water, the adsorption of Pb and Cr at varying biosorbent dosages of *S. serrata* and *P. viridis* shells is significantly lower based on the result of the statistical treatment using one-way ANOVA and Tukey post-hoc analysis as presented in tables 1-4.
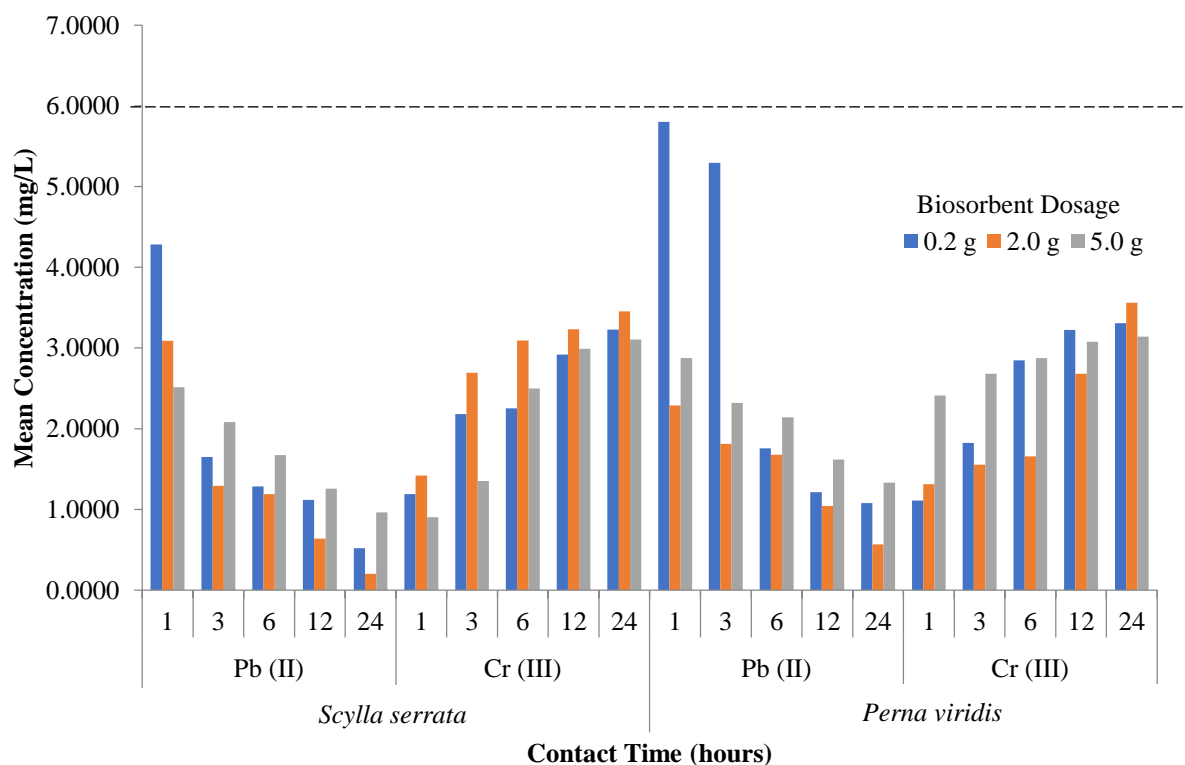

Figure 1. Effects of varying contact time and biosorbent dosage on lead (Pb) and chromium (Cr) uptake using Philippine mud crab (*Scylla serrata*) and tahong (*Perna viridis*) shells (broken lines are the initial heavy metal mean concentration).

Table 1. Analysis of variance of lead between treated and untreated surface water.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	128.179	3	42.726	28.394	0.000
Within Groups	75.238	50	1.505		
Total	203.417	53			

Table 2. Tukey post-hoc analysis of lead between treated and untreated surface water.

Test Group	Mean Concentration	F	*p*-value	Decision	Remarks
Control	6.0100	65.0430	0.0000	Reject H$_o$	Significant
0.2 g	2.4600				
2.0 g	2.1500				
5.0 g	2.8400				

Table 3. Analysis of variance of chromium between treated and untreated surface water.

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	96.787	3	32.262	65.043	0.000
Within Groups	24.801	50	0.496		
Total	121.587	53			

Table 4. Tukey post-hoc analysis of chromium between treated and untreated surface water.

Test Group	Mean Concentration	F	*p*-value	Decision	Remarks
Control	6.0100	65.0430	0.0000	Reject H_o	Significant
0.2 g	2.4600				
2.0 g	2.1500				
5.0 g	2.8400				

For Pb concentrations, the maximum adsorption was observed at 2.0 g biosorbent dosage for both *S. serrata* and *P. viridis* shells. This means whether the biosorbent dosages of *S. serrata* and *P. viridis* shells increases, there was no significant difference in the adsorption of Pb but not in the case of Cr. For *S. serrata* shells, 5.0 g biosorbent dosage was found to be more effective in the adsorption of Cr while *P. viridis* shells it was observed that 2.0 g biosorbent was more effective.

The possible cause of increasing trend in Cr biosorption with dosage is that as Cr reacts with the chitin and chitosan present in the shells, the *S. serrata* and *P. viridis* shells are being degraded since the organic matters react with most of the Cr. If too much Cr is present in the sample, then not all Cr can react with the chitosan. Another factor to be considered is the chitin decomposition. Since chitin serves as a source of energy, carbon, and nitrogen, degradation may take place extracellularly due to the polymer structure of the chitin [13]. Once this occurs, the Cr previously adsorbed can be desorbed. The increase in concentration of Cr may also be attributed to the oxidation and reduction which affects the toxicity of Cr [16]. Many factors affects the reduction of Cr which includes aeration, wetting and drying, iron and manganese status, microbial activity, organic matter, pH and the electron donors and acceptor availability [17]. Chromium occurs in two states, Cr (VI) and Cr (III) with Cr (VI) being known to be highly toxic as compared to Cr (III) which is much less toxic than Cr (VI) [18]. If anions are present in the medium (e.g. soil, groundwater), the adsorption affinity of Cr is reduced due to the competing anions [19]. Therefore, it is possible that Cr is being desorbed from the biosorbent in the presence of competing anions [20]. When the pH is increased, adsorption of Cr (VI) on hydrous metals oxides decreases which explains why chromium is mobile in neutral groundwater environment [18]. Moreover, free chitin (chitin that has not formed complexes) degrades, the metal-chitin complexes can exhibit the same biodegradability, explaining the trend obtained for Cr.

The mean concentration of Pb was significantly lower in both *S. serrata* and *P. viridis* shells. However, two-way ANOVA shown in table 5 proved that there is a significant difference on the mean concentration of Pb in *S. serrata* and *P. viridis* shells.

Table 5. Variation of sample at 0.2 g, 2.0 g, and 5.0 g of lead biosorption.

Mass	Biosorbent		Mean Difference	F	*p*-value	Decision	Remarks
0.2 g	*P. viridis*	*S. serrata*	1.2790	270.6810	0.0000	Reject H_0	Significant
	S. serrata	*P. viridis*	-1.2790				
2.0 g	*P. viridis*	*S. serrata*	1.4770	5.7600	0.0260	Reject H_0	Significant
	S. serrata	*P. viridis*	1.2820				
5.0 g	*P. viridis*	*S. serrata*	2.0580	28.2040	0.0000	Reject H_0	Significant
	S. serrata	*P. viridis*	1.7020				

Two-way ANOVA displayed in table 6 showed that there is no significant difference in the mean concentration of Cr at 0.2 g biosorbent dosage. While the mean concentration of Cr using *S. serrata* and *P. viridis* shells are significantly different in 2.0 g and 5.0 g biosorbent dosages. With that,

Bonferroni post-hoc analysis results showed that using *P. viridis* shells at 2.0 g and 5.0 g biosorbent dosages is more effective in adsorbing Cr in surface water (see table 7). Furthermore, in table 8, Bonferroni post-hoc analysis results showed with *p*-value of 0.000 across the sample dosage shows that adsorption of Pb is more efficient using *S. serrata*. On the other hand, the mean concentration of Cr increases across time using both *S. serrata* and *P. viridis* shells.

Table 6. Variation of sample at 0.2 g, 2.0 g, and 5.0 g of chromium biosorption.

Mass	Biosorbent		Mean Difference	F	*p*-value	Decision	Remarks
0.2 g	*P. viridis*	*S. serrata*	2.4620	1.2030	0.2860	Accept H_0	Not Significant
	S. serrata	*P. viridis*	2.3540				
2.0 g	*P. viridis*	*S. serrata*	2.1540	4.9650	0.0000	Reject H_0	Significant
	S. serrata	*P. viridis*	2.7780				
5.0 g	*P. viridis*	*S. serrata*	0.6670	94.2870	0.0000	Reject H_0	Significant
	S. serrata	*P. viridis*	-0.6670				

Table 7. Bonferroni post-hoc analysis on variation of time for chromium.

Dosage	F	*p*-value	Decision	Remarks
0.2 g	60.4040	0.0000	Reject H_0	Significant
2.0 g	56.9930	0.0000	Reject H_0	Significant
5.0 g	69.9400	0.0000	Reject H_0	Significant

Table 8. Bonferroni post-hoc analysis on variation of time for lead.

Dosage	F	*p*-value	Decision	Remarks
0.2 g	426.144	0.000	Reject H_0	Significant
2.0 g	91.2830	0.0000	Reject H_0	Significant
5.0 g	66.4380	0.0000	Reject H_0	Significant

Table 9. Correlation coefficient of lead and chromium concentration and contact time.

Heavy Metal	Biosorbent	0.2 grams	Verbal Interpretation	2.0 grams	Verbal Interpretation	5.0 grams	Verbal Interpretation
Lead (Pb)	*S. serrata*	-0.7137	Moderate Negative	-0.7936	Moderate Negative	-0.9187	Strong Negative
	P. viridis	-0.7792	Moderate Negative	-0.9610	Strong Negative	-0.8861	Strong Negative
Chromium (Cr)	*S. serrata*	0.8731	Moderate Positive	0.7325	Moderate Positive	0.8324	Moderate Positive
	P. viridis	0.8009	Moderate Positive	0.9813	Strong Positive	0.8604	Moderate Positive

Correlation analysis showed that the mean concentration of Pb and the contact time are negatively correlated as shown in table 9, thus the mean concentration of Pb reduces over time using *S. serrata* as biosorbent which proved to be better than *P. viridis*. The mean concentration of Cr and the contact time are positively correlated, thus the mean concentration of Cr also increases for both biosorbent across time. A study conducted by Berihun [21] utilized coffee husk in order to remove Cr from industrial wastewater. The study showed a trend where the concentration of Cr decreases as time increases; this might indicate that there might be other variables such as other effluents present in wastewater or there are other metals present in the biosorbents used in this study that might have supressed the binding capability of Cr thus the concentration of Cr increases over time. Presence of

organic matter might also be a factor since it reduces the Cr (VI) to Cr (III) thereby reducing the chromium's mobility in its medium. Once reduced, negative functional groups associated with the organic matter adsorb the Cr (III) resulting to lower concentration of Cr in the leachate. Considering low organic matter content, a high concentration of Cr can be observed in the leachate.

4. Conclusion

After the batch adsorption treatment and series of data analyses, the efficiency of Philippine mud crab (*Scylla serrata*) and tahong (*Perna viridis*) shells were compared in terms of its heavy metal (Pb and Cr) uptake by varying the biosorbent dosage and contact time. Based on the mean concentration of the control group, the use of both *S. serrata* and *P. viridis* shells as biosorbent was effective in the adsorption of Pb and Cr due to significant decrease in the mean concentration of Pb and Cr compared to the initial heavy metal concentration of the surface water. *S. serrata* shells were more effective in the adsorption of Pb content in surface water while *P. viridis* adsorbs more Cr.

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