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Demetallization of heavy metals in clam, corbicula fluminea utilizing catalytic chelation technique

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ABSTRACT

Malaysian clams can be contaminated with heavy metals which are cumulative poison through long term ingestion. Currently, there is no commercial technology has been developed for the removal of these heavy metals from contaminated clams especially *C. fluminea* species. Therefore, the main objective for this study is to develop method to remove these heavy metals from clams until achieve the permissible limit set by Malaysia Food Regulation 1985 (MFR) and Commission Regulation of EU (2006). In this study, three types of chelating agents were used which are trisodium citrate, sodium acetate, and disodium oxalate. Meanwhile, three types of catalysts supported on Al_2O_3, which are CaO, MgO, and BaO were used. Heavy metals analyse conducted using Flame Atomic Absorption Spectroscopy (FAAS). The results showed that initial concentration of Pb, Ni, and Cd were 28.73±6.06 (µg/g), 67.36±3.29 (µg/g), and 24.875±0.35 (µg/g) respectively. The optimization chelation technique showed that 300 mg/L of trisodium citrate gave the highest percentage removal of studied heavy metals which were 67.50% (9.34±0.75 µg/g) of Pb, 75.45% (16.54±1.29 µg/g) of Ni, and 74.63% (6.31±0.35 µg/g) of Cd. Then, the addition of catalyst to chelation technique improved the removal of heavy metals in *C. fluminea*. Therefore, among the three-catalyst studied, CaO/Al_2O_3 calcined at 1000°C gave the highest percentage removal of heavy metals which were 93.11% (1.98±0.32 µg/g) of Pb, 98.65% (0.91±0.18 µg/g) of Ni, and 96.10% (0.97±0.24 µg/g) of Cd, with stirring at 29.50±0.50ºC for 1 hour. Therefore, this study showed that catalytic chelation technique at optimum conditions could enhance the removal of heavy metals in C. *fluminea* to achieve the permissible limit set by MFR and EU.

Keywords: Heavy metal, Corbicula fluminea, Chelating agent, Catalyst, Flame Atomic Absorption Spectroscopy (FAAS).

1. INTRODUCTION

Corbicula fluminea (Genus: Corbicula) is known as Asian Clam or called as *Etok* in Malaysia. *C. fluminea* is one of the species of freshwater clam, which are commonly found in the sandy-muddy bottoms of the internal and sublittoral zones of the coastal environment [1]. *C. fluminea* may be found in the east coast of Malaysia such as Kelantan and Terengganu.

Heavy metals such as cadmium and mercury and metals such as arsenic, lead, magnesium, manganese, selenium, vanadium, and essential metals such as copper and zinc could be classified as potentially dangerous heavy metals [2]. These heavy metals contribute to degradation of marine ecosystems by reducing species diversity and abundance and through accumulation of metals in living organisms and food chains [3]. The factors which influence metal concentration and accumulation are bioavailability of metals, season, size, sex, hydrodynamics of the environment, changes in tissue composition and reproductive cycle [4]. Basically, clams focused on the use of total soft tissues of clams rather than the clams shell as a quantitative indicator to reflect the heavy metal contamination in the coastal area. Basically, types of toxic and heavy metals found in clams are Cd, Cr, Cu, Fe, Pd, Ni, Hg and Zn and Fe was the highest concentration accumulated in the soft tissue of clams which having 289 ppm [5], [6].

Table 1.1: Scientific Classification of *C. fluminea*

Kingdom	Animalia
Phylum	Mollusca
Class	Bivalvia
Suborder	Heterodonta
Order	Veneroida
Family	Corbiculidae
Genus	Corbicula
Species	*Corbicula fluminea*

One of the effective ways to treat heavy metals poisoning is through chelating technique [7]. Chelation technique is recommended for heavy metal poisoning and these metals exert their toxic substances by combining with one or more reactive groups essential for normal physiological functions. The chelating agent is the formation of ring-like structure that called as 'chelate' and the chelating agent will be bind to the metal ion and form complexes before excreting out from the flesh. The used of catalysts is needed to enhance the chelation technique. The purpose of the study is to remove toxic and heavy metals (Pb, Ni and Cd) from contaminated *C. fluminea* using several types of chelating agents with addition of catalysts. The result should complement with the permissible limit set by the Malaysian Food Regulations (1985) and Commission Regulation of EU (2006).

2. EXPERIMENTAL

Pb, Ni and Cd metals were analyzed through Flame Atomic Absorption Spectroscopy, FAAS (Perkin Elmer Pin AAcle). All reagents used in the study were analytical grade and were used without any purification. All the solutions were prepared using distilled water. Samples were digested using HNO_3 (QRëC™, 65%). All the plastic and glassware were cleaned by soaking in diluted HNO_3 and rinsed with distilled water. The element standard solutions used for calibration were produced by diluting a stock solution. The chelating agents used were sodium citrate dehydrate, $C_6H_5Na_3O_7.2H_2O$ (QRëC™), disodium oxalate, $Na_2C_2O_4$ (Bendosen) and sodium acetate trihydrate, $CH_3COONa.3H_2O$ (QRëC™). Meanwhile for the catalyst, the chemicals were magnesium acetate tetrahydrate, $C_4H_6O_2Mg.4H_2O$ (Rinting Scientific), barium nitrate, $Ba(NO_3)_2$ (Sigma Aldrich) and calcium nitrate tetrahydrate, $Ca(NO_3)_2.4H_2O$ (Sigma Aldrich). For standard solution for calibration, Pb, Ni and Cd pure single-element standards (Perkin Elmer) were used.

Sampling

Corbicula fluminea was purchased from the wet market in Kelantan. These clam samples were then brought back to laboratory and were stored in refrigerator until treatment.

Heavy Metals Removal

Treatment for heavy metals removal in *C. fluminea* was conducted using three types of chelating agents. *C. fluminea* were put in sack and were soaked in the beaker that contains the chelating agents with stirring for 1 hour. *C. fluminea* was rinsed with distilled water and digested before analyzed using FAAS. Chelation process was optimized using chelating agent (300 to 600 μL/L), for 1, 3 and 5 hours of treatment time and at different treatment temperature (29.5±0.5°C, 32.5±0.5°C and 37.5±0.5°C). For catalytic chelation treatment, samples were soaked in chelating solutions by immersing 0.25 g of prepared catalysts which put in sack, in the solution and left it at the bottom of the solution.

Heavy Metal Analysis

All prepared samples were digested using 65% of HNO_3. The digestion was done until clear solutions were obtained. After the digestion process, the samples would allow to cool and filtered using filter paper and then diluted to 25 mL of volumetric flask with distilled water. The prepared samples were then analysed for Pb, Ni and Cd using FAAS. The concentrations are presented in μg/g. The standard solution and blank were also run for calibration.

3. RESULTS AND DISCUSSION

3.1 Heavy Metal Concentration in *Corbicula fluminea*

The initial concentrations of heavy metals in *C. fluminea* were presented in **Table 3.1.** The trisodium citrate was varied from 300 to 600 mg/L to get the optimum concentration of chelating agent. Based on the results, the average initial concentration for each heavy metal in *C. fluminea* were higher than both the permissible limit stated by Malaysia and EU Standard.

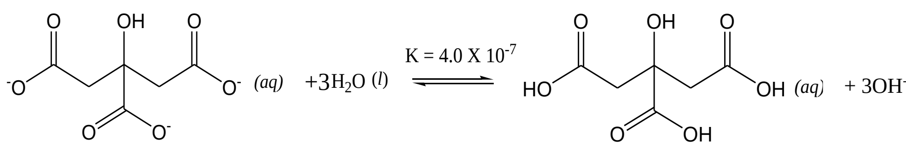
Optimization of Chelating Agents

The optimization treatment condition of chelation treatment by using trisodium citrate were at concentration dosing of 300 mg/L, 29.50±0.50°C of treatment temperature and 5 hours' treatment were initially selected as it gave the highest percentage removal of heavy metals in *C. fluminea.* However, one hour treatment was more practically used in laboratory and consumer's application thus, 1 hour of treatment time was applied for *C. fluminea* treatment with other chelating agents which are sodium acetate and disodium oxalate.

Table 3.1: Initial concentration of heavy metals in *C. fluminea* and the permissible limit of MFR and EU

	Cd (µg/g)	Ni (µg/g)	Pb (µg/g)
Initial Concentration	24.88±0.35	67.36±3.29	28.73±6.06
Permissible Limit:			
Malaysia	1.00	1.00	2.00
EU	1.00	1.00	1.50

The efficiency of trisodium citrate at different concentrations in the removal of heavy metals concentration in *C. fluminea* was presented in **Fig. 1**. From the results, it is revealed that the levels of heavy metals studied were successfully reduced by trisodium citrate treatment (Pb; 62.27%, Ni: 72.73% and Cd: 71.62%) and the concentration of 300 mg/L was found to be the most effective with highest percentage removal of heavy metals. The analysis suggests that there is a trend on heavy metals removal by trisodium citrate as the increased in dosing of chelating agents. The removal of the heavy metals increased and reached optimum at concentration of 300 mg/L. Exceeding this concentration, the percentage removal of heavy metals decreased accordingly. This pattern could be explained by Le Chartelier's principle [8] whereby the increased in concentration of trisodium citrate will enhance the reversible reaction towards the formation of starting material, thus decrease the citrate ion production to chelate the heavy metals.



Further investigating was done in the treatment time with varied to one, three and five hours. Results showed that the percentage removal of heavy metals removal increased as the time increased (**Fig. 2**). Five hours' treatment showed the highest percentage removal of heavy metals (Pb: 66.31 %, Ni: 75.45 %, Cd: 73.27 %). It is most probably the longer period of treatment time allowing the trisodium citrate to remove the heavy metals from *C. fluminea.*

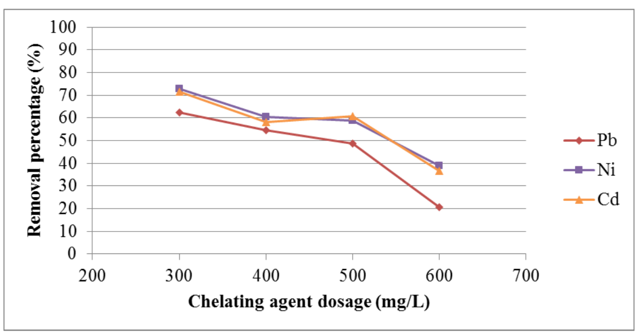


Fig. 1: Efficiency of trisodium citrate at different concentrations towards removal of heavy metals from *C. fluminea* at ambient temperature for 1 hour.

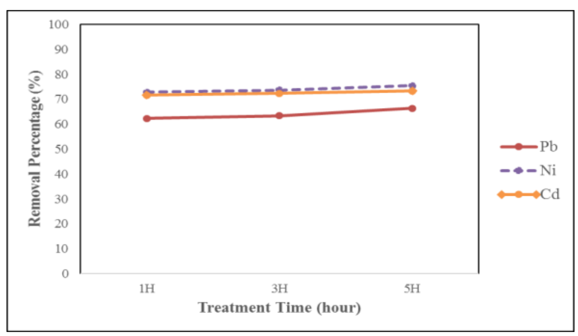


Fig. 2: Effect of treatment time on heavy metals removal in *C. fluminea* using 300 mg/L trisodium citrate at ambient temperature.

Effect of temperature on the efficiency of trisodium citrate was studied and results are presented in **Fig. 3.** From the results, the percentage removal of heavy metals increased at 29.50±0.50°C and decreasing at 32.50±0.50°C and 37.50±0.50°C. Highest percentage removal of heavy metals (Pb: 67.50%, Ni: 75.45%, Cd: 74.63%) was observed at 29.50±0.50°C. The increased with temperature up to 29.50±0.50°C may due to habitat of clams which can survive at 31.11°C thus, increase the mucus gland in clam and the percentage removal of toxic and heavy metals increased. On the other hand, heavy metals removal decreased at 37.50±0.50°C due to the high mucus gland from *C. fluminea* which covered the flesh surface and prevent the chelating agent to remove heavy metals.

The results of the three different chelating agents were presented in **Fig. 4.** The results indicated that trisodium citrate was the most effective chelating agents with the percentage removal of heavy metals (Pb: 67.50%, Ni: 75.45%, Cd: 74.63%) were obtained. The trisodium citrate gave the highest percentage removal of heavy metals in *C. fluminea* followed by sodium acetate and disodium oxalate. This trend showed that the high stability of the ring structured metal-citrate complex produced from chelation, thus increase the removal percentage of heavy metal ions [9].

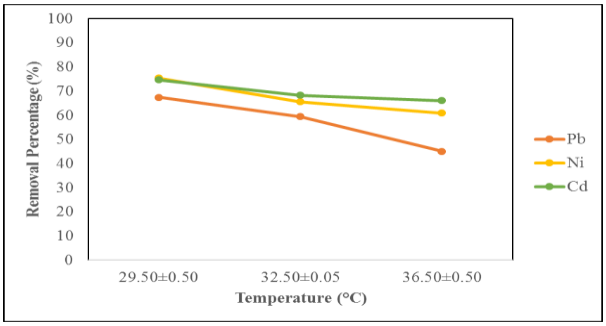


Fig. 3: Effect of reaction temperature on heavy metal removal in *C. fluminea* using 300 mg/L trisodium citrate for 1 hour.

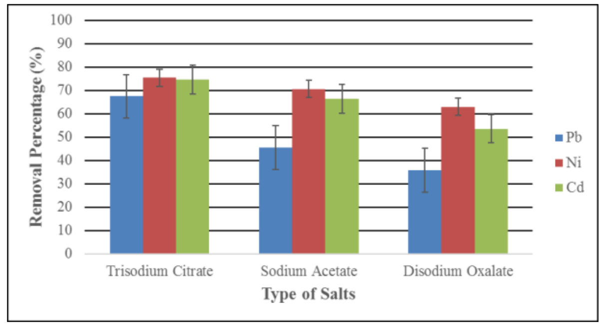


Fig. 4: Effect of chelating agent on heavy metal removal in *C. fluminea* at 300 mg/L trisodium citrate at 29.50±0.50°C for 1 hour.

Catalytic Activity

The study on the catalytic treatments was done to identify the effect of CaO, BaO and MgO supported with Al_2O_3 catalyst with 1000°C calcination temperature towards metals chelation of trisodium citrate. The heavy metals concentration with and without the presence of catalyst was determined. The results are presented in **Table 3.2**. The result showed that CaO/Al_2O_3 gave the highest percentage removal of heavy metals in *C. fluminea.* It indicates, with the presence of CaO/Al_2O_3 catalyst, the percentage removal of heavy metals increased compared without catalyst. Hence, the catalyst was optimized to get the optimum catalytic treatment. The increase in removal percentage of heavy metals probably due to the enhancement the formation of irreversible reaction by catalyst to produce the anion (citrate) which then reacts with the heavy metals in the contaminated *C. fluminea* [10].

The optimization for the treatment time of catalyst, one hour treatment gave the highest percentage removal of heavy metals in *C. fluminea.* The results were presented in **Table 3.3.** The longer treatment duration with catalysts increased the frequency of catalytic chelation cycle and the possibility of the chelate ions to reach out the metal ions for complexation [10]. The removal percentages of heavy metals were not much different between 30 minutes and 45 minutes. Thus, from the results, it can show that chelation technique and catalytic chelation technique can remove toxic and heavy metals in *C. fluminea* especially the catalytic chelation technique which can enhance the removal of heavy metals by having the highest percentage removal of heavy metals.

Table 3.2: Percentage removal of heavy metals in *C. fluminea* after treatment using various catalyst with stirring at 29.50±0.50ºC for 1 hour in trisodium citrate (300 mg/L)

Chelating agents		Pb (µg/g)	Ni (µg/g)	Cd (µg/g)
Initial Concentration		28.73±6.06	67.36±3.29	24.88±0.35
Without Catalyst		9.34±0.75	16.54±1.29	6.31±0.35
		67.50%	75.45%	74.63%
Calcined at 1000°C	CaO/Al_2O_3	1.98±0.32	0.91±0.18	0.97±0.24
		93.11%	98.65%	96.10%
	MgO/Al_2O_3	2.37±0.17	1.88±0.13	1.95±0.27
		91.77%	97.21%	92.14%
	BaO/Al_2O_3	3.45±0.21	2.03±0.26	2.84±0.12
		87.98%	96.98%	88.57%

Table 3.3: Percentage removal of heavy metals in *C. fluminea* after treatment using various catalyst with stirring at 29.50±0.50ºC for 1 hour in trisodium citrate (300 mg/L) at different treatment times.

Chelating agents	Pb (µg/g)	Ni (µg/g)	Cd (µg/g)
Initial Concentration	28.73±6.06	67.36±3.29	24.875±0.35
Treated for 1 hour	1.98±0.32	0.91±0.18	0.97±0.24
	93.11%	98.65%	96.10%
Treated for 45 min	3.06±0.17	7.45±0.16	5.22±0.19
	89.36%	88.95%	79.03%
Treated for 30 min	7.44±0.26	10.64±0.35	8.06±0.37
	74.11%	84.20%	67.60%
Treated for 15 min	9.13±0.21	11.26±0.41	11.65±0.22
	68.21%	83.28%	53.17%

4. CONCLUSION

The chelation method is found to be a potential technique for removal of heavy metals in *C. fluminea.* The optimization treatment conditions were obtained by having 300 mg/L trisodium citrate, one hour of treatment time and 29.50±0.50°C of treatment temperature. The initial concentration of heavy metals, Pb, Ni, and Cd were 28.73±6.06 µg/g, 67.36±3.29 µg/g, and 24.88±0.35 µg/g, respectively. Present investigation illustrates the efficiency of the studied chelation agents in the order of trisodium citrate > sodium acetate > disodium oxalate. The trisodium citrate gave the highest removal percentage of heavy metals which were, 67.50% (9.34±0.75 µg/g) of Pb, 75.45% (16.54±1.29 µg/g) of Ni, and 74.63% (6.31±0.35 µg/g) of Cd. The highest percentage removal of toxic and heavy metals for catalytic chelation technique were achieved in the presence of CaO/Al_2O_3 catalysts, which were 93.11% (1.98±0.32 µg/g) of Pb, 98.65% (0.91±0.18 µg/g) of Ni, and 96.10% (0.97±0.24 µg/g) of Cd at calcination temperature of 1000°C. In conclusion, catalytic chelation technique can enhance the removal of heavy metals in *C. fluminea* to achieve permissible limits set by Malaysian Food Regulation and EU Regulation.

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