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Demetallization of toxic and heavy metal in Razor Clam, *Ensis Arcuatus* utilizing catalytic chelation technique

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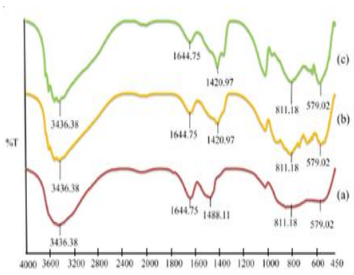
GRAPHICAL ABSTRACT



ABSTRACT

Ensis arcuatus is a local seafood and aquatic catches in Malaysia. The toxic and heavy metals content in *Ensis arcuatus* were recorded below the Malaysian Food Regulation (1985) and EU Food Regulations with the range of initial concentration Pb 0.053±0.030 to.0.074±0.04µg/g, Cd 0.008±0.030 to 0.140±0.240µg/g and Ni 0.003±0.040 to 0.180±0.070µg/g. In this research, the potential chelating agents which is trisodium citrate to remove toxic and heavy metals in *Ensis arcuatus* was determined using chelation method, while the optimum conditions used were 600 mg/L, dosage of chelating agents for 1 hours of treatment at temperature 29.5°C. The percentage removal of Pb, Cd and Ni were 21.62% (0.06±0.03µg/g), 31.73% (0.005±0.00µg/g) and 38.07% (0.001±0.02µg/g) respectively. The experimental data revealed that with the presence of catalyst, the percentage removal of the heavy metals increased. Experimental result showed that with the present of CaO/Al_2O_3, the percentage removal were increased. It is due to the properties of the CaO that have potential towards the removal of heavy metal in *Ensis arcuatus.* Meanwhile, XRD analysis depicted the active sites were due to the presence of orthorhombic Al_2O_3, monoclinic $CaAl_{14}O_7/CaO.2Al_2O_3$ and cubic $Ca_{12}Al_{14}O_{33}$ species. BET surface area study illustrated the surface area of 33.22 m^2/g. Finally, FTIR analysis concluded that CaO/Al_2O_3 catalyst at 1000°C treatment showed total removal of metal nitrate species.

Keywords: Heavy metal, *Ensis arcuatus*, Chelating agent, Catalyst, Flame Atomic Absorption Spectroscopy (FAAS)

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1. INTRODUCTION

The Razor clams *Ensis arcuatus (*Family: pharidae) is a local seafood and also aquatic catches. *Ensis arcuatus* locally known as 'siput buluh' in Kuala Selangor and called them 'ambal' in Sarawak. These species are a recognized well and also act as significant income source for Malaysian fisherman community in Malaysia at certain area. *E.arcuatus* is one of shelled marine catches that are most found in Terengganu, Selangor, Sabah and Sarawak, Malaysia. It is can be proved that razor clams is the one of the main species of economically important bivalves collected from mudflats in the western part of the Sarawak. It can be characterized by two long, narrow shell valves gaping at both ends but connected by hinges, two siphons and one strong foot. Moreover, each species displays different shell outlines as well as arrangement of the adductor scars and pallial muscle scars [1].

Since *E.arcuatus* are the bivalve molluks which is the clams, there are found on the mangrove mudflats and intertidal sandy beaches and are well known accumulator of heavy metals and exposed to toxic and heavy metal such as nickel (Ni), cadmium (Cd), lead (Pb), arsenic (As) and other heavy metal [2]. It will affect its quality and their demand. For evidence, the results shows that the Cu levels in marine catches reaching marketable size are within the limit of 40 ppm specified in the Malaysian Food Regulations 1985, whereas Zn level exceeded the limit of 100 ppm. Therefore, the heavy metals should be removed or lower that concentration due to the human health. Thus, the technique that suitable for removing of toxic and heavy metals is chelation treatment.

The trisodium citrate gets high percentage in removal of heavy metals due to the the citric acid was one of the organic acids and it seemed to be more promising as chemical extracting agents for removal toxic and heavy metals as well as it is biodegradable and can attain a higher metal extraction efficiency even at mildly acidic pH compared to other chelating agents [3]. The invented chelation is able to remove all the toxic and heavy metals comply to food standard and safe for human consumption. Besides that, catalyst play the important role in the catalytic oxidation process and can be used for heavy metals eliminations [4]. The presence of the catalyst causes an acceleration of chemical reaction without being consumed in the reaction. However, it might be deactivated and destroyed by the secondary reaction. Thus the application of catalyst has been conducted on this study to enhance the percentage removal of heavy metals [5].

2. MATERIAL AND METHODS

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 was trisodium citrate $Na_3C_6H_5O_7$ (QRëC™). Meanwhile for the catalyst, the chemicals was calcium nitrate tetrahydrate, $Ca(NO_3)_2.4H2O$ (Sigma Aldrich). For standard solution for calibration, Pb, Ni and Cd pure single-element standards (Perkin Elmer) were used.

2.1 Catalyst Preparation

The catalyst was prepared by dissolving 5 gram of calcium nitrate tetrahydrate salt powder into 5 mL of distilled water and was stir until the powder was completely dissolved. Alumina pallets were immersed into the solution. Later, it was aged at 80°C for 24 hours before further calcined at various temperature 900, 1000 and 1100°C for another 5 hours. All analysis were conducted in three series of replicates.

2.2. Catalyst Characterization

Several techniques were used to characterize the potential catalyst towards the removal of heavy metals from *E.arcuatus*. The obtained information on the chemical and physical properties enables one to determine the effects of its properties towards its catalytic performance. Through this research, the X-ray diffraction spectroscopy (XRD), nitrogen absorption (BET surface area) and Fourier transform infrared spectroscopy (FTIR) were used in the characterization techniques.

2.3. Sampling

Ensis arcuatus was purchased from the wet market in Mersing Johor. These razor clam samples were then brought back to laboratory and were stored in refrigerator until treatment.

2.4. Heavy Metal Removal

Treatment for heavy metals removal in *E.arcuatus* was conducted using the chelating agents. *E. arcuatus* were put in sack and were soaked in the beaker that contains the chelating agents with stirring for 1 hour. *E.arcuatus* was rinsed with distilled water and digested before analyzed using FAAS. Chelation process was optimized using chelating agent (300 to 800 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 was put in sack in the solution and was left it at the bottom of the solution.

2.4. 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 were allowed to cool and filtered using Whatman No 42 filter paper and then diluted to 10 mL 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 *Ensis arcuatus*

The initial concentrations of heavy metals in *E.arcuatus* are presented in Table 1. The trisodium citrate was varied from 300 to 800 mg/L to get the optimum concentration of chelating agent. The obtained results from FAAS showed that the initial *E.arcuatus* samples contain heavy metals meanwhile, Pb, Cd and Ni concentration below the permissible limit stated by Malaysian Food Regulation (MFR) and European Union (EU) as stated in Table 1

Table 1 Initial concentration of heavy metals in P. textile and the permissible limit of MFR and EU

Replicate of inial concentration	Concentration of Heavy Metals (µg/g)		
	Pb	Cd	Ni
E.arcuatus (i)	0.074±0.040	0.028±0.030	0.138±0.040
E.arcuatus (ii)	0.053±0.030	0.028±0.030	0.138±0.040
E.arcuatus (iii)	0.074±0.030	0.008±0.030	0.003±0.040
E.arcuatus (iv)	0.074±0.020	0.140±0.240	0.180±0.070
Average conc	0.068±0.030	0.058±0.100	0.107±0.050
Permissible limit :	Malaysia : Pb (2.00 µg/g); Cd and Ni (1.00 µg/g)		
	EU : Pb (1.50 µg/g); Cd and Ni (1.00 µg/g)		

3.2. Optimization of Chelating Agents

The optimization treatment condition of chelation treatment by using trisodium citrate were at 600 mg/L concentration dosing, 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 *E.arcuatus.* Since one hour treatment was more practically used in laboratory and consumer's application thus, 1 hour of treatment time was applied for *E.arcuatus* treatment.

In this study, the percentage removal of toxic and heavy metals in *E.arcuatus* after treatment using trisodium citrate (600 mg/L) had successfully removed the highest percentage of toxic and heavy metals which were 73.11% for Pb, 53.93% for Cd and 87.0% for Ni. The removal of the heavy metals increased and reached optimum at concentration of 600 mg/L. Exceeding this concentration,

the percentage removal of heavy metals decreased accordingly. This pattern could be explained by Le Chartelier's principle whereby the increased in concentration of trisodium citrate will enhance the reversible reaction towards the formation of citrate acid, 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 removal increased as the time increased (Fig. 2). Five hours treatment showed the highest percentage removal of heavy metals (Pb: 94.96%, Cd: 84.32%, Ni: 73.14%). It is most probably the longer period of treatment time allowing the trisodium citrate to remove the heavy metals from *E.arcuatus* .

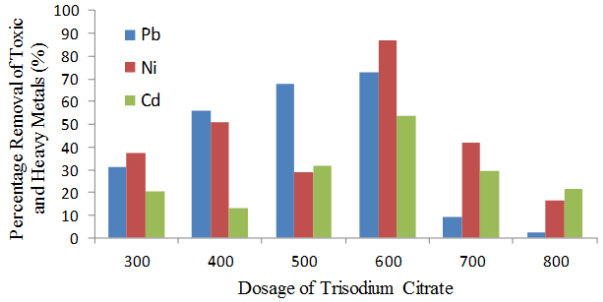

Fig. 1 The percentage removal of toxic and heavy metals in *E.arcuatus* using trisodium citrate (300mg/L -800 mg/L) at ambient temperature 29.50 °C with stirring for 1 hour

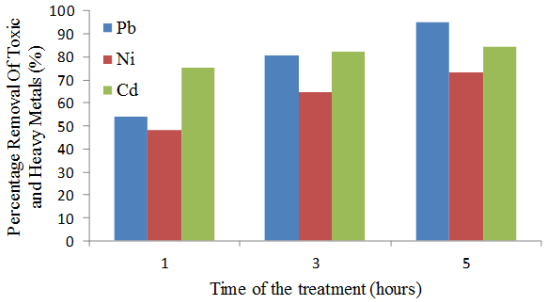

Fig. 2 Percentage removal of toxic and heavy metals in E.arcuatus using trisodium citrate (600mg/L) at ambient temperature (29.5°C) with stirring for 1, 3 and 5 hours

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 from 29.50±0.50°C decrease from 32.50±0.50°C to 37.50±0.50°C. Highest percentage removal of heavy metals (Pb: 21.62%, Cd: 31.73% , Ni: 38.07%) was observed at 29.50±0.50°C The increased with temperature from 29.50±0.50°C may due to habitat of clams which can survive at 29.50°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 32.50±0.50°C to 37.50±0.50°C due to the high mucus gland from *E.arcuatus* which covered the flesh surface and prevent the chelating agent to chelate with heavy metals.

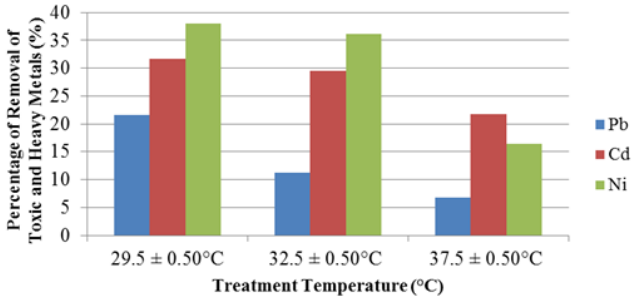

Fig. 3 Percentage removal of toxic and heavy metals in *E.arcuatus* using trisodium citrate(600mg/L) at various temperature of 29.50 ± 0.50°C, 32.50 ± 0.50°C and 37.50 ± 0.50°C with stirring for 1 hours.

3.3. Catalytic Activity

The removal of heavy metals in *E.arcuatus* using chelating agents was successfully proven and explained in previous section. However, the percentage removal of toxic and heavy metals is probably can still be improved by adding catalyst. For further improvement of removal of toxic and heavy metals from *E.arcuatus*, the experiment was continued to be carried out in the presence of catalyst which was CaO. In previous research, CaO was the most suitable catalyst for the treatment catalytic chelation technique due to their enhancement of the formation of irreversible reaction by catalyst to produce the anion (citrate) which react with the toxic and heavy metals that contaminated the razor clams. Therefore, a few characterization of CaO/Al_2O_3 were study to determine their physicochemical at various calcined temperature.

3.4. Characterization of catalyst

XRD analysis was conducted on the CaO/Al_2O_3 catalysts as to examine its crystallinity. The data obtained regarding the analysis was collected and summarized. Figure 4 shows the overlay of XRD diffractograms for CaO/Al_2O_3 catalyst calcined at 900, 1000 and 1100 °C for 5 hours. Based on the XRD diffractogram, CaO/Al_2O_3 catalyst calcined at 900°C shows broad amorphous pattern which indicates that the catalyst has low degree of crystallinity. CaO/Al_2O_3 catalyst calcined at 1000°C shows high degree of crystallinity as shown by the sharp peaks in XRD diffractogram. At the calcination temperature of 1100°C for CaO/Al_2O_3 catalyst, the peaks intensity were observed to be more intensify compared to the calcination temperature at 1000°C. The variation of calcination temperatures for the catalytic activity was defined to be higher at 1000 °C calcined temperature. It could be recognized that the orthorhombic Al_2O_3 species was the best alumina phase for active species besides the presence of active site species in monoclinic $CaAl_4O_7/CaO.2Al_2O_3$ and cubic $Ca_{12}Al_{14}O_{33}$ phases. High crystallinity was shown to promote the existence of the active species as proven by the presence of those species when calcination temperature was increased from 900 C to 1000 °C.

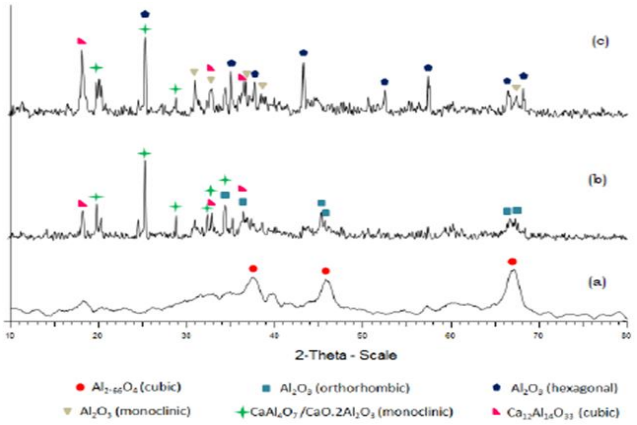

Fig. 4 XRD Diffractogram patterns of CaO/Al2O3 calcined at a) 900˚C, b) 1000˚C, c) 1100˚C for 5 hour

The surface area of the CaO/Al_2O_3 catalyst was determined by BET surface area analysis at different calcination temperature. The data as shown in Table 2 shows that the surface area of CaO/Al_2O_3 catalyst is inversely proportional to the calcination temperature. As the calcination temperature was increase, the surface areas decreased. BET surface area of CaO/Al_2O_3 catalyst (33.22 m^2/g) calcined at 1000°C is lower than that calcined at 900°C (68.16 m^2/g), in agreement with XRD analysis whereby when the degree of crystallinity of catalyst increases, the surface area decreases. However, calcination at 1100°C temperature gave low surface area (11.54 m^2/g) that most probably due to the presence of large aggregation and agglomeration compared to the calcination at 900°C and 1000°C which block the pore of surface area. According to the catalytic activity results the catalyst calcined at 1000°C gave the highest heavy metals removal. This finding implies that the surface area property is not the only main factor contribute to the higher catalytic activity.

Table 2 BET surface area of CaO/Al2O3 catalyst calcined at 900°C, 1000°C and 1100°C for 5 hours.

Catalyst	Calcination Temperature	Surface Area (m^2/g)
CaO/Al_2O_3	900°C	68.16
	1000°C	33.22
	1100°C	11.54

Figure 5 shows the comparison of CaO/Al_2O_3 catalyst FTIR spectra at different calcination temperature. The absorption bands between 579.02 to 811.18 cm^{-1} for CaO/Al_2O_3 catalysts were due to the stretching mode of metal oxide (M=O) groups. Wavelength at 1488.11 cm^{-1} as shown in the CaO/Al_2O_3 catalyst calcined at 900°C indicates the stretching mode of nitrate (NO^{3-}) group which meant that the Ca metal was not completely oxidized from its metal precursors of calcium nitrate tetrahydrate, $Ca(NO_3)_2.4H_2O$. Hence, nitrate group in the CaO/Al_2O_3 catalyst calcined at 1000°C which has a high catalytic activity and in CaO/Al_2O_3 catalyst calcined at 1100°C had been completely removed as indicated with the wavelength at 1420.97 cm^{-1}. Meanwhile, wavelength at 3436.38 cm^{-1} shows the presence of H_2O stretching group

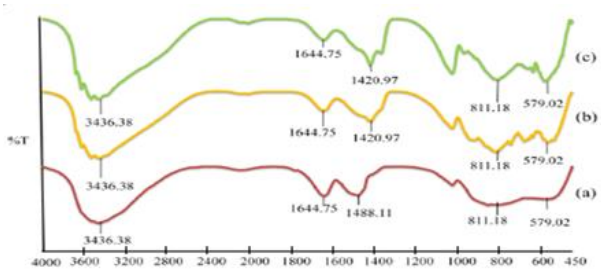

Fig. 5 FTIR spectra of CaO/Al_2O_3 catalyst calcined at (a) 900°C, (b) 1000°C and (c) 1100°C

Table 3 Percentage removal of toxic and heavy metals in *E.arcuatus* using trisodium citrate (600mg/L) with stirring at 29.5 ± 0.50°C in presence of CaO/Al_2O_3 (calcined at 1000°C) for 1 hours using two different initial sample concentration of *E.arcuatus*

Treatment catalyst	Pb (µg/g)	Cd (µg/g)	Ni (µg/g)
Initial Con. Samples (A)	0.074 ±0.02	0.008±0.24	0.003±0.07
Without catalyst	0.06±0.03 (21.62%)	0.005±0.00 (31.73%)	0.002±0.02 (38.07%)
CaO/Al_2O_3	0.05±0.03 (22.97%)	0.001±0.00 (87.50%)	0.001±0.03 (53.30%)

Treatment catalyst	Pb (µg/g)	Cd (µg/g)	Ni (µg/g)
Initial Con. Samples (B)	0.074 ±0.02	0.14±0.24	0.18±0.07
Without catalyst	0.06±0.04 (18.91%)	0.10±0.00 (28.57%)	0.11±0.05 (38.88%)
CaO/Al_2O_3	0.05±0.03 (22.62%)	0.02±0.00 (87.39%)	0.08±0.04 (55.95%)

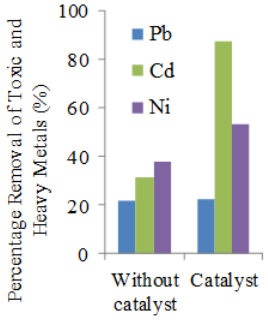

Fig. 6 Percentage removal of toxic and heavy metals in *E.arcuatus* using trisodium citrate (600mg/L) with stirring at 29.5 ± 0.50°C in presence of CaO/Al_2O_3 (calcined at 1000°C) for 1 hours by using Initial sample concentration A

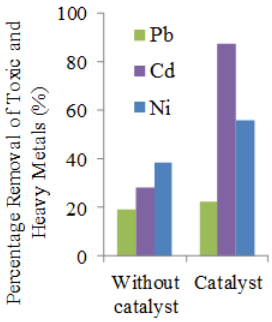

Fig. 7 Percentage removal of toxic and heavy metals in *E.arcuatus* using trisodium citrate (600mg/L) with stirring at 29.5 ± 0.50°C in presence of CaO/Al_2O_3 (calcined at 1000°C) for 1 hours by using Initial sample concentration B

Therefore, from the characterization of CaO/Al_2O_3, it proves that CaO that calcined at 1000°C gives more effective than other temperature calcined. Table 3 shows that the data of percentage removal of toxic and heavy metals from different initial concentration of *E.arcuatus* is conducted with the presence of catalyst that was calcined at 1000 °C. For initial sample A, the percentage removal of toxic and heavy metals in *E.arcuatus* after treatment using trisodium citrate (600 mg/L) in the presence of CaO/Al_2O_3 was 22.62% of Pb, 87.39%of Cd and 55.95% of Ni. For sample Initial

B, the percentage removal with the presence of catalyst were (Pb: 22.97%) (Cd: 87.39%), (Ni: 55.95%). This results shows that the percentage removal were increased with presence of catalyst than the experiment that carried out with absence of the catalyst. The comparison was simplified and are demonstrated in Figure 6 and Figure 7.

The result shows that with presence of the catalyst such as CaO/Al_2O_3, it was increased the percentage of removal of toxic and heavy metals. It was because the catalyst can speed up the reaction and lowering the energy barrier. Therefore, the catalytic chelation method was more effective since it can remove higher percentage of toxic and heavy metals compared with chelation technique only.

4. CONCLUSION

The removal of Toxic and Heavy Metal from *E.arcuatus* was carried out using edible chelating agents which was trisodium citrate. The toxic and heavy metals content in *E.arcuatus* were recorded below the Malaysian Food Regulation (1985) and EU Food Regulations with the initial concentration Pb 0.074±0.04 µg/g, Cd 0.028±0.03 µg/g and Ni 0.138±0.04µg/g. The chelating agent was react with the optimized condition which were 600 mg/L dosage of chelating agents for 1 hours of treatment at temperature 29.5°C. It conclude that the percentage removal of Pb, Cd and Ni were 21.62% (0.06±0.03µg/g), 31.73% (0.005±0.00µg/g) and 38.07% (0.001±0.02µg/g) respectively by using trisodium citrate. However, with the presence of catalyst which was CaO/Al_2O_3, the percentage of removal of heavy metals from *E.arcuatus* getting higher due to the reaction with catalyst toward the heavy metals.

Thus, experimental result showed that with the present of CaO/Al_2O_3, the percentage removal were increased with Pb 22.62% (0.05±0.03µg/g), Cd 87.39% (0.02±0.00µg/g) and Ni 55.95% (0.08±0.04µg/g). In conclusion, it shows that the removal of toxic and heavy metals can be determined by using edible catalytic chelation technique. Meanwhile, XRD analysis depicted the active sites were due to the presence of orthorhombic Al_2O_3, monoclinic $CaAl_4O_7/CaO.2Al_2O_3$ and cubic $Ca_{12}Al_{14}O_{33}$ species. BET surface area study illustrated the surface area of 33.22 m^2/g. Finally, FTIR analysis concluded that CaO/Al_2O_3 catalyst at 1000°C treatment showed total removal of metal nitrate species.

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