DEMETALLIZATION OF TOXIC AND HEAVY METALS IN CLAM, *PAPHIA TEXTILE* UTILIZING CATALYTIC CHELATION TECHNIQUE

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Abstract

This research was carried out to study the toxic and heavy metals removal like lead (Pb), cadmium (Cd) and nickel (Ni) from *Paphia textile.* Three types of chelating agents, namely trisodium citrate, sodium acetate and disodium oxalate and three types catalysts supported on Al_2O_3 namely MgO, CaO and BaO were used. The demetallization treatment screening carried out at a 400 mg/L, one hour treatment time and treatment temperature of 32.5 ± 0.5°C on *Paphia textile*, revealed trisodium citrate was the most potential chelating agent. Metals concentration were analysed using Flame atomic absorption spectroscopy (FAAS). The initial concentration of Pb, Ni and Cd in *Paphia textile* were found to be 1.05±0.18 µg/g, 0.83±0.21 µg/g and 0.56±0.02 µg/g respectively. The results on the optimization chelation technique showed that 400 mg/L of trisodium citrate gave the highest percentage removal of toxic and heavy metals with Pb 84.69% (0.16±0.05 µg/g), Ni with 78.60% (0.18±0.08 µg/g) and Cd with 41.96% (0.33±0.01 µg/g). Among the three catalysts studied, CaO/Al_2O_3 catalysts at an optimum calcination temperature of 1000°C, in the presence of trisodium citrate, gave the highest percentage removal with 87.79% (0.13±0.15 µg/g) of Pb, 83.56% (0.14±0.11 µg/g) of Ni and 76.43% (0.13±0.01 µg/g) of Cd. This study showed that catalytic chelation technique at optimum conditions able to remove further the toxic and heavy metals compared to chelation technique from *P. textile* to achieve permissible limits set by Malaysian Food Regulation (Cd and Ni: 1.00 µg/g; Pb: 2.00 µg/g) and EU Regulation (Cd and Ni: 1.00 µg/g; Pb: 1.50 µg/g).

Keywords: Toxic and heavy metal, Paphia textile, Chelating agent, Catalyst, Flame Atomic Absorption Spectroscopy (FAAS)

INTRODUCTION

Paphia textile (Family: Veneridae) is known as Lala in Malaysia. *P. textile* is an infaunal filter-feeding which feed on phytoplankton, small zooplankton and other organic materials. This bivalve commonly found in the sandy-muddy bottoms of the internal and sublittoral zones of the coastal environment [1]. Basically, *P. textile* was found in Pantai Bagan Panchor until Pantai Remis, Perak of Peninsular Malaysia.

Heavy metals such as cadmium and mercury and toxic 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 is the highest concentration accumulated in the soft tissue of clams [5]. Concentration of heavy metals in clams revealed that Fe gives the high concentration by having 289 ppm [6]. 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 a complexes before excrete out from the flesh. The used of catalysts is needed in order to enhance the chelation technique. The purpose of the study is to remove toxic and heavy metals (Pb, Ni and Cd) from contaminated *P. textile* using several types of chelating agents with addition of catalysts. The result should compliment with the permissible limit set by the Malaysian Food Regulations (1985) and Commission Regulation of EU (2006).

MATERIALS 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 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.

Catalyst Preparation

The catalyst was prepared by dissolving 6.25 gram of magnesium acetate tetrahydrate salt powder into 5 mL of distilled water and stir until the powder was dissolved. Alumina pallets were immersed into the solution. Later, it was aged at 80°C for 24 hours before further calcined at 1000°C for another 5 hours. Similar procedure was repeated to prepare 5 mol calcium nitrate tetrahydrate powder (5 gram in 5 mL distilled water) and 0.5 mol of barium nitrate powder (5 gram in 50 mL distilled water). All analysis was conducted in three series of replicates.

Sampling

Paphia textile was purchased from the wet market in Pasar Taman Universiti, Skudai. These clam samples were then brought back to laboratory and were stored in refrigerator until treatment.

Toxic and Heavy Metals Removal

Treatment for toxic and heavy metals removal in *P. textile* was conducted using three types of chelating agents. *P. textile*s were put in sack and were soaked in the beaker that contains the chelating agents with stirring for 1 hour. *P. textile* 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.

Toxic and 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.

RESULTS AND DISCUSSION

Toxic and Heavy Metal Concentration in *Paphia textile*

The initial concentrations of toxic and heavy metals in *P. textile* are presented in **Table 1.** The trisodium citrate was varied from 300 to 600 mg/L to get the optimum concentration of chelating agent. The obtained results from FAAS showed that the initial *P. textile* samples contain Pb a bit higher than permissible limit stated by European Union (EU) meanwhile, 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 toxic and heavy metals in *P. textile* and the permissible limit of MFR and EU

	Cd (µg/g)	Ni (µg/g)	Pb (µg/g)
Initial Concentration	0.85±0.002	0.82±0.07	1.77±0.09
Permissible Limit:			
Malaysia	1.00	1.00	2.00
EU	1.00	1.00	1.50

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The results of the three different chelating agents are presented in **Fig. 1.** The results indicated that trisodium citrate was the most effective chelating agents with the percentage removal of toxic and heavy metals (Pb: 84.69%, Ni: 78.60%, Cd: 41.96%) were obtained. The trisodium citrate gave the highest percentage removal of heavy metals in *P. textile* 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].

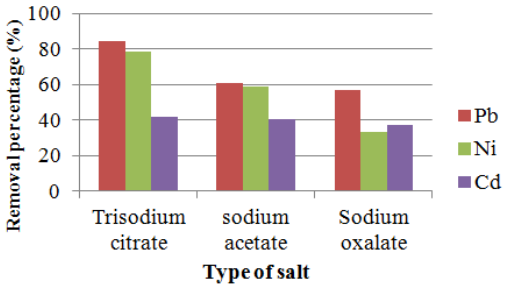


Fig. 1: Effect of chelating agent on toxic and heavy metals removal in *P. textile* at 400 mg/L trisodium citrate at 32.5±0.50°C for 1 hour.

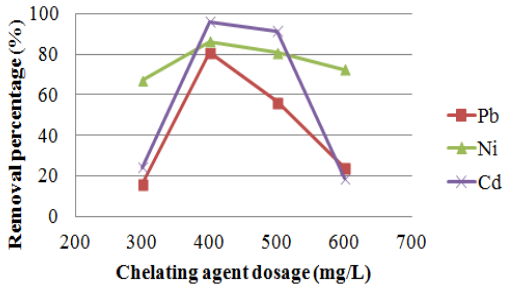
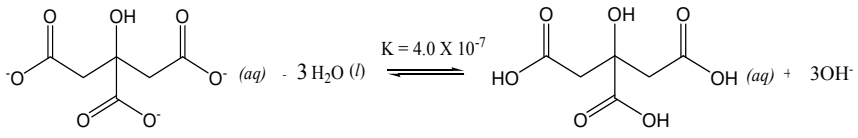


Fig. 2: Efficiency of trisodium citrate at different concentrations towards removal of toxic and heavy metals form *P. textile* at ambient temperature for 1 hour.

Optimization of Chelating Agents

The optimization treatment condition of chelation treatment by using trisodium citrate were at 400 mg/L concentration dosing, 32.50±0.50°C of treatment temperature and 5 hours treatment were initially selected as it gave the highest percentage removal of toxic and heavy metals in *P. textile.* Since one hour treatment was more practically used in laboratory and consumer's application thus, 1 hour of treatment time was applied for *P. textile* treatment with other chelating agents which are sodium acetate and disodium oxalate.

The efficiency of trisodium citrate at different concentrations in the removal of toxic and heavy metals concentration in *P textile* is presented in **Fig. 2**. From the results it is revealed that the levels of toxic and heavy metals studied were successfully reduced by trisodium citrate treatment (Pb; 80.96%, Ni: 86.99% and Cd: 96.20%) and the concentration of 400 mg/L was found to be the most effective with highest percentage removal of toxic and heavy metals. The analysis suggests that there is a trend on toxic and heavy metals removal by trisodium citrate as the increased in dosing of chelating agents. The removal of the toxic and heavy metals increased and reached optimum at concentration of 400 mg/L. Exceeding this concentration, the percentage removal of toxic and 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 toxic and 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 toxic and heavy metals removal increased as the time increased (**Fig. 3**). Five hours treatment showed the highest percentage removal of toxic and heavy metals (Pb: 71.65%, Ni: 57.36%, Cd: 50.70%). It is most probably the longer period of treatment time allowing the trisodium citrate to remove the toxic and heavy metals from *P. textile.*

Effect of temperature on the efficiency of trisodium citrate was studied and results are presented in **Fig. 4.** From the results, the percentage removal of toxic and heavy metals increased from 29.50±0.50°C to 32.50±0.50°C and decreased at 37.50±0.50°C. Highest percentage removal of toxic and heavy metals (Pb: 84.69%, Ni: 78.60%, Cd: 41.96%) was observed at 32.50±0.50°C. The increased with temperature up to

32.50±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, toxic and heavy metals removal decreased at 37.50±0.50°C due to the high mucus gland from *P. textile* which covered the flesh surface and prevent the chelating agent to remove toxic and heavy metals.

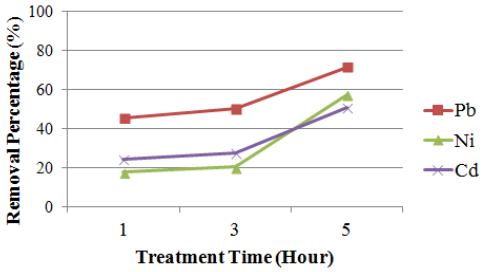


Fig. 3: Effect of treatment time on toxic and heavy metals removal in *P. textile* using 400 mg/L trisodium citrate at ambient temperature.

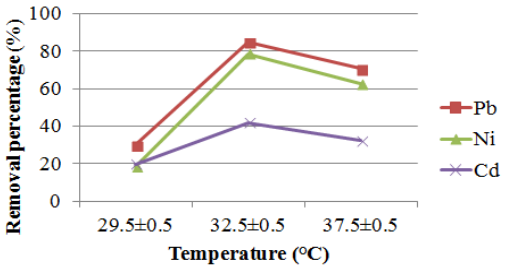


Fig. 4: Effect of reaction temperature on toxic and heavy metals removal in *P. textile* using 400 mg/L trisodium citrate for 1 hour.

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 2**. The result showed that CaO/Al_2O_3 gave the highest percentage removal of toxic and heavy metals in *P. textile*. It indicates, with the presence of CaO/Al_2O_3 catalyst, the percentage removal of toxic and heavy metals increased compared without catalyst. Hence, the catalyst was optimized to get the optimum catalytic treatment. The increase in removal percentage of toxic and heavy metals probably due to the enhancement the formation of irreversible reaction by catalyst to produce the anion (citrate) which then reacts with the toxic and heavy metals in the contaminated *P. textile* [10].

The optimization for the treatment of catalyst, one hour treatment gives the highest percentage removal of toxic and heavy metals in *P. textile.* The results are presented in **Table 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 shown that chelation technique and catalytic chelation technique can remove toxic and heavy metals in *P. textile* especially the catalytic chelation technique which can removed further towards heavy metals by having the highest percentage removal of toxic and heavy metals.

Table 2: Percentage removal of toxic and heavy metals in *P.textile* at 1000°C calcination temperature in trisodium citrate (400 mg/L) for 1 hour.

Chelating agents		Pb (µg/g)	Ni (µg/g)	Cd (µg/g)
Initial Concentration		1.05±0.18	0.83±0.21	0.56±0.02
Without catalyst		0.16±0.05	0.18±0.08	0.33±0.01
		84.69%	78.60%	41.96%
Calcined at 1000°C	CaO/Al_2O_3	0.13±0.15	0.14±0.11	0.13±0.01
		87.79%	83.56%	76.43%
	MgO/Al_2O_3	0.29±0.08	0.26±0.05	0.32±0.02
		72.17%	68.65%	43.09%
	BaO/Al_2O_3	0.54±0.01	0.25±0.19	0.33±0.03
		48.58%	69.59%	41.09%

Table 3: Percentage removal of toxic and heavy metals in *P. textile* at 1000°C calcination temperature in trisodium citrate (400 mg/L) at different treatment times.

Chelating agents	Pb (μg/g)	Ni (μg/g)	Cd (μg/g)
Initial Concentration	1.05±0.18	0.83±0.21	0.56±0.02
Treated for 1 hour	0.13±0.15	0.13±0.11	0.13±0.01
	87.79%	83.56%	76.43%
Treated for 45 min	0.47±0.11	0.14±0.06	0.30±0.00
	55.15%	82.80%	46.39%
Treated for 30 min	0.46±0.08	0.15±0.01	0.31±0.02
	56.34%	82.05%	44.90%
Treated for 15 min	0.52±0.010	0.53±0.12	0.27±0.04
	50.95%	36.05%	52.03%

CONCLUSIONS

The chelation method is found to be a potential technique for the removal of toxic and heavy metals in *P. textile.* The optimization treatment conditions were obtained by having 400 mg/L trisodium citrate, one hour of treatment time and 32.50±0.50°C of treatment temperature. Present investigation illustrates the efficiency of the studied chelation agents in the order of trisodium citrate > sodium acetate > sodium oxalate. The trisodium citrate gave the highest percentage removal of toxic and heavy metals, whereby 84.69% (0.16±0.05 μg/g) of Pb, 78.60% (0.18±0.08 μg/g) of Ni and 41.96% (0.33±0.01 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, namely 87.79% (0.13±0.15 μg/g) of Pb, 83.56% (0.14±0.11 μg/g) of Ni and 76.43% (0.13±0.01 μg/g) of Cd at calcinations temperature 1000°C. In conclusion, both chelation and catalytic chelation technique can remove toxic and heavy metals. However, the catalytic chelation technique offers better removal of toxic and heavy metals from *P. textile* to achieve permissible limits set by Malaysian Food Regulation and EU Regulation.

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