

Demetallization of toxic and heavy metals in squid, *D. Sibogae* utilizing catalytic chelation technique

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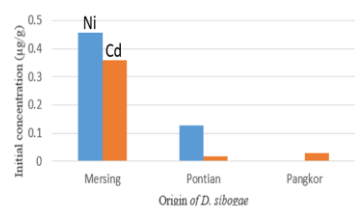
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GRAPHICAL ABSTRACT



Initial concentration of Ni and Cd in squid, *D. sibogae* from different origins

ABSTRACT

Malaysian squid were exposed to contamination of toxic and heavy metals. Continuous uptake of food containing heavy metals may affects human health. Therefore, this study was done to reduce percentage of heavy metals in squid since there is no technology has been developed in removing heavy metals in squid. In this study, the concentration of heavy metals (Ni and Cd) in muscle of *D. Sibogae* were determined. Squid samples were bought from Pasar Awam Taman Universiti in which the squid used were originated from Mersing with size range of 5.5 to 7.5 inch. From this study, the initial concentration of heavy metals found before treatment were 0.4565 and 0.358 µg/g for Ni and Cd respectively. The concentration of heavy metals contain were below permissible limit recommended by Malaysia Food Regulation (1985). Concentrations of chelating agent (trisodium citrate) used in this study were 300, 400, 500, 600, 700 and 800 ppm. After treatment, it was found that the optimum concentration of chelating agent used was 600 ppm with percentage of removal for Ni and Cd were 55.64% and 93.97 % respectively. After treatment for 1 hour, percentage removal of Ni and Cd were 73.67 % and 88.03 % respectively. To enhance the performance of chelation process, catalyst was added in which the catalyst used was CaO supported on Al₂O₃. Using catalytic chelation technique, the percentage removal of Ni and Cd were 89.64 % and 94.95% respectively. It was proven that catalytic chelation technique will give more percentage removal of heavy metals compared to chelation technique itself. From characterization, XRD analysis depicted the active sites were due to the presence of orthorhombic Al₂O₃, monoclinic CaAl₄O₇/CaO.2Al₂O₃ and cubic Ca₁₂Al₁₄O₃₃ species. BET surface area study illustrated the surface area of 33.22 m²/g. Finally, FTIR analysis concluded that CaO/Al₂O₃ catalyst at 1000°C treatment showed total removal of metal precursor's species.

Keywords: *D. sibogae*, heavy metal, chelating agent, catalyst, Flame Atomic Absorption Spectroscopy

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1. INTRODUCTION

Cephalopods are a small class of the phylum mollusca. Currently more than 700 species (octopuses, squids, cuttlefish and nautilus) are recognized inhabiting all the world's oceans, from the intertidal areas to the deep sea. The squid *Doryteuthis sibogae* is a neritic species which is under class: Cephalopoda and order: Teuthoidea (Neethiselvan and Venkataramani, 2002) with geographical distribution is at Eastern Indonesia and China. A study on heavy metals contamination in squid, *loligo* spp. tissues was done by Jamil et al., (2014). The research was using squid from coastal waters of Kedah-Perlis. Heavy metals of cadmium, copper, plumbum and zink in three different tissues collected from the ink-sac, head, and muscle of the squid were determined in this study. From this study, the concentration of Zn (35.06±4.06 mg/kg) was found significantly higher in all of the tissues, followed by Cu (15.10±13.28 mg/kg), Cd (4.76±3.77 mg/kg) and Pb (4.01±0.08 mg/kg).

It was discovered that Zn and Cu concentrations in the tissues were below the maximum limit recommended by the Food and Agriculture Organization, Malaysian Food Regulation (1985), as well as other countries. Meanwhile, Cd and Pb were recorded having higher values from the recommended concentrations [1]. According to the Metal Pollution Index (MPI), the ink-sac indicated medium-range contamination, while the head and muscle tissues showed low contamination levels. 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 *D. sibogae* using trisodium citrate as chelating agents with addition of catalysts.

2. EXPERIMENTAL

Pb, Ni and Cd metals were analyzed through Flame Atomic Absorption Spectroscopy (FAAS) which was carried out on Perkin Elmer Pin AAcle. All reagents used in the study were analytical grade. All the solutions were prepared using

deionized water. Samples were digested using HNO₃ (MERCK, 65%). All the plastic and glassware were cleaned by soaking in diluted HNO₃ (10%) and rinsed with distilled water. The element standard solutions used for calibration were from salt of the interested metal which were lead (II) nitrate, Pb(NO₃)₂ (ACS Reagent), cadmium sulphate, 3CdSO₄·8H₂O (Analar™) and nickel (II) nitrate, Ni(NO₃)₂·6H₂O (QRëc™). The chelating agent used was trisodium citrate dehydrate, C₆H₅O₇·2H₂O·3Na (QRëc™). Meanwhile, calcium nitrate tetrahydrate, Ca(NO₃)₂·4H₂O (Sigma Aldrich) was used for preparation of catalyst.

2.1 Catalyst preparation

The SBA-15 was prepared according to the method reported by Zhao et al. [10]. The triblock copolymer P123 (EO₂₀PO₇₀EO₂₀, Aldrich) was used as the structure-directing agent and tetraethyl orthosilicate (TEOS, Merck) as the silica source. The P123 was dissolved in the solution of deionized water and 2M hydrochloric acid solution and stirred at 40 °C for 1 h. The TEOS was slowly added to the mixture with vigorous stirring at 40 °C for 24 h, and the precipitate product was obtained. The precipitate product was filtered, washed with deionized water and dried overnight at 110 °C. The sample was calcined at 550 °C for 3 h to remove the triblock copolymer.

The Ni/SBA-15 catalyst was prepared by three types of preparation methods which were impregnation (IM), ion exchange (IE) and physical mixing (PM). For impregnation method, an appropriate amount of Ni salt precursor, Ni(NO₃)₂·6H₂O (Merck, 99%) was mixed with SBA-15, and then was heated slowly at 80 °C under continuous stirring and maintained at that temperature until nearly all the water had evaporated. The solid residue was dried overnight at 110 °C followed by calcination at 550 °C for 3 h to give dark grey colored Ni/SBA-15(IM). For the ion exchange method, an appropriate amount of Ni salt precursor, Ni(NO₃)₂·6H₂O (Merck, 99%) was mixed with SBA-15 under continuous stirring for 12 h. The product was filtered and dried overnight at 110 °C followed by calcination at 550 °C for 3 h to obtain Ni/SBA-15(IM). For the physical mixing method, an appropriate amount of Ni salt precursor, Ni(NO₃)₂·6H₂O (Merck, 99%) was calcined at 550 °C for 3 h to obtain black NiO powder. A desired amount of NiO was physically mixed with SBA-15 and calcined for 3 h at 550 °C to give a dark grey colored Ni/SBA-15(PM). In this study, Ni loading was adjusted at 5wt% for all catalysts.

2.2. Catalyst characterization

The catalyst was prepared by dissolving 5 gram of calcium nitrate tetrahydrate powder into 5 mL of deionized water. It was stirred until the powder was dissolved. Alumina pellets were immersed into the solution. Later, it was aged at 80°C for 24 hours before calcined at 1000°C for another 5 hours.

2.3. Sampling

D. sibogae was purchased from Pasar Awam Taman Universiti, Skudai. The squid samples were then brought back to laboratory and were stored in refrigerator until experiment begin.

2.4. Heavy metal removal

Treatment for heavy metals removal in *D. sibogae* was conducted using chelating agent which was trisodium citrate. *D. sibogae* were put in a sack and were soaked in beaker that contains chelating agent with stirring for 1 hour. *D. sibogae* was rinsed with distilled water and digested before analyzed using FAAS. Chelation process was optimized using chelating agent (600 mg/L) for 1 hour of treatment time and at ambient temperature (29.5±0.5°C).

2.5. Heavy metal analysis

All prepared samples were digested using 65% of HNO₃. The digestion was done until clear solutions were obtained. After 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 analyzed for Pb, Ni and Cd using FAAS. The concentrations were presented in µg/g.

3. RESULTS AND DISCUSSION

3.1. Heavy metal concentration in *D. Sibogae*

The initial concentration for both Ni and Cd in *D. sibogae* from Mersing were 0.4565 and 0.358 µg/g respectively. However, initial concentration of Pb was not detected by FAAS. Meanwhile, the initial concentration of Ni and Cd in *D. sibogae* from Pontian were 0.114 and 0.016 µg/g respectively. For *D. sibogae* originated from Pangkor, it was observed that

there was no detection of Ni from FAAS (Fig. 1) However there was present of Cd metal in *D. sibogae* from Pangkor which was 0.028 $\mu\text{g/g}$. It was clearly shown that Ni and Cd content in Mersing's squid, *D. sibogae* were the highest compared to Pontian's and Pangkor's *D. sibogae*. Therefore, samples used for this study were samples of squid, *D. sibogae* from Mersing due to the highest Ni and Cd contamination Results of initial concentrations of toxic and heavy metals in squid showed they were below the permissible limit of Malaysian Food Regulation (MFR) and European Union (EU).

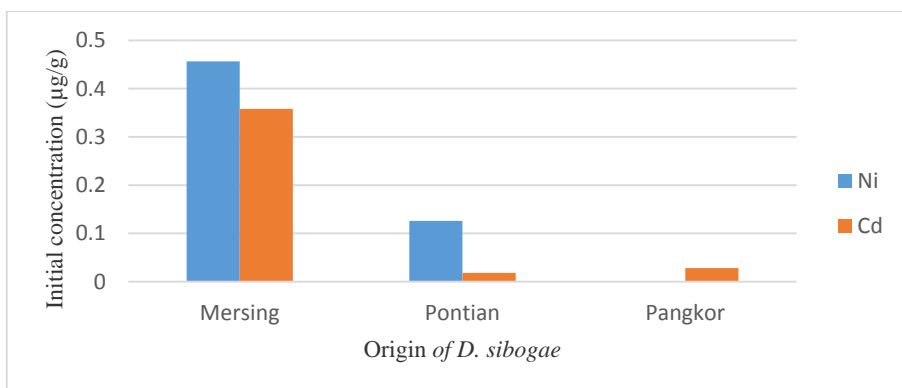


Fig. 1 Initial concentration of Ni and Cd in squid, *D. sibogae* from different origins

3.2. Optimization of chelation reaction

The optimization treatment condition of chelation treatment by using trisodium citrate were at 600 mg/L concentration dosing, $29.50 \pm 0.50^\circ\text{C}$ of treatment temperature and 2 hours treatment. The optimization condition were selected as it gave the highest percentage removal of heavy metals in *D. sibogae*. Since one hour treatment was more practically used in laboratory and suitable for consumer's application thus, 1 hour of treatment time was applied for treatment of *D. sibogae* with present of catalyst. The efficiency of trisodium citrate at different concentrations in the removal of heavy metals in *D. sibogae* is presented in Fig. 2. From the results, it was revealed that the levels of heavy metals studied were successfully reduced by trisodium citrate treatment (Ni: 55.64% and Cd: 93.97%). The optimum concentration of chelating agent found was 600 mg/L.

The analysis suggests that there is a trend on heavy metals removal by trisodium citrate as dosing of chelating agents were increased. The removal of heavy metals were increased as the concentration of chelating agent used increased. The percentage removal of heavy metals had 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 Chatelier's principle 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 [2].

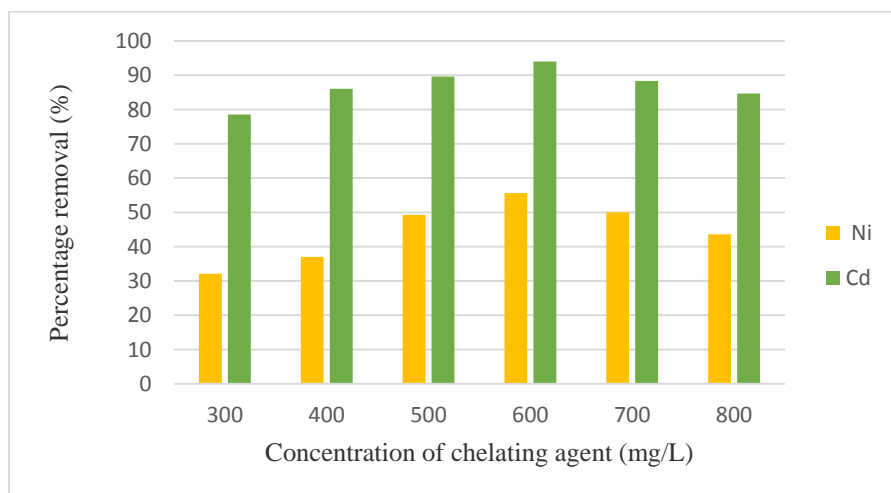


Fig. 2 Percentage removal of heavy and toxic metals in squid, *D. sibogae* at different concentration of chelating agent

Further investigation was done in the treatment time which varied from 30 minutes to 2 hours Results showed that the percentage removal of heavy metals were increased as the time increased (Fig. 3). Two hours treatment showed the highest

percentage removal of heavy metals (Ni: 87.60%, Cd: 92.99%). It is most probably the longer period of treatment time allowing the trisodium citrate to remove heavy metals from *D. sibogae*.

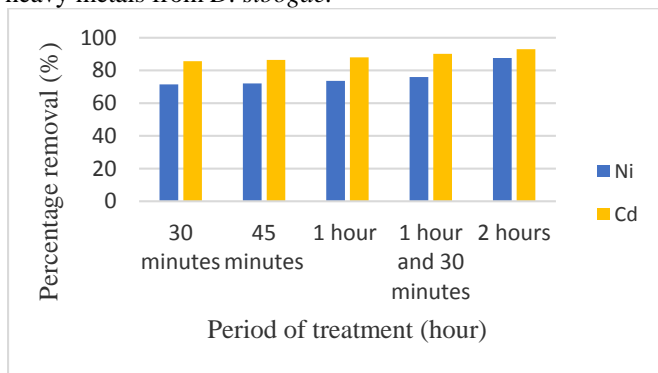


Fig. 3 Percentage removal of toxic and heavy metals using trisodium citrate (600 mg/L) at ambient temperature (29.0±0.5°C) with stirring for 30 minutes, 45 minutes, 1 hour, 1 hour and 30 minutes and 2 hours.

When treatment condition was set to the nature of sample’s habitat, the flesh may easily react to the environment in loosening the attraction of heavy metals in the flesh enabling the chelating agent to penetrate easily and thus chelated heavy metals contained in the flesh. As mean annual habitat temperature of *D. sibogae* habitat is 28.0 °C thus ambient temperature, 29.5 ±0.5 °C was used as the treatment temperature [3].

3.3. Catalytic activity

The study on the catalytic treatments was done on the effect of CaO supported with Al₂O₃ 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 1. It showed that CaO/Al₂O₃ gave higher percentage removal of heavy metals in *D. sibogae*. It indicates, with the presence of CaO/Al₂O₃ catalyst, the percentage removal of heavy metals increased compared to absent of catalyst. The increase in removal percentage of toxic and heavy metals probably due to the enhancement of formation of irreversible reaction by catalyst to produce anion (citrate) which then reacts with the toxic and heavy metals in the contaminated *D. sibogae* [4].

Table 1 The percentage removal of toxic and heavy metals in squid, *D. sibogae* after treatment with catalyst (CaO/Al₂O₃) with stirring at 29.5±0.5 °C for 1 hour in trisodium citrate (600 mg/L)

Chelating agent	Ni (µg/g)	Cd (µg/g)
Initial concentration	0.457±0.015	0.358±0.024
Without catalyst	73.67%	88.03%
With catalyst (CaO/Al ₂ O ₃) calcined at 1000°C	0.120±0.019	0.043±0.011
	89.64%	94.95%
	0.037 ±0.016	0.023±0.023

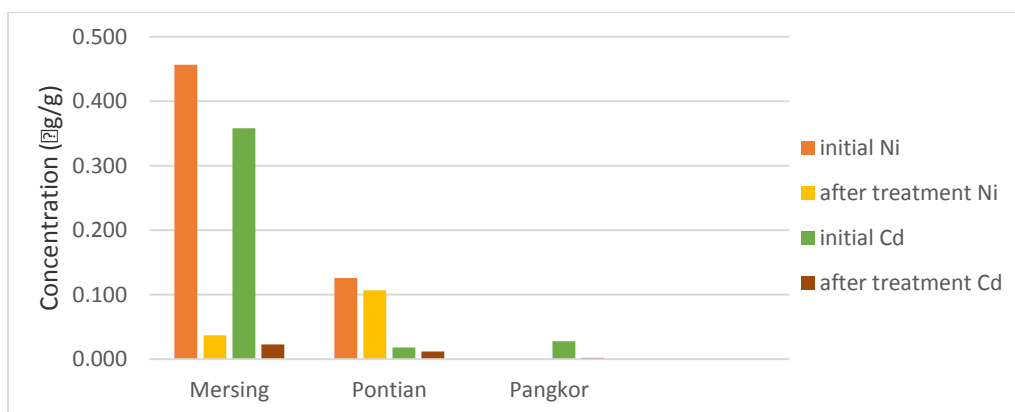


Fig. 4 The comparison of concentration of toxic and heavy metals in squids, *D. sibogae* from different origins before treatment and after treatment with catalyst (CaO/Al₂O₃) with stirring at 29.5±0.5 °C for 1 hour in trisodium citrate (600mg/L)

Comparison of initial concentration of toxic and heavy metals in three squids originated from different places with their concentration after treatment with catalyst can be observed as in Fig. 4. All of the squids showed decrement in concentration of toxic and heavy metals after treated with addition of catalyst.

3.4. Characterization of Catalyst

XRD analysis was conducted on the CaO/Al₂O₃ catalysts to examine its crystallinity. The data obtained regarding the analysis was collected and summarized as in Fig. 5

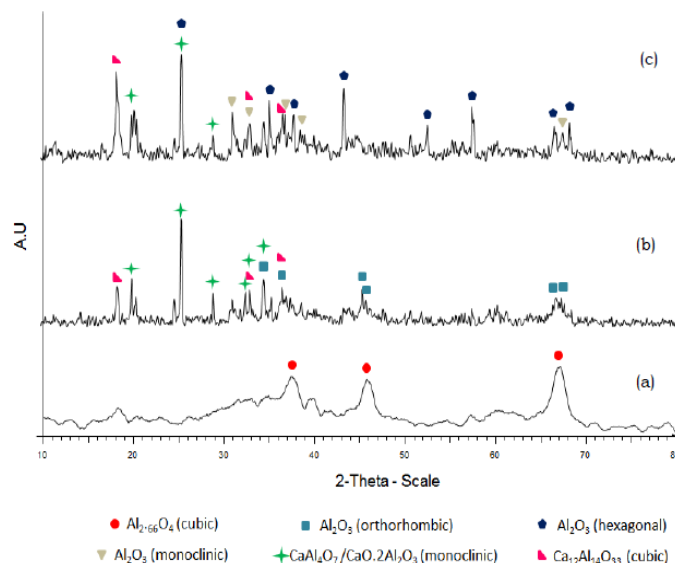


Fig. 5 XRD Diffractogram patterns of CaO/Al₂O₃ calcined at a) 900°C, b)1000°C c) 1100°C for 5 hours.

CaO/Al₂O₃ catalyst calcined at 1000°C shows high degree of crystallinity as shown by the sharp peaks in XRD diffractogram. Three new species which are orthorhombic Al₂O₃, monoclinic CaAl₄O₇/CaO.2Al₂O₃ and cubic Ca₁₂Al₁₄O₃₃ were present at this calcination temperature. The phase which was dominated by alumina support was revealed at 2θ = 67.354°, 66.767°, 45.881°, 45.407°, 34.554° and 36.568° for orthorhombic Al₂O₃. Meanwhile, the other two phases were due to solid state reaction and were observed at 2θ = 25.494°, 34.554°, 19.990°, 28.989°, 30.528° and 33.032° for monoclinic CaAl₄O₇/CaO.2Al₂O₃ while at 2θ = 18.412°, 33.427° and 36.568° for cubic Ca₁₂Al₁₄O₃₃.

The surface area of the CaO/Al₂O₃ 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/Al₂O₃ catalyst is inversely proportional to the calcination temperature. As the calcination temperature was increased, the surface areas decreased. BET surface area of CaO/Al₂O₃ catalyst (33.22 m²/g) calcined at 1000°C is lower than that calcined at 900°C (68.16 m²/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 m²/g) that most probably due to the presence of large aggregation and agglomeration. 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 (°C)	Surface Area (m ² /g)
CaO/Al ₂ O ₃	900°C	68.16
	1000°C	33.22
	1100°C	11.54

Fig. 6 shows the comparison of CaO/Al₂O₃ catalyst FTIR spectra at different calcination temperature. The absorption bands between 579.02 to 811.18 cm⁻¹ for CaO/Al₂O₃ catalysts were due to the stretching mode of metal oxide (M=O) groups.

Wavelength at 1488.11 cm^{-1} as shown in the $\text{CaO}/\text{Al}_2\text{O}_3$ 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, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. Hence, nitrate group in the $\text{CaO}/\text{Al}_2\text{O}_3$ catalyst calcined at 1000°C which has a high catalytic activity and in $\text{CaO}/\text{Al}_2\text{O}_3$ 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.

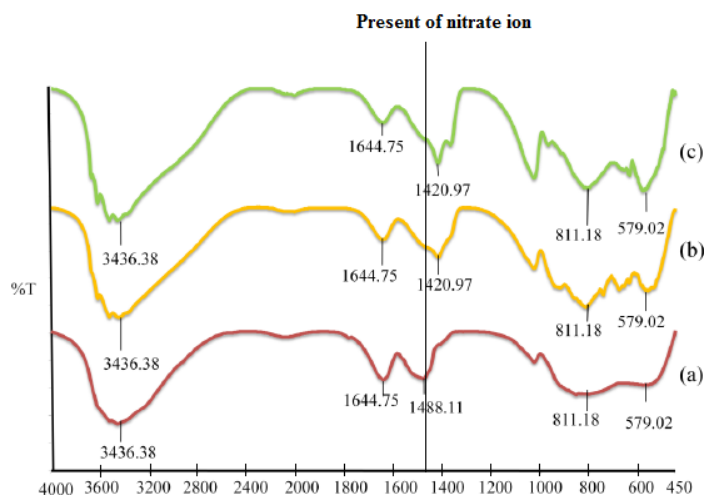


Fig. 6 FTIR spectra of $\text{CaO}/\text{Al}_2\text{O}_3$ catalyst calcined at (a) 900°C , (b) 1000°C and (c) 1100°C .

4. CONCLUSION

The removal of toxic and heavy metals from squid, *D. sibogae* was carried out using edible chelating agent which was trisodium citrate. The treatment conditions were carried out using trisodium citrate at 600 mg/L , one hour treatment time and treatment temperature of $29.5 \pm 0.5^\circ\text{C}$. The initial concentration of Ni and Cd in squid, *D. sibogae* from Mersing were $0.4565\text{ }\mu\text{g/g}$ and $0.358\text{ }\mu\text{g/g}$ respectively. For squid, *D. sibogae* from Pontian, the initial concentration of Ni and Cd were $0.126\text{ }\mu\text{g/g}$ and $0.0182\text{ }\mu\text{g/g}$ respectively while for squid, *D. sibogae* from Pangkor, only Cd was detected which was $0.028\text{ }\mu\text{g/g}$. The results on the optimization chelation technique for squids, *D. sibogae* from Mersing showed that 600 mg/L of trisodium citrate gave the highest percentage removal of toxic and heavy metals, which were 55.64% and 93.97% for Ni and Cd respectively. In the present of $\text{Ca}/\text{Al}_2\text{O}_3$ catalyst, higher removal percentage of toxic and heavy metals in *D. sibogae* from Mersing were achieved which were 89.69% and 94.95% for both Ni and Cd respectively. Meanwhile, XRD analysis depicted the active sites were due to the presence of orthorhombic Al_2O_3 , monoclinic $\text{CaAl}_4\text{O}_7/\text{CaO} \cdot 2\text{Al}_2\text{O}_3$ and cubic $\text{Ca}_{12}\text{Al}_{14}\text{O}_{33}$ species. BET surface area study illustrated the surface area of $33.22\text{ m}^2/\text{g}$. Finally, FTIR analysis concluded that $\text{CaO}/\text{Al}_2\text{O}_3$ catalyst at 1000°C treatment showed total removal of metal precursor's species. In conclusion, both chelation and catalytic chelation technique can remove toxic and heavy metals in *D. sibogae* especially the catalytic chelation technique which can remove further compared to chelation technique.

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