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REMOVAL OF SELECTED HEAVY METALS FROM GREEN MUSSEL VIA CATALYTIC OXIDATION

(Penyigkiran Logam-Logam Tertentu Daripada Kupang Melalui Pengoksidaan Bermangkin)

Faizuan Abdullah, Abdull Rahim Mohd Yusoff, Wan Azelee Wan Abu Bakar*, Razali Ismail, Dwi Priya Hadiyanto

Department of Chemistry,
Faculty of Science,
Universiti Teknologi Malaysia,81310 UTM Johor Bahru, Johor Darul Ta'zim, Malaysia.

**Corresponding author: wazelee@kimia.fs.utm.my*

Abstract

Perna viridis or green mussel is a potentially an important aquaculture product along the South Coast of Peninsular Malaysia especially Johor Straits. As the coastal population increases at tremendous rate, there was significant effect of land use changes on marine communities especially green mussel, as the heavy metals input to the coastal area also increase because of anthropogenic activities. Heavy metals content in the green mussel exceeded the Malaysian Food Regulations (1985) and EU Food Regulations (EC No: 1881/2006). Sampling was done at Johor Straits from Danga to Pendas coastal area for green mussel samples. This research introduces a catalytic oxidative technique for demetallisation in green mussel using edible oxidants such as peracetic acid (PAA) enhanced with alumina beads supported CuO, Fe_2O_3, and ZnO catalysts. The lethal dose of LD_{50} to rats of PAA is 1540 mg kg^{-1} was verified by National Institute of Safety and Health, United State of America. The best calcination temperature for the catalysts was at 1000 ºC as shown in the X-Ray Diffraction (XRD), Nitrogen Adsorption (BET surface area) and Field Emission Scanning Electron Microscopy (FESEM) analyses. The demetallisation process in green mussel was done successfully using only 100 mgL^{-1} PAA and catalyzed with Fe_2O_3/Al_2O_3 for up to 90% mercury (Hg) removal. Using PAA with only 1 hour of reaction time, at room temperature (30-35ºC), pH 5-6 and salinity of 25-28 ppt, 90% lead (Pb) was removed from life mussel without catalyst. These findings have a great prospect for developing an efficient and practical method for post-harvesting heavy metals removal in green mussel.

Keywords: Green mussel **(***Perna viridis)*, heavy metals, catalytic oxidative demetallisation, peracetic acid

Abstrak

Perna viridis atau kupang adalah produk akuakultur penting dan berpotensi tinggi di selatan Semenanjung Malaysia terutama di Selat Johor. Akibat kepesatan peningkatan populasi manusia dan aktiviti pembangunan di sekitar selat tersebut, kesan signifikan yang berlaku menyebabkan hidupan marin terutama kupang turut tercemar dengan peningkatan kandungan logam berat. Kandungan logam tersebut telah melebihi had yang dibenarkan Peraturan Makanan Malaysia 1985 dan Peraturan Makanan Kesatuan Eropah (EU) (EC No:1881/2006). Pensampelan telah dilakukan di Selat Johor dari Teluk Danga hingga ke Pendas untuk sampel kupang. Kajian ini memperkenalkan teknik pengoksidaan bermangkin untuk penyingkiran logam dalam kupang menggunakan agen pengoksidaan yang selamat dimakan seperti asid perasetik (PAA) dimangkinkan oleh CuO, Fe_2O_3, dan ZnO yang disokong pada permukaan manik alumina (Al_2O_3). LD_{50} utk PAA ialah 1540 $mgkg^{-1}$, telah disahkan Institut Keselamatan dan Kesihatan Kebangsaan (NIOSH) Amerika Syarikat. Bagi mangkin yang digunakan, suhu kalsinasi terbaik ialah pada suhu 1000 ºC seperti yang dibuktikan dengan analisa XRD, Analisis Penjerapan Nitrogen (keluasan permukaan BET) dan mikrograf FESEM. Proses penyingkiran logam dari kupang telah dilakukan dengan hanya menggunakan 100 mgL^{-1} PAA dimangkinkan oleh Fe_2O_3/Al_2O_3 untuk penyingkiran hampir 90% merkuri (Hg), dan menggunakan PAA tanpa mangkin untuk penyingkiran hampir 90% plumbum (Pb) dengan masa tindakbalas hanya 1 jam, dalam suhu sekitar 30-35 ºC, pH 5-6 dan kemasinan air 25-28 ppt. Penemuan ini memberikan prospek yang sangat baik dalam membangunkan kaedah yang efisyen dan praktikal untuk menyingkirkan logam dari kupang yang masih hidup sebelum dipasarkan.

Kata kunci: Kupang (*Perna viridis*), logam berat, penyingkiran logam pengoksidaan bermangkin, asid perasitik

Introduction

Asia productions of aquaculture give 90% of fisheries output compared with overall places [1]. The small and traditionally coastal aquaculture that has been practiced 500 years ago is being developed to the high commercial scale with intensive technologies and well-managed system especially in Southeast Asia. Among all sites, coastal aquaculture contributes 66-69% of production [2]. Anthropogenic activities such as effluents, marine and coastal developments, dredging, sewage discharge, oil slicks and spill form shipping activities, agricultural land runoff (fertilizers and pesticides), and climate changes are the prime sources of pollutions that affecting direct and indirectly the aquaculture development as the water quality reduce significantly [2-8]. Land use change for development via dredging activities considered as vital sources of water quality change and heavy metals enrichment in the Peninsular Malaysia coastal water, as the sediment with the secondary pollutants dredge up and mixed with the water body up to the surface, and accumulate pollutants such as heavy metals (Cd, Cu, Pb and Zn) to the fish and mollusk species green mussel [9].The spawning for cultural of this species are mainly from southern coast of Johor Strait, and currently being expanded up to Kedah because of mussel seeds transplantations [10].

Perna viridis (Linnaeus.) or green mussel are widely distributed in the Indo-Pacific coastal waters and also in the shallow waters along west coast of Peninsular Malaysia [10-13]. The coast comprises about 100,000 hectares contribute significantly the coastal productivity including green mussel. Green mussel is one of the mollusk species that is used around the world as an established bioindicator and biomonitoring agent for heavy metals as it is filter feeder type of organism [14]. It is also a commercially popular seafood species worldwide with easily found, and sampled [15-16]. Green mussel could grow up to 83 mm shell length in a year and its marketable size is 50-60 mm shell length, achievable for only approximately for 6 month. Besides, there are potential factors for reproduction, culture and growth of green mussel outside of its natural habitat [17]. Normally, the main food sources of green mussel are phytoplankton or algae such as Isochrysis galbana, Chaetoceros sp., and Pavlova sp. [18-19].

Studied had shown that lysosomal integrity directly affected by exposure of high concentration of copper [20], mercury [21] polyaromatic hydrocarbons (PAH) [22], low dissolve oxygen (DO) and high total ammoniacal-nitrogen (TAN) [23-24], and sudden temperature change and paraquat exposure[24]. Yap et al., 2002 reported that the concentration of heavy metals especially lead (Pb) in green mussel exceeding the permissible limits stated by Malaysian Food regulations (1985) and EU food regulations [9]. Demetallisation is a possible solution to reduce the excess or unneeded compounds. Table 1 shows maximum permissible limit by several regulations globally.

Table 1. Guidelines of maximum permissible limits of heavy metals (µg/g) in seafood from Different countries (Yap *et al.* 2004) [16]

Location	Cd	Pb	Zn
Permissible limits by Malaysian Food Regulation (1985)	1.00	2.00	100.0
EU Food Regulations (EU)	1.00	0.50	-
International Council for the Exploration of the Sea (ICES, 1988)			
Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	5.00	10.0	250
Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	-	6.67	667
Food and Drug Administration of the United State (USFDA, 1990)	3.7	1.7	-
Australian Legal Requirement (NHMRC, 1987)	10.0	-	750
Permissible limit set by the Hong Kong Environmental Protection Department (HK EPD, 1997)	2.00	6.00	-

Materials and Methods

Sampling, Sample Preservations and Preparations

As shown in Figure 1, green mussel samples were collected along Johor Strait started from S1 (Sg. Danga Estuaries) to S11 (Pendas) for heavy metals (Pb and Hg) content analyzing. The present sampling session were conducted three times between January - November in 2011. The green mussel samples were collected and grouped according to size. Before the mussels been shucked, the external shell surface was thoroughly cleaned with a brush and water to remove all the sand and dirt adhering to the shell to prevent the contamination of composite samples. The mussels then thawed and shucked, and the flesh was collected in a clean dish and homogenized by mixing. The green mussels (2g) then were weighed and digested on a wet basis, using a modified reflux system by digesting the samples overnight on a hot plate. The digested samples were filtered and diluted using distilled deionized water (DDW) to ensure the acid in the samples less than 5%. The digestion method used was adopted from the American Public Health Association (APHA) 3030E, which was a nitric acid digestion method. In this study, only the heavy metals content of adult mussels (7 to 9 cm) were analyzed on a dry weight basis. The green mussel samples analyzed for baseline of heavy metals content, and for simulation test in heavy metals removal.

was

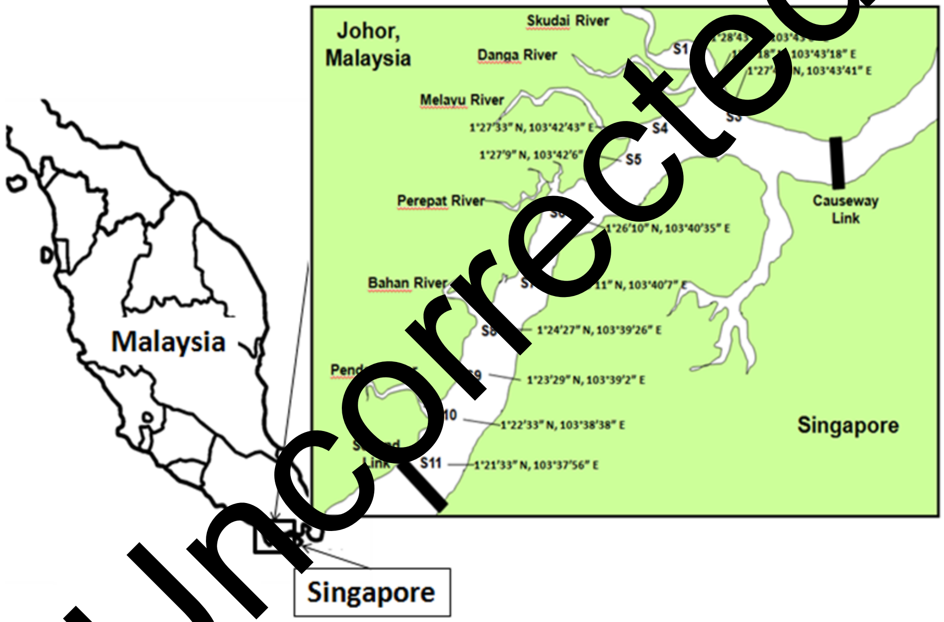


Figure 1. Sampling stations map (Johor Strait)

Development of oxidants-catalyst combination

For this purpose, several combinations of oxidants and catalyst were studied for heavy metals removal. The oxidant that were used at this time for the simulation test using standards metals solutions and dead mussel flesh was peracetic acid (PAA). The preparation of PAA was via the mixture of acetic acid and hydrogen peroxide with the ratio of 1:1. The catalysts that were used for this stage were alumina supported catalyst (granular) for the single transition metals catalyst consisting 5M of CuO, ZnO and Fe_2O_3. These common alumina supported catalysts were prepared by using impregnation method, and calcined for three different temperatures for every metal, which were 900°C, 1000°C, and 1100°C for six hours. The characterization of these catalysts was done by XRD and FESEM.

Demetallisation of heavy metals in green mussel

Pre-harvesting green mussels were collected at Johor Strait and were sustained for living in an aquarium laboratory scale. Mussels were fed twice for a day with *Chatocaerus sp.*, *Pavlova sp.*, and *Isochrysis sp*. The salinity of the water in the aquarium was controlled for 25 to 28ppt using ultrapure sodium chloride salt, and the temperature was maintained at ambient scale which was 30 to 35°C, in pH 5-6 with continuously flowing water using water filter. The demetallisation of selected heavy metals that adsorbed in the mussel flesh removal using oxidation technique was done by using PAA with concentration of 100, 200, 300 and 500 mg L^{-1} with and without catalyst. Mussel flesh were treated using this technique and the heavy metals content were analyzed using AAS for untreated samples as control and treated samples for comparison. For both type of mussel, the treatment process also done with five different times of treatment, with one hour interval.

Instrumentation

All the samples were analyzed using Perkin Elmer Atomic Absorption Spectrophotometer AAnalyst 400 (Flame) for heavy metals content. X-Ray Diffraction Scaterring system for catalyst diffractogram was done using X-RD Bruker model, nitrogen surface adsorption (BET surface area) using Micromeritics ASAP 2010 and micrographs of the catalyst was analyzed using FESEM-EDX Jeol 7600F model.

Results and Discussion

Baseline of Heavy Metals Concentration in Green Mussels

Figure 2 describes the heavy metals content in green mussel flesh that has been analyzed from January to June 2011. The content of mercury varied from 0.343 to 0.988 mg L-1, 0.033 to 0.082 mg L-1 for arsenic, 0.028 to 0.517 mg L-1 for lead, 0.020 to 0.089 mg L-1for cadmium, and 0.058 to 0.103 mg L-1 for nickel. All the heavy metals concentration in water and green mussel were the preference baseline of the heavy metals removal study using the combinations of specific oxidants and catalysts.

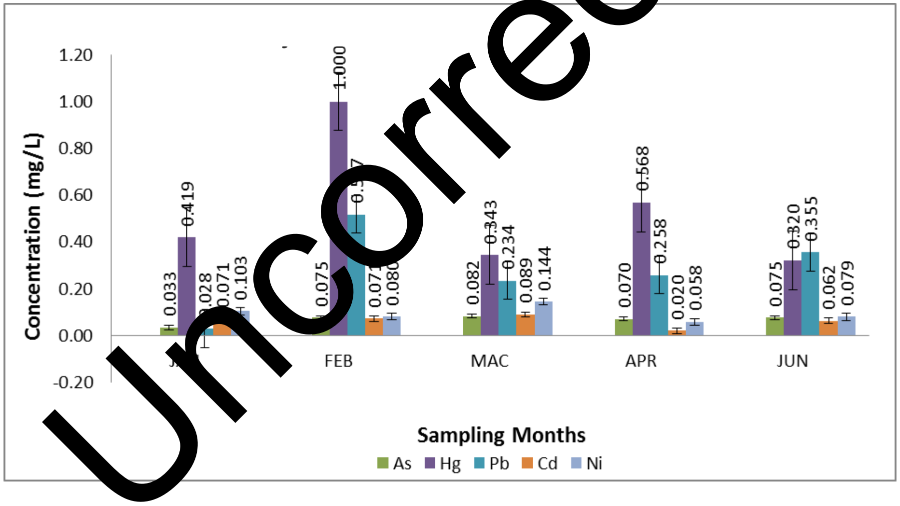


Figure 2. Heavy metals content in green mussel sample from January to June 2011

Results for XRD Analysis of Alumina Supported Transition Metals Catalysts

The X-Ray Diffraction (XRD) analysis was performed to all the alumina supported transition metals oxide catalysts in order to identify the crystalinity and particle size of the catalysts. The following diffractograms were used to be compared with the FESEM-EDX micrographs to implement the best calcination temperature for heat treatment in producing the best catalyst with smallest particle size for enhancing the heavy metals removal process. Three types of catalysts from transition metals group, supported with alumina were synthesized for the enhancement of peracetate ions productions, in order to increase the removal percentage of heavy metals from green mussel. These catalysts were prepared in laboratory scale includes copper, iron (III), and zinc using impregnation methods and calcined with three different temperatures which were 900 °C, 1000 °C, and 1100 °C in scientific laboratory furnace. Figures 3 to 5 described the characterization of the catalysts.

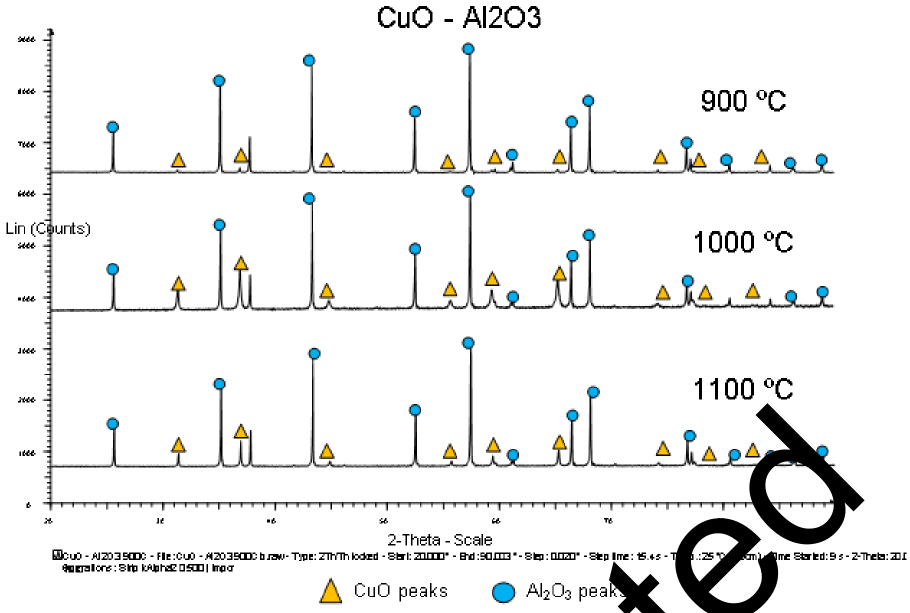


Figure 3. Diffractograms for alumina supported copper oxide catalyst with calcination temperatures of (a) 900 °C, (b) 1000 °C and (c) 1100 °C

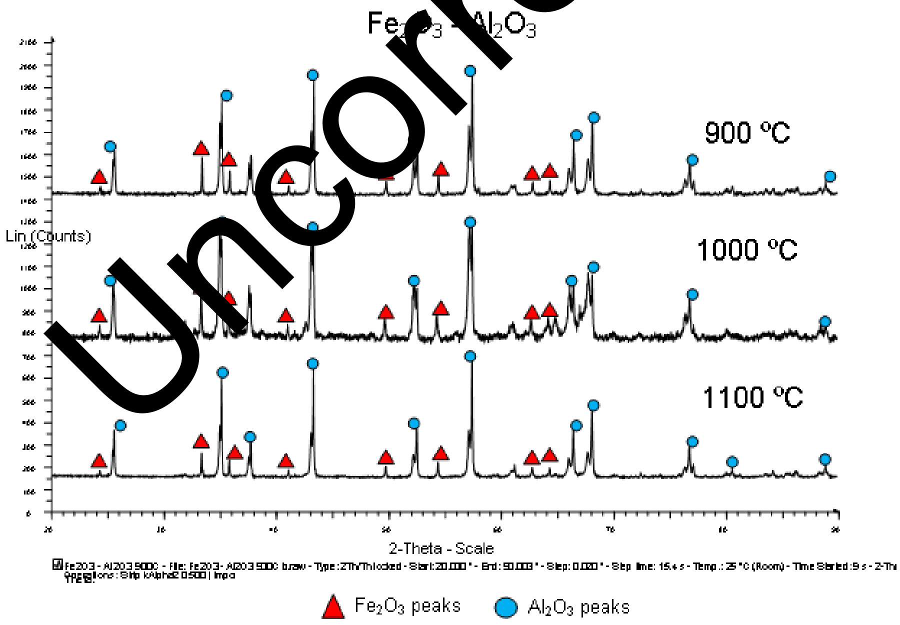


Figure 4. Diffractograms for alumina supported iron (III) oxide (Fe_2O_3) catalyst with calcination temperatures of (a) 900 °C, (b) 1000 °C and (c) 1100 °C

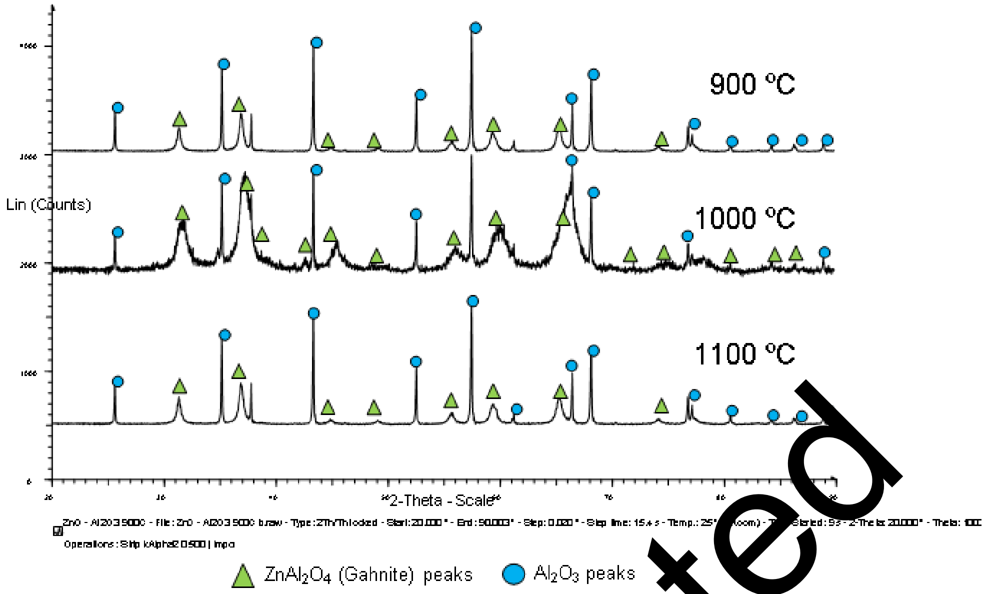


Figure 5. Diffractograms for alumina supported zinc oxide catalyst with calcination temperatures of (a) 900 °C, (b) 1000 °C and (c) 1100 °C

All of the catalysts (CuO/Al_2O_3, Fe_2O_3/Al_2O_3, and ZnO/Al_2O_3) with concentration of 5 Molar were analyzed using XRD for preliminary data of morphology and particle size. From the diffractograms in Figures 3 - 5, all the copper, iron, and zinc oxides catalysts with different calcination temperatures showed the different intensity. However, all the catalyst with heat treatment of 1000 °C showed the diffractograms with broad base width compared to other calcination temperatures, representing the amorphous structure of the catalyst. In amorphous phase, x-ray diffraction was scattered in many directions leading to a large bump distributed in a wide range (2 theta) instead of high intensity narrower peaks. From Figure 5, it was observed that the peaks which represent binary compound $ZnAl_2O_4$ (gahnite phases) were detected with high degree of amorphous could be seen in the diffractogram. The gahnite phase were appeared more in the catalyst structures after calcination at temperature of 1000°C for 5 hours, at 2 theta (31.8°, 37.2°, 39.0°, 45.5°, 49.[illegible]°, 56.4°, 60.0°, 66.0°, 69.5°, 70.5°, 78.3°, 79.4°, 83.6°, and 86.9°). According to Scherrer equation, the τ value of all diffractograms of 1000 °C heat treatment catalysts gave the smallest value indicated the smallest particle size exist for these catalysts. On the other hand, these catalysts were in highly amorphous in structure and this temperature of heat treatment gave the rhombohedral phase of metals oxide with more ordered in structure. The FESEM-EDX micrographs had support the evidence in the form of particle size obtained. These behaviors described by Scherrer in Equation 1.

$$\tau = \frac{K\lambda}{\beta cos\theta} \qquad \text{(Eq.1)}$$

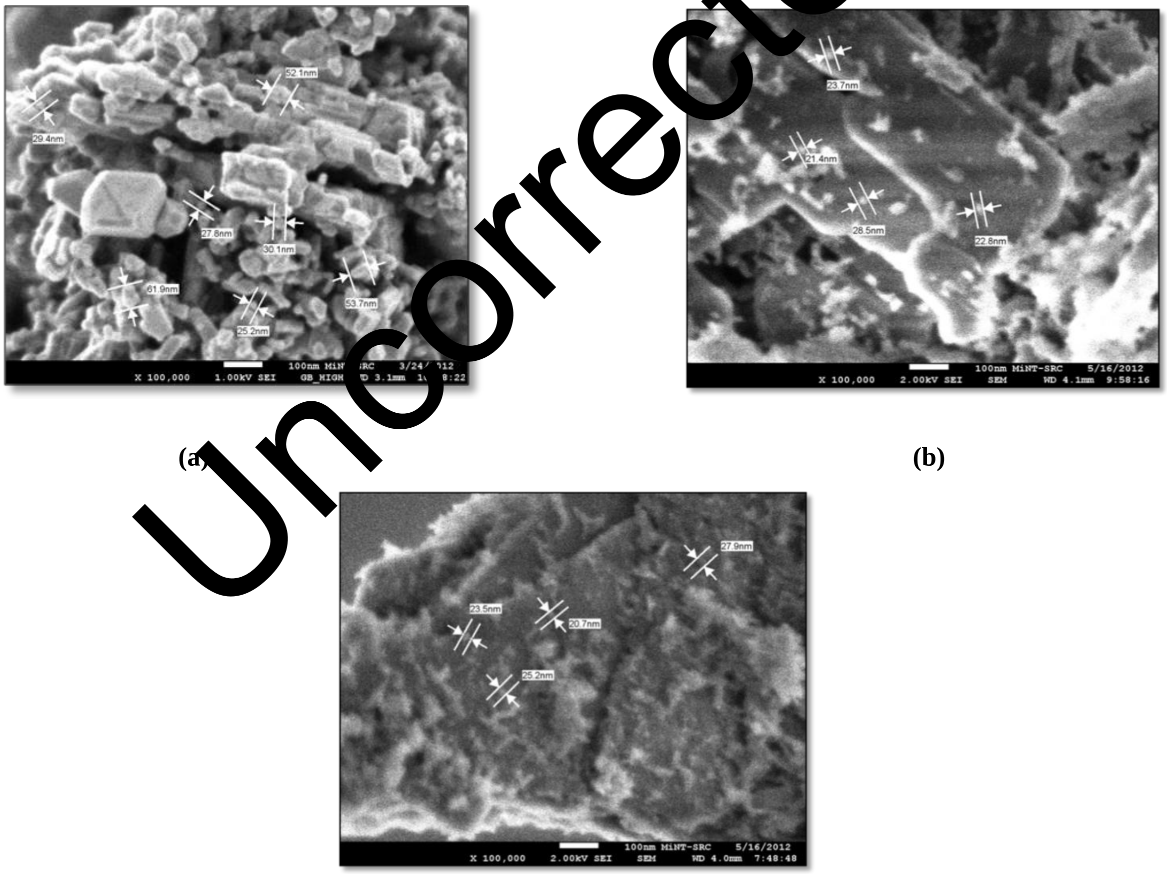
where k is defne as constant, λ is the X-rays wavelength. While θ is the value of Bragg diffraction angle of the plane, and β is the full width at half-maximum of diffraction peak.

Nitrogen Adsorption Analysis (BET surface area)

Micromeritics ASAP 2010 was used for the analysis. About 0.2-0.3 g powder form of catalyst sample was degassed at 120^{o}C using a vacuum pump before the analysis. As an example, the catalyst sample used in this study ZnO/Al_2O_3 surface area was then calculated from the adsorption curve according to the Brunauer-Emmett-Teller (BET) Theory. The data as shown in Table 2 shows that the surface area of ZnO/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 ZnO/Al_2O_3 catalyst (50.93 m^2/g) calcined at 1000°C was lower than that calcined at 900°C (77.33 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 (77.33 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 as shown in FESEM analysis in Figure 6. 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 contributing to the higher catalytic activity.

Table 2. BET surface area of ZnO/Al_2O_3 catalyst calcined at 900°C, 1000°C and 1100°C for 5 hours.

Catalyst	**Calcination temperature**	**Surface Area (m^2/g)**
ZnO/Al_2O_3	900°C	77.33
	1000°C	50.93
	1100°C	24.[illegible]



(a) **(b)**

(c)

Figure 6. The FESEM micrographs with 100,000 magnifications of (a) CuO/Al_2O_3 (b) Fe_2O_3/Al_2O_3 and (c) ZnO/Al_2O_3 calcined at 1000 °C for 5 hours. Scale bar: 100nm.

Field Emission Scanning Electron Microscopy (FESEM) for Analysis of Alumina Supported Transition Metals Catalysts

FESEM analysis was performed to investigate the morphology and particle size of the transition metals oxide catalysts (CuO, Fe_2O_3, and ZnO) with different heat treatment temperatures. This characterization will prove the evidences given by XRD analysis with specific micrographs of the surface particles with size scale down to nanometers. Thus, the combination of analysis method will complement each other. The micrographs of FESEM with 100,000 magnifications in Figure 6 indicates the morphology and particle size of a) CuO/Al_2O_3, b) Fe_2O_3/Al_2O_3, and c) ZnO/Al_2O_3 with the nanosize scale. As shown in the micrographs, the metal oxide with heat treatment of 1000 °C gave the smallest particle sizes in nanometer. The transition metal oxides with rhombohedral phase were homogenously distributed on the supporting material alumina (Al_2O_3). The size of the particles was below 100 nm, proving that most of the catalysts were in nano size.

Removal of Heavy Metals in Green Mussel via Oxidation Technique

Figure 7 shows the percentage of Hg removal using 200, 300, and 500 mg L^{-1} PAA for different time of reactions from 1 hour to 5 hours. The initial concentration of Hg in controlled green mussel was [illegible]911 mg L^{-1}. The removal percentage of Hg using 200 mg L^{-1} of PAA showed a significant increase from 1 to 5 hours of reaction. For the first hour, the removal was 40 %, followed by 50 % for second hour, 60 % for third hour, [illegible]0% for fourth hour and 80% for fifth hour. It can be concluded that the removal percentage was increased with time of reaction. For 300 mg L^{-1} and 500 mg L^{-1} of PAA, the removal percentage was not significantly increased with time, the removal percentage for 300 mg L^{-1} of PAA started with 80 % of removal for the first hour, 8[illegible]% for second hour, dropped to 81% for third hour and increased again to 86 % for fourth hour and 96 % for fifth hour of reaction. By using 500 mg L^{-1} of PAA the removal was 100 % except for second and fifth hour. These results showed that the study for optimum value for PAA concentration used would be much higher using 60[illegible] mg L^{-1} and above to get the comparison of removal percentage with 500 mg L^{-1} of PAA used.

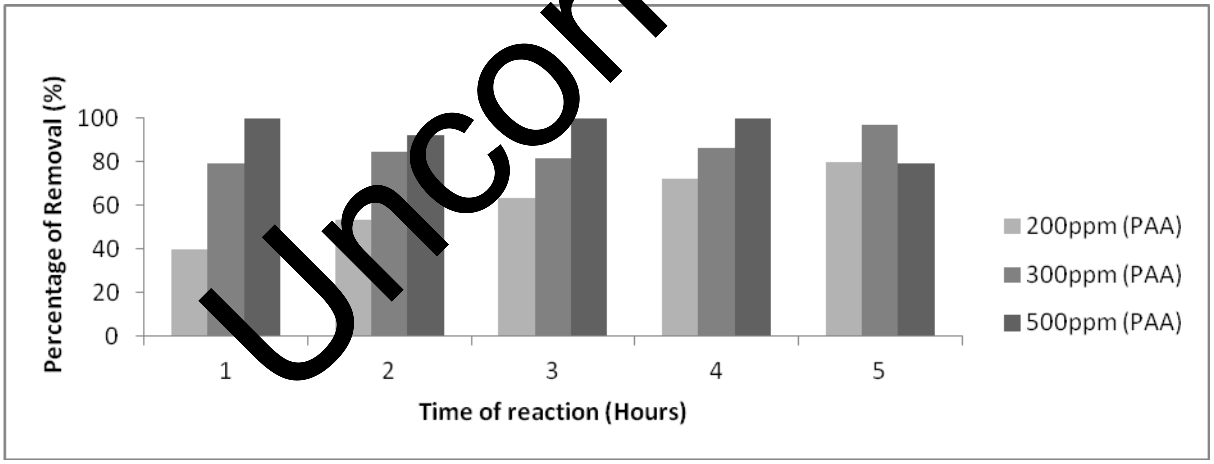


Figure 7. Percentage of mercury (Hg) removal of dead mussel flesh after treatment

For removal percentage of lead (Pb) in the dead mussel flesh, Figure 8 shows that the studied of different PAA concentration and reaction showed that only 200 mg L^{-1} of PAA and 1 hour reaction time was enough to remove 100 % of lead content in the green mussel. The initial concentration in the controlled mussel's flesh was 1.275 µg g^{-1}.

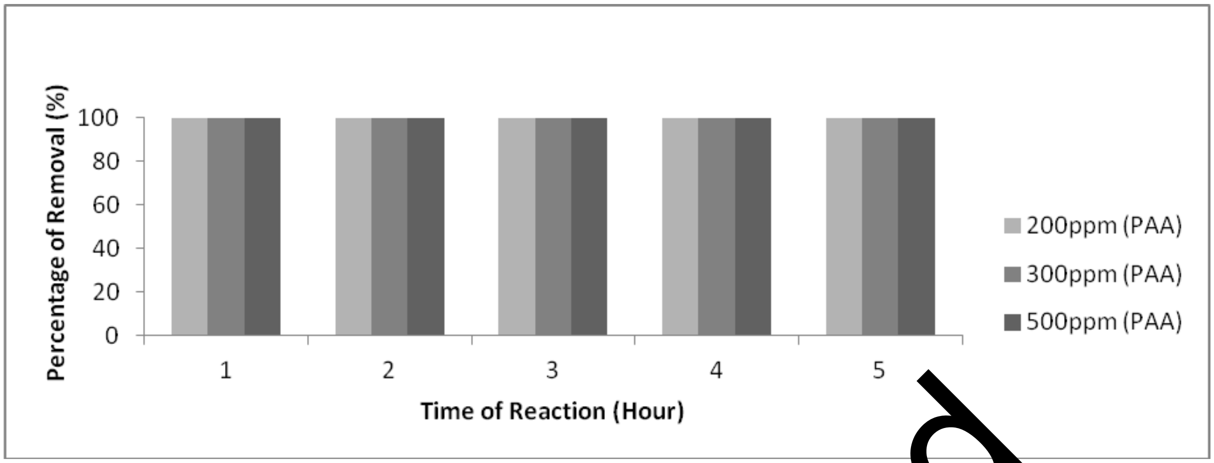


Figure 8. Percentage of lead (Pb) removal from dead mussel flesh after treatment (all concentrations of PAA gave 100 % removal)

Removal of Heavy Metals in Green Mussel via Oxidation Technique Catalyzed with CuO/Al_2O_3, Fe_2O_3/Al_2O_3 and ZnO/Al_2O_3

For preliminary result of Hg and Pb removal in dead mussel flesh using PAA with CuO/Al_2O_3, Fe_2O_3/Al_2O_3, and ZnO/Al_2O_3 catalysts, Figures 9 to 14 represent the percentage of Hg and Pb removal using 100, 200, 300, and 500 mg L^{-1} PAA for 5 hours reaction, with 1 hour interval. In Figure 9, the initial concentration of Hg in controlled green mussel was 1.690 mg $L^{-1,}$ and Pb was 2.400 mg L^{-1}. The reaction was performed in 1 L of water with constant temperature of 32.5 °C and stirred using IKA HS-7 magnetic stirrer. The removal percentage of Hg using 100, 200, 300, and 500 mg L^{-1} of PAA at first hour showed a significant increase as the PAA concentration increased which was 10 % to 100 % except for first and second hour. For the second hour of reaction using all concentrations of PAA, the removal of mercury was at 100 %. These can be concluded that for the reaction of Hg removal using CuO/Al_2O_3 catalyst, only 100 mg L^{-1} of PAA needed to remove mercury with two hours of reaction time.

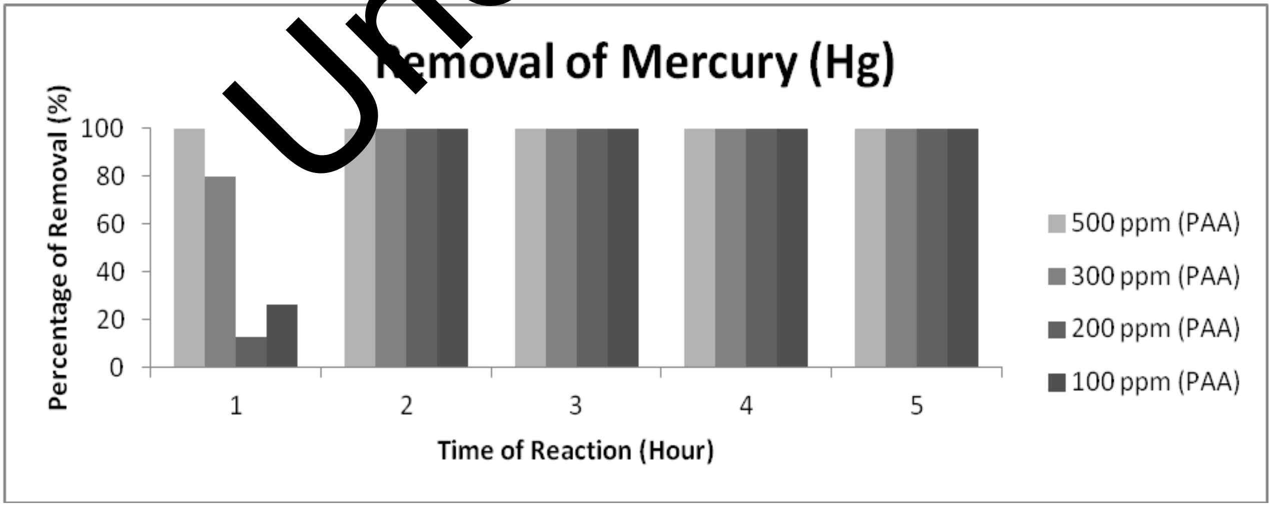


Figure 9. Percentage of mercury (Hg) removal from dead mussel flesh after treatment using PAA catalyzed with CuO/Al_2O_3.

For the removal of Pb in Figure 10, most of the PAA concentrations gave 100 % removal at the fifth hour of reaction time. This indicated that a serious uncertainty has been observed for the Pb removal using PAA catalyzed with CuO.

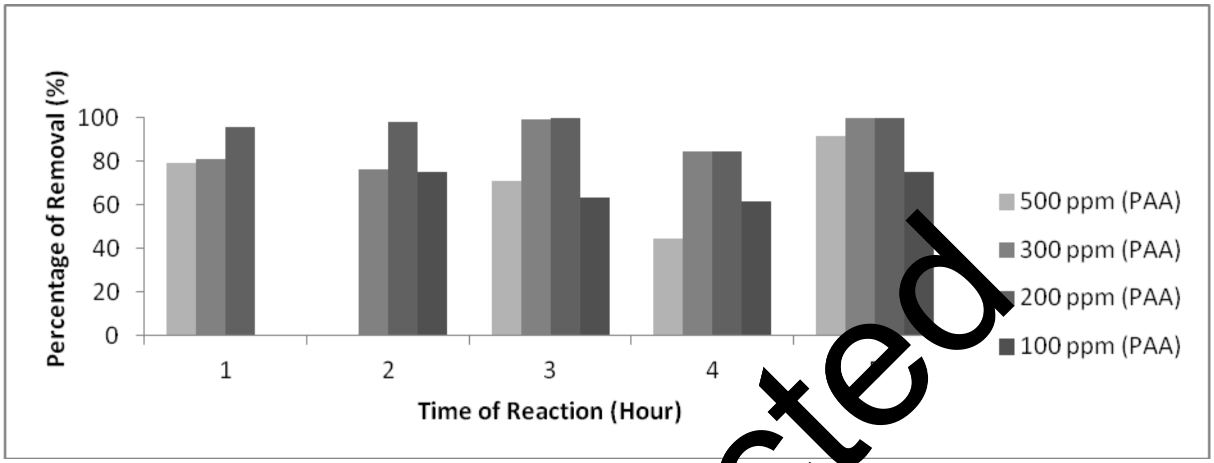


Figure 10. Percentage of lead (Pb) removal from dead mussel flesh after treatment using PAA catalyzed with CuO/Al_2O_3.

For removal percentage of mercury (Hg) in the dead mussel flesh using PAA catalyzed with Fe_2O_3/Al_2O_3, Figure 11 showed that the studied of different PAA concentration and reaction showed that only 200 mg L^{-1} of PAA and 1 hour reaction time was enough to remove 100 % of mercury content in the green mussel.

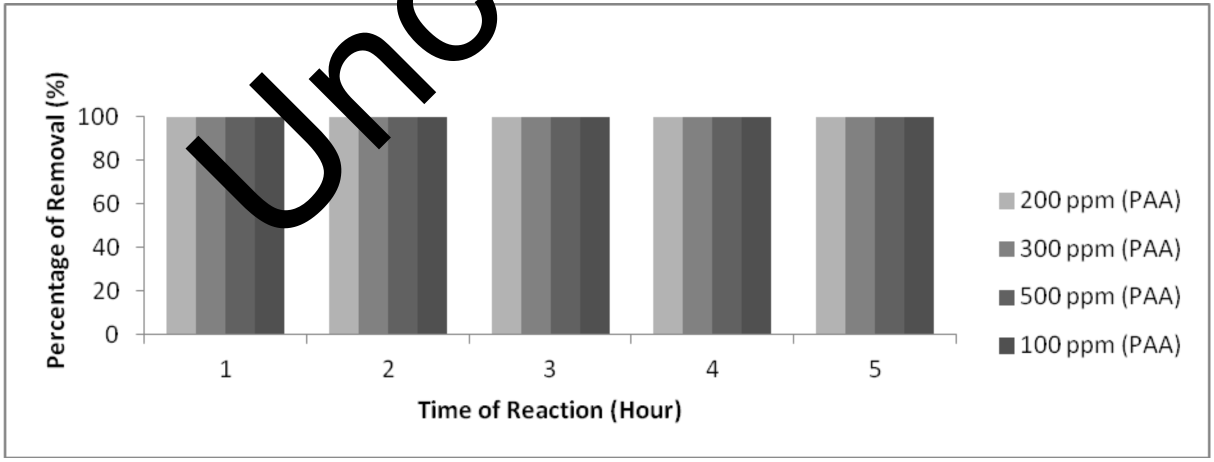


Figure 11. Percentage of mercury (Hg) removal from dead mussel flesh after treatment using PAA catalyzed with Fe_2O_3/Al_2O_3.

Figure 12 describes the percentage of Pb removal from dead flesh of green mussel using PAA catalyzed with Fe_2O_3/Al_2O_3. As the time of reaction increase, the removal percentage was decrease accordingly among all PAA concentrations used, until the third hour the removal percentage stared to increase again for an hour and decrease again up to fifth hour of reaction time. This pattern of graph indicated that the metal that has been removed by peracetate ions from the mussel's flesh react was absorbed again. Fe_2O_3/Al_2O_3 catalyst might not be suitable for the removal of lead from the green mussel's flesh.

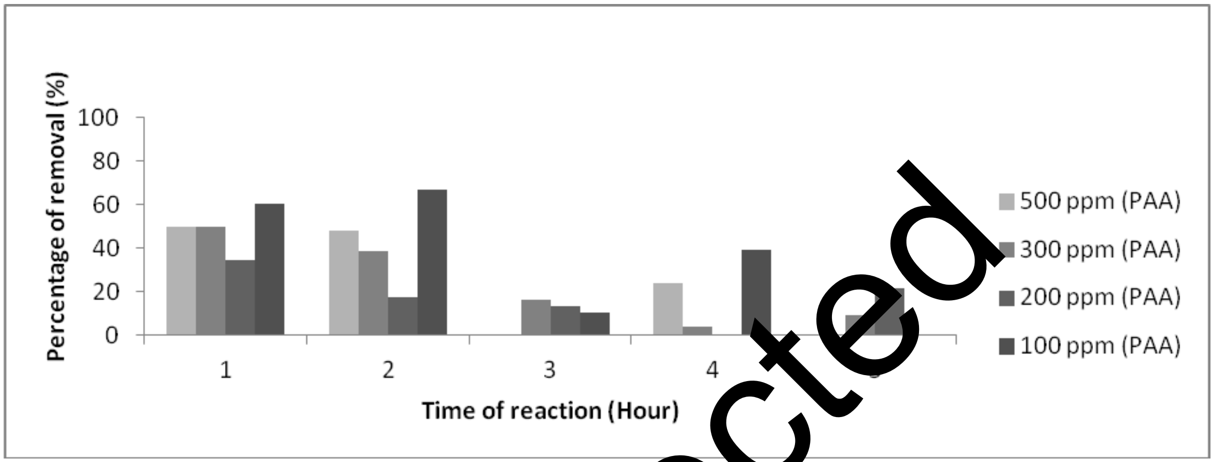


Figure 12. Percentage of lead (Pb) removal from dead mussel flesh after treatment using PAA catalyzed with Fe_2O_3/Al_2O_3

For the removal of mercury from dead mussel's flesh using PAA catalyzed with ZnO/Al_2O_3 as shown in Figure 13, the Hg content was totally removed from the mussel using all concentrations of PAA at all five hour of reaction time, indicates that the minimum concentration of PAA needed was only 100 mg L^{-1} for only one hour of reaction time. However, for the second hour of 200 and 500 mg L^{-1} PAA, the method was not effective. It was observed that ZnO/Al_2O_3 catalyst was efficient for removal of Hg.

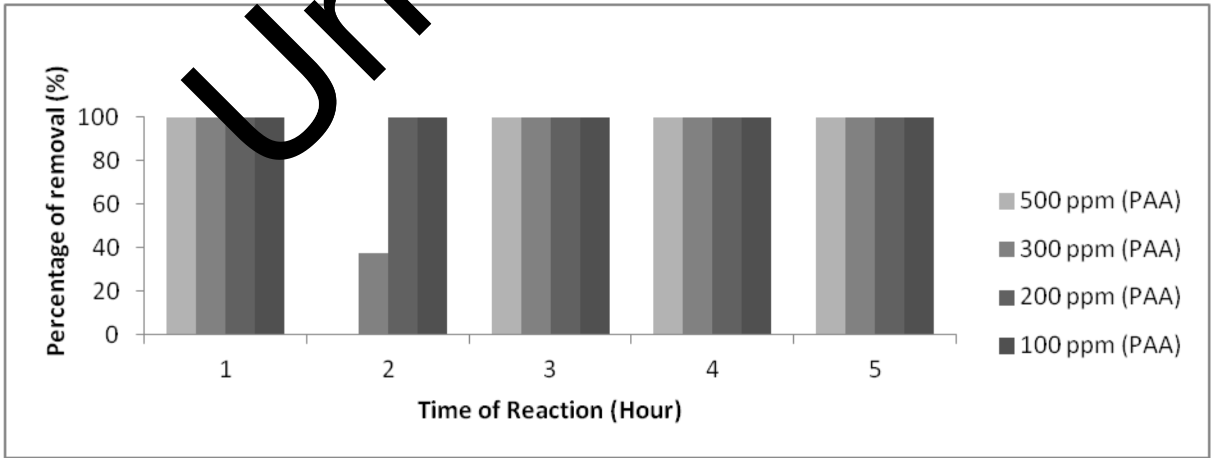


Figure 13**.** Percentage of mercury (Hg) removal from dead mussel flesh after treatment using PAA catalyzed with ZnO/Al_2O_3

The graph in Figure 14 describes on the removal of Pb using PAA catalyzed with ZnO/Al_2O_3. From the graph pattern, there were a lot of uncertain especially involving the PAA concentration used in the reaction. Among all concentrations of PAA, only 200 mg L^{-1} gave 100 % of removal at second hour of reaction. There was no significance pattern in the Pb removal percentage as the PAA concentrations and reaction time increase. It can be conclude that the ZnO/Al_2O_3 catalyst was not practically reliable to remove Pb from the mussel's flesh.

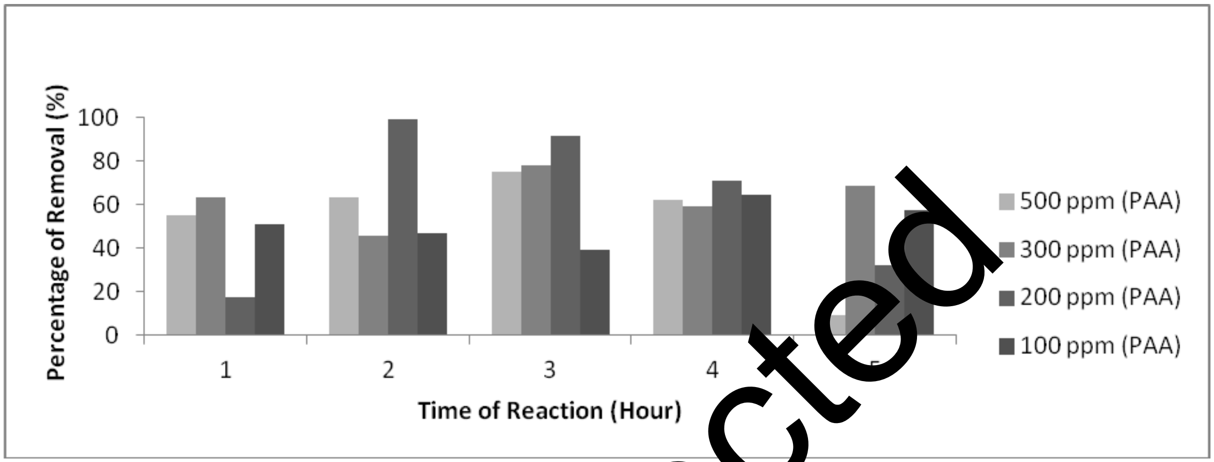


Figure 14. Percentage of lead (Pb) removal from dead mussel flesh after treatment using PAA catalyzed with ZnO/Al_2O_3

showed

Conclusion

The study for heavy metals removal in green mussel, have been done successfully. The best calcination temperature of the catalyst used was 1000 ºC as proven with XRD diffractograms, BET and FESEM micrographs. Thus, the demetallisation process in green mussel was more efficient using only 100 mgL^{-1} of PAA catalyzed with Fe_2O_3/Al_2O_3 for Hg removal, and using PAA without catalyst for Pb removal. However, the other catalyst also shown positive results in removal of Hg and Pb especially ZnO/Al_2O_3 that give almost 100% removal of Hg in 3 to 5 hours of reaction using only 100 mg L^{-1} of PAA and could remove up to 90% of Pb at 5 hours of reaction.

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