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Determination of Selected Heavy Metal Contents in Fresh and Smoked *Corbicula Fluminea* (Muller, 1774) Collected from a Vendor in Kampung Kasar, Pasir Mas, Kelantan, Malaysia

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Abstract

Smoked C. fluminea is most popular traditional snack in Kelantan. Kampung Kasar, Pasir Mas is one of the popular selling area for this traditional snack. Therefore, it is crucial to determine the heavy metal contents in "etak" tissue supplied by vendor in Kampung Kasar. Thus, this study aims to determine and evaluate the concentration of selected heavy metal (Cr, Zn, Mn and Cu) contents in fresh and smoked C. fluminea which were collected from a vendor in Kampung Kasar, Pasir Mas, Kelantan, Malaysia. Wet digestion method was used to extract the samples and tested with the air acetylene flame atomic absorption spectrometry (AAS). Result shows that, fresh C. fluminea has higher concentrations of heavy metals as compared to smoked C. fluminea processed by the vendor in all the measured parameters except for Cr. However, further assessment needs for cultural sustainability and safety consumption of smoked C. fluminea.

Keywords: *Pasir Mas, chromium, zinc, manganese, copper, Atomic Absorption Spectrometer (AAS)*

1. Introduction

Corbicula fluminea (C. fluminea) is also known as "etak" in Kelantan dialect. Smoked "etak" is one of the popular snacks among local people in Kelantan. The preparation of smoked "etak" involved washing, purging, marinating and smoking processes. The *C. flumineas* is marinated and mixed well with a paste consists of blended lemongrass, garlic, onion and chillies for an hour. Then, the marinated *C. fluminea* is arranged on a platform made of the bamboo stick placed above the embers and left for about 30 to 45 minutes. Fresh "etak" was washed and soaked in water to clean all the mud and sand particles. The soaking process takes at least three hours or overnight to ensure it is clean. This smoked "etak" can be a source of income to local people. The market price of fresh "etak" is between RM5 to RM7 per kilogram depending on the demand and transportation cost. The fresh *C. fluminea* can be stored in the refrigerator for two or three days [12] before the smoking or roasting process. According to [21], the carbohydrate in Asian clam is lower compare to oyster and smoked *C. fluminea* has high crude protein, ash and carbohydrate compared to fresh C. *fluminea*. This also supported by [4], where high protein value found in smoked C. fluminea as smoking process use low temperature which helps to avoids protein denatured during the process occurs.

Asian clam or *C. fluminea,* is originally distributed in Asiatic ecosystems but now a common inhabitant of American and European freshwater habitats. The size of *C. fluminea* usually less than 25 mm but it can reach up to 50 to 65 mm in length with life span about one to seven years. It is a small clam that has an inflated round to triangular-shaped shell. The shell is usually pale brownish or yellowish-brown. This species can be found in lakes and streams of all sizes with silt, mud, sand and gravel substrate. It also usually can be found in flowing water because it requires high levels of dissolved oxygen and generally intolerant to pollution. It can tolerate up to 13 ppt for short periods with the high salinity of water, and the temperature is between 2 and 30°C [5]. *C. fluminea* is a filter feeder that removes particles from the water column and feeds primarily on phytoplankton as well as particulate organic matter (POM), this indirectly purify water column. *C. fluminea* can also be as an anthropogenic pollutant because it accumulates and stores organic and inorganic chemicals in its tissue [14]. Not only that, being a filter feeder, *C. fluminea* can also concentrate microbes present in the surrounding waters, which can cause severe illness in humans. Gastrointestinal infections and related symptoms such as diarrhea, fever, nausea, abdominal cramping, dehydration, headache, and vomiting are always blamed on the consumption of raw, semi-cooked or coliform bacteria-contaminated shellfish [28]. *C. fluminea* is also capable of deposit-feeding wherein the clam transports sediments through its labial palps by their ciliary tracts on its foot. *C. fluminea* is hermaphrodite where both sexes are found in an organism and also capable of self- fertilisation. The sperm is released into the water, caught by another clam, and brooded in the gills. The larvae are released through the excurrent siphon and sent out into the water column.

Spawning can continue year round in water temperature higher than 16°C, whereas to release their larvae, the water temperature must be above 16°C [3]. *C. fluminea* grows rapidly due to its high filtration and assimilation rates. The major part of its energy is allocated to growth and reproduction, and only a small portion is devoted to respiration. This species has the highest net production efficiencies recorded for any freshwater bivalve, reflected by short turnover times of only 73 – 91 days. *C. fluminea* has a high fecundity but low juvenile survivorship and a high mortality rate throughout the life span. This low adult survivorship leads to populations dominated by high proportions of juveniles. In some ecosystems, this population domination by immature juveniles is not so effective, and the presence of adults in high abundance and having large sizes have been reported [22].

Heavy metal can be defined as any metal or chemical element with a specific gravity at least five times or greater than water. The toxicity of heavy metal will depend on several factors such as the dose, route of exposure, and chemical species. Because of their high degree of toxicity, As, Cr and Pb are ranked among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that can induce multiple organ damage, even at lower levels of exposure. In recent years, the increase of these metals in environment has caused a rise in global public health concern associated with environmental contamination. Human exposure to heavy metals also has increased unexpectedly for the exponential results of their involvement in industrial, agricultural, domestic and technological applications [25]. A long-term exposure to heavy metal will cause in slow progressive physical, muscular and neurological degenerative processes [9]. The dangers posed by heavy metals is currently a prominent environmental issue. Heavy metals can accumulate in the body of an organism, and become poisons when they remained in the body for a prolonged time. The danger of heavy metal contamination was widely publicise in media. For example, pollution of mercury (Hg) which caused Minamata disease in Japan, and pollution of cadmium (Cd) which caused the Itai-itai disease and liver cancer of Jinzo River on the island of Honshu Japan. Lead

poisoning led to high levels of lead in the aorta, liver, kidneys, pancreas, lungs, bones, spleen, testes, heart and brain [20]. Some heavy metals are essential elements to organisms, including humans but can be toxic in excess. Cu is an essential trace element, but exposure to excess Cu can result in anaemia, developmental problems, immunotoxicity, and liver and kidney damage [24]. The presence of these heavy metals in the environment, especially aquatic ecosystems, could lead to health problems if being consumed by living organisms such cockles over a long time. The metals in the aquatic biota such as cockles are in constant interaction with the metals in the surface water, suspended matter, sediment and the interstitial water [32].

C. fluminea (Asian clam) comes from rivers and lakes as it is a freshwater species. Since it is a filter feeder, every pollutant that goes into river or lake will absorb into clam tissue. In Kelantan, "etak" is processed by a vendor. Hence, this study aims to determine and evaluate the concentration of selected heavy metal (Cr, Zn, Mn and Cu) contents in fresh and smoked *C. fluminea* which were collected from a vendor in Kampung Kasar, Pasir Mas, Kelantan, Malaysia.

2. Materials and Methods

Fresh and smoked *C. fluminea* were collected from a vendor in Kampung Kasar, Pasir Mas, Kelantan, Malaysia. Four heavy metal parameters were chosen for the study, namely Cr, Zn, Mn and Cu. Wet digestion method was used to extract the samples and tested with the air acetylene flame atomic absorption spectrometry (AAS). All the results were compared with three standards namely the Malaysia Food Regulation, 1985 [16], the European Union Commission Standard, 2016 [10] and the Food Standard Australia and New Zealand, 1991 [11]. The research site for fresh and smoked *C. fluminea* that has been chosen for this study is Kampung Kasar, Pasir Mas, Kelantan as shown in Figure 1.

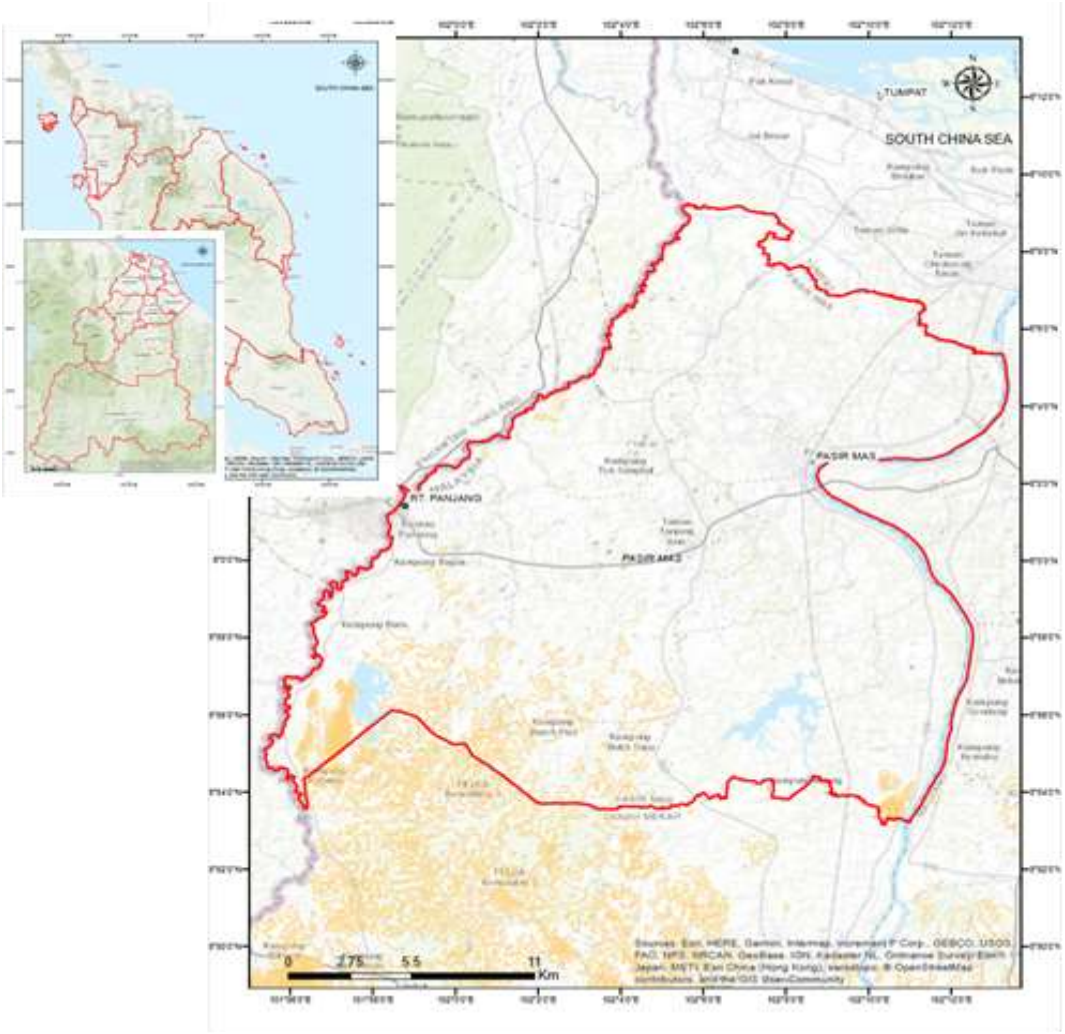

Figure 1: Study Area

A total of 200 pieces of fresh and smoked C. fluminea were collected from a vendor in Kampung Kasar, Pasir Mas, Kelantan. The tissues were collected and dried in the oven for three days at 60°C to obtain a constant weight. After that, the dried sample was ground by using pestle and mortar to obtain a homogeneous sample. The sample was kept in a zipper bag and stored in desiccator (for storage). In order to get an accurate heavy metal concentration, the sample preparation must be repeated for three times [29].

A total of 5.0 gram of sample was digested by using hot plates for both fresh and smoked *C. fluminea* tissues. The dry sample was mixed with 5 mL of H_2SO_4 and 5 mL of HNO_3 in a beaker. Then, the beaker was placed on a hot plate at 60°C for 30 minutes. After the reaction occurred, the beaker was covered with a watch glass. After 30 minutes when the beaker has cooled, 10 mL of HNO_3 was added into the beaker. The temperature was slowly increased until it reached 150°C. After all the tissues were completely digested, the sample were let to cool at room temperature and 1 mL of 30% H_2O_2 was added for every half an hour until the clear solution appeared. After clear solution was obtained, the sample then filtered through Whatman® qualitative filter paper, Grade 1. Then, the filtering process continued using syringe filter before it was transferred it into 50 mL of falcon tube and followed with dilution with deionised water. The filtrate was stored in the refrigerator at 20°C before undergoing further analysis. Filtered sample was

analysed for Cr, Fe, Pb and Zn using a Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer [29].

3. Results and Discussion

Chromium (Cr) is a trace mineral needed by the body in a small amount for healthy functioning. People are generally exposed to Cr via the food they eat, especially clam because it can be accumulated in aquatic animals and can cause toxicity [18]. Chromium (Cr) is naturally present in the earth's crust with oxidation states. For the human and animals, a certain level of Cr is an essential nutrient which may present in glucose, fat and protein metabolism. Table 1 showed the concentration of Cr in fresh and smoked "etak" compared to national and international standards. Results showed that the concentration of Cr in smoked *C. fluminea* is slightly higher than in fresh *C. fluminea,* which is 0.27 mg/kg and 0.16 mg/kg, respectively. Cr concentrations in fresh and smoked C. fluminae were well below the Malaysia Food Regulation, 1985 (0.50 mg/kg). The concentration of Cr in fresh "etak" is lower than smoked "etak" was believed to be due to the release of Cr during the burning of solid fuel (wood) during smoking or roasting process. The finding was in-line with [23] findings, where they found that waste burning is one of the primary sources of chromium. This was further supported by [31], where they reported that, combustion of wood material released 8.14 mg/kg of total heavy metals.

Zinc (Zn) is a mineral and an essential trace element where a small intake of Znwould give benefit to human health. It is a crucial micronutrient for the human body. Zn is a trace element that has indispensable role in human health and diseases. It is the most abundant intracellular metal ion found in cytosol, vesicles, organelles and the nucleus. However, a small deficiency of Zn will affect human health [8]. Three major routes of Zn for the human body are by inhalation, through the skin or by ingestion. Zn is likely safe for adults when ingested through the mouth with the lower amount or not larger than 40 mg/day. Taking in a high amount of Zn might cause fever, coughing, nausea, vomiting, diarrhea, kidney and stomach pain and other problems [19]. Based on the results in Table 1, Zn concentration is 73.89 mg/kg and 27.49 mg/kg in fresh and smoked *C. fluminea,* respectively. Zn concentrations in fresh and smoked *C. fluminae* were well below the Malaysia Food Regulation, 1985 (100 mg/kg) and Food Standard Australia and New Zealand standards, 1991 (100 mg/kg). This result confirmed to the findings from [7], [27], where they found that, thermal processing applied to the shellfish affect significantly the content of macro and microelements as well as the content of heavy metals except for potassium and manganese. But it was not in-line with [2] findings, where based on their study they found that zinc concentrations in fresh oyster (0.11 ppm) is lower as compared to smoked oyster (0.86 ppm).

Manganese (Mn) is an essential nutrient for the human body and a small daily intake of manganese is important to stay healthy. However, if it is consumed more than what is required, it will cause a negative effect. Mn is one of the metals that enter the body via inhalation, taking more than the allowed limit can damage the nervous system and respiratory tract as well as other adverse effects. Other than that, excessive uptake of Mn can also cause neurological effect such as parkinsonian-like syndrome (Marilena Kampa, 2008). The absorption of Mn into the human body is transported through the blood into the liver, kidney, and pancreas. Based on a study by [30], the daily manganese intake was 4.2 mg/day for the males and 4.1 mg/day for the females. Results of the study show that, Mn concentration is 46.18 mg/kg and 40.64 mg/kg in fresh and smoked *C. fluminea,* respectively (Table 1). Mn concentrations in fresh and smoked *C. fluminea* were far higher than the Malaysia Food Regulation, 1985 (10 mg/kg) and the Food Standard Australia and New Zealand standards, 1991(10 mg/kg).

Copper (Cu) is an active redox metal, and it is an essential nutrient for all species. However, an excessive amount of Cu in the body can pose a risk. Cu will give diseases to human health such as cardiovascular and diabetes. However, heart vessels are vulnerable to Cu deficiency. Cu deficiency has been proposed to induce cardiac damage via relatively low oxidant defence enzyme activities in the heart compared to other tissues. Cu deficiency can alter the blood lipid profiles and associated with cardiovascular disease [26]. Cu is also an element that contributes to neurological defects. Cu has been documented in cancer patients suffering from breast, cervical, ovarian, lung, prostate, stomach cancer and leukaemia. Cu is also essential for promoting angiogenesis, a process of the growth of any tumour beyond a few millimetres. In the process of angiogenesis, new blood supplies are formed to feed the malignant cells. Based on a study by [30], the daily copper intake was 1.3 mg/day for the males and 1.2 mg/day for the females. Results in Table 1 showed that, Cu concentration is 14.25 mg/kg and 23.64 mg/kg in fresh and smoked *C. fluminea*, respectively. The concentrations of Cu in fresh and smoked *C. fluminea* were higher than the European Union Commission Standard, 2016 (10 mg/kg) and lower than the Malaysia Food Regulation, 1985 (30 mg/kg). This result confirmed to the findings from [7], [27], where they found that thermal processing applied to the shellfish has significantly affect the content of macro and microelements as well as the content of heavy metals except for potassium and manganese. However, it was not in-line with [2] findings, where based on their study they found that copper concentrations in fresh oyster (0.98 ppm) is lower as compared to smoked oyster (1.32 ppm).

Table 1: The concentration of heavy metals content in fresh and smoked *Corbicula fluminea* (etak) samples collected from smoking/roasting point in Kampung Kasar, Pasir Mas, Kelantan, Malaysia

Samples	Chromium (Cr) mg/kg	Zinc (Zn) mg/kg	Manganese (Mn) mg/kg	Copper (Cu) mg/kg
Fresh	0.16	73.89	46.18	14.25
Vendor(smoking/roasting point)	0.27	27.49	40.64	23.64
Standards				
Malaysia Food Regulation, (1985)	0.50	100	10	30
European Union Commission Standard, (2016)	-	-	-	10
Food Standard Australia and New Zealand (FSANZ), (1991)	-	100	10	-

4. Conclusion

This study revealed that chromium and zinc are below the permissible limit set by the Malaysian Food Regulation, 1985 as well as international standard namely the European Union Commission Standard, 2016 and Food Standard Australia and New Zealand, 1991. However, the concentration of manganese and copper is higher than the standard, especially manganese. Although copper concentration exceeded the European Union Commission Standard, 2016, it did not exceed the Malaysian Food Regulation, 1985 standard. Therefore, based on the results obtained from this study and study by [30], smoked "etak" bought from the vendor in Kampung Kasar, Pasir Mas, Kelantan, Malaysia cannot be consumed more than 100g/day/person to avoid negative health impact because the manganese and copper concentrations are high and exceeded the allowed limit. However, this can be a guidance and preliminary picture for authorities and vendors to

improve the smoking method to ensure this traditional snack sustainability and for safety food consumption in future.

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