

## Determination of Selected Heavy Metal Contents in Fresh and Smoked *Corbicula Fluminea* (Muller, 1774) Collected from Hawkers in the District of Tumpat, Kelantan, Malaysia Running Head: Heavy Metal Content in *C.fluminea* Tumpat's Hawkers

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### Abstract

Apart being contaminated at the habitat, *Corbicula fluminea* (*C. fluminea*) was also believed to be contaminated at the selling point caused by the dust from the roadside as well as old newspaper wrapping used by hawkers. This situation can cause discomfort among smoked *C. fluminea* also known as "etak salai" (as per Kelantanese dialect) enthusiasts because it is commonly associated with poor hygiene and health problem. Therefore, 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* collected from hawkers in the district of Tumpat, Kelantan, Malaysia. Wet digestion method was used to extract the samples and the samples were tested using air acetylene flame atomic absorption spectrometry (AAS). Result shows that, fresh *C. fluminea* has higher concentrations as compared to smoked *C. fluminea* which are sold at the stall of all measured parameters except for Cr (fresh: 0.14 mg/kg and smoked: 0.22 mg/kg). However, Zn is the highest concentrations heavy metal in *C. fluminea* (fresh: 74.39 mg/kg and smoked: 35.41 mg/kg) compared to other parameters. Cr and Zn concentrations in fresh and smoked *C. fluminea* are below the stipulated standard of Malaysia Food Regulation, 1985 (0.50 mg/kg for Cr; 100 mg/kg for Zn) and Food Standard Australia and New Zealand standards, 1991 (100 mg/kg). However, Mn concentrations in *C. fluminea* for fresh (45.58 mg/kg) and smoked (16.32 mg/kg) are far higher than Malaysia Food Regulation, 1985 (10 mg/kg) and Food Standard Australia and New Zealand standards, 1991 (10 mg/kg). On the other hand, the concentration of Cu in fresh *C. fluminea* (13.75 mg/kg) and smoked *C. fluminea* (12.60 mg/kg) are higher than the European Union Commission Standard, 2016 (10 mg/kg) and lower than the Malaysia Food Regulation, 1985 (30 mg/kg). Based on the results obtained, it shows that smoked *C. fluminea* collected from hawkers in the district of Tumpat are safe in terms of Cr and Zn but it is unsafe for the Mn and Cu parameters if consume more than 100 g/day/person.

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

## 1. Introduction

*Corbicula fluminea* (*C. fluminea*) is also known as Asian clam or golden freshwater clam from Cyrenidae family. The population size of this *C. fluminea* is unknown but it is very common in its native and introduced range. This species can be found in rivers, lakes, ditches and ponds of freshwater. This species is used for human food. The shell can be used for producing lime and it's also as a material of traditional Chinese medicine [1]. *C. fluminea* was first discovered on the Pacific coast of Canada, possibly introduced by Chinese immigrants as a food source. The North American distribution of *C. fluminea* now extends from coast to coast in the United States. This species is now considered a problem in North America because it passively dispersed within watersheds by water currents [2]. The *C. fluminea* normally can be found in temperate to tropical southern Asia, western to the eastern Mediterranean like Africa and except for Sahara Desert [3]. It is a filter feeder that can remove particles from the water column. It also can be found in the sediment surface or slightly buried in sand. It has the ability to reproduce and has low tolerance of cold temperature in between 2°C - 30°C. It is also able to reproduce by self-fertilization at different levels. The life span of *C. fluminea* has a positive relationship with the latitudinal gradient. Populations from higher latitudes have slower growth, reflecting a slower metabolism and a longer life span. This type of relationship has been described for other mollusk species, including freshwater bivalves. Furthermore, the availability of food may be impaired at higher latitudes due to shorter sunlight periods [4]. The life span of this species is about one to seven years. The life cycle and reproductive stage of *C. fluminea* are related in a large ecological spectrum [3]. Populations of *C. fluminea* seem to negatively be affected by the combination of hypoxia and high temperatures. The species show a clear preference for well-oxygenated sandy substrates. Despite being usually describes as a freshwater bivalve, it can tolerate salinities up to 10-14 ppm, allowing the species to colonize the upstream areas of estuaries [4]. As for reproduction, their fertilization process occurs in the mantle cavity and larvae and the eggs of *C. fluminea* are rich with nutrients to develop embryos. After the protective period, larvae will be released into water, settled and buried into the substratum. When the *C. fluminea* juveniles are released, they have small dimensions around 250 µm, with a well-developed shell, adductor muscles, foot, statocysts, and digestive system. After the water column release, juveniles anchor to sediments, vegetation or hard surfaces due to the presence of a mucilaginous byssal thread. These juveniles can also be re-suspended by turbulent flows and dispersed for long distances, principally in the downstream direction. The maturation period occurs within the first three to six months when the shell length reaches 6 to 10 mm [5].

*C. fluminea* is the most harvested freshwater shellfish for consumption in several states in Malaysia such as Kelantan, Terengganu, Perak, and Pahang. In general, there is a growing demand for *C. fluminea* in the Malaysian market. In Kelantan, smoked *C. fluminea* or smoked 'etak' is one of the most popular snacks among Kelantanese. Traditionally, the preparation of smoked Asian Clam is very simple where they were gathered and cooked by smoking after marinated in a paste made of salt, blended with lemongrass, shallots, ginger and garlic. The marinating process takes at least one hour. According to the smoked "etak" vendors, before marinating, "etak" must be washed and soaked at least three hours in clean water so that it will purge out all mud and sand particles from its body. Then, the marinated 'etak' will be placed on a platform raised 2.5 feet above the floor. The platform is made from bamboo sticks with a space between one stick to another is about 1 – 2 cm to allow heat to pass through to the "etak" during the smoking or roasting process. Over a slow fire, "etak" is constantly turned with a piece of plank attached to a wooden handle. The constant turning over is necessary to ensure evenness of exposure to the heat (smoked "etak" vendors, personal communication).

Normally, 30 to 45 minutes of smoking or roasting is sufficient before they are ready for consumption. Smoked “etak” can be taken as a snack or a meal with rice [6]. Due to its nature of feeding through filter feeder, “etak” tissue normally will be contaminated with heavy metals depending on the quality of the water of the habitat. Additionally, smoked “etak” is also believed to be contaminated at the selling point.

Heavy metals can be defined as metallic elements that have high density compared to water such as arsenic that can induce a low level of exposure [7]. There has been an increasing ecological and global public health concern related to environmental contamination caused by these metals. Naturally, these elements are introduced in the aquatic environment through metal corrosion, atmospheric deposition, soil erosion metal ions and leaching of heavy metals [8]. Heavy metals can enter our body through air, food, and water and can cause long-term effects on human health. They are natural constituents of the earth’s crust because they cannot be degraded or destroyed by micro-organisms; they are instead enriched in bio-accumulated by living organisms [9], [30]. *C. fluminea* was shown to have significantly greater levels of Zn and Cu within its body tissue when compared to surrounding sediments. These findings suggest a possible transport route for nutrients and metals from pelagic to benthic environments in shallow reservoirs. *C. fluminea* also can be used as a bio-indicator for pollutants such as heavy metals and carcinogens [10], [11].

## 2. Materials and Methods

Fresh and smoked *C. fluminea* were collected from hawkers in the district of Tumpat, Kelantan, Malaysia. Sampling point was determined based on available hawkers in the district of Tumpat and five stalls were selected for this study. Four stalls are located by the roadside except one in the Wakaf Baharu wet market. Each station is in each locality in the district of Tumpat namely Kampung Laut, Kampung Kok Keli, Kampung Delima, Wakaf Baharu wet market and Pasir Pekan. 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 the samples were tested using air acetylene flame atomic absorption spectrometry (AAS). All the results were compared with three standards namely Malaysia Food Regulation 1985 [12], European Union Commission Standard 2016 [13], and Food Standard Australia and New Zealand 1991 [14].



Figure 1: Study site

A total of 300 pieces of fresh and smoked 'etak' were collected from five hawkers in Tumpat district with 60 pieces from each hawker (30 pieces fresh and 30 pieces smoked). The tissues and shells were carefully separated by using forceps and knives. The sample

(etak tissue) was dried in an oven with a temperature of 60°C for 72 hours minimum or until the dried weight is constant. A pestle and mortar was used to grind the sample. The preparation of the sample has to be repeated three times to get accurate results. The digestion of the sample was conducted based on the acid digestion technique. About 5.0 grams of the sample was placed into three 200mL beakers. After that, 5 mL of concentrated nitric acid and 5 mL of sulfuric acid were added into each beaker. All the beakers were placed on the hot plate for 30 minutes at 40 °C. After that, the temperature was increased to 140°C for another one hour. About 1 mL of Hydrogen peroxide was added until the solution is clear. After the digestion process completed, the sample was cooled at room temperature. Next, it continued with the filtration process. All the samples were filtrated with filter paper into the beaker. The filtrates were transferred into a 50 mL falcon tube by using the syringe filter. The solutions were diluted to 50 mL with deionized water. The 40 mL of deionized water was added to 50 mL beaker. After that, the samples were transferred into a 15 mL falcon tube and make the serial dilution for each sample. Then, the filtrates were stored in the refrigerator at 4°C before undergoing further analysis. The filtered sample was analyzed for Cu, Fe, Pb, and Zn by using an air acetylene flame atomic absorption spectrometry [15].

### 3. Results and Discussion

Chromium (Cr) is a stable compound in the trivalent [Cr (III)] form and occurs in nature. Cr enters various environmental matrices such as air, water, and soil from variety of natural and anthropogenic sources. The largest contributor to chromium released into environment comes from industries that include metal processing and stainless-steel welding. The increase of the chromium released will affect the environmental concentration that has been linked to air and wastewater [16].

The effects of Cr to human health can cause chromosomal abnormalities and DNA strand breaks [17]. It also causes irritation and ulcers in the stomach and small intestine [18]. Carcinogenicity appears with the inhalation of less soluble or insoluble Cr (VI) compounds exposure to Cr compounds that have been reported can cause epidemic. Epidemiological investigations have been reported respiratory cancers in workers occupationally exposed to Cr compounds [16]. Chromium (Cr) occurs naturally and present in the earth's crust with oxidation states. For human and animals, a certain level of Cr is an essential nutrient that has a role in regulating glucose, fat and protein metabolism. Table 1 shows the concentration of Cr in fresh and smoked "etak" compared to national and international standards. Results showed that, Cr concentration is 0.14 mg/kg and 0.22 mg/kg in fresh and smoked *C. fluminea* respectively. Cr concentrations in fresh and smoked *C. fluminea* were below 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 and it was in-line with [19] findings, where they found that waste burning is one of the primary sources of chromium. This was supported by [20], where they reported that, combustion of wood material released 8.14 mg/kg of total heavy metals of Pb, Ni, Cu, Cd, Cr, and Zn.

Zinc (Zn) is an essential element for the human body. It is an essential component of various enzymes and zymoexcitators that supports growth, tissue generation and the immune systems. Zn is the source of pollution of heavy metal released into the environment through mining and smelting activities. From the mining industries, it will cause a high risk of pollution from wastewater associated. The major pollutants are Pb, Zn, and Cd that absorbed into sediments which act as a secondary source of environmental pollution. Due to the mining activities, migrate and accumulate may

directly affect plants, animals, and humans. Zinc is the element of the human body with a function to support growth, tissue regeneration and immune system. Zn can affect cholesterol balance and infertility [19]. Generally, zinc (Zn) compounds are the metal that relatively non-toxic to human if the uptake is within the recommended standard (100 mg/kg). However, if the uptake is higher, it will cause toxic to the body. The immediate symptoms after taking a toxic amount of Zn include abdominal pain, nausea and vomiting. The additional effects are anemia and dizziness. Based on the results in Table 1, Zn concentration is 74.39 mg/kg and 35.41 mg/kg in fresh and smoked *C. fluminea* respectively. Zn concentrations in fresh and smoked *C. fluminea* were below 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 made by [21]-[22], 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 [23] where they found that the concentrations zinc 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. A small intake of Mn each day is important to stay healthy but if it is consumed more than what is required it will cause negative effects. Mn is one of the metals that enter the body via inhalation which can cause damage the nervous system and respiratory tract as well as other adverse effects. Mn will cause a neurological effect such as parkinsonian-like syndrome [24]. Mn is a chemically active element that will become reactive when pure and will burn in oxygen when it is a powder. It is also toxic when it is in too high concentration that presents in the human body. Based on study by [25], the daily manganese intake was 4.2 mg/day for the males and 4.1 mg/day for the females. The absorption of the Mn into the human body will be transported through the blood into liver, kidney, and pancreas. The results of the study show that, Mn concentration is 45.58 mg/kg and 16.32 mg/kg in fresh and smoked *C. fluminea* respectively (Table 1). Mn concentrations in fresh and smoked *C. fluminea* were far higher than Malaysia Food Regulation, 1985 (10 mg/kg) and Food Standard Australia and New Zealand standards 1991 (10 mg/kg).

Copper (Cu) is a redox-active metal. It is an essential nutrient for all the species. However, an excessive amount of Cu in the body also can pose a risk. Cu will cause diseases to human health such as cardiovascular and diabetes. The heart vessels are vulnerable to Cu deficiency. Cu deficiency has been proposed to induce cardiac damage via relatively low oxidant defense enzyme activities in the heart compared to other tissues. Blood lipid profiles are also affected if a person is experiencing Cu deficit as it has been proposed to be associated with cardiovascular disease [26]. Cu is the element that contributes to neurological defects. Cu has been documented in cancer patients suffering from breast, cervical, ovarian, lung, prostate, stomach cancer and leukemia. Cu is also important for angiogenesis, a process of the growth of any tumor beyond a few millimeters. In the process of angiogenesis, new blood supplies that feed the malignant cells are formed. Based on study by [25], the daily copper intake was 1.3 mg/day for the males and 1.2 mg/day for the females. The results in Table 1 show that Cu concentration is 13.75 mg/kg and 12.60 mg/kg in fresh and smoked *C. fluminea* respectively. The concentration 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 [21]-[22], as they found that thermal processing applied to the shellfish has significantly affected the content of macro and microelements as well as the content of heavy metals except for potassium and manganese. However, it was not similar to the findings reported by [23]

where they found that the concentrations copper in fresh oyster (0.98 ppm) is lower as compared to smoked oyster (1.32 ppm).

The findings from the research show that, “etak” was contaminated at the habitat, meaning that, it was contaminated through polluted water. Therefore, in order to control heavy metals concentration in “etak”, it is important to control the river water quality where the “etak” habitat is. The common method to control pollution from entering the water system (river) is by treating it before entering the river either via biological, chemical or physical treatment. Activated carbon is the most widely used adsorbent for wastewater treatment as this material has a large surface area to adsorb all pollutants including heavy metals [27]-[29].

**Table 1: Heavy metals content in fresh and smoked etak collected from hawkers in Kampung Laut, Tumpat Kelantan**

<b>Samples</b>	<b>Chromium (Cr) mg/kg</b>	<b>Zinc (Zn) mg/kg</b>	<b>Manganese (Mn) mg/kg</b>	<b>Copper (Cu) mg/kg</b>
Fresh	0.14	74.39	45.58	13.75
Selling	0.22	35.41	16.32	12.6
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 discovered that chromium and zinc are below the permissible limit set by Malaysian Food Regulation, 1985 as well as international standard namely the European Union Commission Standard 2016 and Food Standard Australia and New Zealand (FSANZ) [14]. However, the concentration of manganese and copper is higher than the standard, especially manganese which is far higher. Although copper concentration exceeded European Union Commission Standard, 2016, it did not exceed the Malaysian Food Regulation, 1985 standard. So, based on the results obtained from this study and study by [25], smoked “etak” sold in Tumpat district cannot be consumed more than 100 g/day/person in order to avoid negative health impact because the manganese and copper concentrations are high and exceeded the allowed limit.

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