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Determination of mercury (Hg) and lead (Pb) content in selected milkfish from fishponds around Pampang – Makassar River

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Abstract

Heavy metals are naturally embedded in aquatic environment, but their levels are increasing due to industrial, agricultural and mining activities. Mercury (Hg) and lead (Pb) are two of the most common toxicants that can be found in aquatic environment. Behavioural and neurological changes are associated with overexposure to Hg poisoning, while Pb might cause high blood pressure and damage to the reproductive organs. Fish is a common table food consumed by humans for protein nourishment; therefore, it is important to study heavy metal contamination in it. The aim of the present work was therefore to determine the content of Hg and Pb in milkfish (*Chanos chanos Forsk*) from Pampang fishponds, Makassar, Indonesia. Dithizone was used as the reagent for the qualitative analysis of Hg, while KCN was used for Pb. Quantitative analysis performed by Atomic Absorption Spectrophotometry at 253.5 nm for Hg and 283.3 nm for Pb confirmed the presence of Hg and Pb. The results showed that the average content of Hg in milkfish was 1.01 µg/g while Pb was undetected. Therefore, it could be concluded that milkfish from the Pampang fishponds were contaminated with Hg thus poses high risk for human health.

Introduction

In most Asian countries, fish, as a source of protein, fats and vitamins, serves as the main dish eaten with carbohydrates such as rice (Alina *et al.*, 2012; Taweel *et al.*, 2013). Fish has the largest population in aquatic environment and feeds on anything available in the waters. Therefore, if the water is polluted, fishes are at risk of feeding on them (Agusa *et al.*, 2005; Oyoo-Okoth *et al.*, 2010; Taweel *et al.*, 2013). For example, if there is heavy metal pollution in the water, the fishes could be contaminated and transfer the toxicity to humans that frequently consume them (Jordao *et al.*, 2002; Mora *et al.*, 2004).

Heavy metals are naturally embedded in aquatic environment, but their levels are increasing due to uncontrolled industrial and mining activities. If this continues without any control measure, the pollution will be more severe and threaten the fish population, which is detrimental to the surrounding communities (Sivaperumal *et al.*, 2007).

Pampang is one of the rivers in the heart of Makassar City and primarily used as source of water for fishponds around it. The water that flows to it is connected to Tello and Toll rivers, which are both located around the industrial area of the city. However, the wastes from these industrial area contaminate the environment including the rivers and all the aquatic animals. Therefore, the present work was undertaken to investigate the current heavy metal contamination in milkfish tissue (*C. chanos*) from Pampang fishponds, Makassar, Indonesia.

Materials and methods

Chemicals and standard solution

Mercury (Hg) and lead (Pb) were purchased from Sigma-Aldrich. Pro analysis grades of nitric acid (HNO_3), potassium permanganate ($KMnO_4$), and sulfuric acid (H_2SO_4) were purchased from Merck. The stock solution of Hg (1,000 µg/L) was prepared by dissolving 100 µg of $Hg(NO_3)_2$ in 1 mL

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HNO_3 and diluted with 100 mL distilled water. The stock solution of Pb (1,000 µg/mL) was prepared by dissolving 100 mg of Pb in 1 mL HNO_3 and diluted with 100 mL distilled water.

Fish sample

Milkfishes were purchased from a Pampang pond at harvest time in 2015. The selected milkfishes had the same size (43 ± 3 cm) and weight (360 ± 5 g). These were chosen based on the average weight and length of the milkfishes in the pond.

Sample preparation for Hg analysis

Milkfish tissues were dissected, thoroughly washed with distilled water, and dried. Next, 15 g dried fish sample was weighed and 5 mL 65% HNO_3, 72% $KMnO_4$, and 97% H_2SO_4 were added. The mixture was stored at room temperature for 1 d. The sample was later placed into the heater at 60-80°C for 3 h for destruction. After this, it was allowed to cool in a 50 mL volumetric glass, and was later diluted with distilled water to reach 50 mL and then filtered (Falco *et al.*, 2006).

Sample preparation for Pb analysis

Firstly, 10 g dried fish sample was measured and 10 mL 65% HNO_3 was added to it. The mixture was then placed on a hot plate until the vapour colour changed from brown to white. Next, it was stored in volumetric glass, and was later diluted with distilled water to reach the 50 mL mark and then filtered.

Determination of heavy metals

All the plastic and glassware were cleaned by soaking them in 2 M HNO_3 for 48 h, rinsed five times with distilled water, and then another five times with deionised water before they were used. Stock standard solutions of Hg and Pb (1,000 µg/mL in distilled water) were used to prepare the calibration standards (Stancheva *et al.*, 2013). The concentrations of Hg were 10; 20; 30; 40; and 50 parts per billion (µg/L), while the concentrations of Pb were 0.5; 1.0; 1.5; 2.0; and 5.0 parts per million (µg/mL). These were measured using Atomic Absorption Spectroscopy at 253.6 nm for Hg and 283.3 nm for Pb.

Data analysis

Atomic Absorption Spectrophotometer was used to obtain the calibration standard curve by plotting absorbance against concentrations. The best fit for the line curve was calculated using equation of line. Evaluation of linearity was carried out through the use of correlation coefficient (R^2). The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation:

$$y = bx + a \tag{1}$$

where, y = absorbance, b = slope, x = concentration, and a = intercept at *y*-axis.

Results and discussion

Fish is one of the best dishes required in a healthy diet. It contains high-quality protein, essential nutrients, low in saturated fat, and omega-3 fatty acids. Balanced intake of fish can repair some organs that have been damaged. It is also important for the growth and development of children due to its many nutritional benefits (U.S. FDA, 2004).

However, some rivers, such as Pampang, are already polluted by industrial wastes and there are many fish ponds around this river that rely on it for water supply. Milkfish, a favourite of local residents, is widely cultivated in these ponds. There is thus possibility that the fishes have also been contaminated with the wastes. Therefore, further assessment was warranted.

Table 1. Level of mercury (Hg) and lead (Pb)

Reagent	Reaction with sample	Information	Level (µg/g)	
			Hg	Pb
Dithizone	Layer 1: Brownish red	Positive Hg	1.05	≤ 0.01
	Layer 2: Grey		1.03	
	Layer 3: Green		0.94	
KCN + Dithizone	Yellowish white solution	Negative Pb		
	Reddish orange			
Average			1.01	

The qualitative analysis carried out confirmed the presence of heavy metal in the sample as shown in Table 1. The sample was also subjected to quantitative analysis using Atomic Absorption Spectroscopy. This instrument was used because of its selectivity and accuracy in determining the presence of metals even at trace concentration. Standard reference used include Hg in parts per billion (µg/L) and Pb in parts per million (µg/mL) as shown in Table 2. The equation of linear regression was obtained from both the data measured and standard references. The resulting graph showed that there was a linear relationship between concentration and absorbance (Figures 1 and 2). This linearity was not only evidenced by the line formed on the graph but also by the R^2 values which were almost 1.000. This proves that the method of measurement used was valid.

Table 2. Reference standard calibration

Mercury (Hg)		Lead (Pb)	
Concentration (µg/L)	Absorbance (253.6 nm)	Concentration (µg/mL)	Absorbance (283.3 nm)
0	0.0022	0.0	0.0009
10	0.0801	0.5	0.0037
20	0.1816	1.0	0.0072
40	0.3607	1.5	0.0088
50	0.4520	2.0	0.0116
		5.0	0.0278

Based on the data obtained, the average level of Hg content in milkfish from the Pampang fishponds was found to be 1.01 µg/g and this is twice the maximum limit of 0.5 µg/g prescribed by the Indonesia National Standard (BSN, 2009).

According to the U.S. Food and Drug Administration (FDA), the risks from Hg in fish and shellfish depend on the quantity of fish and shellfish consumed and the levels of Hg in it. Therefore, the FDA and the Environmental Protection Agency (EPA) advice women who might get pregnant, pregnant women, nursing mothers, and young children to eat fish and shellfish that has lower Hg content (U.S. FDA, 2004).

The FDA and EPA recommend that people should consume about 12 ounces of varieties of fish and shellfish that are lower in Hg in a week and there are five types that fall within this category: shrimp, canned light tuna, salmon, pollock, and catfish. In addition, fish outside the recommended ones should be eaten twice lower than those that are recommended. Local advisory information about safety of fish in local lakes, rivers, and coastal areas should also be considered, and if no advice is provided, it is enough to eat six ounces per week (U.S. FDA, 2004).

With respect to FDA recommendation, the Hg content level of milkfish analysed in the present work was higher than the threshold; therefore, six ounces is recommended per week while consideration is also given to the advice of local residents. This is important because excessive consumption could result in poisoning, permanent brain and kidney damage and when consumed by pregnant women, it could inhibit the development of foetus (Castro-González and Méndez-Armenta, 2008).

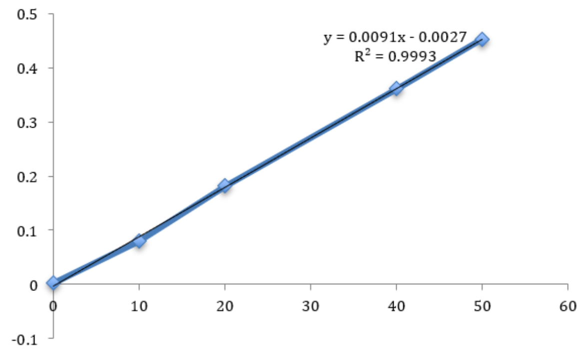

Figure 1. Calibration curve of mercury (Hg)

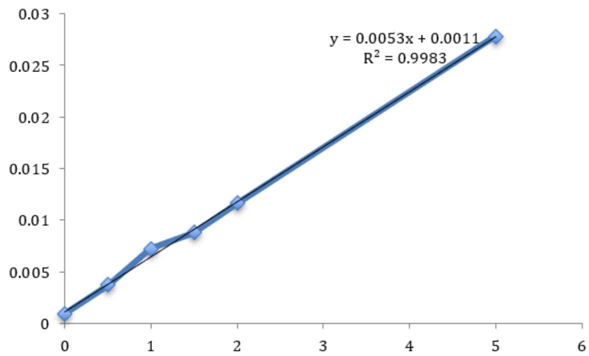

Figure 2. Calibration curve of lead (Pb)

The present work however did not detect Pb (Table 1). Previous research found that sixbar grouper (*Epinephelus sexfasciatus*), Spanish mackerel (Scomberomorini) and Indian mackerel (*Rastrelliger kanagurta*) contained Pb at 0.11 µg/g, 0.1 µg/g and 0.09 µg/g, respectively, and that these are lower than the ones found in samples from Langkawi Island (Irwandi and Farida, 2009). Investigation on Pb contamination is important because based on different research results, excessive exposure to Pb could cause headaches, brain tissue damage and various symptoms relating to nervous system (Jarup, 2003). The variation in the levels of heavy metals in samples might be influenced by the environment, sex, number of samples and species of fish (Tuzen, 2003).

Contamination of milkfish in Pampang ponds by heavy metals could occur as a result of industrial activity, iron processing, processing of plastic and wastes coming from the Industrial Area of Makassar (KIMA). In addition, it could also be due to the sewage contamination caused by household and domestic wastes around the river. Previous researches explained that ecological factors such as season, place of development, nutrient availability, temperature and salinity of the water could also contribute to the inconsistency of metal concentration in fish tissue (Clearwater *et al.*, 2002; Tuzen, 2003). Nevertheless, the metabolism and biological system of several types of fish could also affect the level at which they accumulate heavy metals in their body (Law and Singh, 1991).

The permitted level of heavy metals' contamination in fish and shellfish are usually compared with the recommendation of the Food and Agriculture Organisation and World Health Organisation (FAO/WHO, 2004), and also that of U.S. FDA and EPA. Locally, the Indonesia National Standard also has set the standard for food, and as can be seen in Table 1, the levels of Hg found in the fish were higher than the permitted level.

This result shows that there is a need for strict regulations regarding waste disposal by the industries because of its harmful side effects on the general health of the society. It is also important to educate the industries about the dangers of heavy metal contamination to water resources, including watershed residents. The same information should also be conveyed to the public so that they will know the type of fish they should be consuming.

Conclusion

Milkfish from Pampang fishponds contained heavy metal mercury (Hg) at 1.01 µg/g which was twice the allowed quantity prescribed by the Indonesia National Standard for Food of 0.5 µg/g. The quantity of lead (Pb) in the sample was not significant enough for attention.

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