Jurnal Teknologi

Full Paper

HEAVY METAL IN FISH: ANALYSIS AND HUMAN HEALTH-A REVIEW

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Article history
Received
9 March 2015
Received in revised form
12 April 2014
Accepted
1 October 2015

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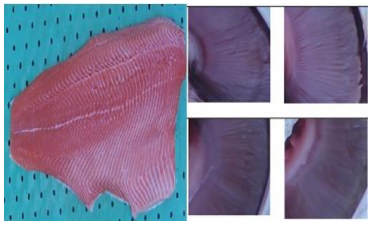
Graphical abstract



Abstract

Living organisms require trace amounts of heavy metals, including cobalt, copper, manganese and zinc to survive. However, the excessive levels of the metal can be detrimental to the organism. Other heavy metals such as mercury, lead and cadmium have no vital on organisms, and their accumulation in long time period in the bodies can cause serious illness or death. The consumption of fish is recommended because fish is a basic and good nutritious food that has omega-3 fatty acids due to its cardio-protective effects. This present mini-review accounts for the description of heavy metal in fish and the effect of toxic metals on the human health. Besides, the acid digestion method was also discussed in order to identify the best method for applying in the laboratory analysis. The best method used can reduce the contamination error in the results.

Keywords: Heavy metal, fish consumption, acid digestion method, toxic metal, human health

Abstrak

Organisma hidup memerlukan sejumlah logam berat seperti kobalt, tembaga, mangan dan zink untuk meneruskan kehidupan. Walau bagaimanapun, logam yang berlebihan boleh memusnahkan organism. Logam berat lain seperti merkuri, plumbum dan cadmium tidak mempunyai sebarang kepentingan terhadap organism dan kehadiran mereka dalam jangka masa yang panjang boleh menyebabkan penyakit yang serius atau membawa kepada kematian. Pengambilan ikan adalah disyorkan kerana ikan mengandungi omega 3-asid lemak yang berkhasiat. Mini kajian ini menbincangkan perihal logam berat dalam ikan dan kesannya pada kesihatan manusia. Selain itu, kaedah-kaedah penghadaman asid juga dibincangkan untuk mengenalpasti kaedah terbaik bagi diaplikasikan di dalam analisis makmak. Kaedah terbaik mampu mengurangkan ralat dalam keputusan yang diperolehi.

Kata kunci: Logam berat, pengambilan ikan, kaedah penghadaman asid, logam toksik, kesihatan manusia

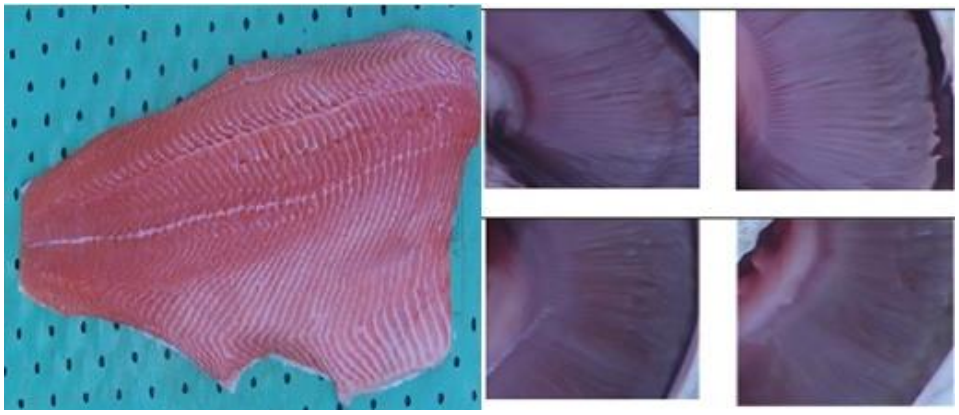
1.0 INTRODUCTION

Many marines life were becoming destroyed due to the impact of human activities that discharge their waste such as heavy metals into the marine ecosystems. In 2014, East coast areas in Malaysia have been heavily impacted by major discharges from industrial outflows and also municipal especially at the Paka River after flooding season and gave impact toward aquatic organism especially fish. This is due to the rapid development of industry such as chemical manufacturing, oil and gas and others [1-3].

Fish is a basic and important food for human nutrition [4-6] such as fatty acid in fish that can reduce the risk of heart diseases and stroke due to their contribution in lowering the cholesterol levels in blood and also provides minerals and vitamins [7]. The present of heavy metal in fish gives impact to the human health. Besides, fish is a very suitable bioindicators of heavy metal contaminations [8-10]. The parts of fish that take up heavy metals from the gills, body surface, digestive tract, muscle and liver [11-14]. Figure 1 shows the example of edible part of fish that use for the analysis.

Moreover, gill is a modest accumulation of heavy metal even though it has low metabolic activity due to the high adsorption of metal on the gill surface of higher metals [15]. Besides, water becomes the main source of contamination due to the high heavy metal contents in the gills [16]. While, the concentration of heavy metal in fish muscle is always low compared to the other tissues of fish [17-19] due to their low metabolic activity [20].

However, it is still important to compare muscles in order to determine the safe levels. This is because the consumption of fish muscle is the greatest mass compared to the other part of the fish [21, 22]. The higher intake of heavy metal in our body can cause bad impact towards health [23-25].



(*Source:* Dowlati *et al* [26]; Stien *et al* [27])
Figure 1 Edible part of fish use for the analysis a) muscle and b) gill

2.0 PREPARATION AND ANALYSIS OF SAMPLE

2.1 Microwave Digestion Method

The microwave digestion technique was widely used as a new alternative for conventional acid digestion. This is due to the retention of the volatile compounds in the solution (keeping volatile compounds in the solution), shorter time, and less acid consumption [28, 29].

The homogenized samples were digested in a microwave oven digestive system using nitric acid (65 % HNO_3) and hydrogen peroxide (30 % H_2O_2) in Teflon vessels. Based on Bashir *et al* [11], inductive coupled plasma mass spectrometry (ICP-MS) was used to identify the concentrations of iron (Fe), zinc (Zn), aluminium (Al), arsenic (As), cadmium (Cd) and lead (Pb) in two species of fish. They also used the standard reference material of the marine biota sample (SRM2976, freeze- dried mussel tissue, National Institute of Standards and Technology, USA) in order to control the performance of this method.

In India, Sivaperumal *et al*. [30] used 10 ml of nitric acid–Perchloric acid with a ratio of 10:4 in order to digest the samples using a microwave digester. Atomic absorption spectrophotometer was used to analyze the level of Zn, Cu, Cd, Pb, Co, Ni, Mn and Cr. While, hydride generation and cold vapor techniques were used for the determination of As, Se and Hg. For determination recovery, 2.5 g of samples were spiked with three different concentrations of heavy metals, in triplicate.

In Morocco, Chahid *et al*. [31] used microwave oven where digested with 5 ml of HNO_3 in Teflon vessels and added 2 ml of 30%H_2O_2. Then, 4 ml of concentrated nitric acid, 2 ml of concentrated sulphuric acid and 1 ml of concentrated hydrochloric acid was slowly added. The digestion of each sample was made in triplicate and blanks were also performed to check for any possible contamination. Automated Mercury Analyzer was used to analyze the total concentration of mercury. Analysis of lead and cadmium was determined by a graphite furnace atomic absorption spectrophotometer.

In Turkey, Turkmen *et al*. [32] performed microwave oven to digest a tissue sample with 10 ml of 1 N HNO_3 in Teflon vessels. Then, 1.5 ml of 30% H_2O_2 was added to break down organic matter that may be undigested during the acid digestion. Analytical blanks were also prepared. All samples were analyzed for three times. Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn were analyzed using ICP-AES. Standard solutions were prepared from stock solutions. A Dorm-2 certified dogfish tissue was used for calibration. To validate the calibration, the percentage of recovery ranged between 90- 110% were accepted.

In a study performed by Velusamy *et al*. [33], the microwave accelerated digestion system was performed to digest a tissue sample with 1 ml of HNO_3 and 3 ml of HCl. Each sample was prepared with a blank, spike and certified reference material. Mercury was analyzed with a flow Injection Mercury System whereas Graphite Furnace Atomic Absorption Spectrometry and an Inductively Coupled Plasma Optical Emission Spectrophotometer to analyze Cr, Zn, Mn, Cu and Fe. By using various spiked metal concentrations, 96% to 101% of recovery percentage was obtained.

In another study, Ozparlak *et al*. [29] studied 1g of the homogenate was digested by the microwave digestion system. Digestion conditions for microwave system for the samples were applied as 2 min for 250 Watt, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, and 8 min for 550 W. The concentrations of heavy metal (Pb, Cd, Zn, Cu, Ni, Cr, Mn, Fe, Co, Mg, Sr, Bi, Na, K, Li and Ca) were carried out using atomic absorption spectrophotometer.

Furthermore, Murthy *et al*. [34] had studied about fish meal and digested in Teflon containers using a microwave digester. Dried powder sample (3 g) was weighed into Teflon vials (100 ml) and digested overnight with 7mL of pure HNO3 and 3 ml of H_2O_2. Then, Atomic Absorption Spectrophotometer following the Association of Analytical Communities (AOAC) method [35] was performed to analyze the heavy metal content in the fish samples. Mercury content was estimated using the mercury analyzer. According to AOAC [36], proximate composition of fish meal had been measured.

2.2 Conventional Digestion Method

Method of acid digestion was performed to digest and homogenize the tissue sample. In Bangladesh, Ahmad *et al*. [37] used conventional acid digestion method by adding 1.0 ml 70% HClO4, 4mL concentrated HNO3 and 1.5 ml concentrated H2SO4 into the test tube. Then, the samples were analyzed by using AAS. Standard reference materials; NBS-SRM-1566 for Oyster tissue prepared by the National Bureau of Standards, Washington, DC, USA was used to check the quality of the work. The average recovery ranged between 94 to 107% was achieved by calibrating the analytical procedures against the above standard reference materials.

Kamaruzzaman and his group [12,38] also used the conventional method of sample tissue digestion by adding the concentrated H_2O_2, HNO_3, HCl and H_2SO_4 at 60 °C using Teflon beaker. All samples were analyzed by ICP-MS for three times for each metal. Standard reference material (DORM-2 National Research Council, Canada) and a blank in replicates were performed in order to control and achieve good accurateness of this work. The average percentage of recovery was between 95% and 105% [12, 38].

Elnabris *et al*. [39] taken out about 4 g for each specimen of fish sample and then placed into 300 ml kjeldahl digestion tubes. High purity of nitric acid, 2 ml of 10 M HCl and 4 ml of 35% H_2O_2 were mixed and added to each tube [40]. Thermoelement Atomic Absorption Spectroscopy was used to analyze heavy metal levels in all samples. Before running the analysis, the blank standard was prepared to ensure the samples and chemicals used does not contain any contaminants.

In Nigeria, Olawusi-Peters *et al*. [41] used FAO methods cited by Dybem [42] in order to dissect fish samples. 0.5 g of each fish sample was ashed in electric muffle furnace at 550 °C for 24 h and diluted in 5 ml of mixture of concentrated HNO_3 and concentrated HCl with ratio 1:3. The sample then used Atomic Absorption Spectrometer flame emission spectrometer fitted with GFA-4B Graphite Furnace performed to analyze the level of Fe, Cu, Pb and Zn.

In another study in Nigeria, Farombi *et al*. [43] investigated about heavy metal in organs of fish such as liver, heart, gills and kidney. Teflon Homogenizer was used to homogenize the sample tissues in 4 volumes of homogenizing buffer (50 Tris - HCl mixed with 1.15% KCl and pH adjusted to 7.4). The levels of Cu, As, Zn and Cd of the tissues (kidney, liver, gills and heart) were analyzed using Atomic Absorption Spectrophotometer.

Moreover, Arslan & Secor [44] studied about right and left pairs of otolith fish and were placed in a Teflon beaker. The samples were dissolved in concentrated HNO_3 and heated at 80 °C until no solution remained. Then, the residue was dissolved in 10 ml of 0.1% HNO_3 using 15 ml polystyrene centrifuge tube. According to Yoshinaga *et al*. [45], certified reference material No. 22 obtained from the National Institute of Environmental Studies, Japan had been applied in this study. The level of heavy metal was analyzed using ICP-MS.

In other research, Bat *et al*. [46] has performed their study by using about 20 g of fish samples to digest the samples in hot concentrated HNO_3. All samples were prepared in triplicate. Blanks were also performed to check for any possible contamination. The AAS (modified by Bernhard [47]) was used to analyze the concentration of heavy metal. Serial dilution of concentrated stock solutions of 1000 mg/L was also prepared as calibration verification standard.

Dalman *et al*. [48] studied about the procedure of sample digestion in the Digestion Applications Manuel by Anon [49]. 0.5 g of fish sample was placed in a Teflon vessel with 10 ml water, 5 ml HNO_3, 4 ml HF and

l ml HCl. Digested samples were treated using 2g of crystalline H_3BO to neutralize excess HF. Flame atomic absorption spectrometer (FAAS) was used for analysis of Zn and Cu, while graphite furnace atomic absorption spectrometer (GFAAS) was used for analysis of Cd and Pb. Analysis of standard reference material (fish: DORM-2, National Research Council, Canada) was used to check the quality of the method.

Furthermore, Oyakhilome *et al*. [50] reported that approximately 5 g of each homogenized samples were weighed into a 200 ml Kjeldahl digestion flask and 20 ml of mixed concentrated nitric acid and 62% Perchloric acid (ratio 2:1 v/v) were used. AAS performed in order to determine the concentration of metal in each sample. Standard procedure established by USEPA [51] was used in determination of heavy metal in fish.

Another study performed by Tao *et al*. [52], used 0.5 g of fishes and put into 50 ml Bunsen beakers and added to 10 ml nitric acid and 1 ml Perchloric acid. Then, 5 ml of HCl was added and transferred to 50 ml volumetric flasks for analysis. Analysis of Cu, Zn, and Cr had been done by using flame AAS, whereas Ni, Pb and Cd use ICP-MS. National Certified Reference Material- prawns (GSB-28) with known concentration of metals were also prepared. The recovery percentage for all tested metals was 87 % to 107 %.

Besides, Ashraf *et al*. [53] also studied about classical acid digestion and the methods used according to Moller *et al*. [54]. The samples were ashed for 4 h until a white ash residue was obtained. Then, the residue was dissolved in 5 ml of 25% HNO_3, transferred to a 25 ml volumetric flask and made up to volume. ICP-OES was used to analyze the level of heavy metals in fish tissue samples. Standard stock solutions of heavy metal (As, Cu, Pb, Zn) was prepared from Titrasolm (1000 mg/L). In addition, certified reference materials (fish: DORM-2, National Research Council, Canada) were also used to ensure the accuracy of the procedure.

Other researchers, Mohammadi *et al*. [55] performed in Teflon beakers with 5 ml of 65% ultrapure HNO_3 for 4h at 100 °C. Determination of heavy metal (Cd, Ni and Pb) was measured by using graphite furnace atomic absorption spectrophotometry. Hg concentrations were analyzed with a mercury analyzer coupled to a Perkin–Elmer 4100 spectrophotometer. The analytical procedure was checked using reference material [MESS-1, the National Center of Canada and CRM 277, the Community Bureau of Reference, Brussels, Belgium, and details were discussed in [56, 57]].

2.3 Comparison of Microwave and Conventional Digestion System

Table 1 Difference between conventional and microwave digestion method

Conventional digestion	Microwave Digestion	Reference
Inexpensive technique	Expensive technique	[58]
High time duration of digestion	Less time duration of digestion	[28, 29, 59]
High possibility of contamination	Less possibility of contamination	[59, 60]
High acid consumption	Less acid consumption	[28, 29]

Based on the above consideration (Table 1), microwave digestion method gave the best performance in terms of time consuming and less contamination occurs. However, in order to reduce cost, the conventional method is suggested. The contamination occurs when using the conventional method can be avoided by placing a small screw cap vial in the Teflon digestion system for closed systems. For open system, the graphite block digestion system was preferred. [59, 60]

3.0 HEAVY METAL AND HUMAN HEALTH

Food consumption is the main route of exposure of non-occupationally individuals [61]. The presence of heavy metal in commercial fish can pose potential health risks to human [62-64]. Hence, it is important to identify the level of heavy metal contents in fish in order to ensure that it does not expose any hazard to the human and maintain concentration under permissible level [30, 65, 66].

Many regulatory bodies from various countries have been established about the maximum permitted concentration of heavy metals in foodstuffs such as the World Health Organization (WHO), Food and Agriculture Organization (FAO) and European Union (EU) [67, 68]. For example, according to EU [69] stated that the maximum tolerable limit (MTL) of lead in fish meat is 0.3 mg/kg where cadmium and mercury were about 0.05-0.3 and 0.5-1.0 mg/kg wet weight respectively depends on the type of fish.

Basically, heavy metal is divided into two categories, which are essential metals and non-essential metals. Heavy metal such as lead, mercury and cadmium are categorized as non-essential metals and they are a toxic and harmful to organisms, even in small amount, over a long period of time [70-71]. While, nickel, copper and manganese is an essential metal due to their important role in biological systems [72-74]. The dose-response curve for essential

metal is U-shaped due to those metals that have both deficiency and copper excess which produce adverse health [74].

Manganese (Mn) is an element of low toxicity that has considerable biological significance due their ability to prevent heart attack, stroke and cardiac arrest. Deficiency of Mn gave congenital malformations in offspring, poor growth performance and low efficiency of the reproductive system [75]. However, it's become dangerous and toxic at high concentrations and usually may lead to the neurologic and psychologic disorder [14, 76].

Normally, nickel (Ni) is an essential metal and occurs at very low levels in the environment. However, a deficiency of Ni in humans has not been documented [77]. Ni is known to be carcinogenic [78]. Moreover, fibrosis, tumours, lung inflammation and emphysema occur also caused by Ni [79].

Iron (Fe) is a mineral essential for every living cell and necessary for the synthesis of myoglobin, haemoglobin and certain enzymes. Deficiency of Fe results in weakness, susceptibility and inability to concentrate [80]. Anderson and Fitzgerald [81] studied that one of the most common nutrient deficiencies in the world is Fe deficiency in anaemia such as malaria. Anaemia disease gives poor performance in circulatory transport and also reduces oxygen supply to muscle, less efficiency due to the decreasing of myoglobin content and impairing endurance capacity [82].

Chromium (Cr) is an essential trace element in some animals and humans. Cr may reduce body fat and also improve lean body mass. However, their effects are small compared to those of a well-balanced diet and exercise [83, 84]. But it could have an undesirable fatal effect in excess amount. Lack of Cr can affect the growth and disturbances in glucose, lipid and protein metabolism [80]. According to Stipanuk and Caudill [85], they found that 12 out of 15 studies showed a positive effect on the relationship between Cr and impaired glucose tolerance based on a meta-analysis.

Zinc (Zn) is a ubiquitous trace element and one of an essential element that important to human and plants. Zn is known as a cofactor to more than 300 enzymes that involved in RNA and DNA metabolism. Zn is also important in the structure stabilization of a large amount of proteins [86-88]. When exceeding amounts are present, Zn becomes toxic [73] but a deficiency of Zn can lead to several disorders [89] such as results in poor pregnancy outcomes [90, 91] and development of chronic diseases, including cardiovascular disease [92-93] and also cause cancer [94].

Copper (Cu) is an essential metal of enzymes and necessary for the haemoglobin synthesis [30]. Impaired delivery of Cu can result in decreased cuproenzyme activity, the skeletal and vascular systems [95] and also cause anaemia, neutropenia and osteoporosis [96]. Impaired metabolism of Cu could cause two genetic diseases which are Mense disease and Wilson disease. Accumulation of Cu can expose to the Mense disease which is about a fatal disorder [97]. Wilson disease also could occur due to Cu accumulates in the brain and eyes in the form of Kayaer-Fleischer ring [98, 99]. Excessive intake of Cu also could cause kidney damage and even death [100].

Besides, mercury (Hg) is a non-essential element. The levels of Hg increase due to the increases of fish size [101]. Toxicity of Hg can damage the organ in fish [73, 102]. While, in human, Hg can cause the development of fetus destroyed due to their toxicity and also considered as a carcinogen [103]. While, Vettori *et al*. [104] studied that neuronal loss in the cerebellum granule layer and damage of discrete visual cortex area occurs in adult brain due to the Hg poisoning. Emami Khansari *et al*. [105] also stated that Hg is a human toxicant and become primary sources of human by eating fish.

Food consumption is a main source of exposure cadmium (Cd) in the human body. Cd is known as an endocrine disturbing substance and it is well documented that Cd can cause to develop breast cancer and prostate cancer in humans [14]. Cd also causes damage in kidney, hypertension, tumours, poor reproductive performance and hepatic dysfunction [7, 106, 107].

In addition, lead (Pb) is a naturally-occurring and industrially-produced element that is very toxic to the human, especially children [108]. Children are the most vulnerable to Pb because having less effective renal excretion and greater absorption of gastrointestinal. The fetal brain presents a greater sensitivity to the toxic effects of Pb compared to the mature brain [109]. Umar *et al*. [110] stated that symptoms of intestinal cramps, anaemic condition and fatigue caused by poisoning of Pb. Pb also can cause nephrotoxicity and neurotoxicity [111].

Nowadays, Arsenic (As) is widely spread in the environment due to both natural and anthropogenic processes [112]. As is a carcinogen and potent toxicant. As also has potential to destroy communities of ecological system [113]. Toxicity of As depends on the speciation [114] and trivalent As (III) has the greatest toxicity. According to ATSDR [115], mono and dimethyl arsenics have low toxicity.

4.0 CONCLUSION

In summary, we studied about the application of different analytical technique to perform modern chemical tests for indicating the presence of heavy metals in fish. We also consume a large number of fish in order to detect amounts of heavy metals easily. However, the maximum permissible limits of heavy metal for intake of fish should be a concern. Health institutions, public and private organizations must have a continuous communication about risk and benefit of fish consumption in order to control the quality and improve the balance between risk and

benefit of the fish consumption towards human health.

Acknowledgement

This work was supported by East Coast Environmental Research Institute (ESERI), Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, Kuala Terengganu, Terengganu.

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