Journal of Chemical Health Risks

Journal of Chemical Health Risks (2017)7(2), 133–138

ORIGINAL ARTICLE

Assessment of Four Heavy Metals Mercury, Lead, Copper and Cadmium Levels in Muscles of Imported Tilapia to Iran

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(Received: 2 July 2016 Accepted: 5 September 2016)

KEYWORDS

Heavy metals;
Seafood safety;
Tilapia

ABSTRACT: This study was conducted to determine the residues of mercury (Hg), lead (Pb), copper (Cu) and cadmium (Cd) in the imported tilapia fillets. Thirty random samples from imported tilapia fillets were collected from different markets in Isfahan City, central Iran. They were analyzed using Graphite Furnace Atomic Absorption Spectrometer (Perkin Elmer 800) for Pb, Cu, Cd and flow injection mercury system (Perkin Elmer 400) for Hg. Out of the 30 tested samples, concentration of Hg, Pb, Cu and Cd in the tilapia fillets samples as mean± standard deviation were 0.083±.016, 0.638±0.067, 0.521± 0.081 and 0.136 ± 0.025 mg/kg, respectively. Among these, amounts obtained for all metals except for lead were lower than the permissible level specified by WHO (*P*<1%). The Pb concentrations in all examined samples were higher than WHO standards. The continuous consumption of these contaminated fish regularly for long time may lead to health troubles.

INTRODUCTION

Tilapia, as a common name, is applied to various cichlids from three distinct genera: *Oreochromis*, *Sarotherodon* and *Tilapia*. Similar to the grass carp, most tilapia species are herbivores that have the potential to alter aquatic plant populations and ecosystems [1]. Heavy metals are persistent contaminants in the environment causing serious illness in fish, animals and human [2]. Considerable amounts of various metals may be deposited in fish tissues without causing mortality [3].Based on toxicity of aquatic ecosystems and assemblage in biota and fish, heavy metals attract significant focus to themselves [4]. Heavy metals are persistent type of pollutants and cannot be destroyed by heat treatment, so their persistence enhances their potential to reach and affect human being [5].

In recent years, tilapia fillets have been imported into Iran. Sometimes there has been a discussion on the presence of harmful drug residues like heavy metals in this fish, which is necessary to these fish products,

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periodically is examined according to world heath quality standards. Many studies have been done in the field of measurement and detection of heavy metals as for Tilapia [6-9].

Copper under ionic forms Cu^{2+}, Cu_2OH^+ and $CuOH^+$ is toxic to fish [10]. The presence of copper (II) ions causes serious toxicological concerns.It is usually known to deposits in brain, skin, liver, pancreas and myocardium. Free cupric ions are much more toxic than most organic and inorganic copper complexes and precipitates [11]. Occurrence of copper in large amounts is extremely toxic to living organisms [12]. Pb poisoning is generally ranked as themost common environmental health hazard [13]. Pb absorption may constitute a serious risk to public health. Pb may induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular diseases in adults.By passing decade, the levels in food have considerable reduction due to the consciousness of lead, the health problem and sources which leads to decline the emission [14]. Cd is a non-essential element that can be toxic and carcinogenic. Sources of Cd for freshwater and saltwater include atmospheric deposition, direct and via runoff, as well as direct discharges into water or watersheds. Much of the Cd added to aquatic systems accumulates in sediments where it presents a risk to benthic biota and under certain conditions may reenter the water column. Cd has a high bioavailability in the aquatic organisms, which some factors consisting of salinity, hydrogen ion concentration and dissolved organic matter can influence on the chemical forms of Cadmium. [15]. Mercury is known as a pollutant in the world due to potential to human health risks. Although it is located within the compartments and comes from natural sources, but there are many factors related to pathway and receptor linkages that are still not obviously identified. [16].

The aim of the present study was assessment of concentration of different heavy metals such as Hg, Pb, Cu and Cdin tilapia fillets. The results obtained from this study would provide information for background levels of human consumption.

MATERIALS AND METHODS

Collection of samples

Thirty random fillet samples of tilapia were collected from different markets in Isfahan City, central Iran for determination of Hg, Pb, Cu and Cdlevels. The fish samples were then transported to the lab in an ice cooler kept at about 4°C.

Digestion of samples

Fish samples were analyzed by a modified procedure from the Association of Official Analytical Chemists. Five grams wet weight each of muscle for each of the fish sample was dried for 8h at 180°C. 0.3g of each dried sample was placed separately in labeled test tubes and 5ml of a mixture of chloroform-methanol-water (2:1:0.5) was added to each tube and allowed to stand overnight at room temperature for lipid extraction. The samples were subsequently washed in deionized water before the addition of 5ml of nitric acid to each tube for sample digestion. Thereafter, 20 ml of de-ionized water was added to each tube to give a final nitric acid concentration of 20%. Digested samples were analyzed for levels of Pb, Cu and Cd using Graphite Furnace Atomic Absorption Spectroscopy (AA Perkin Elmer 800).

For mercury analysis, 0.3 g of the tilapia samples was weighed in 300 mL Biochemical Oxygen Demand (BOD) bottles. To each bottle, 1 ml of 70% HNO_3 and 4 ml of H_2SO_4 was added and mixed well. The BOD bottles were then incubated in a water bath set at 80 ± 5°C for 30 min. The bottles were later taken from the water bath and allowed to cool. To the BOD bottles, the following two solutions were added; 15 ml of $KMnO_4$ and 8 ml of 5% $K_2S_2O_8$. The BOD bottles were then incubated in a water bath for 90 min at 30 ± 5°C. Then, 10 ml of the solution from the BOD bottles were transferred to 15 ml polypropylene centrifuge tubes. To each centrifuge tube, 750 µL of 12%

hydroxylamine solution was added. The solution was then tested for mercury concentration in triplicates using the Perkin Elmer 400 Flow Injection Mercury System (USA).The system is compact; easy-to-operate mercury analyzes, and works based on flow injection techniques.

STATISTICAL ANALYSIS

Mean values of residues of heavy metals among sample location of the fish were compared using one-way analysis of variance (ANOVA). *P*-values less than 0.01 were considered significant. All analysis was carried out using the SPSS, version 17 (Chicago, IL, USA).

RESULTS

The concentrations of Hg, Pb, Cu and Cd in muscle samples are presented in Table1 along with the statistical parameters. Statistical analysis of the data showed significant differences among all of the samples. The mean residue levels of heavy metals varied among all samples. This variation is significantly different for each tested residue (P<0.01). Hg, Pb, Cu and Cdlevels were 0.136±0.025, 0.638±0.067, 0.083±0.016 and 0.521±0.081 mg/kg, respectively. Except lead, means of all metals in muscles of fish are lower than the maximum permissible limit set by the WHO (P<0.01). In Table 2, recovery and detection limit of assessed metals is listed.

Table 1. Concentration of heavy metals in muscles of tilapia (mg/kg)

Type of metal	Number of samples	Mean ± SD	The maximum permissible limit	*P* value
Cd	30	0.136±0.025	≤0.2	0.001**
Pb	30	0.638±0.067	≤ 0.5	0.001**
Cu	30	0.521± 0.081	≤ 1.0	0.001**
Hg	30	0.083±.016	≤ 0.1	0.001**

**The difference of obtained means is significant with the maximum permitted limit standard

Table 2. Percent of recovery and detection limit were obtained in measured heavy metals

Sample	Percent of recovery	Detection limit (ppb, μ/kg)
Cu	98.8	1.2
Cd	98.6	0.7
Hg	98.1	0.1
Pb	99.1	1.4

DISCUSSION

The term of heavy metals originated with reference to the harmful effects of metals like Hg, Pb, Cu and Cd, all of which are denser than iron. Cu commonly encountered toxic heavy metal. The heavy metal could be harmful to human and fish health. This study was performed for assessment of four heavy metals (Hg, Pb, Cu and Cd) for tilapia fillets imported from China to Iran.

Hg, Cd and Pb are toxic at low concentrations; non-essential heavy metals and have no role in biological processes in living organisms. Cu is an essential trace nutrient that is required in small amounts (5-20 μg/g)) by fish and shellfish. But the effects of copper on aquatic organisms can be direct or indirect lethal. Copper is used in the rainbow trout farms as disinfectants compounds which can impact on healthy of fish. Moreover all aquatic populations and ecosystems can be individually impressed by toxic effect of it [17]. Results of present study revealed that all heavy metals residues detected in muscles samples. Cu, Cd and Hg concentration determined were below the maximum permissible limits set by WHO [18]. In contrast, we showed high concentration of Pb residues in all samples. In the literature, heavy metal levels in the tissue of freshwater fish vary considerably among different studies. Some heavy metals in environmental water and tissues of Nile tilapia (*Oreochromisniloticus*) in Egypt were measured. Significant higher amounts of some heavy metals such as Fe, Pb, Cd, Cu, Mn and Zn have been found in polluted waters. Concentrations of different traces of heavy metals in various tissues of fish caught from polluted area were greatly dependent on the concentrations of these elements in the raw water [6].

Badr et al. found significant higher amounts of some heavy metals such as Fe, Pb, Cd, Cu, Mn and Zn in polluted waters and investigated that concentrations of different traces of heavy metals in various tissues of fish caught from polluted area were greatly dependent on the concentrations of these elements in the raw water [6].

The obtained data in present study illustrated that high accumulation of Pb in muscles that is similar to [19] where the highest concentration of Pb was in kidney and liver of Nile tilapia, from Nile River at Assiut region. In comparison of heavy metals in catfish and tilapia in Densu River of Ghana, Cd and Iron accumulated by the two fish species exceeded the maximum permissible limits prescribed WHO guideline well as the Food and Agriculture Organization standard. The present results agreed with another study which found that Cd, As and Hg showed lower concentrations in muscle of imported Tilapia Fillets from China to USA [9]. But unlike our study, concentration of lead levels were below the maximum permissible limit set by the United States Food and Drug Administration (US FDA). In addition, presence of Pb and Cd in freshwater fish (*O.niloticus* and *L. niloticus*) in Assiut City markets in Egypt was measured andwas lower than the Egyptian Organization of Standardization and Quality Control [20]. High levels of lead may be attributed to presence of industrial and agricultural discharges, motor boat traffics and also from mine and smelting operations. Pb is toxic even at low concentrations. It is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. [21]. The accumulation patterns of contaminants in fish depends on both uptake and elimination rates [22]. Since seafood is used extensively for human consumption, understanding of heavy metals content in muscle is very important. Though in comparison among such organs, muscles have lowest and bones, gonads and brain have highest concentration of metals. In fish, chemical pollutants commonly lead to genotoxicity through their aquatic environments [23].

Generally, pollution of waters to some heavy metals causes to accumulate of them in fish tissues. Those factors that contribute in accumulation of metals are time of exposure, metal concentration, way of uptake and environmental conditions. Fish age and way of feeding are considered as others significant factors. Various metals show different affinity to fish tissues. Most of them accumulate mainly in liver, kidney and gills. Fish muscles, compared to the other tissues; usually contain the lowest levels of metals. Metal distribution in various organs is time-related. Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances. Accumulation of metals in fish living in the polluted waters can be affected by kind of fish species [3].

In aquatic organisms, lead concentrations are usually higher in benthic organisms and algae and lowest in upper trophic level predators (e.g., carnivorous fish). Exposure of a fresh-water fish to several sub lethal concentrations of Pb for a period of 30 days showed significant accumulation of lead in the blood and tissues. The lead accumulation in tissues was found to increase with lead in water up to a concentration of 5 mg/L, although this was not seen at 10 and 20 mg/L of Pb accumulation in the tissues [24]. Tilapia is one of the most consumed seafood in the world, and it is a good model for measurement the impacts of different environmental pollutants on aquatic ecosystems. Previous studies have shown this fish has a strong immune system that is able to tolerate different types of stress [25]. Tilapia has a surface feeder omnivorous non-predatory fish that this dietary behavior might reduce their possibility of contacting numerous types of pollutants when compared to bottom feeder fishes [22].Therefore, it is thought that tilapia among other teleost fish have a high food safety.

CONCLUSIONS

All tested metals except Pb are in permissible limits and their use is allowed for consumers. But if these were continuously consumed in small quantities, they could lead to poisoning or harmful effects in humans. Higher levels of heavy metals accumulated by the fishes might be due to increase in the agricultural influx waters, domestic wastes and some anthropogenic activities which, merit further investigation. In Iran, all fish or shellfish products are examined under food quality standards before any human consumption.

ACKNOWLEDGEMENTS

The authors declare that there is no conflict of interest.

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