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Assessment of Heavy Metal Concentration in Fish Meat of Wild and Farmed Nile Tilapia (*Oreochromis Niloticus*), Egypt

Mohamed G. Hamada[1], Zakaria H. Elbayoumi[1], Reda A. Khader[2], Abdel Rahman M. Elbagory[3]
[1]Department of Food Hygiene & Control, Faculty of Veterinary Medicine, University of Sadat City, Egypt
[2]Veterinarian, Directorate of Veterinary Medicine, Sadat City, El-Menofia Governorate, Egypt
[3]Dean faculty of veterinary medicine, Menofia University Egypt

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***Correspondence to:**
zico76us@yahoo.com

ABSTRACT

Subjection to Heavy metals is an imperative normal issue coming to fruition because of various human exercises. The aim of this study was the assessment of some heavy metals and its possible hazards on fish and consumers. Eighty Nile tilapia fillet samples were collected from Nile canals and markets in Menoufia, Egypt for analysis of Mercury (Hg), lead (Pb), and cadmium (Cd), using atomic absorption flame. In general, wild tilapia samples contained higher mercury, lead and cadmium than those of farm tilapia samples. A positive correlation between heavy metals concentration and the fish size was observed, as the large sized tilapia samples had higher heavy metal residues than those of the small sized tilapia samples. Heavy metal levels in some fish samples in our investigations exceeded the maximum permissible limits of Egyptian standards.

1. INTRODUCTION

Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins. During the last few decades, great attention has been paid to the possible dangers of many environmental pollutants due to the consumption of contaminated fish (Marzouk et al., 2016). Industrial and agricultural discharges such as coal and oil combustion, phosphate fertilizers, plastics and pesticides are considered the major sources of heavy metal pollutants of water. Fish absorbed heavy metals from water through the gills, skin and digestive tract. The heavy metals of the widest spread concern to human health are lead, mercury and cadmium (Kris-Etherton et al., 2003; Chen et al., 2007; Din et al., 2008). It is thus evident that heavy metal toxicity is a subject that requires the attention of scientists and policy makers to increase public understanding of the severity of conditions caused by toxic elements and help at minimizing heavy metal related illnesses and deaths (Achparaki, et al., 2012). This has encouraged researchers worldwide to study heavy metals pollution in air, water, and foods to avoid their harmful effects and to determine their permissibility for human consumption. Heavy metals from natural and anthropogenic sources continuously enter the aquatic ecosystem where they pose a serious threat because of their toxicity, long time persistence, bioaccumulation, and biomagnifications in the food chain (Karadede-Akin and Ünlü, 2007). Toxicity with heavy metals is due to disrupt the function of essential biological molecules such as protein, enzymes and DNA as metals lead to displacement of an essential metal cofactor of the enzyme and interaction of the metallic ions with DNA which proven to be carcinogenic to humans and animals (Hodgson, 2011). In the body, heavy metal resist chemical and biological transformation and accumulated in the tissues including, liver, kidney and nerve to cause toxicity. Uptake of heavy metals through food chain in aquatic organisms may cause various pathological disorders like hypertension, sporadic fever, renal damage or cramps in human (Gabriel et al., 2006). The growth, reproduction rate, mortality and physiological functions of fish has affected by toxic effect of heavy metals. Biomagnifications, sediments, contaminated water and contamination in food web is

also cause of deposition of heavy metal in fishes (Javed, 2005). But still, there are some traces minerals which is very beneficial and nutritive to health (Rehman et al., 2013). Harmful metal is characterized as that metal which shows extreme toxicological side effects at low levels. Heavy metals are categorized as: essential and non-essential heavy metals. Essential metals are (Cobalt, Chromium, Copper, Iron, Nickel, Zinc), and nonessential are (Arsenic, Cadmium, Mercury, Lead) (Umer, et al., 2017). Although heavy metals are considered as the oldest known toxins harmful to humans, heavy metal toxicity remains a very general subject due to the variety of symptoms caused by heavy metal poisoning. Lead (Pb), Mercury (Hg) and Cadmium (Cd) are some of the most commonly found metals associated with several adverse effects to humans due to their accumulation in the human body caused through any dietary products especially fish (Achparaki, et al., 2012). Lead, due to its vast use for industrial purposes is a very dangerous toxic element that can induce oxidative stress to human tissues and DNA. Mercury is easily absorbed by the human body and is extremely harmful for all groups of people, especially women of reproductive age and children as it affects the fetus and the normal development of young individuals. Cadmium is easily accumulated by plants and animals and reaches humans through the food chain affecting many organs and causing serious illnesses. It is thus evident that heavy metal toxicity is a subject that requires the attention of scientists and policy makers to increase public understanding of the severity of conditions caused by toxic elements and help at minimizing heavy metal related illnesses and deaths (Achparaki, et al., 2012). Heavy metals may accumulate in fish either through direct consumption of water or by uptake through epithelia like the gills, skin, and digestive tract (Burger et al., 2002). Eventually, dietary intake of these metals poses risk to human health as fish occupied a significant part of human diet (Turkmen et al., 2005). For these reasons, heavy metals load in fish has become an important worldwide concern, not only because of the threat to fish but also due to the health risks associated with fish consumption (Begum et al., 2013). So, this study assessed and compared heavy metals contamination (Hg, Pb and Cd residues) in edible muscle tissues of farmed and wild caught fish, then compare such residues with the safe permissible limits stipulated by the Egyptian Organization for Standardization and quality "EOSQ".

2. MATERIALS AND METHODS

2.1. Collection of samples:

Eighty fillet samples of Nile tilapia (farmed and wild, 40 samples of each, 20 large sized fish (205:275gm) and 20 small sized fish (90:125gm) for each group) were collected from Menoufia Governorate for determination of Hg, Pb and Cd levels based on wet weight (mg/Kg). The fish samples were then transported to the lab in an ice cooler kept at about 4°C.

2.2. Washing procedures (AOAC, 2006):

Instruments were thoroughly cleaned with deionized water and soaked in hot diluted HNO_3 (10%) for 24 hours and rinsed several times with deionized water then dried to be free from heavy metals. The digestion instruments were soaked in water and soap for 2 hours and then rinsed several times with tap water. They were rinsed once with distilled water, once with the mixture (250 ml deionized water, 200 ml conc. HCl and 80 ml H_2O_2) and once with 10% HNO_3. Finally, they were thoroughly washed with deionized water and air-dried in incubator away from any contamination.

2.3. Digestion technique:

Concerning mercury, 0.5 g of each sample was macerated by sharp scalpel and digested in 10 ml of digestion mixture (60 ml of 65% Nitric acid and 40 ml of 70% perchloric acid) at 45^o C for 15 hours (Voegborlo and Akagi, 2007). Regarding lead and cadmium, 2 g of each macerated sample was digested by 10ml of such digestion solution in screw capped tubes. (Deng et al., 2007). The tubes were tightly closed, and the contents were vigorously shaken and allowed to stand overnight at room temperature. Moreover, the tubes were heated for 4 hours in water bath starting from 60^{o}C till reach 110^{o}C ensure complete digestion of the samples. The digestion tubes were vigorously shaken at 30 minutes intervals during the heating period. The tubes were left to cool at room temperature and diluted with 1ml deionized water (30%) as well as reheated in water bath at 70^{o}C to ensure complete digestion of the samples. At this point, all organic matrixes have been destroyed. Each tube was diluted with deionized water till reach 25 ml and the digest was filtered with Whatman filter paper No. 42. The filtrates were collected in Pyrex glass test tubes capped with polyethylene film and kept at room temperature until analyzed for their mercury, lead and cadmium concentrations (Hassan et al., 2015).

2.4. Preparation of blank and standard solutions:

Blank and standard solutions were prepared in the same manner as applied for wet digestion and by using the same chemicals by Shibamoto and Bjeldanes (2000) and according to the operator manual of the Atomic Absorption Spectrophotometer. Blank solution consists of 10 parts of nitric acid and 1 part of H_2O_2 and was diluted with 25 parts of deionized water and was filtered. The blank was used to determine the metal contamination which may be present in the chemicals and its value was discounted from the end calculated results. Furthermore, the standard solutions using pure certified metal standards at different strengths were prepared by 10 parts of nitric acid and 1 part of H_2O_2 and diluted with 25 parts of deionized water (Hassan et al., 2015).

2.5. Analysis:

The digest, blanks and standard solutions were aspirated by Flame Atomic Absorption Spectrophotometer (AAS) (Germs and Stennenberg, 1978) (VARIAN, Australia, model AA240 FS) and analyzed for mercury, lead and cadmium concentration. The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the conditions recommended by the instrument instruction. Mercury absorbency was recorded directly from the digital scale of AAS and its level was calculated according to the equation: $C_1 = (A_1/A_2) \times C \times (D/W)$ mg/kg, Where**,** C_1=concentration of mercury (mg/kg) wet weight. A_1=absorbency reading of sample solution. A_2=absorbency reading of standard solution. C=concentration of mercury on the standard solution. D=dilution factor of sample. W=weight of each sample. Lead and cadmium was estimated according to the equation: C=R x (D/W). Where, C=concentration of lead (mg/kg) wet weight. R=reading of digital scale of AAS. D= dilution of prepared sample. W=Weight of the sample. The concentration of each heavy metal in the blank solution was also calculated and subtracted from each analyzed sample (AOAC, 1990, Hassan et al., 2015).

2.6. Statistical Analysis: The obtained results were statistically evaluated by application of student t-test according to Feldman et al., (2003).

3. RESULTS AND DISCUSION

Heavy metals are naturally occurring elements that become contaminants when human activities increase their concentrations above normal levels in the environment (Unger, 2002). Heavy metals enter the environment through various natural methods and human activities and can accumulate in fish and other organisms (Kalay and Canli, 2000), causing a serious and widespread environmental problem due to their toxicity. The untreated industrial and sewage wastes arising from industries and metropolitan activities polluted River Nile. The prevalence of renal failure and liver cirrhosis in human, was markedly increased in the last few years, which could be linked with heavy metal pollution in Egypt (Salem et al., 2000; Kamel and El-Minshawy, 2010). According to WHO data published in April 2011, the fourth and third cause of death in Egypt were due to kidney and liver diseases which reached 5.19% and 7.34% of the total death, respectively (Hosnia et al., 2015). Analysis of heavy metal levels in examined muscle tissue (fillets) provide information on potential risk to the fish themselves and the consumers of these fish. Pollution by heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem (Malik, 2010).

4.1. Mercury:

The results recorded in Table (1) showed that the mean value of total mercury concentration in the examined muscle samples of wild Nile tilapia was 0.73±0.09 mg/kg in small size fish, while the mean value of total mercury concentration in large size fish samples was 1.18±0.12 mg/kg. The obtained results were comparatively lower than that obtained by Hussien et al., (2011), who reported that mean concentration was 1.87 mg/kg, and relatively higher than obtained by Madiha (2009), who reported that mean concentration was 0.49±0.05 and 0.81±0.05mg/kg for small and large size fish samples, respectively. The mean value of total mercury concentration in the examined muscle samples of farmed Nile tilapia was 0.45± 0.06 mg/kg in small size fish, while the mean value of total mercury concentration in large size fish samples was 0.94±0.10 mg/kg. The obtained results were agreed with that recorded by Hassan and Salem (2003) who noted that mean concentration was 0.45±0.03 mg/gm. The obtained results are relatively higher than that reported by Marzouk et al., (2016) who noted that mean concentration was 0.105±0.005ppm, and Eboh et al., (2006) and Hashim et al., (2008) who cited that mean

concentration was 0.013± 0.001mg/kg. High results were recorded by Moustafa et al., (2011) who cited that mean concentration of mercury in tilapia were1.9 mg/kg. EOSQ (2010) specified a maximum permissible limit of mercury in fish meat to be 0.50 mg/kg. According to this permissible limit 20% and 45% of examined small and large wild tilapia samples and 10% and 25% of examined small and large farmed tilapia respectively were unaccepted samples and unfit for human consumption (table 1). So, the concentration pattern of mercury residues in tilapia muscle were large wild > large farmed > small wild > small farmed tilapia, this pattern due to the ability of fish to bioaccumulate mercury in their muscle tissues. Airborne mercury is transported over variable distances, then deposited in rivers converted to a more bioavailable form (methyl mercury), then bioaccumulated in fish. This linkage, from air to water to fish and other biota must controlled to produce safe fish (Kathleen et al., 2012). The high percentage of mercury in wild tilapia than in farmed tilapia may be attributed to industrial effluents discharged into Nile river directly without treatment, these effluents contain large amounts of mercury, lead, cadmium (Madiha, 2009). The disposal of dead animals and birds in Nile River and its branches lead to animal's decay which release and increase load of heavy metals into the aquatic environment (Levensen and Barnard, 1988). The most famous mercury compounds in the environment are monomethyl and dimethyl salt of mercury which are soluble salts that produced from inorganic mercury in sediment by anaerobic bacteria and get into natural water (Manahan, 1989). The average (88.9%) of total mercury in fish meat was in the form of methyl mercury (Bishop and Neary, 1974) which is lipid soluble and easily absorbed and distributed through biological system.

Table (1): Statistical analytical and acceptability of Mercury levels (mg/kg) in the examined samples of wild and farmed *Oreochromis niloticus* (n=20).

Size / Fish meat	Small Mean ± S.E*	Large + Mean ± S.E*	° EOSQ Unacceptability % Small	Large
Wild *O.niloticus* +	0.73 ± 0.09	1.18 ± 0.12	20	45
Farmed *O.niloticus*	0.45 ± 0.06	0.94 ± 0.10	10	25

S.E* = standard error of mean
+ = Significant differences (P<0.05) as indicated by student t-test.
° Maximum Permissible Limit of Mercury according to "EOSQ" (2010) is 0.50 mg/Kg meat.

Table (2): Statistical analytical and acceptability of Lead levels (mg/kg) in the examined samples of wild and farmed *Oreochromis niloticus* (n=20).

Size / Fish meat	Small Mean ± S.E*	Large + Mean ± S.E*	° EOSQ Unacceptability % Small	Large
Wild *O.niloticus* +	0.34 ± 0.05	0.54 ± 0.07	25	45
Farmed *O.niloticus*	0.25 ± 0.04	0.29 ± 0.03	10	30

S.E* = standard error of mean
+ = Significant differences (P<0.05) as indicated by student t-test.
°Maximum Permissible Limit of lead according to "EOSQ" (2010) is 0.10 mg/Kg meat.

Table (3): Statistical analytical and acceptability of Cadmium levels (mg/kg) in the examined samples of wild and farmed *Oreochromis niloticus* (n=20).

Size / Fish meat	Small Mean ± S.E*	Large + Mean ± S.E*	° EOSQ Unacceptability % Small	Large
Wild *O.niloticus* +	0.10 ± 0.01	0.15 ± 0.02	10	20
Farmed *O.niloticus*	0.05 ± 0.01	0.08 ± 0.03	5	15

S.E* = standard error of mean
+ = Significant differences (P<0.05) as indicated by student t-test.
°Maximum Permissible Limit of cadmium according to "EOSQ" (2010) is 0.05 mg/Kg meat.

4.2. Lead:

The mean value of lead concentration in the examined muscle samples of wild Nile tilapia was 0.34±0.05 mg/kg in small size fish, while it was 0.54± 0.07 mg/kg in large size fish (Table 2). The obtained results proved to be lower than that obtained by Hussien et al., (2011) who reported that mean concentration of lead was 1.43 mg/kg, and Malhat (2011) who cited that mean concentration of lead was 1.864 mg/kg. higher than that obtained by Madiha (2009) who reported that mean concentration was 0.11±0.01 and 0.16±0.03 mg/kg in small and large sized fish samples and Abdel-Mohsien and Mahmoud (2015) who noted that mean concentration of lead was 0.25±0.03 mg/kg. The mean value of lead concentration in the examined muscle samples of farmed Nile tilapia was 0.25±0.04 mg/kg in small size fish, while the mean value was 0.29±0.03 mg/kg in large size fish examined samples (Table 2). This result is lower than that obtained by Ali and Abdel-Satar (2005) who reported that mean concentration was 6.5 mg/kg in Fayoum farms and Kaoud and El-Dahshan (2010) who reported that mean concentration of lead was 1.52mg/kg. Similar result noted by Marzouk et al., (2016) who reported that mean concentration of lead was 0.25±0.014 ppm and Lamada (2003) who reported that mean concentration (ppm) was 0.219±0.011, while lower results were obtained by Seddek et al., (1996), and Hashim et al., (2008) who reported that mean concentration was 0.058± 0.002mg/kg. EOSQ (2010) stipulated a maximum permissible limit of lead in fish muscles as 0.1 mg/kg., according to that 25% and 45% of examined small and large wild tilapia and 10% and 30% of examined small and large farmed tilapia respectively were unaccepted samples and unfit for human consumption where they exceed this permissible limit (table 2). So, the concentration pattern of lead residues in tilapia muscle were large wild > small wild > large farmed > small farmed tilapia. High levels of lead in examined samples could be attributed to the presence of industrial and agricultural discharges, direct sewage bonds discharge, motor boat traffics and using of leaded gasoline, phosphate fertilizer and some herbicides in agricultural activities. In general, fish can concentrate the heavy metals in their tissues in rate higher than those in water. So, fish can bioaccumulate mercury and cadmium dissolved in water, but they cannot bioaccumulate lead (Rehulka, 2002) due to the low binding ability of lead with sulphdryl group in fish muscles as well as low solubility of lead salts (Moore and Ramamoorthy, 1984). Lead is non-essential element and higher concentrations can occur in aquatic organisms close to anthropogenic sources. It is toxic even at low concentrations and has no known function in biochemical processes (Burden et al., 1998). Lead exposure has been associated with reduced bone growth in fetuses and children and resulting in reduced head circumference and stature**.** Lead interferes also with bone formation, maturation and resorption and may also be a potential risk factor for osteoporosis. Lead may exert both indirect and direct action on bone turnover. Signs and symptoms of acute lead poisoning in adults may include abdominal pain, anorexia, nausea, severe vomiting, intestinal cramps, epigastric, colic, constipation**,** headache, joint and muscle pain convulsions and hemolytic anemia (CDC, 2005). Lead toxicity in human includes abnormal size and hemoglobin content of erythrocytes, hyperstimulation of erythropoiesis inhibition of both haeme synthesis and some enzyme activity in anemia and permanent damage of the brain, liver and central nervous system (Qiao-Qiao et al., 2007).

4.3. Cadmium:

The mean value of cadmium concentration in the examined muscle samples of wild Nile tilapia was 0.10±0.01 mg/kg in small size fish, while it was 0.15±0.02 mg/kg in large size fish (Table, 3). This results were lower than that obtained by Moustafa et al., (2011) who reported that mean concentration was 1.9 mg/kg and Hussien et al., (2011) and Lasheen et al., (2012) who reported that mean concentration was 1.16±0.24 and 1.2±0.3 mg/kg respectively, and nearly similar result with that of Madiha (2009) reported that mean concentration of cadmium in small size Nile tilapia was 0.08±0.01 and 0.12±0.02 mg/kg for large size and Reham (2011) who reported that mean concentration of cadmium was 0.14±0.02 mg/kg. The mean value of cadmium concentration in farmed Nile was 0.05±0.01 mg/kg in small size fish, while the mean value was 0.08±0.03 mg/kg in large size examined fish samples (Table, 3). This result is lower than that obtained by Ali and Abdel-Satar (2005) who reported that mean concentration was 2.2 mg/kg in Fayoum farms, and like that obtained by Laz and Abou El-Magd (2006) and Marzouk et al., (2016) who cited that cadmium concentration ranged from 0.0466 to 0,0686 ppm**.** While it was relatively higher than that obtained by Eletta et al., (2003) and Hashim et al., (2008) who reported that mean concentration was 0.036 ± 0.004 ppm. EOSQ (2010) determined a maximum permissible limit of cadmium as 0.05 mg/kg.

According to the permissible limit, 10% and 20% of examined small and large wild tilapia samples respectively and 5% and 10% of examined small and large farmed tilapia samples respectively were unaccepted samples and unfit for human consumption where they exceed this permissible limit. So, the concentration pattern of cadmium residues in tilapia muscle were large wild > small wild > large farmed > small farmed tilapia. The toxic effect of cadmium is exacerbated by the fact that it has an extremely long biological half-life and is therefore retained for long periods of time in organisms after bioaccumulation (Webb, 1975), Also it may exert toxic effects to man including kidney damage and severe pain in bones which called itai-itai in Japan (Tsuehiya, 1978). Although these residues occurred at very low concentrations in fish, they may accumulate to higher levels in human beings who consume it.

The effluents which are directly thrown down to these water by many factories found along the course of Nile river canals in Menoufia governorate are considered the main source of these metals, so it was advised that these effluents should be drained away of the water streams to avoid its bioaccumulation in fish tissues and aquatic organisms. Agriculture constitutes one of the very important sources of metals pollutants, as it comes from impurities in fertilizers, pesticides and sewage sludge. Nile river pass through agricultural and industrial fields, since most activities in Egypt are around it, thus it is subjected to contamination with different pollutants. Drainage water is pumped into several major drains that finally discharged their waters into the river Nile or lakes. The intensive feeding of tilapia under farm condition and adding of anabolic agents lead to rapid growth of fish to marketed size (large size) on short time in comparative with wild (large size) tilapia that spend long time to reach the same size which give times to heavy metals to bioaccumulated on wild fish than farmed fishes, moreover, wild tilapia feed on water biota, algee and phytoplanketons that accumulated with heavy metals in water lead to biomagnification of heavy metals in wild *O.niloticus.* Feeding habits of *O.niloticus* is shallow feeding from surface water that lead to gaseous and heavy metal problem in fish that lead to high residues in wild *O.niloticus* than farmed *O.niloticus.* So, the public health hazard from consumption of large size farmed tilapia may be considered less than large size farmed tilapia.

5. Conclusion

It can be concluded that tilapia samples collected from Menoufia Nile canals and markets were partially have different heavy metal residue as mercury, lead and cadmium in high values exceeding the safe Egyptian permissible limits for human consumption. The examined wild tilapia samples contained higher mercury, lead and cadmium than those of farm tilapia. The result proved that the large size wild and farmed tilapia samples have high residues of mercury, lead and cadmium than those of small size tilapia samples. To control the heavy metal residues in fish and to ensure the safety of consumers, it is imperative that a monitoring system should be put in place to address the concerns.

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