Evaluation of some heavy metals’ residues in tilapia
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ABSTRACT
In the present study, heavy metal (mercury, lead, and cadmium) residues were appraised in various body weights of Tilapia fish gathered randomly from various regions at Cairo and Giza markets utilizing Atomic Absorption Spectrophotometer (AAS). The average estimations of mercury, lead, and cadmium residues in tilapia at weight up to 200 gm were 0.07±0.01, 0.1±0.02 and 0.0005±0.0001 ppm, respectively. The average values of mercury, lead, and cadmium residues in tilapia at weight 200-400 gm were 0.3±0.05, 0.2±0.02 and 0.03±0.01 ppm, respectively. The mean values of mercury, lead, and cadmium deposits in tilapia at weight 400-600 gm were 0.6±0.06, 0.3±0.03 and 0.05±0.01 ppm, respectively. HeNNDavy metal residues were positively correlated with fish size. Lead was the most elevated metal in tilapia up to 200gm. While mercury was the highest metal in tilapia 200-600 gm. The outcomes were assessed by Egyptian Organization for Standardization EOS (NO. 7136/2010) and the public health hazards of heavy metal residues was debated.

1. INTRODUCTION
Tilapia is the major fish species consumed in Egypt, particularly due to its high nutritive value, palatability and relatively low price compared with other kinds of fishes or red meat (Morshdy et al., 2018). The environmental pollutants in aquatic ecosystems was evaluated in Tilapia as a good model fish (Badr et al., 2014). Heavy metal is easily embedded in body tissues and affected human health, so it classified as "the unknown killers" (Abdullahi, 2013). Heavy metal has a direct toxic effect on organisms and indirect effects from the consumption of food contaminated with metal (Gbogbo et al., 2018). Domestic sewage and industrial sources such as galvanizing, electrical conductors, batteries, cement, pigments and mining activities are the natural and anthropogenic sources of elevated heavy metal in fish (Mohamed et al., 2016). The age, size, gender, fish species, feeding habits of the fish, time and area of fishing, level of heavy metal in water and duration of exposure are several factors affecting bioaccumulation of heavy metals in fish (Zhang and Wong 2007). Fish expose to heavy metal through the gills, skin and ingestion of contaminated food via the alimentary tract (Sfikianakis et al., 2015). The concentration of heavy metal residue in fish was higher than in water, sediment and food (Osman et al., 2007). Consumption of fish contaminated with toxic elemental contaminants are results in serious deterioration effects on human health (Alimor and Obiji 2010), when exceeding the recommended safety concentrations (Basiony, 2014). A sheat treatment cannot destroyed them (Onimma and Aboud 2010). Heavy metals have a significant harmful impact on public health, it may result in mutation in the genetic materials, influence the biochemical processes by impairment the synthesis and metabolism of carbohydrates, protein and lipids (Azmat et al., 2008). Three metals are of primary concern to human health mercury, lead and cadmium because of their known toxicity to human being and their high concentrations in wastes disposed in aquatic environment in which the fish live (Hassan and Salem, 2003). Neurological disorder, kidney damage, skin damage, circulatory system problems, and increased risk of cancer are pathological conditions affecting human health from toxicity of heavy metals as Hg, Pb, and Cd (WHO, 2010). Exposure to high levels of organic, inorganic and metallic mercury can damage the kidney, brain and developing fetus (Alina et al., 2012). There are two forms of lead toxicity; Acute form: headache, loss of appetite, abdominal pain, fatigue, hallucinations, vertigo, renal dysfunction, hypertension and arthritis, and chronic form: birth defects, mental retardation, autism, psychosis, allergies, paralysis, weight loss, dyslexia, hyperactivity, muscular weakness, kidney damage, brain damage, coma and may even death (Martin and Griswold, 2009). Exposure to cadmium causes bone and lung damage (Bernard, 2008), also development of prostate and breast cancer (Oyek/e et al., 2017). According to data published in 2011 from WHO, 5.19% and 7.34% of the total death in Egypt were due to kidney and liver diseases (Abdel-Mohssen et al., 2015). Therefore, this study aimed to evaluate heavy metals (Hg, Pb and Cd) residues in tilapia fish at different weights then comparing such residues with the safe permissible limits stipulated by the Egyptian Organization for Standardization (EOS, 2010).

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2. MATERIAL AND METHODS
2.1. Collection of samples
One hundred random samples of tilapia fish were divided into 3 groups according to their weights (Group A: up to 200 gm, n = 30), (Group B: 200-400 gm, n=40) and Group C: 400-600gm, n=30 and collected from different localities at Cairo and Giza markets for estimation of heavy metals residues Hg, Pb and Cd levels in muscle tissues.

2.2. Washing procedures (AOAC, 2006)
Instruments were thoroughly cleaned with deionized water and soaked in hot diluted HNO₃ (10%) for 24 hours and rinsed several times with deionized water then dried to be free from heavy metals. The processing instruments were splashed in water and cleanser for 2 hours and afterward washed a few times with tap water then washed for once time of each distilled water, with the mixture (250 ml deionized water, 200 ml conc. HCl and 80 ml H₂O₂) and with 10% HNO₃, eventually, washed with deionized water and air-dried in incubator away from any contamination.

2.3. Digestion technique (Staniskiene et al., 2006):-
To determined Pb and Cd residues, one gram of each sample was macerated by sharp scalpel then add 10ml of digestion mixture (60ml of 65% HNO₃ and 40ml of 70% HCl) in screw capped tube. Whereas, mercury residues, 0.5 g of macerated sample was digested in 10 ml of concentrated H₂SO₄/ HNO₃ solution (1:1). The tightly closed tubes were vigorously shaken and allowed to stand overnight at room temperature. To ensure complete digestion of the samples, the tubes were heated for 4 hours in water bath starting from 60 °C till reach 110°C. During the heating period, the digestion tubes were vigorously shaken at 30 minutes intervals, then left to cool at room temperature and diluted with 1ml deionized water (30%) as well as reheat in water bath at 70°C to ensure complete digestion of the samples. At this point, all organic matrixes have been destroyed at this point. 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 estimation of their heavy metals content.

2.4. Analysis:
The digest, blanks and standard solutions were aspirated by Flame Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS) and analyzed for mercury, lead and cadmium concentrations. The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the following conditions recommended by the instrument instruction.

2.5. Quantitative determination of heavy metal residues:
Lead and cadmium concentrations was measured according to the following equation: 
\[ C = R \times (D/W) \]
Where, R=reading of element concentration, ppm from digital scale of AAS; D= Dilution of prepared sample and W= Weight of the sample. Whereas, absorbance of mercury was directly recorded from the digital scale of AAS and the concentration was calculated according to the following equation:

\[ C_{\text{max}} = (A_{\text{std}}/A_{\text{smp}}) \times C \times (D/W) \] mg/kg, Where C=concentration of mercury (mg/kg) wet weight. A=Absorbency reading of sample solution. Astd=Absorbency reading of standard solution C=Concentration of mercury on the standard solution D=Dilution factor of sample W=weight of each sample. The heavy metal concentration in blank solution was calculated and subtracted from each analyzed sample.

3. RESULTS
According to table (1) statistical analytical results and acceptability of mercury residues(mg/kg) in Tilapia fish with different body weights :total mean values of mercury residues in tilapia were 0.32 ±0.03ppm with minimum and maximum values of ND and 1.26 ppm. The mean values of mercury residue in tilapia at weight up to 200 gm were 0.07 ± 0.01mg/kg with minimum and maximum values of ND and 0.19 ppm. While the mean values at weight 200-400gm was 0.30 ± 0.05 mg/kg with minimum and maximum values of ND and 0.79 ppm. Whereas the mean values at weight 400-600 gm was 0.60 ± 0.06mg/kg with minimum and maximum values of ND and 1.26 ppm. There was a high significant difference (P<0.05) between different weights tilapia. According to mercury permissible limit stipulated by EOS (ES No. 7136/2010) in fish meat to be 0.5 mg/kg, 30% and 60% of examined samples in group B and C respectively were unacceptable samples and unfit for human consumption.

According to table (2) Statistical analytical results and acceptability of lead residues(mg/kg) in Tilapia fish with different body weights:the total mean values of lead residue in tilapia were 0.21 ±0.02ppm with minimum and maximum values of ND and 0.63 ppm. The mean values of lead residue in tilapia at weight up to 200 gm were 0.10 ± 0.02mg/kg with minimum and maximum values of ND and 0.22 ppm. While the mean values at weight 200-400gm was 0.20 ± 0.02 mg/kg with minimum and maximum values of ND and 0.37 ppm. Whereas the mean values at weight 400-600 gm was 0.34 ± 0.03mg/kg with minimum and maximum values of ND and 0.63 ppm. There was a high significant difference (P<0.05) between different weights tilapia. According to lead permissible limit stipulated by EOS (ES No. 7136/2010) in fish meat to be 0.3 mg/kg, 22.5% and 40% of examined samples in group B and C, respectively were unacceptable samples and unfit for human consumption.

According to table (3) statistical analytical results and acceptability of cadmium residues(mg/kg) in Tilapia fish with different body weights :total mean values of cadmium residues in tilapia with mean value 0.03 ±0.01ppm with minimum and maximum values of ND and 0.22 ppm. The mean values of cadmium residue in tilapia at weight up to 200 gm were 0.005 ± 0.001mg/kg with minimum and maximum values of ND and 0.02 ppm. While the mean values at weight 200-400gm was 0.03 ± 0.01 mg/kg with minimum and maximum values of ND and 0.13 ppm. Whereas the mean values at weight 400-600gm were 0.05 ± 0.01mg/kg with minimum and maximum values of ND and 0.22 ppm. There was a high significant difference (P<0.05) between different weights tilapia. According to cadmium permissible limit stipulated by EOS (ES No. 7136/2010) in fish meat to be 0.05 mg/kg, 12.5% and 20% of examined samples in group B and C, respectively were unacceptable samples and unfit for human consumption.
Mercury, lead and cadmium are the most common heavy metals affecting human health (Jarup, 2003). Cancer and nervous system damage are a serious effect on adults and children health due to ingestion of fish contaminated with these heavy metals (Vieira et al., 2011). The small fish had more total mean value was 0.21±0.02 ppm. Lower results were obtained by Bayomy et al. (2016)(0.24±0.03 ppm). Higher results were obtained by Oyeleke et al. (2018)(6.69±3.89 ppm). While, El-Batroawy et al. (2018) (0.46 ppm). Shokr et al. (2019) (0.49±0.01 ppm). Hamada et al. (2018) cited the mean value of total lead concentration in muscle of small and large size farmed Nile tilapia was 0.34±0.05 and 0.54± 0.07 ppm, respectively. Whereas the mean value of total lead concentration in the muscle of small and large size farmed Nile tilapia was 0.25±0.04 and 0.29±0.03 mg/kg, respectively. Lower results were obtained by Bayomy et al. (2015) (0.02 ppm). Whereas, Shaltout et al. (2015) (0.08 ± 0.02 ppm). Toxicity of lead incorporates with abnormal size and hemoglobin content of erythrocytes, hyperstimulation of erythropoiesis inhibition of both some enzyme activity in anemia and haeme synthesis, permanent damage of the liver, central nervous system and brain are the lead toxicity in human (Qiao et al., 2007). Chronic exposure to lead in children related to growth retardation, behavior disorders and death (Rossi, 2008).

Cadmium in the current study, total mean value was 0.03±0.01 ppm. Similar results were recorded by Hashim et al. (2016) (0.00±0.01 ppm). Marzouk et al. (2016) (0.105±0.005ppm), Abdel-Baki et al.(2011) (0.003±0.002 ppm) and Hashim et al. (2008) (0.013± 0.001 ppm). Cadmium has a great affinity for thiol or sulphydryl groups of proteins and compete with essential metals zinc and copper binding sites, so it is considered a cumulative poison and a highly toxic metal (Obiri et al., 2010). Exposure to high level of mercury affected central nervous system includes tremors, headaches, dysarthria, incoordination, hallucinations and death also it affected cardiovascular system lead to hypertension(Azevedo et al., 2012).Women of reproductive age and children are highly susceptible people for mercury toxicity (Hamada et a.l.,2018). Lead in the current study, total mean value was 0.21±0.02 ppm. Similar results were recorded by Ali et al.(2016)(0.24±0.03 ppm). Higher results were obtained by Oyeleke et al. (2018) (6.69±3.89 ppm). While, El-Batroawy et al. (2018) (0.46 ppm). Shokr et al. (2019) (0.49±0.01 ppm). Hamada et al. (2018) cited the mean value of total lead concentration in muscle tissue of O. niloticus of small and large size wild Nile tilapia 0.34±0.05 and 0.54± 0.07 ppm, respectively. Whereas the mean value of total lead concentration in the muscle of small and large size farmed Nile tilapia was 0.25±0.04 and 0.29±0.03 mg/kg, respectively. Lower results were obtained by Bayomy et al. (2015) (0.02 ppm). Whereas, Shaltout et al. (2015) (0.08 ± 0.02 ppm). Toxicity of lead incorporates with abnormal size and hemoglobin content of erythrocytes, hyperstimulation of erythropoiesis inhibition of both some enzyme activity in anemia and haeme synthesis, permanent damage of the liver, central nervous system and brain are the lead toxicity in human (Qiao-Qiao et al., 2007). Chronic exposure to lead in children related to growth retardation, behavior disorders and death (Rossi, 2008).

4. DISCUSSION

Toxicity and bioaccumulation of heavy metals in biota may have adverse effects on humans(Malik and Maurya, 2014). Mercury, lead and cadmium are the most common heavy metals affecting human health (Jarup, 2003).Cancer and nervous system damage are a serious effect on adults and children health due to ingestion of fish contaminated with these heavy metals (Vieira et al., 2011). According to Table (1) there were high significant differences between different weights of tilapia fish for heavy metals residues (mercury, cadmium and lead). Also, there was a positive relationship between weight of fish and heavy metals residues. The small fish may have lower contaminant than the large one, as the large fish had more time to accumulate contaminants in their bodies, especially mercury which has the ability of bioaccumulation and biomagnifications through the food chain (Ali et al., 2016).Moreover, the metals have great affinity to combine with fat content in muscle tissues (Elghobashy et al., 2001). Mercury: in the current study, total mean value was 0.32±0.03 ppm. Higher results were obtained by Hashim et al. (2019) (0.89±0.01 ppm), Hamada et al. (2018) (0.73±0.09 and 1.18±0.12 ppm) and Shaltout et al. (2015) (0.45± 0.06 and 0.94±0.10 ppm). Whereas, Lower results were recorded by Ali et al.(2016) (0.04±0.01 ppm), Marzouk et al. (2016) (0.105±0.005ppm), Abdel-Baki et al.(2011) (0.003±0.002 ppm) and Hashim et al. (2008) (0.013± 0.001 ppm). Mercury has a great affinity for thiol or sulphydryl groups of proteins and compete with essential metals zinc and copper binding sites, so it is considered a cumulative poison and a highly toxic metal (Obiri et al., 2010). Exposure to high level of mercury affected central nervous system includes tremors, headaches, dysarthria, incoordination, hallucinations and death also it affected cardiovascular system lead to hypertension (Azevedo et al., 2012). Women of reproductive age and children are highly susceptible people for mercury toxicity (Hamada et al., 2018).
al. (2008) (0.036 ± 0.004 ppm). Whereas, higher results were recorded by Hamada et al. (2018) determined mean value of cadmium concentrations in the muscle of small and large size wild Nile tilapia was 0.10±0.01 and 0.15±0.02 ppm, respectively. Oyeleke et al. (2018) (1.30±0.71 ppm). Ali et al. (2016) (0.05±0.01 ppm). Lower findings were reported by Bayomy et al. (2015) (0.0002 to 0.0003 ppm). Whereas, Badr et al. (2014) measured the levels cadmium in muscle tissues of Nile tilapia from two locations in Egypt reference area (El-Zamalek, Area 1) and polluted area (El-Tebeen, Area 2) were 0.009±0.002 and 0.024±0.005 ppm. Cadmium toxicity causes bone damage through direct and indirect effect on bone tissue through renal dysfunction. It is also a nephrotoxic causing kidney tubular damage (Wang et al., 2008). Biodiversity and human health are affected with bioaccumulation of heavy metals in the aquatic environment (Annabi et al., 2013). The large tilapia accumulates more cadmium which related to feeding habit of tilapia (Wei et al., 2014). Biomagnification of toxic metals in fish affect food chain so regular overlooking and monitoring must applied (Mohanty et al., 2013).

5. CONCLUSION

From the present study, it could be concluded that there wasa positive relationship between different weights of tilapia fish and heavy metals residues in its tissue. The large weight tilapia samples contained higher mercury, lead and cadmium than those of small weight tilapia. So, people should choose tilapia samples contained higher mercury, lead and cadmium and heavy metals residues in its tissue. The large weight was

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