

**ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN *MUGIL
CEPHALUS* AND *CLARIAS LAZERA* FISHES IN KAFR
EL SHEIKH, EGYPT**

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ABSTRACT

Fish are considered as an important source of protein, essential minerals, vitamins and unsaturated fatty acids. However, nutritional and economic importance of fish it may be a main source of heavy metals which can counteract their beneficial effects and may cause health hazards for human if consumed for long time. Therefore, eighty fish samples *Clarias lazera* and *Mugil cephalus* were collected randomly from different sources (markets, captures and farms) in Kafr-El-sheikh governorate, Egypt. Fish samples were collected from and were analyzed for heavy metals residues (Total Mercury, Lead, Cadmium and Zinc) in their flesh using Atomic Absorption Spectrophotometer (AAS). Fish samples were collected from market revealed that, the percent of samples exceeded the safe permissible limits for Hg, Pb and Cd that established by **EOSQC (2010)** were 35, 20 & 20% and 65, 55 & 60% for *Mugil cephalus* and *Clarias lazera*. Even though, fish samples were collected from capture showed that, the percent of samples exceeded the safe permissible limits that recommended by **EOSQC (2010)** were 30, 20 & 20% and 50, 40 & 60% for Hg, Pb and Cd residues of *Mugil cephalus* and *Clarias lazera* respectively. The results also showed that, the percent of farmed fish samples exceeded the safe permissible limit were 30, 0, 10% & 70, 40 & 60% for Hg, Pb and Cd residues of *Mugil cephalus* and *Clarias lazera*. The results also clarified that Zn concentration levels of both *Mugil cephalus* and *Clarias lazera* fish were higher than those of other examined heavy metal residues. Moreover, there were non- significant differences ($p > 0.05$) in heavy metal concentration levels in flesh of *Mugil cephalus* and *Clarias lazera* which were collected from captures and farms.

Key words :

Heavy metals, *Clarias lazera*, *Mugil cephalus*, Spectrophotometer.

INTRODUCTION

In the recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. Furthermore, fish are considered as important source of protein, essential minerals, vitamins and unsaturated fatty acids (**Medeiros et al., 2012**). Therefore, American Heart Association recommended eating fish at least twice per week to reach the daily intake of omega-3 fatty acids (**Kris-Etherton et al., 2002**). In Egypt, fish are considered one of the main sources of the national income, stimulating local market economics and important source of foreign exchange (**Sadek, 2000**). However, nutritional and economic importance of fish, it may be a main source of heavy metals which can counteract their beneficial effects and may cause health hazards for human if consumed for long time (**Petre et al., 2012**).

Heavy metals are persistent type of pollutants and cannot be broken down or destroyed over long time of heat treatment, their persistence enhances their potential to reach and affect human beings (**Levensen and Barnard, 1988**). Heavy metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes (**Tarvainen et al., 1997; Stephen et al., 2000**). Fish have ability to accumulate heavy metals in their tissues by absorption along the gill surface and gut tract wall to higher levels several hundred times more than the concentration of metals in their surrounding water medium. (**Nammalwar, 1983**). It has been reported that fresh water fish are more sensitive to heavy metals than marine species especially for lead, cadmium and mercury which are considered as the most toxic metals (**Sorensen, 1991**). *Clarias lazera* was reported as more dangerous fresh water fish contained high level of heavy metals in their tissue where they live on the bottom of the Nile River searching on their foods in the mud which contains high concentrations of heavy metals (**Levensen and Barnard, 1988**). Not all heavy metals are toxic for human, where some of them such as zinc and copper, are biologically essential and natural constituents of aquatic ecosystems, and generally only become toxic at very high concentrations (**Munoz-Olivas and Camara, 2001**). Zinc has a multitude of biological functions in the human body. It is an important constituent of over 100 enzymes involved in a variety of fundamental metabolic processes. It is involved in the production and function of several hormones. However, excessive intake of zinc causes abdominal pain, violent vomiting, collapse, and degenerative

changes in the liver (Celik and Oehlenschläger, 2007). Copper is probably a functional constituent of all cells. Copper toxicity can result from excessive intake, which results in gastrointestinal disturbance, headache, cirrhosis, necrosis, and liver failure. Cadmium and lead are considered the most toxic element to human life. Cadmium toxicity causes a bone disease similar to rickets "itaiitai", cardiac enlargement, anemia, gonadal atrophy, kidney failure, and pulmonary emphysema. Even though, lead poisoning causes anemia, encephalopathy, weight and coordination loss, abdominal pain, vomiting, constipation, and insomnia (Khallaf *et al.*, 1998). Therefore, many international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al.*, 2010).

In the last few decades, the concentrations of heavy metals in fish have been extensively studied in different parts of the world (Elnabris *et al.*, 2013). Most of these studies concentrated mainly on the heavy metals in the edible part (Fish muscles) however, few studies reported the distribution of metals in different organs. Factors that can influence metal uptake such as sex, age, size, reproductive cycle, swimming patterns, feeding behavior and living environment (i.e., geographical location) also have been discussed (Mustafa and Guluzar, 2003). To the best of our knowledge, the studies on the comparison of heavy metals concentration in specific fish species in one governorate collected from different sources are limited. Therefore, the main objectives of the current study were determination of some heavy metal concentrations (lead, mercury, zinc and cadmium) in flesh of *Mugil cephalus* and *Clarias lazera* fish which were collected from different sources "market, farm and capture" in Kafr-El-sheikh governorate, and compare these limits with the permissible limits established by EOSQC (2010).

MATERIAL AND METHODS

Fish samples:

Eighty fish samples were collected randomly from two types of fish (Mugil cephalus and Clarias lazera) in Kafr-El-sheikh governorate, Egypt. Forty samples from each fish were collected from different sources (20 from markets, 10 from captures and 10 from farms). The fish sample weight ranged from 100 to 600 for Mugil cephalus and from 160 to 2000 gram for Clarias lazera. The fish samples were washed with deionized water and wrapped separately in acid washed polyethylene bags and stored frozen at -20°C until analysis.

Spectrophotometric method for estimation the heavy metals levels in fish:

The collected fish samples were examined for determination of mercury, lead, cadmium and zinc levels in their flesh "axial muscle" on the basis of wet weight (ppm).

Preparation and analysis of fish samples:

Washing procedures.

Washing of all equipment used in analysis is an important process to avoid contamination with the analyzed element. Glasswares and vessels 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 ascertain that all the equipment were metal free. Even though, the digestion vessels were soaked in water and soap for 2 hours and then rinsed several times with tap water. The digestion vessels were rinsed once with distilled water, once with the mixture of 250 ml deionized water, 200 ml conc. HCl and 80 ml H₂O₂ and once with 10% HNO₃. Finally, all containers were thoroughly washed with deionized water and air-dried in incubator away from any contamination or dust (Järup, 2003).

Digestion technique:

Accurately, one gram of each fish sample was macerated by sharp scalpel and digested by 10 ml of digestion mixture (60 ml of 65% Nitric acid and 40 ml of 70% perchloric acid) in screw capped tube for determination of cadmium, lead and zinc. In regard to mercury, half gram of macerated sample was digested in 10 ml of concentrated H₂SO₄/ HNO₃ solution (1:1). The tubes were tightly closed and the contents were vigorously shaken and allowed to stand over night at room temperature. After that, the tubes were heated for 4 hours in water bath starting from 60°C till reach 110°C to ensure complete digestion of the samples. The digestion tubes were vigorously shaken at 30 minutes intervals during the heating period. The tubes

ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN

were then left to cool at room temperature and diluted with 10 ml deionized water and reheated again in water bath at 70°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 Whattman 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, cadmium and zinc concentrations (Staniskiene *et al.*, 2006).

Preparation of blank and standard solutions:

Blank and standard solutions were prepared in the same manner as applied for wet digestion of samples and by using the same chemicals. Blank solution consisted of 10 parts of nitric acid and 1 part of H₂O₂ then was diluted with 25 parts of deionized water and 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 H₂O₂ then was diluted with 25 parts of deionized (Andreji *et al.*, 2005).

Analysis:

The digest, blank and standard solutions were aspirated by ASS "Atomic Absorption Spectrophotometer" and analyzed for their concentrations of such elements "ppm".

The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the following conditions recommended by the instrument instruction.

Condition	Hg	Pb	Cd	Zn
Lamp wave length (nm)	253.7	217.0	228.8	213.9
Lamp current (mA)	4	5	4	5
Slit width (nm)	0.5	1.0	0.5	1.0
Used gas	AC/N ₂ O	AC/A	AC/A	AC/N ₂ O

AC/N₂O= Acetylene / Nitrous oxide

AC/A= Acetylene / Air

Quantitative determination of heavy metals:

Absorbance of mercury, lead, cadmium and zinc was directly recorded from the digital scale and their concentrations were calculated according to the following equation:

$$C=R \times (D/W)$$

Where,

C= Concentration of the element (wet weight).

R= Reading of digital scale of AAS.

D= Dilution of the prepared sample.

W= Weight of the sample.

Statistical analysis:

The data of heavy metal concentrations between the different fish groups (Each fish species from different sources) was statistically analyzed using one-way analysis of variance ANOVA and multiple comparisons between groups (post hoc) LSD using **SPSS version (24), 2017**. Statistical comparison between the mean of the different groups (different fish species from the same source) was made by independent-Samples T test. A probability (P value) of ≤ 0.05 was assumed for statistical significance.

RESULTS

Table (1): Incidence of heavy metal residues in flesh of *Mugil cephalus* and *Clarias lazera* fish were collected from market, capture and farm (n = 40).

Heavy metals	Permissible limits (PL)	<i>Mugil cephalus</i>					
		Market (n=20)		Capture (n=10)		Farm (n=10)	
		No. of samples exceeded PL	%	No. of samples exceeded PL	%	No. of samples exceeded PL	%
*Total Hg	0.5	7	35	3	30	3	30
*Pb	0.3	4	20	2	20	0	0
*Cd	0.05	4	20	3	30	1	10
**Zn	100	0	0	0	0	0	0
<i>Clarias lazera</i>							
		Market (n=20)		Capture (n=10)		Farm (n=10)	
*Total Hg	0.5	13	65	5	50	7	70
*Pb	0.3	11	55	4	40	4	40
*Cd	0.05	10	50	6	60	6	60
**Zn	100	0	0	0	0	0	0

*EOSQC (2010)

** WHO (1989)

ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN

Table (2): Mean values of heavy metal residues "ppm" in examined flesh of *Mugil cephalus*

<i>Mugil cephalus</i>									
Heavy metal residues	Market			Capture			Farm		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Total Hg	0.24	1.32	0.69 ± 0.114 ^a	0.09	1.44	0.58 ± 0.193 ^a	0.21	0.75	0.53 ± 0.087 ^a
Organic Hg	0.14	0.60	0.33 ± 0.059 ^a	0.03	0.70	0.24 ± 0.099 ^a	0.12	0.31	0.23 ± 0.039 ^a
Inorganic Hg	0.10	0.72	0.36 ± 0.062 ^a	0.06	0.74	0.34 ± 0.101 ^a	0.09	0.44	0.30 ± 0.053 ^a
Pb	0.03	0.52	0.24 ± 0.063 ^a	0.02	0.57	0.22 ± 0.083 ^a	0.03	0.27	0.15 ± 0.041 ^a
Cd	0.01	0.33	0.13 ± 0.042 ^a	0.01	0.20	0.10 ± 0.041 ^a	0.01	0.15	0.06 ± 0.031 ^a
Zn	1.86	3.87	2.66 ± 0.127 ^a	1.38	3.18	2.17 ± 0.167 ^b	1.67	2.88	2.33 ± 0.134 ^{ab}
<i>Clarias lazera</i>									
Heavy metal residues	Market			Capture			Farm		
	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
Total Hg	0.36	1.97	1.09 ± 0.139 ^a	0.29	1.73	0.94 ± 0.211 ^a	0.31	1.53	0.88 ± 0.140 ^a
Organic Hg	0.16	0.86	0.54 ± 0.068 ^a	0.13	0.74	0.41 ± 0.101 ^a	0.11	0.64	0.43 ± 0.069 ^a
Inorganic Hg	0.20	1.11	0.55 ± 0.076 ^a	0.16	0.99	0.53 ± 0.111 ^a	0.20	0.89	0.45 ± 0.074 ^a
Pb	0.05	1.23	0.63 ± 0.109 ^a	0.14	1.05	0.46 ± 0.137 ^a	0.11	0.92	0.33 ± 0.081 ^a
Cd	0.03	0.49	0.26 ± 0.047 ^a	0.06	0.40	0.19 ± 0.055 ^a	0.05	0.31	0.15 ± 0.035 ^a
Zn	1.6	3.18	2.38 ± 0.115 ^a	1.54	3.10	2.23 ± 0.167 ^a	1.84	3.28	2.42 ± 0.164 ^a

and *Clarias lazera* fish were collected from market, capture and farm.

Data represented as mean ± Standard error "SE"

* ^{a-b}: Means within the same row carrying different superscripts are significantly at $P < 0.05$.

Table (3): Comparison between the mean values of heavy metal residues "ppm" in examined flesh of *Mugil cephalus* and *Clarias lazera* fish.

	Market		Capture		Farm	
	<i>Mugil cephalus</i>	<i>Clarias lazera</i>	<i>Mugil cephalus</i>	<i>Clarias lazera</i>	<i>Mugil cephalus</i>	<i>Clarias lazera</i>
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Total Hg	0.69 ± 0.114 ^a	1.09 ± 0.139 ^b	0.58 ± 0.193 ^a	0.94 ± 0.211 ^a	0.58 ± 0.193 ^a	0.94 ± 0.211 ^a
Organic Hg	0.33 ± 0.059 ^a	0.55 ± 0.076 ^b	0.28 ± 0.099 ^a	0.41 ± 0.101 ^a	0.28 ± 0.099 ^a	0.41 ± 0.101 ^a
Inorganic Hg	0.36 ± 0.062 ^a	0.54 ± 0.068 ^a	0.34 ± 0.101 ^a	0.53 ± 0.111 ^a	0.34 ± 0.101 ^a	0.53 ± 0.111 ^a
Pb	0.24 ± 0.063 ^a	0.63 ± 0.109 ^b	0.22 ± 0.083 ^a	0.46 ± 0.137 ^a	0.22 ± 0.083 ^a	0.46 ± 0.137 ^a
Cd	0.13 ± 0.042 ^a	0.26 ± 0.047 ^a	0.10 ± 0.041 ^a	0.19 ± 0.055 ^a	0.10 ± 0.041 ^a	0.19 ± 0.055 ^a
Zn	2.66 ± 0.127 ^a	2.38 ± 0.115 ^a	2.17 ± 0.167 ^a	2.23 ± 0.167 ^a	2.17 ± 0.167 ^a	2.23 ± 0.167 ^a

Data represented as mean ± Standard error "SE"

* ^{ab}: Means within the same row carrying different superscripts are significantly at $P < 0.05$.

DISCUSSION

Incidence of heavy metal residues in *Mugil cephalus* and *Clarias lazera* fish.

Fish samples were collected from market revealed that, the percent of samples exceeded the safe permissible limits for Hg (0.5 ppm), Pb (0.3 ppm) and Cd (0.05 ppm) that established by **EOSQC (2010)** were 35, 20, 20% and 65, 55 and 60% for *Mugil cephalus* and *Clarias lazera* respectively (Table 1). Even though, fish samples were collected from capture showed that, the percent of samples exceeded the safe permissible limits that recommended by **EOSQC (2010)** which were 30, 20, 20% and 50, 40 and 60% for Hg, Pb and Cd residues of *Mugil cephalus* and *Clarias lazera* respectively. The results also showed that the percent of farmed fish samples exceeded the safe permissible limit were 30, 0, 10% and 70, 40 and 60% for Hg, Pb and Cd residues of *Mugil cephalus* and *Clarias lazera*. Determination of Zn concentration in different fish samples collected from different sources revealed that all fish samples were within the safe permissible limit (100 ppm) that established by **WHO (1989)**.

Level of heavy metal residues "ppm" in flesh of *Mugil cephalus* and *Clarias lazera* fish.

Mean values of heavy metal residues in *Mugil cephalus* showed that there were non-significant differences ($p>0.05$) in organic Hg, inorganic Hg, Pb and Cd concentration levels among different sources of fish collection (Table 2). Moreover, there were non-significant differences ($p>0.05$) in concentration levels of different examined heavy metals residues among marketed, captured and farmed *Clarias lazera* (Table 2). However, *Mugil cephalus* were collected from market had significantly ($p<0.05$) higher Zn concentration levels than those collected from capture and farm. The results also clarified that Zn concentration levels of both *Mugil cephalus* and *Clarias lazera* fish were higher than those of other examined heavy metals residues (Table 2). These results were in agreement with those recorded by **Muzyed (2011)** who reported that Zn concentration levels were the highest metal residue detected in all examined fish species in Gaza strip Market. Furthermore, **Chen and Chen (2001)** and **Huang (2003)** reported that, the highest Zn concentration levels were detected in common benthic fish followed by copper and cadmium. The results also fixed with those reported by **Bahnasawy et al., (2009)** who established that, the average Zn concentration levels in fish tissues from Lake Manzala, Egypt were significantly higher ($p<0.05$) than their Cu, Pb and Cd content. Higher Zn concentration in fish tissues may be referred to Zn is an element that normally found in meat and organ of fish and large food

animals where, it has a multitude of biological importance as production and function of several hormones and enzymes (Celik and Oehlenschläger, 2007). It has been observed that, the total mercury concentration levels in flesh of *Mugil cephalus* and *Clarias lazera* fish had the second order after Zn followed by lead and cadmium levels (Table 2). These results were in agreement with those of El-Safy and Ghannam, (1996) who recorded lower levels of cadmium in flesh of fish. However, Ali, (2017) mentioned that, the higher concentration levels of heavy metal residues were observed in African catfish, *Mugil cephalus* in Menofia and Kafr El-Sheikh governorate, Egypt were arsenic and cadmium, and the lower heavy metal concentration levels in examined fish samples were lead and mercury.

The results also showed that concentration levels of inorganic Hg were higher than organic form in two fish species were collected from different sources (Table 2). However, WHO (1989) established that, the content of organic Hg in fish tissues should be higher than inorganic form where, the organic or inorganic mercury enters the fish body from the contaminated water through the gills and most of it accumulates in fish tissues in the organic form. There were great variations in each examined heavy metal residue even in the same fish species (Table 2). For example total Hg concentration levels ranged from 0.24 to 1.32 with mean value of 0.96 ppm and from 0.36 to 1.97 with mean value 1.09 ppm for *Mugil cephalus* and *Clarias lazera* collected from market. These variations in this study may be explained by the examination of heavy metal residues in fish flesh were carried on different sizes of the same species. There are many factors affected the concentration levels of heavy metals in fish tissue such as size, genetic composition and age of fish Kamaruzzaman *et al.*, (2010). Incidence and levels of heavy metals residues in *Mugil cephalus* and *Clarias lazera* fish were collected from different sources indicated that occurrence of water pollutions that used for fish breeding. These pollutions may be attributed to the discharge of sewage, industrial and agricultural activities in water and the higher levels of cadmium observed in fish samples were due to their common presence in drainage of factories that polluted the water and fish. Mierio *et al.* (2012) and Dsikowitzky *et al.* (2012) established that higher doses of dietary heavy metals causes' severe dangerous effect on the internal organs of the fish and increasing the concentration of heavy metals residues in fish flesh especially in area contaminated with heavy metals.

Comparison between the mean values of heavy metal residues in flesh of *Mugil cephalus* and *Clarias lazera*.

It is well known that it is very difficult to compare the metal concentrations even between the same tissues in different species. This is because of the difference in many factors as, the aquatic environments, concerning the type and the level of water pollution; feeding habits whether omnivorous or carnivorous, and level of fish presence in water, whether pelagic or benthic fish **Canli and Atli, (2003)**. Taking all these factors into account it was very difficult to compare metal concentration levels between the examined two fish species in the present study, because of the difference in environmental medium and habits, so the interested was in metals levels in fish muscles regardless of fish type or fish environment.

Data in (Table 3) recorded that there were non- significant differences ($p > 0.05$) in heavy metal concentration levels in flesh of *Mugil cephalus* and *Clarias lazera* collected from captures and farms. However, fish samples collected from market showed significantly higher ($p < 0.05$) concentration levels for total Hg, organic Hg and Pb in flesh of *Clarias lazera* than *Mugil cephalus*. These results were in agreement with recorded by **Levensen and Barnard, (1988)** who reported that *Clarias lazera* was considered as more dangerous fresh water fish contained high level of heavy metals in their tissue where they live on the bottom of the Nile River searching on their foods in the mud which contains high concentrations of heavy metals. However, **Chen and Chen (1999) and Yilmaz (2003)** established that *Mugil cephalus* muscles contained higher heavy metal concentration levels than most of fish species because it is considered as a filter and detritus-mud feeder, which means that it can accumulate metals from both water and sediment. The results were substantiated with the results of incidence of heavy metal residues (Table 1) which presented that, the percent of marketed, captured and farmed *Clarias lazera* samples exceeded the safe permissible limits for different examined heavy metals were higher than those of *Mugil cephalus* samples. That mean consumption of *Clarias lazera* especially of large size may cause serious hazards for human beign.

CONCLUSION

Assessment of heavy metals in examined fish indicated that occurrence of water pollution where flesh of most examined fish samples had mercury, lead and cadmium levels exceeded the permissible limits established by **EOSQC(2010)** while, their zinc content were within permissible limit established by **WHO(1989)**. *Clarias lazera* collected from market showed

significantly higher ($p < 0.05$) concentration levels for total Hg, organic Hg and Pb than *Mugil cephalus*. Therefore, Consumption of *Clarias lazera* especially of large size may cause serious hazards for human being than *Mugil cephalus*.

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