Menoufia Veterinary Medical Journal (MVMJ)

Menoufia Veterinary Medical Journal
https://vmmj.journals.ekb.eg

MVMJ. Vol. 1 (1): … September 2025

Menoufia University

Heavy metals (Hg, Pb and Cd) assessment in Nile Fish *Oreochromis niloticus* and *Clarias lazera*

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Key words:
Heavy metal residues, *Oreochromis niloticus*, *Clarias lazera*, Acetic acid.

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Article History
Received: 18 Aug 2024.
Accepted: 9 sep 2024

ABSTRACT

Heavy metals are one of the most important pollutants that affect the aquatic environment and fish. The present study was carried out on 40 random samples of Nile fish represented by *Oreochromis niloticus* and *Clarias lazera* (20 of each) were collected from several fish markets in Shibin El-kom, El-Menoufia, Egypt. These samples were promptly assigned to the laboratory for analysis of their mercury (Hg), lead (Pb), and cadmium (Cd) content. The mean contents of metal, expressed in mg/kg wet weight, varied from 0.46 ± 0.05 in *Oreochromis niloticus* and 0.69 ± 0.07 in *Clarias lazera* for Hg, 0.29 ± 0.03 in *Oreochromis niloticus* and 0.44 ± 0.05 in *Clarias lazera* for Pb and 0.13 ± 0.01 in *Oreochromis niloticus* and 0.21 ± 0.01 in *Clarias lazera* for Cd. The permissible limit by E.O.S in mg/kg for Hg is 0.50 ,for Pb is 0.30 and for Cd is 0.10 .Treatment of fish fillet by different acetic acid concentrations (1%), (3%), (5%) for 5 min; resulted in noticeable reduction of heavy metals by percentage in treated group fish compared with the untreated group, mercury reduced by8.1, 17.4 and 32.6, respectively, while lead reduced by 12.3, 26.2 and 38.5, respectively, and finally cadmium reduced by 17.3, 28.9 and 46.2, respectively. The public health importance of heavy metals was discussed.

1. INTRODUCTION

Fish play a crucial role in aquatic ecosystems and serve as a vital source of animal protein production in Egypt, helping to meet the growing demand for protein (Konsowa., 2007) and in Africa it is one of the cheapest sources of animal protein (Claucas et al., 1996) . Fish protein is highly preferred in the human diet due to its low cholesterol levels. Fish and its products are rich in high-quality proteins, essential vitamins, minerals and fatty acids, making them crucial sources of nutrition. (Metin et al., 2000). The risks associated with heavy metal residues and their effects on public health are a critical concern for those in the field of food hygiene. The main source of toxic heavy metals that enter the aquatic system are anthropogenic impacts including industrial discharge, non-point source runoff, domestic sewage and atmospheric precipitation (Langston et al., 1999). However, metals can also naturally occur in small quantities and enter aquatic systems via ore-bearing rocks, wind-blown dust, forest fires, and vegetation (Fernandez and Olalla ., 2000). Metals that are absorbed readily and eliminated slowly in fish tissues tend to accumulate at higher levels (Kalay et al.,2000).

High concentrations of mercury have been identified in certain fish species (Abrefah et al., 2011).

The health risks of mercury may encompass lung damage, neurological and psychological disorders, kidney damage and congenital abnormalities (Järup.,2003).

Lead (Pb) can enter water systems from various sources, including industrial and smelter emissions, the breakdown of old lead plumbing, pesticides containing lead, precipitation, lead dust deposition, street runoff and municipal wastewater (Sepe et al., 2003). When ingested, lead can accumulate in the human body, substituting calcium in bones, which can lead to numerous health issues. These issues may include reduced intellectual abilities, kidney failure, miscarriage, high blood pressure, lung cancer and even death. (Awuah.,2016).

The kidneys are significantly affected by prolonged exposure to cadmium, along with liver toxicity, skeletal damage, and neurological effects (Misra et al., 1998). Long-term exposure to cadmium is linked to numerous diseases, such as heart disease, anemia, weakened bones, reduced immune system response, and disorders of the kidneys and liver (Codex., 2001).

Acetic acid is a weak organic acid that can lower the pH of fish tissue and form complexes with metal ions, thus reducing their solubility and bio-accessibility (Chan et al., 2021). The reduction of metal loads increases as the acid concentration rises and the immersion period lengthens (Atta et al., 2015). The decrease in heavy metals in fish treated with acetic acid may result from the formation of insoluble acetate salts of these metals (Elnimr., 2011).

So, the aim of the study is to determine the heavy metals content in Oreochromis niloticus and Clarias lazera and comparing with the stipulated permissible limits by (EOS., 2010).

Additionally, the beneficial effect of acetic acid in reducing the levels of these heavy metals in edible fish tissue was studied.

2. MATERIALS AND METHODS

2.1 Sampling:

A total of 40 random samples of Oreochromis niloticus and Clarias lazera (20 of each) were compiled from various fish markets in Shibin Elkom. The collected samples were then directly transferred to the laboratory to determine their contents of mercury, lead, and cadmium as heavy metal residues.

2.2. Detection of heavy metals:

All collected samples were analyzed to determine the levels of heavy metals (mercury, lead, and cadmium) based on wet weight (mg/Kg).

2.2.1. Washing procedures according to AOAC (AOAC., 2006).

2.2.2. Digestion technique (Staniskiene et al., 2006).

2.2.3. Preparation of blank and standard solutions (Andreji et al., 2005).

3: - Detection and analysis:

By using AAS (ATOMIC ABSORPTION SPECTROPHOTOMETER, VARIAN, Australia, model AA240 FS) which was adjusted at 217.0 nm. for lead, 228.8 nm. for cadmium and 253.7 nm. for mercury

4:- Quantitative detection of heavy metals:

Examined samples were calculated on wet weight according to the following equation:- $C=R \times (D/W)$. Where, C = Concentrations of element (mg/ kg) wet weight, R = reading of digital scale of AAS, D = Dilution of prepared sample, W= Weight of the sample.

5:- Experimental part :-

Effect of different concentrations of acetic acid (1%), (3%) and (5%) for 5 min to control heavy metal residues in fish fillet (Atta et al., 2015).

6:- Statistical Analysis:

The results obtained from the examined fish samples were statistically analyzed using the methods (ANOVA) suggested by (Feldman et al., 2003).

3. RESULTS

The results presented in table (1) showed that the mean mercury concentratio in the examined samples was 0.53 ± 0.08 mg/kg for Oreochromis niloticus and 0.70 ± 0.09 mg/kg for Clarias lazera. Additionally, 40% of Oreochromis niloticus and 65% of Clarias lazera samples exceeded the maximum permissible limit (0.5 mg/kg), according to (EOS., 2010).. Table (2) indicated that the mean lead concentration was 0.40 ± 0.06 mg/kg for Oreochromis niloticus and 0.52 ± 0.06 mg/kg for Clarias lazera. Based on the permissible limit (0.30 mg/kg) stipulated by (EOS., 2010)., 50% of Oreochromis niloticus and 70% of Clarias lazera samples were found unacceptable. Table (3) revealed that the mean cadmium concentration in the examined samples was 0.08 ± 0.02 mg/kg for Oreochromis niloticus and 0.15 ± 0.03 mg/kg for Clarias lazera. Furthermore, 30% of Oreochromis niloticus and 50% of Clarias lazera samples were deemed unfit for human consumption due to their exceeding content on the maximum permissible limit (0.1 mg/kg), according to (EOS., 2010). After immersing fish fillet in different concentrations of acetic acid for 5 minutes, table (4), revealed that mercury level reduced from 0.86 ± 0.06 in controlled samples to 0.79 ± 0.04 (8.1%) by using 1% acetic acid, while with 3% acetic acid it reduced to 0.71 ± 0.04 (17.4%), and with 5% acetic acid it reduced to 0.58 ± 0.03 (32.6%). Meanwhile, lead concentrations reduced from 0.65 ± 0.05 in controlled samples to 0.57 ± 0.04 (12.3%) by using 1% acetic acid, while with 3% acetic acid it reduced to 0.48 ± 0.04 (26.2%) , and with 5% acetic acid it reduced to 0.40 ± 0.03 (38.5%),the cadmium level reduced from 0.52 ± 0.04 in controlled samples to 0.43 ± 0.03 (17.3%) by using 1% acetic acid, while with 3% acetic acid it reduced to 0.37 ± 0.02 (28.9%), and finally with 5% acetic acid it reduced to 0.28 ± 0.02 (46.2%).

Table (1): - Statistical analytical results of mercury concentration (mg/kg) in the examined samples of Nile fish and their acceptability according to their mercury content (n=20).

Fish species	Mean ± SE*	MRL mg/kg*	Accepted samples		Unaccepted samples	
			No	%	No	%
Oreochromis niloticus	0.46 ± 0.05	0.50	13	65	7	35
Clarias lazera	0.69 ± 0.07	0.50	11	55	9	45

S.E* = standard error of mean

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization (EOS, 2010).

Table (2): - Statistical analytical results of lead concentration (mg/kg) in the examined samples of Nile fish and their acceptability according to their lead content (n=20).

Fish species	Mean ± SE*	MRL mg/kg*	Accepted Samples		Unaccepted Samples	
			No	%	No	%
Oreochromis niloticus	0.29 ± 0.03	*0.30*	15	75	5	25
Clarias lazera	0.44 ± 0.05	*0.30*	13	65	7	35

S.E* = standard error of mean

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization (EOS, 2010).

Table (3): - Statistical analytical results of cadmium concentration (mg/kg) in the examined samples of Nile fish and their acceptability according to their cadmium content (n=20).

Fish species	Mean ± SE*	MRL mg/kg*	Accepted Samples		Unaccepted Samples	
			No	%	No	%
Oreochromis niloticus	*0.13 ± 0.01*	*0.10*	*16*	*80*	*4*	*20*
Clarias lazera	0.21 ± 0.01	*0.10*	*14*	*70*	*6*	*30*

S.E* = standard error of mean

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization (EOS, 2010).

Table (4): - Effect of immersing fish fillet in different concentrations of acetic acid for 5 minutes on mercury, lead and cadmium residues (n=5).

Treatment	Mercury levels mg/Kg	Reduction %	Lead levels mg/Kg	Reduction %	Cadmium levels mg/Kg	Reduction %
Untreated control	**0.86 ± 0.06**	**-------**	**0.65 ± 0.05**	**-------**	**0.52 ± 0.04**	**-------**
1% acetic acid	**0.79 ± 0.04**	**8.1**	**0.57 ± 0.04**	**12.3**	**0.43 ± 0.03**	**17.3**
3% acetic acid	**0.71 ± 0.04**	**17.4**	**0.48 ± 0.04**	**26.2**	**0.37 ± 0.02**	**28.9**
5% acetic acid	**0.58 ± 0.03**	**32.6**	**0.40 ± 0.03**	**38.5**	**0.28 ± 0.02**	**46.2**

4. DISCUSSION

Continuous exposure to high levels of these metals presents potential health hazards to livestock and wildlife, including fish, birds, mammals and even humans. Their tendency to bioaccumulate in various fish tissues leads to toxic effects (Javed., 2012), (Hounkpatin et al., 2012). It has been established that mercury, lead and cadmium are among the most toxic metals (Fernandes et al., 2008). The results of this study indicated that the *Clarias lazera* samples had higher levels of mercury, lead, and cadmium compared to the *Oreochromis niloticus* samples. The obtined results of mercury level in table (1) were slightly agree with those recorded by (Sohsah., 2009) (0.49±0.05) for small size *Oreochromis niloticus* and in small size *Clarias lazera* were (0.72±0.04); also with (Shaltout et al., 2015), (0.46± 0.03) in *Oreochromis niloticus* ,(0.52±0.04) in *Claries lazera* ; and with (Abd El Maksod et al., 2023), (0.57±0.08) in *Oreochromis niloticus*;, While higher results were recorded by (Sohsah., 2009), for large size *Oreochromis niloticus* were (0.81±0.05) and for large size *Clarias lazera* were (0.98±0.08) ;and obtained by (Helmy et al., 2018), (1.29±0.12)in *O.niloticus* ; also recorded by (Shokr., 2019), (0.89 ± 0.01) in *O. niloticus* ;and by (AbouZeid et al., 2023), (1.13 ± 0.01) in *O. niloticus* ,(1.44 ± 0.03) in *C. lazera* ; and recorded by (Elhefnawy et al., 2023), (0.94 ± 0.01) in *O. niloticus* ,(1.28 ± 0.02) in *C. lazera*, but lower results were obtained by (El-Zahaby., 2007), (0.013 ± 0.001)in *Oreochromis niloticus* and (0.040 ± 0.001)in *Claries lazera*; and finally recorded by (Nasser et al., 2016), (0.16± 0.02) in Menoufia governorate and (0.08± 0.04) in Kafr El- Shaikh for *O. niloticus.*

Mercury compounds pose significant health risks due to their high toxicity, fat solubility, and ability to accumulate in erythrocytes and the central nervous system (CNS) (Castoldi et al., 2003). Exposure to mercury can manifest in various symptoms, including oral inflammation (redness of lips, tongue, and throat), tooth loss, and skin abnormalities such as swelling, redness, and pink-red fingertips. Neurological effects, such as irritability, are also commonly associated with mercury poisoning (Mert., 1987). While mercury occurs naturally in the environment at low levels, industrial activities have been the primary source of mercury pollution (Clarkson., 2002).

Regarding to the lead level, Table (2), lower results were obtained by (Sohsah., 2009),(0.11 ± 0.01)mg/kg for small size *Oreochromis niloticus* and (0.16 ± 0.03) mg/kg for large size one and (0.15 ± 0.02)mg/kg for small size *Clarias lazera* and(0.27 ± 0.04) mg/kg for large size one ; and by

(Shaltout et al., 2015), (0.08± 0.02) in *Oreochromis niloticus* ,(0.14± 0.02) in *Claries lazera* , also (Nasser et al., 2016), reported in Kafr El- Shaikh (0.08 0.03) in *O. niloticus,* while in Menoufia reported results were nearly similar (0.40 0.05) in *O. niloticus* ;also slightly same results were also obtained by (Abd El Maksod et al., 2023), (0.36±0.06) in *Tilabia niloticus* , while higher results were recorded by (Helmy et al., 2018), (0.53 ± 0.06) in *O. niloticus* ;and by (Shokr., 2019), (0.49 ± 0.01) in *O. niloticus* ;and finally by (AbouZeid et al., 2023), (0.78 ± 0.01) and (0.61 ± 0.01) mg /kg in examined *C. lazera* and *O. niloticus.*

Lead is a toxic substance that accumulates in the body due to its slow elimination process. Common symptoms of lead poisoning include abdominal pain, colic, anemia, and encephalopathy. Additionally, lead is regarded as an immunosuppressive agent in humans (Chisolm., 1973). On the other hand, lead induces renal tubular dysfunction, as evidenced by proteinuria or aminoaciduria in humans. Additionally, lead inhibits heme synthesis, resulting in weakened cell walls of red blood cells, which leads to anemia (Jones and Hunt., 1983).

During the 20th century, cadmium production, consumption and emissions to the environment have increased dramatically due to its industrial uses, such as in batteries, electroplating, plastic stabilizers, and pigments. This increase has consequently led to the contamination of aquatic habitats (Järup., 2003). Cadmium is a toxic element that can cause insomnia, nausea, testicular atrophy, prostate cancer and kidney failure (Kikuchi et al., 2002). The obtained results of cadmium levels in Table (3) were similar to those reported by (Sohsah., 2009), with (0.11 ± 0.01) (mg/kg) for small-sized *Clarias lazera* and lower results for larger-sized ones at 0.20 ± 0.03 (mg/kg). In *Oreochromis niloticus*, the cadmium concentration was (0.08 ± 0.01) (mg/kg) for small-sized fish, while larger-sized fish had a lower concentration of 0.12 ± 0.02(mg/kg) , and slightly the same as the result reported by (Shaltout et al., 2015), (0.14± 0.02) for *Clarias lazera* and (0.10± 0.01) for *Oreochromis niloticus* ,but the results were lower than (AbouZeid et al., 2023), (0.43± 0.01) and (0.26± 0.01) (mg/ kg) in examined *C. lazera, O. niloticus,* respectively, and (Shokr., 2019), (0.25±0.01) in *Clarias lazera* and (0.17±0.01) in *O. niloticus.*

Treatment of fish with different concentrations of acetic acid reduced the level of heavy metals content (Elnimr., 2011). The obtained results of mercury after immersion in acetic acid in table (4) slightly agree with (Elnimr., 2011), as mercury reduced by 75% after immersion in 5%acetic acid for 15 min, and with (Elhefnawy et al., 2023) as the mercury level reduced by (52.8%) after immersion in 5% acetic acid for 30min. Moreover, the results of lead level in table (4) slightly agree with (Elnimr., 2011), as lead level reduced by 76% after immersion in acetic acid 5% for 15 min, also agree with (Atta et al., 2015), as lead level reduced by (31.5%) after immersion in acetic acid 5% for 3 min. Finally, the results of cadmium in table (4) agree with (Elnimr., 2011), as cadmium level reduced by (41.7%) after immersion in acetic acid 5% for 15 min, also with (Atta et al., 2015), cadmium level reduced by (25%), and with (Elhefnawy et al., 2023), as cadmium level reduced by (43.5%).

5. CONCLUSIONS

Examined fish samples, somewhat, were contaminated with heavy metal residues exceeding the safe permissible limits for human consummation. Addition of acetic acid to fish aid in reducing the level of heavy metals content, so it is recommended to try to minimize the level of heavy metals in river Nile and the aquatic creatures to protect the human and fish.

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