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Residues of some heavy metals and their effects on the biochemical alteration and nutritional value of Tilapia Fish in El Temsah Lake

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ABSTRACT

This work aimed to assess residues of some heavy metal in Tilapia fish (*Oreochromis niloticus*) of varying weights captured from El Temsah Lake in Ismailia City, as well as evaluating their potential impacts on serum biochemical parameters, antioxidant state of liver and nutritional values of fish meat. One hundred random samples of Tilapia fish were collected in total (distributed into 3 different body weights groups) and were analyzed. The results displayed the presence of some heavy metal residues as lead, cadmium and copper in meat and liver, and their concentrations were directly correlated with fish size as residues were found to be higher in the larger Tilapia compared to the smaller ones. Regarding **Egyptian (No. 7136/2010) and European Commission (EC, 2006), and FAO/WHO (2002)**, 12% and 8% of total meat samples exceeded the permissible limits of lead and cadmium, respectively. While all samples of meat were within the permissible limit of copper concentration. In large sized fish, heavy metal residues led to significant rise in the levels of serum alanine and aspartate aminotransferases, creatinine, uric acid, indicating hepatic and renal dysfunction. Moreover, the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), in large sized Tilapia fish exhibited an increase. However, the proximate analysis of the examined tilapia meat showed that the heavy metals concentrations in meat were not high at a level that affected the nutritive values of fish meat. Totally, significant efforts and coordination among various authorities are required for treating sewage, industrial and agricultural wastes. It is also crucial to regularly check for toxic residues in fish.

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INTRODUCTION

Fish, recognized as a pivotal and nutrient-rich food choice for humans, occupy an elevated status in the food chain, particularly at the tertiary level. Beyond their role as a significant protein source, fish boast an abundance of essential minerals and vitamins **(Habib et al. 2022a)**. El Temsah Lake stands as one of Egypt s paramount lakes. It holds a pivotal role in the activities of Ismailia City and services as a vital source for the local fishing industry **(El-Azim et al. 2018)**. The lake receives diverse water sources, such as freshwater from the outlet of the Ismailia Canal and wastewater produced by various activities in the city of Ismailia **(Hassan and Kandil 2022)**.

The Nile tilapia, scientifically known as *Oreochromis niloticus*, stands out as the most favored and widely consumed fish species among the Egyptian populace. Its popularity is attributed to its rich nutritional content, enjoyable taste, and comparatively affordable price when compared to other fish varieties or red meat **(Khalifa et al. 2020)**. In recent decades, considerable attention has been directed towards the potential dangers posed by various environmental pollutants arising from the consumption of contaminated fish **(Hamada et al. 2018)**. The swift urbanization and industrialization processes have introduced numerous potentially hazardous pollutants, such as heavy metals, into the aquatic ecosystem. Three primary sources contribute to contamination by heavy metal in El Temsah Lake: industrial discharge from shoreline workshops, agricultural runoff, , and untreated sewage effluent from network of the city **(El-Azim et al. 2018)**.

Heavy metals propensity for bioaccumulation and biomagnification in marine environments renders them harmful to both fish and consumers **(Morshdy et al. 2021)**. They have the capacity to accumulate in body tissues and adversely impact human health, earning them the designation of "the unknown killers" **(Abdullahi 2013)**. Generally, food consumption serves as the main pathway for heavy metals to enter the human body **(Taweel et al. 2013)**. The transition from beneficial to toxic occurs when heavy metal intake surpasses acceptable thresholds. Prolonged or recurrent exposure to heavy metals can result in adverse effects on the human body **(Engwa et al. 2019)**. Heavy metals like cadmium (Cd) and lead (Pb) have no known biological significance in the human body and can induce harmful impacts even at minimal levels of consumption, leading to adverse health outcomes in humans **(Habib et al. 2022b)**. This suggests that the existence of certain heavy metals in aquatic ecosystems and organisms, even in small amounts, can pose ecotoxicological risks **(Burch 2022)**.

Assessing serum biomarkers can be particularly valuable in pinpointing target organs affected by toxicity, and the health condition of fish is mainly assessed through the analysis of their blood biochemical parameters **(Al-Hasawi and Hassanine 2022)**, and recommended for detecting early signs of harmful alterations in stressed organisms **(Jacobson-Kram and Keller 2001)**. Changes in fish blood biochemistry indicate unfavorable environmental conditions or the presence of stressors **(Kamal and Omar 2011)**, as well as the significant rise in the utilization of oxidative stress indicators within ecotoxicology. Various heavy metals are known for their induction abilities of oxidative stress that fosters the production of reactive oxygen species (ROS), including oxygen radicals **(Flora et al. 2008)**. Monitoring changes in antioxidant enzymes is established as a validated method for assessing oxidative stress biomarkers **(Morshdy et al. 2021)**. The antioxidant defense system can serve as crucial parameters for testing natural waters for sublethal levels of toxicants. As a result, biomarkers have become increasingly significant for monitoring metal toxicity in recent years **(Eroglu et al. 2015)**.

From another point of view, the substantial demand for fish predominantly stems from its nutritional value, which is determined by its proximate composition **(Njinkoue et al. 2016)**, which can vary based on alterations in environmental conditions. Among these conditions, water pollution which stands out as the most significant element influencing the quality of fish production in their natural environments **(Talab et al. 2016)**. Continued exposure to these water pollutants, even at minimal levels, has been documented to trigger biochemical composition in fish tissues. These alterations could significantly impact the quality and market value of fishes **(Kaoud and El-Dahshan 2010)**. Therefore, profiling the proximate composition of fish is essential for adhering to food regulations and commercial standards. Understanding this composition is particularly vital for consumers as it offers crucial insights into the nutritional content of fish and its suitability for different dietary requirements **(Ayanda et al. 2019)**.

For consumers, toxic heavy metals accumulation in fish meat beyond permissible limits poses health risks to humans. These metals have the potential to induce impairment to the nervous system, liver and kidney **(Ali and Khan 2019)**. Hence, ongoing monitoring and screening of toxic metal residues in fish are essential requirements for the food safety sector **(Morshdy et al. 2021)**.

Based on the preceding facts, the objective of the current work was to assess the existence of some toxic heavy metals (Pb, Cd and Cu) residues in both the meat and liver of Tilapia fish of varying weights, which were captured from El Temsah Lake in Ismailia city with comparing these residues levels with their safe permissible limits. Furthermore, the current investigation aimed to study the impact of heavy metal residues on specific serum biochemical parameters, such as liver and kidney function, as well as antioxidant indicators. Additionally, the study sought to evaluate the impact of these residues on the proximate composition of fish meat.

MATERIAL METHODS

Collection of Samples:

A total of 100 random samples of live Tilapia fish (*Oreochromis niloticus*) were collected weekly from different local fishermen caught fish from El Temsah Lake at Ismailia city over a trial period of three months from October to December, 2023. Collected fish were transported to the laboratory in large boxes filled with aerated lake water. Then fish were divided into 3 groups consistent with their weights; Group A: up to 150 gm/ fish (n = 40), Group B: 150-300 gm/ fish (n=30) and Group C: over 300 gm/ fish (n=30).

Determination of biochemical parameters in serum:

A blood sample was extracted from each fish s caudal vein using a sterile disposable syringe (5 ml) to minimize stress. The samples were promptly placed into blood collection tubes and left to clot at room temperature for 30 minutes. Subsequently, they were centrifuged at 3,000 rpm for 20 minutes **(Osman et al. 2018)**. The obtained sera were frozen and stored at -25°C until they were prepared for various biochemical analyses. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the serum were evaluated calorimetrically according to **Reitman and Frankel (1957)**. Serum uric acid and creatinine were determined according to **Barham and Trinder (1972)** and **Henry (1974)**. All assay kits that were utilized were bought from Bio-diagnostic Co., Giza, Egypt.

Detection of antioxidant enzymes:

Liver tissues were prepared for antioxidant level assessment by trimming, weighing, and homogenizing them with a potassium phosphate buffer solution (50 mM, pH 7.5). After centrifugation at 1500g for 10 minutes at 4°C, the supernatant was collected and kept on ice for immediate biochemical analysis. All analyses were conducted at room temperature (25-27°C). Catalase (CAT) activity was determined using the procedure described by **Aebi (1984)**. while superoxide dismutase (SOD) activity was determined following the procedure detailed by **Nebot et al. (1993)**. The activity of glutathione peroxidase (GPx) was assessed in accordance with the method described by **Chu et al. (1993)**.

Heavy metals residues assessments:

The concentrations of heavy metals (Cd, Pb and Cu) in muscles and liver were determined by using atomic absorption spectrophotometry (AAS) after dry aching according to Official

Method 999.11 **(AOAC, 2002)**, which expressed as mg/kg wet weight (ppm) to compare them with **EOS (No. 7136/2010)**, **EC (2006)** and **FAO/WHO (2002)**.

Proximate analysis of fish meat:

Meat samples of previously collected fish samples were analyzed for determining the proximate composition by using the standards methods of Analytical Methods Committee **(AOAC 2006)**.

Statistical Analysis

The data were analyzed and presented as means ± standard error (SE) utilizing One-way analysis of variance (ANOVA). Subsequently, a post-hoc comparison was conducted using Duncan's multiple comparisons test, considering a statistically significant p-value of $P < 0.05$, to identify significant differences among the means. All statistical analyses were performed using **SPSS software package, version 19.00 (SPSS Inc., Chicago, Ill)**.

RESULTS

The data in **table 1** revealed results of some serum biochemical parameters of Tilapia fish (*Oreochromis niloticus*) with different body weights caught from El Temsah Lake according to their heavy metal residues. Additionally, results of some liver antioxidant enzymes of Tilapia fish (*Oreochromis niloticus*) with different body weights caught from El Temsah Lake were presented in **table 2.** Moreover, the data in **table 3** showed the statistical analytical results of lead, cadmium, and copper residues (mg/kg) in meat and liver samples of Tilapia fish *(Oreochromis niloticus)* with different body weights. In addition, results recorded in **table 4** showed the acceptability of examined Tilapia fish meat samples according to their heavy metals residues. Finally, the data in **table 5** showed the proximate composition of meat samples of examined Tilapia Fish *(Oreochromis Niloticus)* with different body weights

Table 1. Serum Biochemical Parameters of Tilapia Fish *(Oreochromis niloticus)* with Different Body Weights, Caught from El Temsah Lake.

	Group A (n=40) (up to 150 gm)	Group B (n=30) (150-300 gm)	Group C (n=30) (over 300 gm)	Total (n=100)
AST (U/I)	25.77 ± 0.35^{b}	29.17 ± 0.37^{ab}	35.45 ± 0.37^{a}	30.01±0.46
ALT(U/I)	24.75 ± 0.31^{b}	28.04 ± 0.36^{ab}	32.01 ± 0.45^{a}	27.31±0.43
Creatinine (mg/dl)	1.34 ± 0.01^{b}	1.40 ± 0.03^{b}	1.83 ± 0.03^{a}	1.50±0.02
Uric acid(mg/dl)	2.61 ± 0.05^{c}	3.06 ± 0.08^{b}	3.87 ± 0.03^{a}	3.12±0.06

Values with the same superscript within the same row show no significant difference while those with different superscript within the same row show significant difference.

Table 2. Liver Antioxidant Activities of Tilapia Fish *(Oreochromis niloticus)* with Different Body Weights Caught from El Temsah Lake.

	Group A (n=40) (up to 150 gm)	Group B (n=30) (150-300 gm)	Group C (n=30) (over 300 gm)	Total (n=100)
SOD (U/g)	2285.325±22.96^{b}	2264.93±25.00^{b}	2716.5±27.71^{a}	2408.56±24.83
CAT (U/g)	226.92±4.09^{b}	227.84±3.23^{b}	267.04±2.02^{a}	239.24±2.69
GPx (mU/g)	117.60±3.67^{b}	120.65±4.78^{b}	312.80±4.65^{a}	177.08±9.26

Values with the same superscript within the same row show no significant difference while those with different superscript within the same row show significant difference.

Table 3. Statistical analytical results of some heavy metals residues (ppm=mg/kg) in meat and liver samples of Tilapia fish (*Oreochromis niloticus)* with different body weights Caught from El Temsah Lake.

Heavy Metal Detected	Sample Type	Tilapia Fish Group	Positive Samples		Min.	Max.	Mean ± S.E
			No	%			
Lead (ppm)	Meat	Group A(n=40)	13	32.5	0.06	0.12	0.03±0.01^{b}
		Group B (n=30)	11	36.7	0.09	0.58	0.12±0.03^{b}
		Group C (n=30)	15	50	0.10	1.12	0.32±0.08^{a}
		Total	39	39	0.06	1.12	0.14±0.03
	Liver	Group A(n=40)	18	45	0.20	0.87	0.16±0.04^{B}
		Group B (n=30)	14	46.7	0.92	1.87	0.53±0.09^{A}
		Group C (n=30)	17	56.7	1.13	2.19	0.62±0.13^{A}
		Total	49	49	0.20	2.19	0.40±0.05
Cadmium (ppm)	Meat	Group A(n=40)	12	30	0.001	0.004	0.001±0.00^{c}
		Group B(n=30)	10	33.3	0.009	0.12	0.018±0.01^{b}
		Group C(n=30)	16	53.3	0.009	0.18	0.037±0.01^{a}
		Total	38	38	0.001	0.18	0.017±0.00
	Liver	Group A (n=40)	16	40	0.01	0.04	0.018±0.00^{B}
		Group B(n=30)	13	43.3	0.05	0.20	0.073±0.02^{A}
		Group C(n=30)	18	60	0.04	0.30	0.103±0.02^{A}
		Total	47	47	0.01	0.30	0.058±0.01
Copper (ppm)	Meat	Group A (n=40)	40	100	0.09	0.29	0.18±0.01^{c}
		Group B(n=30)	30	100	0.24	0.51	0.38±0.01^{b}
		Group C(n=30)	30	100	0.51	0.69	0.58±0.01^{a}
		Total	100	100	0.09	0.69	0.36±0.02
	Liver	Group A (n=40)	40	100	1.24	3.98	2.95±0.12^{C}
		Group B(n=30)	30	100	2.58	5.98	4.26±0.17^{B}
		Group C(n=30)	30	100	5.68	7.77	6.72±0.09^{A}
		Total	100	100	1.24	7.77	4.47±0.17

% was calculated according to total number of samples. Means on the same column of the same sample and same metal carrying different superscripted letters are significant different ($p<0.05$).

Table 4. Acceptability of Examined Tilapia Fish (*Oreochromis Niloticus)* with Different Body Weights according to Maximum Permissible Limits (MPL) of Heavy Metals Residues in Meat Samples set by EOS, WHO/FAO and EC.

Heavy metals detected	MPL (mg/kg)	Group A (n=40)				Group B (n=30)				Group C (n=30)				Total (n=100)			
		Within MPL		Exceeded MPL		Within MPL		Exceeded MPL		Within MPL		Exceeded MPL		Within MPL		Exceeded MPL	
		No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)	No	(%)
Lead (mg/Kg)	0.3	40	100	0	0	25	83.3	5	16.7	22	73.3	8	26.7	88	88	12	12
Cadmium (mg/Kg)	0.05	40	100	0	0	27	90	3	10	25	83.3	5	16.7	92	92	8	8
Copper (mg/Kg)	30	40	100	0	0	30	100	0	0	30	100	0	0	100	100	0	0

MPL: Maximum Permissible Limits
MPL of lead stipulated by Egyptian Organization for Standardization EOS (7136/2010) and EC (2006) is 0.3 mg/Kg meat.
MPL of Cadmium stipulated by Egyptian Organization for Standardization EOS (7136/2010) and EC (2006) is 0.05 mg/Kg meat.
MPL of Copper stipulated by FAO/WHO (2002) is 30 mg/Kg meat.

Table 5. The Percentage Mean Proximate Composition of Meat Samples of Examined Tilapia Fish *Oreochromis Niloticus)* with Different Body Weights**.**

	Group A (n=40) (up to 150 gm)	Group B (n=30) (150-300 gm)	Group C (n=30) (over 300 gm)	Total (n=100)
Moisture %	78.78 ± 0.09^{a}	78.38 ± 0.12^{b}	78.18 ± 0.16^{b}	78.42 ± 0.08
Protein%	15.87 ± 0.08^{b}	16.63 ± 0.06^{a}	16.86 ± 0.12^{a}	16.40 ± 0.07
Fat%	1.80 ± 0.08^{c}	2.42 ± 0.07^{b}	2.81 ± 0.10^{a}	2.28 ± 0.07
Ash%	1.22 ± 0.03^{b}	1.32 ± 0.02^{a}	1.35 ± 0.02^{a}	1.29 ± 0.01

Values with the same superscript, within the same row, show no significant difference while those with different superscript within the same row shows significant difference.

DISCUSSION

Industrial and domestic wastes are often disposed of indiscriminately into water bodies, posing significant environmental and public health risks **(Aly et al. 2023)**. Reports indicate that these wastes contain toxic and hazardous substances, including metals. Consumption of fish meat contaminated with toxic heavy metals resulted in serious adverse effects on human health, when their level exceed the recommended safety concentrations **(Basiony 2014)**.

Heavy Metals Residues in Liver and Serum Biochemical Response

Assessing the biological tissue levels of heavy metals is relevant to the management of ecosystems and the health of human populations **(Roméo et al. 1999)**. In **Table 3** our results revealed that high levels of lead and cadmium were found in livers of large and medium sized Tilapia fish caught from Lake Temsah. Heavy metals mainly accumulate primarily in fatty tissues **(Omar et al. 2014)**, and metabolically active tissues **(Tiwari and Dwivedi 2014)**, such as liver, which serves as the primary metabolic organ, and the quantity of pollutants found in fish livers correlates directly with the level of pollution present in the aquatic environment **(Tapia et al. 2012)**. Though, this bioaccumulation relies on their intake, storage, and elimination from the body **(Authman et al. 2015)**. This suggests that fish tissues, when subjected to high uptake and low elimination rates, may accumulate heavy metals at elevated concentrations **(Idriss and Ahmad 2015)**. Numerous experimental and field investigations have demonstrated that the liver serves as the primary organ that accumulates various metals. It is highly involved in the uptake and storage of heavy metals due to its functions in storing, redistributing, detoxifying and transforming contaminants **(Zhao et al. 2012)**.

Exposure of fish to toxic substances induces detectable alterations in both serum biochemistry and the histological structure of the liver **(Salamat and Zarie 2012)**. Fish serum can serve as an indicator of various biochemical processes within their metabolism. Environmental stressors such as heavy metals have the potential to change serum biochemical parameters in fish **(Öner et al. 2008)**. The toxins disrupt the fish s physiological equilibrium, impacting enzyme function and causing abnormalities in cell organelles. Consequently, this disturbance can result in increased enzyme activity **(ara and N 2009)**. Regarding lead and cadmium residues in fish liver, in current study there were significant increase in serum AST and ALT levels in large sized Tilapia fish caught from El Temsah Lake compared to small sized fish as shown in Table **1**. There existed a direct correlation between the weight of fish and the presence of heavy metal residues. Smaller fish tended to exhibit lower levels of contaminants compared to larger ones, likely due to the extended duration large fish have to accumulate contaminants in their bodies **(Khalifa et al. 2020)**. Enzymes such as aminotransferases may elevate in the bloodstream due to leakage from cells in damaged tissues, Therefore, they serve as indicators for specific or multiple organs damage **(Boyd 1983)**. It is suggested that ALT and AST serve as commonly utilized enzymes in diagnosing damage induced by pollutants in various tissues including the liver, gills and muscles (**De la Torre et al. 2000**). The finding is in line with **Mohamed et al. (2020); Giri et al. (2021); and Kumar et al. 2021)**. Additionally, **Ibrahim et al. (2021)** reported high ALT and AST levels in fish exposed to Cd, it may be correlated with hepatocellular injury or cellular damage in the liver. Similarly, **Hassan et al. (2020)** observed that lead (Pb), copper (Cu), and cadmium (Cd) were accumulated to higher levels in the liver of Nile Tilapia fish (*Oreochromis niloticus*) across all sites of sampling.

Plasma levels of creatinine and uric acid provide a general indication of glomerular filtration rate and renal impairment **(Ismail and Mahboub 2016)**. Lower concentrations of creatinine and uric acid may not be significant, but an increase in their levels indicates the presence of numerous dysfunctions within the kidney **(Maxine and Benjamine 1985)**. The rise of renal parameters (uric acid and creatinine) could be a consequence of oxidative damage **(Prusty et al. 2011)**. The results in **Table1** regarding creatinine and uric acid levels showed a significant increase in large-sized fish compared to medium and small ones. These findings aligned with **Salem et al. (2021)**; and **Hassan et al. (2020)**. These observations could stem from damage to muscle tissue, disruptions in nitrogen metabolism, and renal dysfunction, resulting in reduced excretion of these compounds and an elevation in their blood levels **(Osman et al. 2018)**. In a study by the National Kidney foundation 2002, **Levey et al. (2002)** observed that creatinine was regarded as a more reliable indicator of kidney disease in contrast to urea. **Omar et al. (2013)** elucidated that the rise in plasma creatinine levels in the fish can be attributed to pathological alterations in the kidney induced by the accumulation of heavy metals in the renal tubules.

Liver Antioxidant Activities

Various categories of environmental pollutants or their metabolites can amplify the intracellular generation of Reactive Oxygen Species (ROS), which result in oxidative harm in fish **(Alak et al. 2013)**. Many heavy metals triggered oxidative stress by generating ROS **(Vinodhini and Narayanan 2009)**. ROS serves as a pivotal marker of oxidative stress in fish, culminating in various molecular or cellular alterations, including modifications in proteins, lipids, carbohydrates, and antioxidants **(Lushchak 2011)**. ROS causes disruption to cellular membranes, rendering them permeable and causing physiological changes in fish. Ultimately, this process leads to necrosis and apoptosis **(Ullah et al. 2019)**.

The escalated activity of antioxidant enzymes could mirror the oxidative stress induced by heavy metals. As heavy metals accumulate in the fish s body, they generate superoxide anions, prompting Superoxide Dismutase (SOD) to convert these radicals into hydrogen peroxide (H_2O_2) **(Farombi et al. 2007)**. As the SOD-CAT system is considered the primary defense against oxidative stress, elevated SOD and CAT activity is commonly noted in the presence of environmental contaminants **(Dautremepuits et al. 2004)**. The heavy metals accumulation in the liver is a well-known outcome of aquatic organisms exposure to these elements. Major antioxidant enzymes in the body, such as SOD, TAC, GPx, and CAT, can neutralize undesirable O2− and H_2O_2, as well as ROOH produced by free radicals **(Abdel-Tawwab et al. 2017)**.

According to the finding showed in **Table 2** the study unveiled a notable rise ($P < 0.05$) in SOD activity of large sized Tilapia fish compared to other groups. In contrast, the levels of SOD showed a non-significant increase between two other groups. The increase in SOD levels could potentially represent a physiological adaptation aimed at neutralizing the generation of reactive oxygen species (ROS), as suggested by **Haredi (2018)**. Persists metal pollution triggered cellular oxidation, ultimately resulting in increased SOD activity and significant alterations in the antioxidant defense system of *Cirrhina mrigala* **(Bano et al. 2017)**. In the current study, large sized Tilapia fish contained lead, cadmium and copper displayed an elevation in the SOD activity than other groups. This finding is supported by previous data observed by **Farombi et al. (2007); Mahboob et al. (2014)** that reported an increased SOD activity in different fish species. Additionally, **Vinodhini and Narayanan, (2009)** reported that the increased activity of hepatic superoxide dismutase suggests the development of chronic stress caused by heavy metals.

The CAT level displayed a significant increase ($P < 0.05$) in large sized Tilapia fish when compared to medium and small ones, whereas there was no significant rise ($P > 0.05$) in its level between the medium and small groups of Tilapia. The increase in the CAT level could be attributed to the presence of an efficient antioxidant defense mechanism aimed at counteracting the oxidative stress induced by exposure to metals. Additionally, it may serve to compensate for the decline observed in other antioxidant enzymes, such as GPx **(Hasanein et al. 2022)**. Additionally, **EL-Gazzar et al. (2014)** showed an elevation in the CAT activity in Nile Tilapia fish subject to cadmium (Cd) at both 21 and 42 days of exposure. Increased CAT activity was also noted in various studies involving different fish species following exposure to heavy metals, including Pb, Cd, Hg, Zn, and Cu **(Sampaio et al. 2008)**.

The present investigation unveiled a notable increase ($P < 0.05$) in hepatic GPx levels in large sized Tilapia fish evaluated to other groups. Nevertheless, there was no significant rise ($P > 0.05$) in its level in the two other groups of Tilapia. The observed increase in GPx levels suggests elevated hydroperoxide levels in the fish liver. Additionally, activities of GPx might be induced as a protective response against the toxicity of water pollutants, including domestic, industrial, and agricultural sewage that enters the Lake **(Hasanein et al. 2022)**. The results were in line with **Alak et al. (2013)** who demonstrated that, glutathione peroxidase (GPx) was higher in rainbow trout exposed to lead during the trial period when compared with control ($p<0.05$), and **Khalil et al. (2017)** who showed that during winter, when water pollution levels tend to be higher compared to summer, SOD, CAT and GPx activities in *O. niloticus* exhibited significant increases. Also, **Haredi (2018)** documented a notable rise in the liver GPx level of Nile Tilapia collected from Edku drain in comparison to fish from the recovery group and El-Maadyah region of Lake Edku, Egypt. Additionally, **Lin et al. (2018)** observed a significant increase in the activity of GPx, GST, and CAT in response to nitrite pollution. The liver, being a primary target organ for ingested oxidants, experiences heightened glutathione peroxidase activity. This probably reflects an adjustment to the oxidative circumstances the fish have faced **(Lenartova et al. 1997)**.

The elevation in antioxidant enzyme activities signifies the adaptive responses of fish aimed at counteracting the oxidative effects of generated ROS and resisting the toxicity of water pollutants. This helps mitigate the harm caused by excessive amounts of oxidative stress and oxygen free radicals **(Carvalho et al. 2012)**.

Vaseem and Banerjee (2016) showed elevated activities of SOD, CAT, and elevated levels of MDA were observed in fish caught from the heavily polluted river. These findings led to the conclusion that the oxidative stress experienced by the fish was induced by pollutants present in the environment. Heavy metals could form molecular complexes with cell protein thiols, leading to detrimental effects on cellular function. On the other hand , this impact is mitigated by the generation of antioxidants, which work to neutralize free radicals and shield the cells from oxidative harm **(Vinodhini and Narayanan, 2009)**.

Romeo et al. (2000) and **Abdel-Kader and Mourad (2020)** recorded a decline in CAT, and GPx activity in *O. niloticus*. The decrease in antioxidants could be linked to the potential binding of metal ions directly to –SH groups within enzyme molecules, leading to rise formation of free radicals and distortion of mitochondrial function **(Sanfeliu et al. 2001)**. **Kanak et al. (2014)** observed decline in GPx activities could be attributed to the direct influence of metal ions on the active sites of enzyme molecules.

Heavy Metals Residues in Meat

Heavy metals can be classified into two groups: essential and non-essential **(Umer et al. 2017)**. The essential, including copper (Cu), serve important functions in biological systems and are required by the human body in small amounts. However, excessive levels of them can become toxic. On the other hand, non-essential heavy metals like cadmium (Cd) and lead (Pb) have no beneficial role in living organisms and even trace amounts of them can be toxic **(Kortei et al. 2020)**.

Regarding results of lead residues in tilapia fish meat **(table 3)**, there were significant increases in the large sized Tilapia fish than the smaller sized ones. The weight of the fish can indeed have a direct relationship with the accumulation of heavy metal residues. Larger fish have a greater capacity to accumulate contaminants due to their longer lifespan, which increases chances of consumption of more contaminants. As these contaminants move up the food chain, they can undergo both bioaccumulation and biomagnification processes **(Khalifa et al. 2020)**. Similarly, several previous studies reported average concentrations of lead residues found in different sizes of Nile tilapia meat as **Hamada et al. (2018)** and **Khalifa et al. (2020)**.

In the current study, the total mean value of lead residue in all meat samples was 0.14±0.03 mg/kg. These results were nearly consistent with those of **Taha et al. (2019)** (0.17 ppm) and **Rizk et al. (2022)** (0.10 ppm). While other studies found lower concentrations as **Bayomy et al. (2015)** (0.02 ppm) and **Kortei et al. (2020)** (0.04 to 0.08 ppm). However, many others reported higher concentration as **Morshdy et al. (2021)** (0.38 to 0.72 ppm), **Hasanein et al. (2022)** (1.74 ppm), **Burch (2022)** (2.85 ppm) and **Osman et al. (2023)** (0.36 ppm).

According to **the Egyptian Organization for Standardization (EOS, No. 7136/2010)** and **European Commission (EC, 2006)**, which stated that the maximum allowed level of lead in fish meat is 0.3 mg/Kg, 12% of total examined fish meat were surpassed the limits. Similarly, **Burch (2022)** reported that all tested fish exceeded the recommendations, as well as **Morshdy et al. (2021)** reported that 100% of fish samples violated lead safety standards in Damietta and El-Husseiniya, while only 60% exceeded the limits in Abbassa. Additionally, **Hamada et al. (2018)** reported that 25% of small and 45% of large wild tilapia samples had lead levels above the safety threshold. However, **Rizk et al. (2022)** reported that all samples were within permissible limits.

Chronic lead exposure puts children at risk of slowed growth, behavioral issues, and in severe cases, death **(Rossi 2008)**. Excessive lead consumption has been associated with an elevated risk of various cancers, including stomach, intestinal, ovarian, kidney, lung, blood (myeloma and leukemia) and lymphatic (lymphoma) cancers **(Bhuloka Reddy et al. 2004)**.

According to cadmium residues in tilapia fish meat **(table 3)**, there were significant differences ($P<0.05$) between the three different weights of Tilapia fish. Larger tilapia exhibit increased bioaccumulation of cadmium, possibly attributable to their dietary preferences **(Wei et al. 2014)**. Similarly, **Hamada et al. (2018)** and **Khalifa et al. (2020)** showed significant variations in cadmium content based on tilapia size.

The current results revealed cadmium total mean value was 0.017±0.00 ppm. These findings were nearly in agreement with those recorded by **Taha et al. (2019)** (0.023 ppm). While, lower results were achieved by **Bayomy et al. (2015)** (0.0002 to 0.0003 ppm) and not even detected by **Ghannam et al. (2015)**. However, other studies found higher concentrations as **Morshdy et al. (2021)** (0.042 to 0.114 ppm), **Burch (2022)** (0.74 ppm), **Hasanein et al. (2022)** (1.00 ppm), and **Osman et al. (2023)** (0.12 ppm).

Regarding to **the Egyptian Organization for Standardization (EOS, No. 7136/2010)** and **European Commission (EC 2006)**, the maximum allowed level of cadmium in fish meat is 0.05 mg/Kg. The total percentage of the examined fish meat samples exceeded permissible limits was 8%. Similarly, **Morshdy et al. (2021)** found that all fish examined in Damietta and El-Husseiniya had cadmium levels above the permissible limit, compared to 30% in Abbassa. However, **Hamada et al. (2018)** reported that 10% of small and 20% of large wild tilapia samples were deemed unsafe for consumption due to exceeding the permissible limit.

Cadmium displays relatively low bioavailability, but once absorbed, it slowly eliminated and gradually accumulated in kidneys causing renal damage even at low levels. Additionally, it can harm the lungs, potentially leading to cancer. Its exposure exhibits both direct and indirect bone damage through renal dysfunction. Additionally, research suggests carcinogenic, neurotoxic, cardiotoxic and reproductive effects **(Kortei et al. 2020)**.

Concerning copper residues in tilapia fish meat **(table 3)**, there were significant differences ($P<0.05$) between the three different weights of Tilapia fish. The present results revealed that the copper total average values in meat was 0.36±0.02 mg/kg. These findings were almost the same outcomes as those reported by **Yaakub et al. (2020)** (0.47 to 0.50 ppm), **Burch (2022)** (0.40 to 0.60 ppm) and **Simukoko et al. (2022)** (0.25 ppm),. While, higher results were achieved by **Oyeleke et al. (2018)** (1.30 ppm) and **Rizk et al. (2022)** (1.2 to 9.17 ppm).

The maximum allowable limit of copper residues was 30 mg/Kg in fish flesh according to **FAO/WHO (2002)**. All the fish examined were within the permissible limits, and these findings were consistent with **Rizk et al. (2022)** and **Burch (2022)** who reported that Cu concentration in fish meat was within the permissible limits.

Generally, dietary copper plays a vital role in human health, influencing key biological processes like iron metabolism, collagen formation, and antioxidant defense **(Mitra et al. 2022)**. While excessive copper intake from food sources can trigger oxidative stress, potentially leading to cellular damage and various health concerns. These harmful effects of copper exposure can manifest at levels even slightly above recommended limits, and once these effects occur, they may be irreversible **(Abdel-Khalek et al. 2015)**. Its toxicity is a silent threat, silently attacking crucial organs as digestive system, heart, kidneys and brain **(Pandey and Madhuri 2014)**.

Proximate Analysis of Fish Meat

Proximate analysis of fish is essential for confirming their quality, safety and adherence to standards outlined in national and international food regulations **(El-Sherif et al. 2023)**. Biological differences, seasonal changes and environmental factors like water quality and pollution can affect fish biochemical composition **(El Shehawy et al. 2016)**. The current results in **table 5** showed that the examined fish were within the typical ranges of proximate composition for fish as outlined by **FAO (1992)** who revealed that the typical fish composition is 66-81% water, 16-21% protein, 0.2-25% fat and 1.2-1.5% ash. Therefore, it can be concluded that levels of heavy metals detected in the analyzed fish from El Temsah Lake were not high enough to influence their nutritional values.

Regarding moisture contents, the results showed that the total examined fish contained 78.42% moisture. The small sized fish revealed significant increase ($p < 0.05$) in moisture contents than the larger sized ones. Generally, the level of moisture in fishes is varied according to the age, season, environment **(Küçükgülmez et al. 2010)**. Water constitutes the primary component of the edible portion of fish **(Ayanda et al. 2019)**.

The current study nearly agreed with those of **El-Sherif et al. (2023)** who reported that the ranges of moisture were 78.24% to 79.11% and **Haredi et al. (2020)** found the ranges 77.44% to 80.42%. While **Ayanda et al. (2019)** revealed the moisture content of fish as 76.4 %. On the other side, nearly lower results were those reported by **Ayanda et al. (2018)** which was 72.22% moisture content.

Concerning protein contents, the results showed that the total examined fish contained 16.40% protein. The small sized Tilapia fish revealed a significant reduction ($p < 0.05$) in the protein content when compared to the other larger ones. Fish protein stands out for its excellent digestibility and rich amino acid composition. It supplies all essential amino acids your body needs, along with non-essential ones, and contains notably high levels of cysteine compared to many other protein sources **(Astawan 2004)**.

The current findings nearly consistent with those of **El-Sherif et al. (2023)** who found the protein as 16.50% and **Talab et al. (2016)** who demonstrated the ranges were 16.10% to 17.88%. While the results were almost lower than those reported by **Burch (2022)** who reported 18.93% protein. Additionally, **Ayanda et al. (2019)** showed that protein content was 17.6%, and **Ayanda et al. (2018)** reported 23.75% protein.

While regarding fat content, the results showed that the total examined fish contained 2.28% fat. There were significant variations ($p < 0.05$) between the three different sized fish with significant increasing in the large sized. Based on fat content classification system proposed by **Ackman (1989)** who categorized fish into four groups which are lean fish (<2 %), low fat fish (2–4 %), medium fat fish (4–8%)

and high fat fish (>8%), the studied tilapia falls into the "low fat" category, containing between 2-4% fat.

Studies have found that the fat content of fish tissues contain adequate amounts of two beneficial polyunsaturated omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have numerous health benefits **(Rasoarahona et al. 2005)**. Some Studies suggested that consuming these polyunsaturated fatty acids may be associated with a reduced risk of developing certain diseases like cancer, heart disease, rheumatoid arthritis, and inflammation **(Raatz et al. 2013)**.

The current results nearly agreed with **El-Sherif et al. (2023)** who found the range of fat contents 2.12% to 2.87% and **Burch (2022)** who reported 2.42% fat content. However, the results were almost higher than those reported by **Ayanda et al. (2019)** who illustrated fat % in Tilapia fish 1.89%, **Ayanda et al. (2018)** reported 1.42% fat content, **Talab et al. (2016)** found that the ranges of fat were 1.10% to 1.95%.

Concerning ash content, the results exhibit that the total examined fish contained 1.29% ash. The small sized Tilapia fish revealed a significant diminsh ($p < 0.05$) in the ash contents when compared to the other larger ones. Ash content, the inorganic residue remaining after burning organic matter, serves as a proxy for an organism s mineral composition. This metric can fluctuate due to several factors, including seasonal cycles, age and size of the organism, its food source, and environmental variables like water composition, salinity, temperature, and contaminant presence **(Ayanda et al. 2018)**.

The current results nearly agreed with those of **El-Sherif et al. (2023)** who showed that ash contents ranged from 1.46% to 1.25%, **Talab et al. (2016)** reported the ranges of ash from 0.55% to 1.50%. However, the results were almost lower than those reported by **Ayanda et al. (2019)** who displayed ash % as 3.84% and **Ayanda et al. (2018)** reported 2.43% ash contents.

CONCLUSION

The investigation focused on Tilapia fish from El Temsah Lake. Some of these Tilapia specimens were found to contain levels of lead and cadmium that exceeded safe permissible limits for human consumption. However, copper concentrations remained within acceptable bounds. Interestingly, the weight of Tilapia was positively correlated with heavy metal residues in their tissues, indicating that larger fish had higher levels than smaller ones. Heavy metals bioaccumulation of in the liver was significantly greater than in the muscles. This phenomenon was associated with increased liver and kidney enzymes in the serum, as well as elevated antioxidant enzymes in the liver. Despite This evidence, the detected levels of heavy metals in the examined Tilapia fish were not sufficiently high to impact their meat nutritive value.

Ethical approval

The study received approval no. ARC-AHRI-80-23, from institutional animal care and use committee (ARC-IACUC), Agricultural Research Center, Giza, Egypt.

Recommendations

Collaborate with research institutions, health authorities, and environmental agencies to conduct further studies on fish quality in Temsah Lake. Investigate the effect of heavy metals on long-term health and explore sustainable fishing practices

Regular assessments of liver health in Tilapia fish populations are crucial. Monitoring elevated liver enzymes and bioaccumulation patterns can provide valuable insights into potential health risks. By understanding these dynamics, management strategies can be tailored to maintain a healthy fish population.

Strengthen regulations and enforcement mechanisms to ensure that Tilapia fish sold in markets and restaurants meets safety standards. Regular inspections and testing can help identify and address any violations promptly.

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