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Evaluate the Health Condition of Some Economic Fish Species After Purification and Development Operations in Burullus Wetland

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ABSTRACT

Burullus Wetland has recently witnessed major cleansing and development operations to rehabilitate the lake from severe pollution. Therefore, the current study aimed to evaluate the health and physiological status of some economic fish species caught from the wetland. The present investigation determined the content of Cd, Pb, As, and Cr in the muscle tissues of eight fish species caught from the studied area, *Oreochromis niloticus, Sarotherodon galilaeus, Oreochromis aureus, Coptodon zillii, Chelon auratus, Liza ramada, Dicentrarchus punctatus,* and *Poecilia reticulata* by using ICP-OES inductively coupled plasma atomic emission spectroscopy. The results showed that the accumulations of Cd, Pb, As and Cr in muscle tissue were 0.005-0.3, 0.012-1.4, 0.12-1.25, and 0.8-2.15µg/ g wet weight tissues, respectively. The fish metal pollution index (MPI) decreased in the order, *O. aureus*> *C. auratus* > *O. niloticus* > *P. reticulata* > *S. galilaeus* > *L. ramada* > *D. punctatus*> *C. zillii*. The activities of Na^+/K^+ and Ca^{2+}ATPases enzymes in muscle, and gill showed significant differences ($P<0.05$) between the examined species and non-significant differences in the liver ($P>0.05$) among examined species. The minimum activity appeared in *O. aureus* in muscle and gill tissues. Moreover, antioxidant defense systems (SOD, CAT, and total thiol) showed significant differences ($P<0.05$) in the muscle tissue of the examined species. Conclusively, this study highlighted the state of the health of some fish species after purification and development operations in Burullus Wetland.

INTRODUCTION

The second-largest ecosystem in the northern wetlands of the Egyptian Mediterranean coast is the Burullus Wetland. The wetland is located in the area between the two branches of the Nile, Rosetta, and Damietta Branches, and is connected to the Mediterranean Sea by an inlet known as El-Boughaz (Boughaz El-Burullus). Recently, the wetland is considered the most productive Egyptian lake yielding around 103.8 thousand tons in 2021 **(GAFRD, 2021)**. The wetland is characterized by a wide diversity of fish species ranging from salt, and brackish to freshwater species (Fig. 1). The wetland was a home to more than 35 species of which family Cichlidae is the most abundant followed by the family Mugilidae. Moreover, moronids, soles, seabream, European eel, catfishes, shrimp, anchovy, and small sardines have inhabited the lake. Among the most profitable species in fish production are four species from the family Cichlidae *Coptodon zillii* (Gervais, 1848), *Oreochromis niloticus* (Linnaeus, 1758), *Sarotherodon galilaeus* (Linnaeus, 1758), and *Oreochromis aureus* (Steindachner, 1864). Following these are two moronid species *Dicentrarchus punctatus* (Bloch, 1792) and

Dicentrarchus labrax (Linnaeus, 1758), as well as two species from family mugilidae *Chelon auratus* (Risso, 1810) and *Liza ramada* (Risso, 1827). The wetland receives a combination of drainage water from fish farms, sewage, agriculture, and industry through eight drains. Additionally, the Brenbal Canal on the western side of the wetland supplies fresh Nile water **(El-Adawy *et al.*, 2013; Alzeny *et al.*, 2024)**.

Even though the phrase heavy metal is not well defined, it is frequently used to refer to metals that have a density higher than that of water. Heavy metals like cadmium (Cd), chromium (Cr), arsenic (As), zinc (Zn), mercury (Hg), copper (Cu), nickel (Ni), and lead (Pb) are defined as elements that have atomic masses higher than 20 and densities more than $5.0 g.cm^{-3}$. From a biological perspective, heavy metals even at extremely low concentrations may pose a threat to plants and animals. Due to the severity of their detrimental effects, the United States Environmental Protection Agency (USEPA) designated Cd, As, Hg, Pb, and Cu as primary concern pollutants that require close monitoring **(Kumar *et al.*, 2024)** for their danger to human health and exhibiting an extreme toxicity even at deficient metal exposure levels **(Järup, 2003)**. Toxic effects occur when excretory, metabolic, storage, and detoxification mechanisms can no longer counter uptake **(Obasohan *et al.*, 2008)**, eventually leading to physiological and histopathological changes **(Georgieva *et al.*, 2014)**.

Since fish is a major food source for the human population, the levels of non-essential trace elements in fish are significant and it has been reported that fish from freshwater bodies receiving industrial effluents are unsafe for human consumption due to high tissue levels of some heavy metals **(Maitera *et al.*, 2012; Tyokumbur & Okorie, 2014)**. Determining the amounts of trace element contamination through chemical biomonitoring and biomarker evaluation which serves as an early indicator of biological effects is essential for safeguarding aquatic biota **(Annabi *et al.*, 2013)**, and consequently human health.

Fish toxicity brought on by heavy metals is complex. But every metal has distinct properties that result in a different toxicological mechanism of action. Fish biochemical and physiochemical characteristics, along with the bioaccumulation of these metals, can serve as useful markers for tracking and evaluating the effects of this particular kind of pollution. Therefore, this may facilitate the timely and efficient management of water pollution, protect aquatic ecosystems from damage, avoid biomagnification, preserve the integrity of the food chain, and reduce the likelihood of extinction **(Kumar *et al.*, 2024)**.

Since 2010, lake Burullus (Burullus wetland) has been subjected to major cleansing and developmental processes. The current study aimed to regard the impact of the recent development of the lake on the health conditions of the commercial fish species caught from salt (around the Boughaz), brackish, extending to fresh water at a depth reaching 900 meters inside the lake.

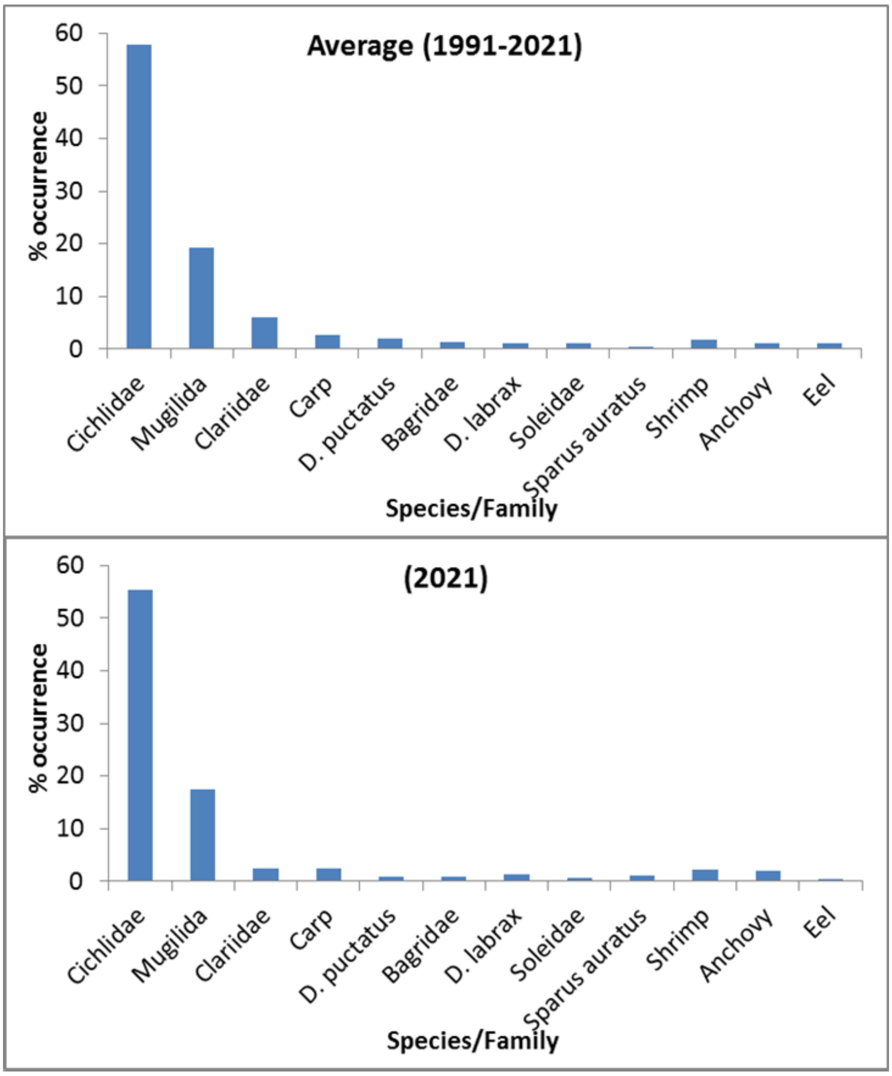


Fig. 1. Commercial fish species in Burullus Wetland

MATERIALS AND METHODS

1. Location of study and sampling

Samples were collected during the winter of 2024 from Burullus Wetland; the collection of the sample started from El-Boughaz region and extended up to 900m depths in the wetland to obtain the brackish species and the freshwater samples in the lake (Fig. 2; yellow circles).

Eight fish species caught from the studied area, *Oreochromis niloticus*, *Sarotherodon galilaeus*, *Oreochromis aureus*, *Coptodon zillii*, *Chelon auratus*, *Liza ramada*, *Dicentrarchus punctatus*, and *Poecilia reticulata*, were transported in ice-cold boxes for the laboratory analysis.

2. Fishery data

The fishery data concerning the annual catch composition and amount from Burullus Wetland was obtained from both field trips and GAFRD statistical yearly book from 1991 to 2021.

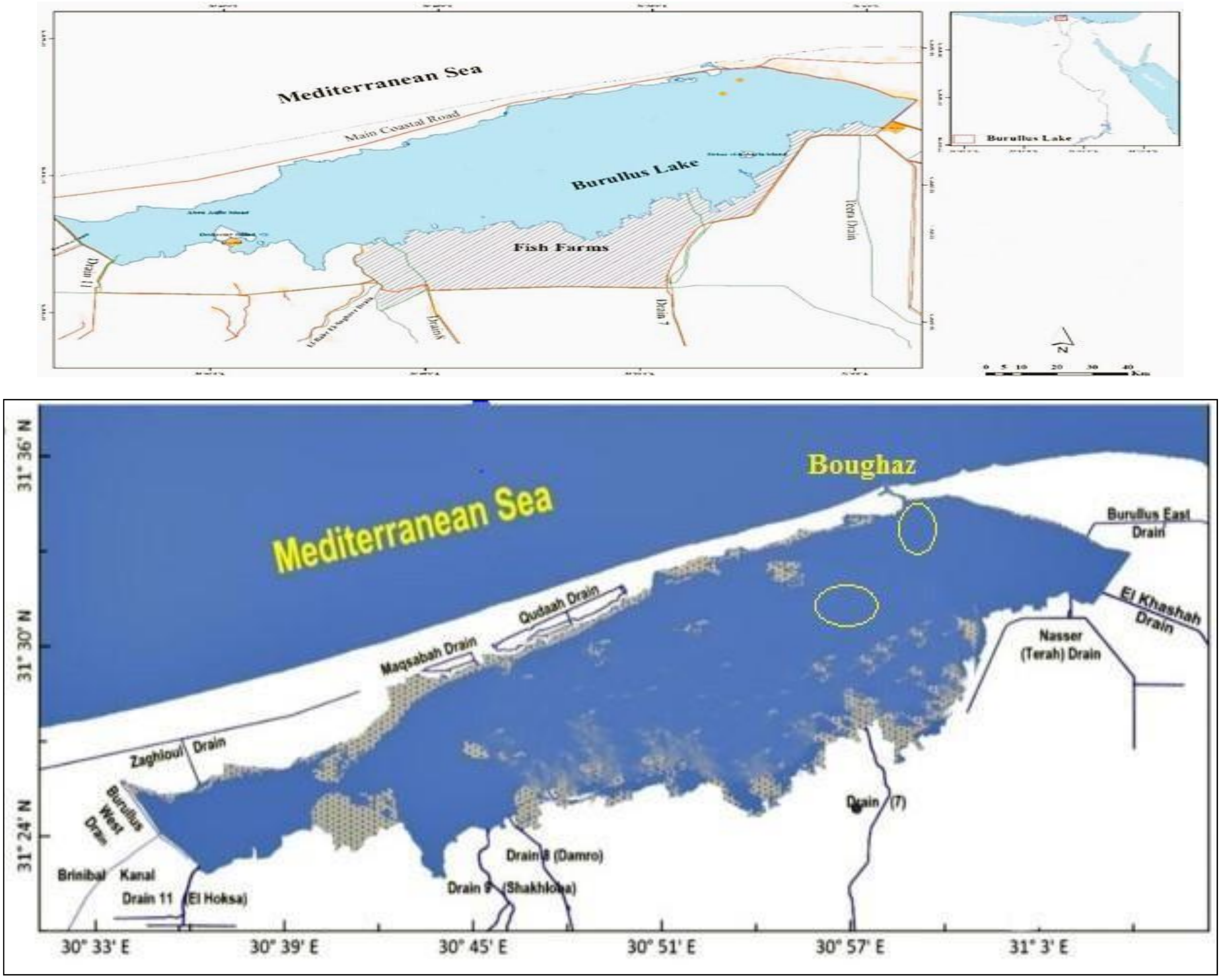


Fig. 2. The map of Burullus Wetland showing the sites of samples collection (yellow circles)

3. Samples identification, sorting, and preparation

Samples were identified and sorted into species according to ideal routine work. The frozen samples were defrosted and washed in deionized water before the skin was swiftly and gently peeled off. Isolation of the liver, gills, and dorsal muscles was done swiftly and completely; the tissues were washed and weighed in an isotonic saline solution. The tissue homogenate (10% w/v) was made in a ratio of 1–9 times ice-cold phosphate buffer (0.1M), pH 7.4 for an antioxidant test.

4. Estimate the metal pollution index (MPI) and the content of muscles heavy metals

The content of heavy metals was calculated as 0.5g of the muscle tissue samples that dissolved in 5ml conc. HNO_3 solution at 85˚C till complete muscle digestion and then filtered and diluted the filtrate up to 25ml of distilled water **(Mohamed *et al.*, 2017)**. Metals evaluated by ICP-OES's inductively coupled plasma atomic emission spectroscopy, and the equation of **(Usero *et al.*, 1997)** was applied as follows:

$$MPI= (M_1XM_2XM_3X………XMn)\ 1/n$$

Where, Mn is the metal content (µg/g wet tissues), and n is the number of examined metals.

5. Evaluation of the activity of Na^+/K^+ and Ca^{2+} ATPases

The method of **Üner *et al.* (2005)** was used to evaluate the activity of Na^+/K^+ and Ca^{2+} ATPases in the tissues of the liver, gill, and muscle by quantifying the amount of inorganic phosphate (Pi) at 25ºC, and the incubation media was prepared following the method of **Ames and Dubin (1756)**.

6. Calculation of the content of muscle total thiol and antioxidant activity

The level of muscle content of total thiol (T-SH) was assessed based on the method of **Sedlak and Lindsay (1968)**, as a non-enzymatically antioxidant. Evaluation of enzymatic antioxidant activity was conducted adhering to the guidelines of **Paoletti and Mocali (1990)** for superoxide dismutase (SOD) activity. However, catalase (CAT) activity was evaluated using the method of **Aebi (1984)**.

7. Statistical analysis

One-way ANOVA was performed for data analysis, and the GraphPad Prism program, Version 5.0, was used for multiple Tukey test comparisons. *P*-values were considered significant at a value< 0.05. Each reading reflects the mean values ± SD.

RESULTS

1. The bioaccumulation of heavy metal

Table (1) shows the bioaccumulation of heavy metal concentrations (µg/g wet weight) in the muscle tissue of fish species with the MPI and the standard maximum permissible limits (MPLs). The present results showed significant differences (P<0.05) in the accumulation of Cd, Pb, As, and Cr among the examined species.

The accumulation of Cd in muscle tissue ranged from 0.005 to 0.3 µg/g wet weight tissues and decreased in the order, *O. aureus* > *C. auratus* > *O. niloticus* > *C. zillii* > *P. reticulata* > *S. galilaeus* > *D. punctatus* > *L. ramada.* The accumulation of Pb in muscle (0.012-1.4 µg/g wet weight tissues) was decreased in the order *O. aureus* > *O. niloticus* and *P. reticulata* > *C. auratus* > *S. galilaeus* > *D. punctatus* > *L. ramada* > *C. zillii.* The means of accumulation of As in muscle tissues (0.12-1.25 µg/g wet weight tissues) decreased in the order *P. reticulata* > *O. aureus* > *O. niloticus* > *C. auratus* > *L. ramada* > *S. galilaeus* > *zillii* > *D. punctatus.* The accumulation of Cr in muscles (0.8 -2.15 µg/g wet weight tissues) decreased in the order *O. aureus*> *P. reticulata* >*C. auratus* > *O. niloticus* > *S. galilaeus and D. punctatus* > *L. ramada* > *C. zillii.*

On the other hand, MPI decreased in the following order: *O. aureus*> *C. auratus* > *O. niloticus* > *P. reticulata* > *S. galilaeus* > *L. ramada* > *D. punctatus* > *C. zillii.*

2. The activity of Na^+/K^+ and Ca^{2+}ATPases

The activities of Na^+/K^+ ATPase enzyme in muscle, and gill showed significant differences (*P*<0.05) among the examined species, but non-significant differences was shown in the liver (*P*>0.05) (Fig. 3). The activity of Na^+/K^+ ATPase enzyme in the liver tissue was >

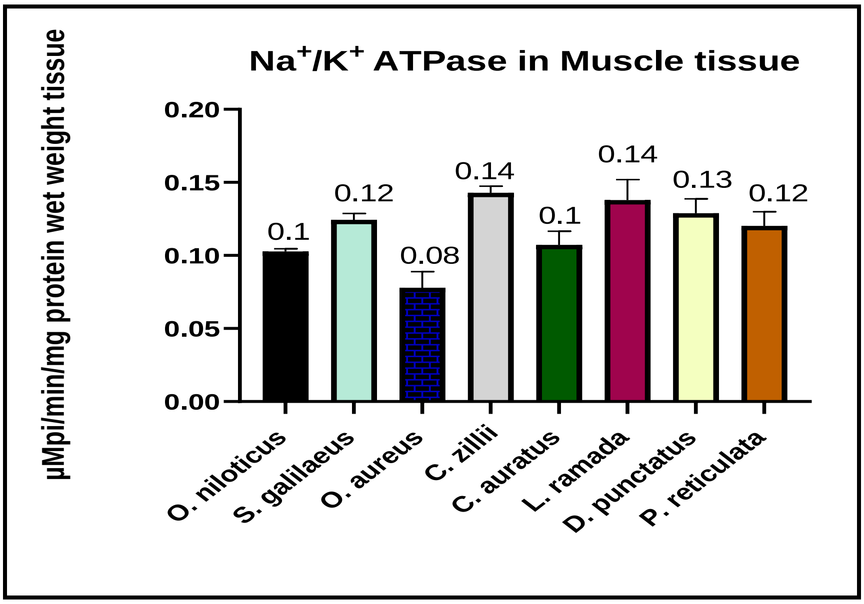
in both the muscle, and gill activity among the examined species. The minimum activity of Na^+/K^+ ATPase enzyme appeared in *O. aureus* in muscle and gill tissues. The activity of Ca^{2+}ATPase in muscle and gill showed significant differences ($P < 0.05$) among the examined species, but non-significant differences in the liver ($P > 0.05$) (Fig. 4). The minimum activity of Ca^{2+}ATPase appeared in *O. aureus* in the muscle and gill tissues of *O. aureus* and *C. auratus.*

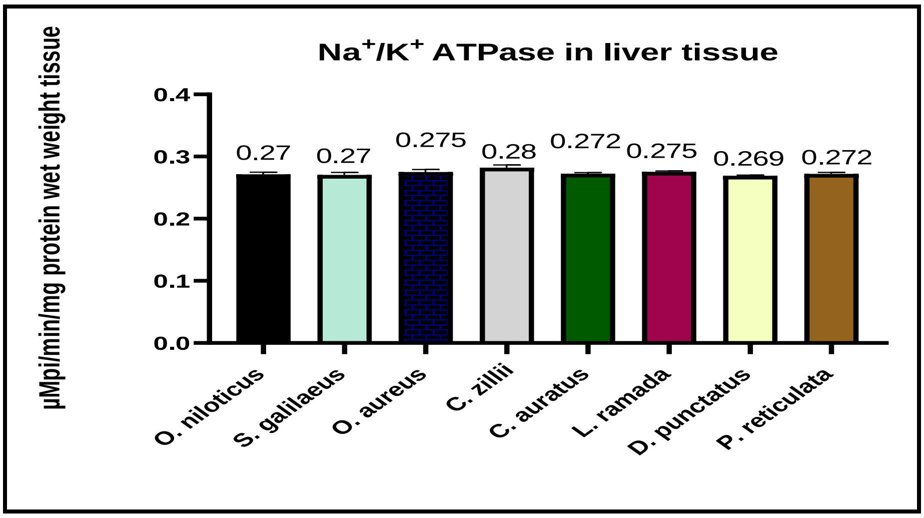
Table 1. The bioaccumulation of heavy metal concentrations (µg/g wet weight) in muscle tissue of some fish species with the metal pollution index (MPI) (Values represent the mean±SD, * significant differences)

Species	Cd	Pb	As	Cr	MPI
Dicentrarchus punctatus	0.006±0.001	0.035±0.004	0.12±0.01	0.95±0.1	0.07
Chelon auratus	0.25±0.001	1.2±0.005	0.8±0.12	2.05±0.2	0.83
Liza ramada	0.005±0.001	0.034±0.004	0.7±0.11	0.85±0.1	0.1
Poecilia reticulata	0.008±0.001	1.3±0.008	1.25±0.19	2.1±0.18	0.41
Oreochromis niloticus	0.2±0.002	1.3±0.007	0.9±0.11	1.3±0.11	0.74
Sarotherodon galilaeus	0.007±0.001	1.0±0.002	0.5±0.001	0.95±0.1	0.24
Oreochromis aureus	0.3*±0.001	1.4*±0.009	1.0±0.17	2.15*±0.22	0.97
Coptodon zillii	0.009±0.001	0.012±0.003	0.14±0.002	0.8±0.1	0.06

Table 2. The maximum permissible limit MPL of bioaccumulation of the investigated heavymetals

M.P.L	Cd	Pb	As	Cr
FAO (1983)	0.05	0.5	0.12-1.0	1.0
FAO/WHO (1989)	0.1	0.5	-	-
FAO/WHO (1993)	0.05	0.5		
USFDA (1993)	-	-	-	12-13
EOSQC (1993)	0.1	0.1	-	-
FAO/WHO (1999)		0.214		
FAO(2003)	2.0	0.2	-	-
EPA (2003)	0.5-1	-	-	-
FAO/WHO (2004)		-	2.0	-
EC (2006)	0.05	0.3	-	-
WHO (2006)	0.5	-	-	-
EC 2008, EC 2011)	0.050	0.3		
WHO (2006)	0.5	-	-	-
FAO (2010)	0.1	-	-	-





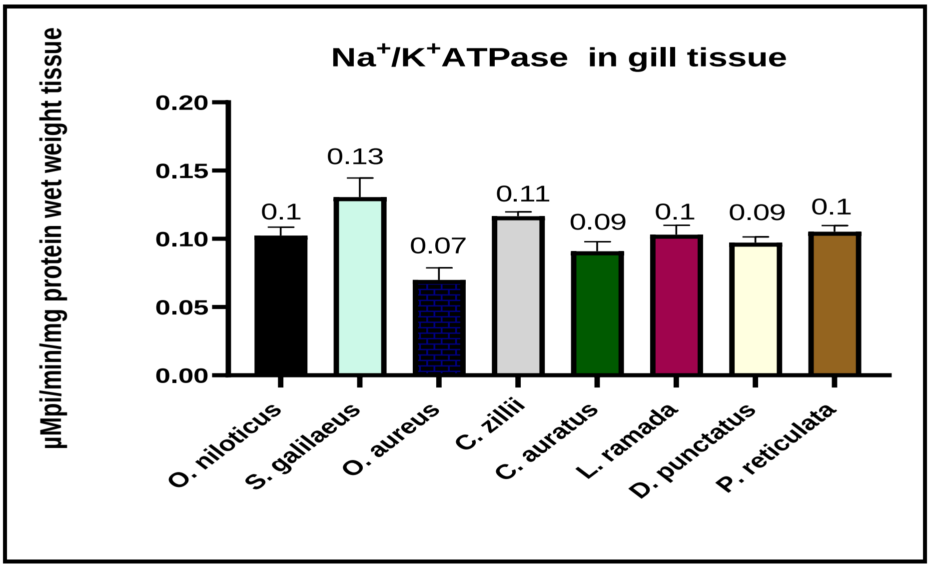
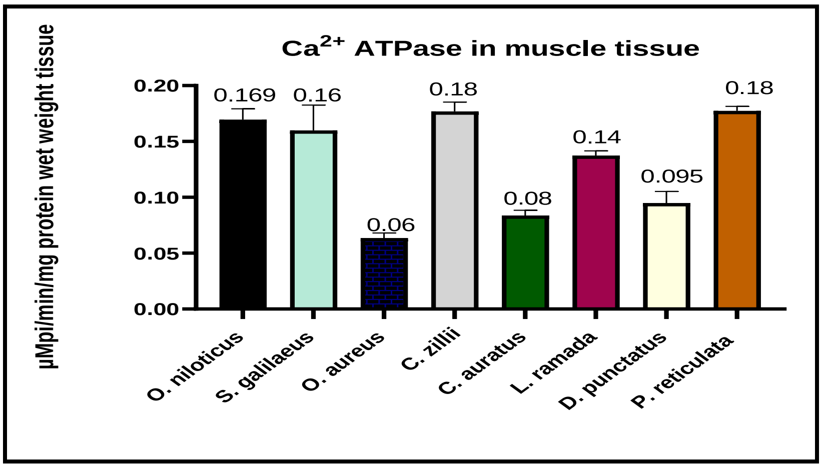


Fig. 3. The activity of Na$^+$/K$^+$ ATPase in muscle, liver and gill tissue for eight fish species fromBurullus Wetland. (Each value represents the Mean ± SD)



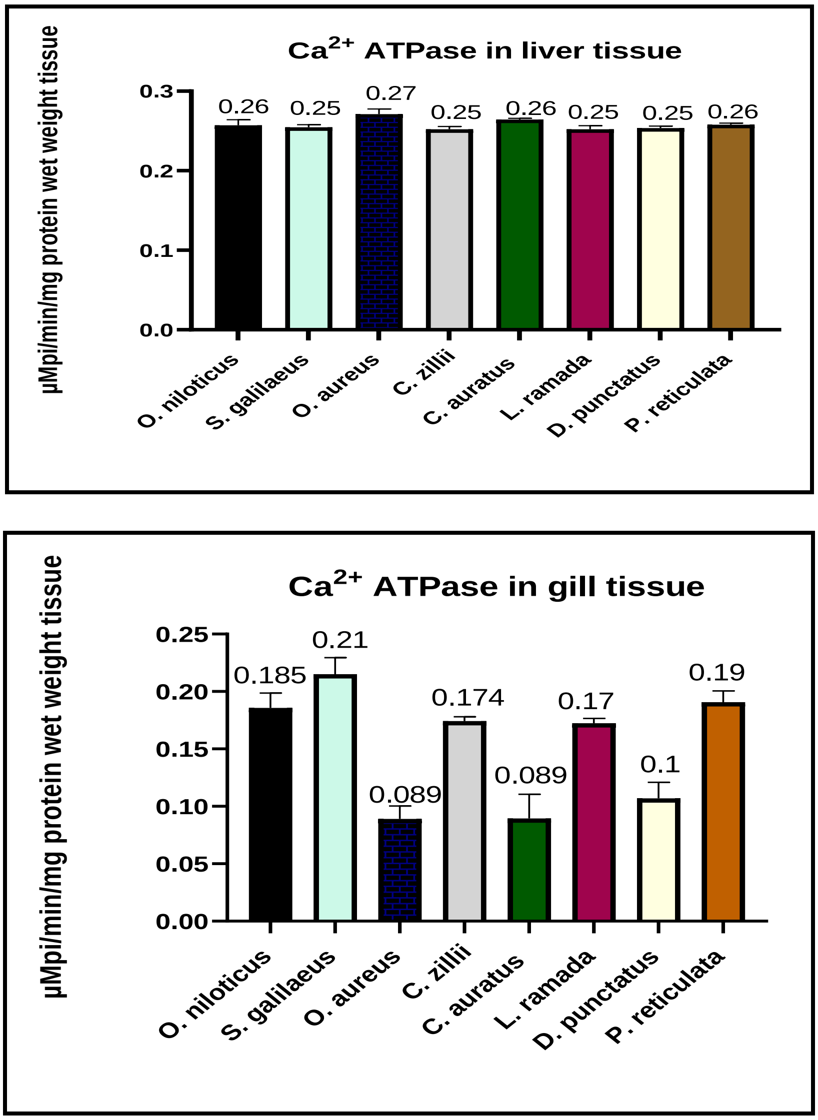


Fig. 4. The activity of Ca^{2+}ATPase in muscle, liver, and gill of eight fish species from BurullusWetland (Each value represents the Mean ± SD)

3. Antioxidant defense system

The SOD showed significant differences ($P<0.05$) in the muscle tissue of the examined species (Fig. 5). The maximum activity of SOD appeared in *O. aureus* and *C. auratus*. The enzymatic activity of CAT was significantly elevated ($P< 0.05$) in the muscle tissue of *O. aureus* and *C. auratus* compared to other examined species (Fig. 5). The nonenzymatic antioxidants, total thiol revealed significant differences among the examined species ($P< 0.05$). The minimum means value appeared in *O. aureus* (Fig. 5).

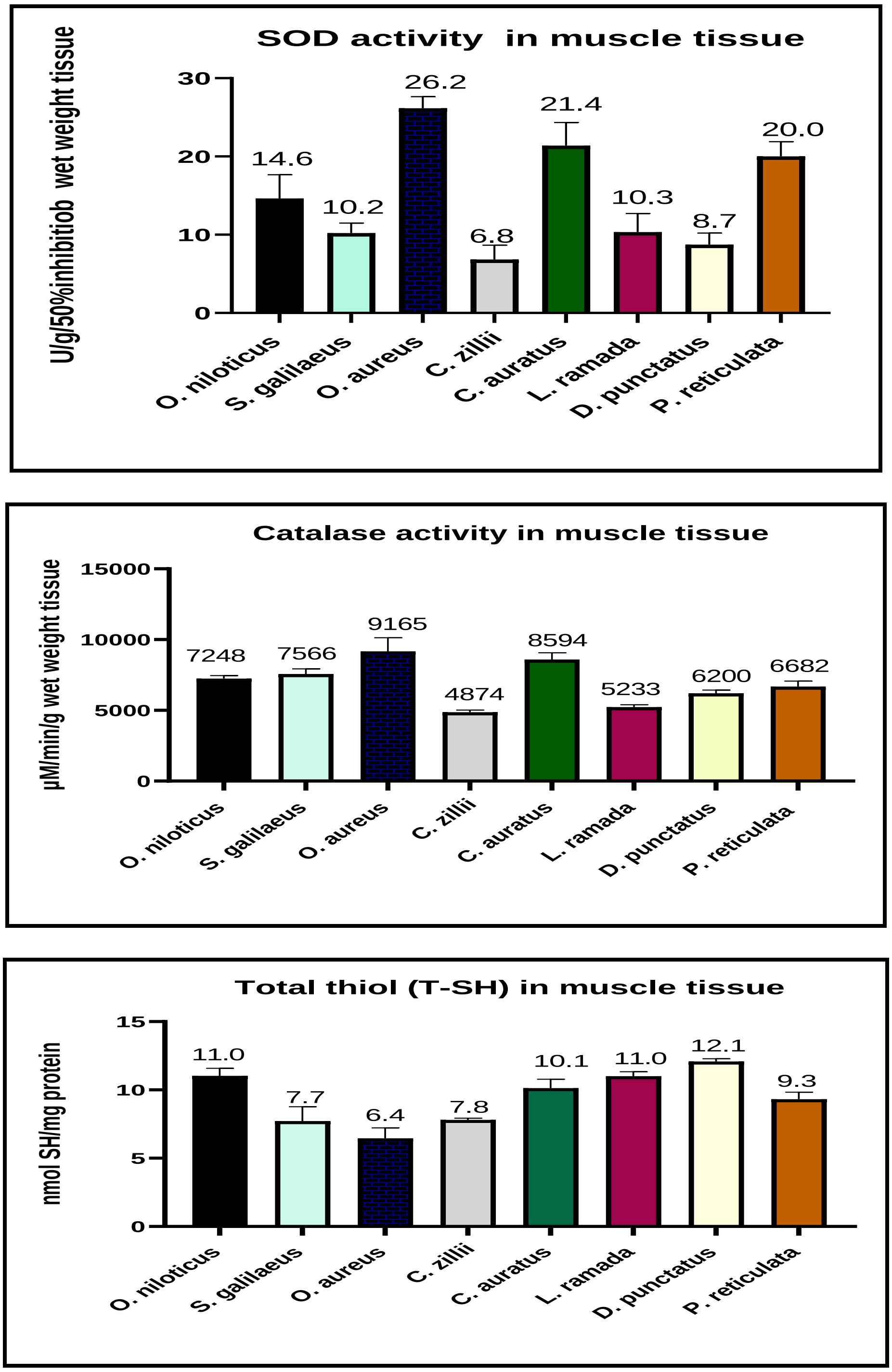


Fig. 5. Antioxidant status in muscle tissues of eight fish species from Burullus Wetland (Eachvalue represents the Mean ± SD)

DISCUSSION

Muscle tissue is the primary edible portion of fish and is important for human nutrition. Therefore, the concentration of metals in this edible tissue is crucial **(El-Sappah *et al.*, 2012)**. The factors that influence the bioaccumulation of heavy metals in different species are the size of the animal **(Yi & Zhang, 2012)**, feeding patterns and preferred habitats **(Jiang *et al.*, 2018)**, adaptability to metal loads, the physical and chemical properties of the aquatic environment; all affect the concentrations of the bioaccumulation of metals **(Liu *et al.*, 2018)**. The present investigation showed a significant variation in the means concentrations of heavy metals among the examined species. This result revealed a decrease in the range of bioaccumulation of metals in the muscle tissue of the examined species from the previous studies performed in this lake. Our result concerning the Cd concentration (0.005-0.3µg/ g) is below the maximum permissible limit of **FAO (2003)**, **EPA (2003),** and **WHO (2006)**, while remaining above the permissible level of **FAO (2010)**. Cd concentration in the current study is below that of **Ezzat *et al.* (2024)** recorded in the muscles of *O. niloticus* at Burullus Lake during 2021. Moreover, it is below the result of **Abdel-Kader and Mourad (2020)** in 2018. Cd is the most poisonous metal even when found with low concentrations **(Flora *et al.*, 2008; Govind & Madhuri 2014)**. The primary source of Cd in the ecosystem is thought to be phosphate fertilizer, but excessive Cd levels can also be linked to mining, industrial, and other activities **(Dimari *et al.*, 2008)**. Cd caused irregular swimming, anemia, fractured vertebrae, and growth fish deficiencies **(Larsson, 1977)**, lower digestive efficiency **(Sastry & Gupta, 1979)**, and osmoregulatory problems **(Reid & McDonald, 1988)**. Moreover, it caused mortality of fish **(Peterson *et al.*, 1983; Eisler, 1985)**, and hematological and biochemical consequences **(Haux & Larsson, 1984)**. Due to its exceptionally long biological half-life and prolonged retention in organisms during bioaccumulation, Cd has a toxic effect and causes harmful consequences on human health **(Webb, 1975; Tsuehiya, 1978)**.

In this study, Pb level in the muscles exceeds the permissible limit recommended by **EOSQC (1993)**, **FAO/WHO (1993)**, **FAO/WHO (1999)**, and **EC (2006)** amended by **EC (2008, EC 2011)**. Our results remained below the level recorded in the study of **Ezzat *et al.* (2024)** conducted on the muscles of *O. niloticus* from Burullus Wetland. Moreover, it is below the result of **Abdel-Kader and Mourad (2020)** on the muscle tissue of some species collected from Burullus Wetland in 2018. Lead (Pb) has no known function in biological processes and is toxic even at low concentrations **(Flora *et al.*, 2008)**. Lead fish poisoning in Egypt is mostly caused by industrial and agricultural wastes **(El Nabawi *et al.*, 1987)**. Growth retardation brought on by prolonged Pb exposure has been related to learning and behavior impairments that may be permanent in children as well as death **(Schwartz *et al.*, 1986; Rossi, 2008)**. Pb concentrations higher than 0.1mg/ l are harmful to developing brain issues in both children and fetuses **(Taupeau *et al.*, 2001)**.

The accumulation of As in muscle tissues remains below the permissible limit recommended by **FAO/WHO (2004)**. The present results also are below that determined in the previous study by **Abdel-Kader and Mourad (2020)**. As is poisonous and high intakes lead to adverse gastrointestinal symptoms, serious disorders of the heart and nervous system, and ultimately death **(Roberts, 1999; Flora *et al.*, 2008)**.

Accumulation of Cr in muscles is below the maximum permissible limit of **USFDA (1993)**. Anthropogenic sources, such as the runoff from agriculture, cities, and industries, can be responsible for high concentrations of Cr in aquatic environments **(Bakshia & Panigrahi, 2018)**. Fish that are exposed to Cr may experience severe issues such as impaired swimming, disturbed eating, erosion of the fin rays, wounds, and even mortality **(Abbasi & Soni, 1984)**. Since it is carcinogenic and has an endocrine-disrupting effect on people, Cr is a worry for the environment and human health **(Goering *et al.*, 1994)**. Post-implantation loss is more common in areas with higher Cr levels, lower number of live fetuses, decreased ossification, and decreased fetal body weight **(ATSDR, 2000, 2008)**.

In the present study, the activity of Na^+/K^+ and Ca^{2+}-ATPases showed a significant decrease in *O. aureus* in muscle and gill tissues but no significant alteration in the liver among the examined species. This may be related to the high content of bioaccumulation of metal in this species. This reduction of Na^+/K^+- and Ca^{2+}- ATPases activities may be due to the suppression of the metabolic rate to control the shutdown of processes involved in membrane ion movement. This finding agrees with that of the previous study of **Monteiro *et al.* (2005)**, which showed that metal poisoning can slow down metabolism in *O. niloticus* exposed to Cu demonstrating decreased gill Na^+/K^+-ATPase activity, as well as decreased plasma Na^+ and Cl^- levels. After being exposed to 10µg/ L Cd for 24 and 96 hours, juvenile freshwater teleosts (*Prochilodus lineatus*) showed dramatically decreased Na^+/K^+–ATPase activity in both the kidneys and gills, but only in the gills, the Ca^{2+}–ATPase activity was significantly decreased; this was demonstrated in the study of **Da Silva and Martineza (2014)**. In the rainbow trout (*Oncorhynchus mykiss*), an electrolyte-imbalanced diet causes a chronic stressor that increases the energy required to maintain the acid-base balance **(Magnoni *et al*., 2018)**. Metal exposure can result in extra energy consumption for both organismal and cellular processes, such as detoxification, metal homeostasis regulation, and compensating reactions involving ionic regulation. For instance, fish with high respiratory activity in conditions of both heat pollution and metal pollution **(Gashkina *et al.*, 2022)** have been found to have an active metabolism of Na, K, and Mg as an adaptive strategy. The previous study of **Hegazi and Hasanein (2010)** showed the effects of chronic exposure to total ammonia nitrogen (TAN) concentrations on the brain Na^+/K^+- and Ca^{2+}- ATPases of *O. niloticus* for 75 days at pH 7.8 and 26°C exposed to 2.5 (low), 5 (medium), and 10 (high) mg TAN $L-1$ concentrations. The levels of Na^+/K^+- and Ca^{2+}-ATPase activities were not significantly altered in fish exposed to low TAN concentration. However, there was a significant decrease in Na^+/K^+- and Ca^{2+}-ATPases activities of fish exposed to medium TAN and a significant increase in high TAN concentrations in comparison with the control.

In the present study, the highest contents of the examined non-essential metals appeared in *O. aureus* followed by *C. auratus*. The antioxidant defense system consists of enzymatic antioxidants such as SOD and CAT and non-enzymatic antioxidants as thiol compounds. In our investigation, the activity of SOD and CAT in *O. aureus* significantly increased in comparison with other examined species. The content of total thiol was significantly reduced in *Oreochromis aureus* compared to the other examined species. This reduction is attributed to the fish's evolved adaptive responses aiming at mitigating the oxidative impact of reactive oxygen species (ROS) or addressing the toxicity of water contaminants. These adaptive mechanisms help counteract the damage caused by excess oxygen free radicals and oxidative stress, as reported by **Carvalho *et al.* (2012)**. Our result agrees with the finding of **Khalil *et al*. (2017)**, who reported that *O. niloticus* white muscles from the Rosetta Branch of the Nile have higher activity of the antioxidant enzymes SOD, CAT, GPX, GST, and GR. Previous studies showed that the exposure of fish to pollutants (agricultural, industrial, and sewage) affects the antioxidative defense system enzymes such as SOD, CAT, GST, and GR **(Hegazi *et al*., 2010)** for *O. niloticus*, and **Hasanein *et al*. (2022)** for the tilapia species and catfish caught from Lake Mariut. **Atli and Canli (2007)** showed that *O. niloticus* exposed to Cd, Co, Zn, and Pb for 14 days, the activity of CAT and SOD in response to metal oxidative stress increased. It is suggested that some of these enzymes can constitute good molecular bioindicators for oxidative stress and can indicate the magnitude of response in vertebrate populations chronically exposed to contaminants, such as metals and other xenobiotics **(Gad, 2009)**.

ROS production and elimination are out of balance, which leads to oxidative stress. In aquatic habitats, fish commonly encounter hazardous and damaging contaminants, which can cause environmental oxidative stress. Fish scavenge ROS with the help of antioxidant defense mechanisms. Increased ROS causes an excessive activation of mitochondrial channels and compromises mitochondrial function **(Chen *et al*., 2021)**. Fish health is endangered when mitochondria produce too much O_2 outflow. Within the antioxidant system, SOD changes $O_2•^-$ into H_2O_2, which CAT and GSH can then decrease. An antioxidant system consists of both non-enzymatic (GSH) and enzymatic (SOD, CAT, and GPx) components to keep ROS below physiological bounds **(Jerome *et al.*, 2017)**. Changes in these enzymes have been reported in a range of fish tissues under toxicant stress when they have been exposed to different pollutants **(Ishaq *et al*., 2023)**. The protection of cells against heavy-metal-induced oxidative stress can occur via non-enzymatic antioxidant systems **(Salbitani *et al.*, 2023)**. Thiol compounds (SH groups), according to **Freitas Souza *et al*. (2019)**, are necessary for the healthy functioning of cells and tissues. On the other hand, the reduction of thiols or their decrease leads to oxidative stress and the development of illnesses. Any low-molecular-weight thiol compounds with a sulfhydryl group (-SH) in their structure are classified as protein thiols or non-protein thiols **(Nassar *et al*., 2014)**. Most plants, microorganisms, and human tissues **(Ferrat *et al*., 2003)**, as well as fish **(Freitas Souza *et al*., 2019)**, contain these chemicals.

CONCLUSION

Burullus Wetland has received much attention due to its significance as an economic and environmental source of fish production in Egypt. The study monitored the health state and physiological conditions of the most abundant fish species in Burullus Wetland. This study revealed that the level of accumulation of toxic metal decreased compared to the previous studies, and this may have been attributed to the cleaning processes that started in 2010. Still, it is necessary to treat wastewater before it is drained into the wetland and monitor the health conditions of fish regularly.

REFERENCES

Abbasi, S.A. and Soni, R. (1984). Toxicity of Lower than Permissible Levels of Chromium (VI) in the Freshwater Teleost Nuria Denricus. Environmental Pollution Series A, 36: 75-82.

Abdel-Kader, H. H. and Mourad, M. H. (2020). Trace elements exposure influences proximate body composition and antioxidant enzyme activities of the species tilapia and catfish in Burullus Lake—Egypt: human risk assessment for the consumers Environmental Science and Pollution Research 27:43670–43681.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol., 105: 121–126.

Alzeny, A.; Abdel-Aziz1, N. E.; El-Ghobashy, A. E. and El-Tohamy,W.S. (2024). Diet Composition and Feeding Habits of Fish Larvae of Five Species in the Burullus Lake, Egypt. Thalassas: An International Journal of Marine Sciences.

Ames, B. N. and Dubin, P. T. (1956). Determination of inorganic phosphate in biological systems, Anal. Chem., Vol.28, 1956, 1756.

Annabi, A.; Said, K. and Messaoudi, I. (2013). Cadmium: bioaccumulation, histopathology and detoxifying mechanisms in fish Am. J. Res. Commun., 1: 60-79.

Atli, G. and Canli, M. (2007). Enzymatic responses to metal exposures in a freshwater fish *Oreochromis niloticus* Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology., 145(2): 282-287.

ATSDR, Agency for Toxic Substances and Disease Registry (2000). Toxicological Profile for Manganese. US Department of Health and Human Services Public Health Service, Atlanta.

ATSDR, Agency for Toxic Substance and Disease Registry (2008). Toxicological Profile for Chromium. US Department of Health and Humans Services, Public Health Service, Centers for Diseases Control, Atlanta.

Bakshia, A. and Panigrahi, A. K. (2018). A comprehensive review on chromium induced alterations in fresh water fishes A. Toxicology Reports, 5: 440-447.

Carvalho, C. S.; Bernusso, V. A.; De Araújo, H. S.; Gaeta Espí ndola, E. L. and Fernandes, M. N. (2012). Biomarker responses as indication of contaminant effects in *Oreochromis niloticus*. Chemosphere., 89(1): 60–69.

Chen, L.; Kaneko, G.; Li**, Y.; Jun** Xie**, J.; Wang, G.; Li, Z. ; Tian, J. Zhang, K. Gong, W. Yun Xia,Y. and Yu, E.** (2021). Reactive oxygen species (ROS)-mediated regulation of muscle texture in grass carp fed with dietary. oxidants. Aquaculture 544, 737150.

Da Silva, A. O. F. and Martineza, C. B. R. (2014). Acute effects of cadmium on osmoregulation of the freshwater teleost *Prochilodus lineatus*: Enzymes activity and plasma ions. Aquat. Toxicol., 156: 161–168.

Dimari, G. A.; Abdulrahman, J. C. and Garba, S. T. (2008). Metals concentrations in tissues of *Tilapia Galli*, *Clarias lazera* and Osteoglossidae caught from Alau Dam, Maiduguri, Borno State, Nigeria. Am J Environ Sci., 4: 373–37.

EC (2006). European Commission Regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 20.12.

EC (2008). Commission regulation no.629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Off J Eur Communities L173: 6–9.

EC (2011) Commission regulation no.420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs (Text with EEA relevance). Off J Eur Communities L111:3–6

Eisler, R. (1985). Cadmium Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. US Fish and Wildlife Service Report 85(1.2), Washington DC.

El Nabawi, A. G.; Heinzow, B. and Kruse, H. (1987). As, Cd, Cu, Pb, Hg and Zn in Fish from the Alexandria Region,Egypt. Bulletin of Environmental Contamination and Toxicology, 39: 889-896.

El-Adawy, A.; Negm, A. M.; Elzeir, M. A.; Saavedra, O. C. El-Shinnawy, I. and Nadaoka, K. (2013). Modeling the hydrodynamics and salinity of El-Burullus Lake (Nile Delta, northern Egypt). J Clean Energy.

El-Sappah, A. H.; Shawky, A. S.; Sayed-Ahmad, M. S. and Youssef, M. A. H. (2012). Nile tilapia as bioindicator to estimate the contamination of water using SDS-PAGE and RAPD-PCR techniques. Egypt J Genet Cytol, 41: 209–227.

EOSQC (1993). Egyptian Organization for Standardization and Quality Control, maximum residue limits for heavy metals in food. Ministry of Industry No. 2360/1993, 5.

EPA (Environmental Protection Agency) (2003). Risk assessment: technical background information. RBG table

Ezzat, N.; Al-Hawary, I.; Elshafey, A.; Althobaiti, N. and Elbialy, Z. I. (2024). Effect of Seasonal Changes in Heavy Metals on the Histomorphology of the Liver and Gills of Nile Tilapia (*Oreochromis niloticus* L.) in Burullus Lake, Egypt., 55 (6): 1705-1716.

FAO (1983). Compilation of legal limits for hazardous substances in fish and fishery products Fishery Circular No. 464, Food and Agriculture Organization.

FAO (2003). Legal notice No 66/2003 Heavy metals regulations, PART I.

FAO (2003). Fisheries and aquaculture topics. Quality and safety of fish and fish products. Topical fact Sheets. In: FAO Fisheries and Aquaculture department.

FAO (2010). The international fish trade and world fisheries Accessed 1.

FAO/WHO (1989). Evaluation of certain food additives and the contaminants mercury, lead, and cadmium. WHO Tech. Rep. Ser No. 505.

FAO/WHO(1993) Evaluation of certain food additives and contaminants (Fourth-First Report of Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 837, WHO, Geneva

FAO/WHO (1999). Expert Committee on Food Additives. Summary and conclusion, 53rd meeting, Rome, 1-10 June.

FAO/WHO (2004). Summary of evaluations performed by the joint FAO/WHO Expert Committee on Food Additives (JECFA 1956-2003), (First through Sixty First Meetings).ILSI Press International Life Sciences Institute.

Ferrat, L.; Gnassia-Barelli, M.; Pergent-Martini, C. and Roméo M. (2003). Mercury and Non-Protein Thiol Compounds in the Seagrass *Posidonia Oceanica*. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol., 134: 147–155.

Flora, S. J. S.; Mittal, M. and Mehta, A. (2008). Heavy metal-induced oxidative stress & its possible reversal by chelation therapy. Indian J. Med. Res., 128: 501–523.

Freitas Souza,C. D.; Baldissera, M. D.; Verdi, C. M.; Santos, R. C. V.; Izabel, M.; Da Rocha, U. M.; Marcelo, L.; da Veiga, M. L. D.; Aleksandro, S.; da Silva, A. and Baldisserotto, B. (2019). Oxidative stress and antioxidant responses in Nile tilapia *Oreochromis niloticus* experimentally infected by *Providencia rettgeri*. Microbial Pathogenesis, 131: 164-169**.**

Gad, N. S. (2009). Determination of glutathione related enzymes and cholinesterase activities in *Oreochromis niloticus* and *Clarias gariepinus* as bioindicator for pollution in Lake Manzala. Global Vet., 3(1): 37-44.

GAFRD, (2021) The General Authority for fishery resources development: Summary production statistics. Cairo, Egypt.

Gashkina, N. A.; Moiseenko, T. I.; Shuman, L. A.; Koroleva, I. M. (2022). Biological responses of whitefish (*Coregonus lavaretus* L.) to reduced toxic impact: Metal accumulation, haematological, immunological, and histopathological alterations. Ecotoxicol. Environ. Saf.**,** 239: 113659.

Georgieva, E.; Velcheva, I.; Yancheva, V. and Stoyanova, S. (2014).Trace metal effects on gill epithelium of common carp *Cyprinus carpio* L. (cyprinidae) Acta Zool.Bulgarica, 66: 277-282.

Goering, P. L.; Waalkes, M. P. and Klaassen, C. D. (1994). Toxicology of Cadmium. In: Goyer, R.A. and Cherian, M.G., Eds., *Handbook of Experimental Pharmacology*, Springer, New York, 189-214.

Govind, P. and Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. Res. J. Ani, Vet and Fish Sci., 2(2): 17–23.

Hasanein, S. S.; Mourad, M. H. and Haredi, A. M. M. (2022). The health risk assessment of heavy metals to human health through the consumption of Tilapia spp and catfish caught from Lake Mariut, Egypt .Heliyon, 8(7), e0980.

Haux, C. and Larsson, A. (1984). Long-Term Sublethal Physiological Effects on Rainbow Trout, *Salmo gairdneri*, during Exposure to Cadmium and after Subsequent Recovery. Aquatic Toxicology, 5: 129-142.

Hegazi, M. M.; Attia, Z. I. and Ashour, O. A. (2010). Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. Aquat. Toxicol., 99(2): 118-125.

Hegazi, M. M. and Hasanein, S. S. (2010). Effects of chronic exposure to ammonia concentrations on brain monoamines and ATPases of Nile tilapia (*Oreochromis niloticus*) Comparative Biochemistry and Physiology, Part C 151: 420–425.

Ishaq, S.; Jabeen, G.; Arshad, M.; Kanwal, Z. Un Nisa, F.; Zahra, R.; Shafiq, Z.; Ali, Bakht, K. Samreen and Manzoor, F. H. (2023). Heavy metal toxicity arising from the industrial effluents repercussions on oxidative stress, liver enzymes and antioxidant activity in brain homogenates of *Oreochromis niloticus.* Sci Rep. 15;13(1):19936.

Järup, L. (2003). Hazards of heavy metal contamination Br. Med. Bull., 68: 167-182.

Jerome, F. C.; Hassan, A.; Omoniyi-Esan, G. O.; Odujoko, O. O. and Chukwuka, A. V. (2017). Metal uptake, oxidative stress and histopathological alterations in gills and hepatopancreas of *Callinectes amnicola* exposed to industrial effluent. Ecotoxicol. Environ. Safety, 139: 179–193.

Jiang, Z.; Xu, N.; Liu, B.; Zhou, L.; Wang, J.; Wang, C.; Dai, B. and Xiong, W. (2018). Metal concentrations and risk assessment in water, sediment and economic fish species with various habitat preferences and trophic guilds from Lake Caizi, Southeast China. Ecotoxicol Environ Saf. 15;157:1-8.

Khalil, M. T.; Gad, N. S.; Nasr, A. M. Ahmed and Mostafa, S. S. (2017). Antioxidant Defense System Alternations in Fish as a Bio-Indicator of Environmental Pollution. 21(3): 11-28.

Kumar, P. M.; Singh, S.; Jain, A.;Yadav, S. Dubey, A. and Trivedi,S. P. (2024). A review on heavy metal-induced toxicity in fishes: Bioaccumulation, antioxidant defense system, histopathological manifestations, and transcriptional profiling of genes. J. of Trace Elements in Medicine and Biology, 83, **127377.**

Larsson, A. (1977). Some Experimentally Induced Biochemical Effects of Cadmium on Fish from the Baltic Sea. Ambio Special Report. 5: 1-67.

Liu, H.; Liu, G.; Wang, S.; Zhou, C.; Zijiao Yuan, Z. and Da, C. (2018). Distribution of heavy metals, stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and risk assessment of fish from the Yellow River Estuary, China. Chemosphere:731-739.

Magnoni, L. J.; Eding, E.; Leguen, I.; Prunet, P.; Geurden, I.; Ozório, R. O. A.; Schrama, J.W. (2018). Hypoxia, but not an electrolyte-imbalanced diet, reduces feed intake, growth and oxygen consumption in rainbow trout (*Oncorhynchus mykiss*). Sci. Rep., 8, 4965.

Maitera, O. N.; Ogugbuaja, V. O. and Barminas, J.T. (2012). Determination of trace metal levels in water and sediments of River Benue in Adamawa State Nigeria J. Ecol. Nat. Environ., 3: 149-156.

Mohammed, E.; Mohammed, T. and Mohammed, A. (2017). Optimization of an acid digestion procedure for the determination of Hg, As, Sb, Pb and Cd in fish muscle tissue. MethodsX: 513-523.

Monteiro, S. M.; Mancera, J. M.; Fontaínhas-Fernandes, A. and Sousa, M. (2005). Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*. Comp. Biochem. Physiol. C Pharmacol. Toxicol., 141: 375–383.

Nassar, M.; Hiraishi, N.; Shimokawa, H.; Tamura, Y.; Otsuki, M.; Kasugai, S.; Ohya, K. and Tagami, J. (2014). The Inhibition Effect of Non-Protein Thiols on Dentinal Matrix Metalloproteinase Activity and HEMA Cytotoxicity. J. Dent.,42:312–318.

Obasohan, E.E.; Oronsaye, J. A. O. and Eguavoen, O. I. (2008). A comparative assessment of the heavy metal loads in the tissues of a common catfish (*Clarias gariepinus)* from Ikpoba and Ogba Rivers in Benin City Nigeria Afr. Sci., 9: 13-23.

Paoletti, F. and Mocali, A. (1990). Determination of superoxide dismutase activity by purelychemical system based on NADPH oxidation. J. Methods Enzymol., 186: 209–220.

Peterson, R. H.; Metcalfe, J. L. and Ray, S. (1984). Effects of Cadmium on Yolk Utilization, Growth, and Survival of Atlantic Salmon Alevins and Newly Feeding Fry. Archives of Environmental Contamination and Toxicology, **12**: 37-44.

Reid, S. D. and McDonald, D. G. (1988). Effects of Cadmium, Copper, and Low pH on Ion Fluxes in the Rainbow Trout, Salmo gairdneri. Canadian Journal of Fisheries and Aquatic Sciences, **45**: 244-253.

Roberts, J. R. (1999). Metal toxicity in children. In: Training Manual on Pediatric Environmental Health. Children's Environmental Health, Emeryville Technol., 1:157–163.

Rossi, E. (2008). Low Level Environmental Lead Exposure—A Continuing Challenge. The Clinical Biochemist Reviews, **29:** 63-70.

Salbitani, G.; Maresca,V.; Cianciullo, P.; Bossa, R.; Carfagna, S. and Basile, A. (2023). Non-Protein Thiol Compounds and Antioxidant Responses Involved in Bryophyte Heavy-Metal Tolerance. Int. J. Mol. Sci., 24(6): *5302.*

Sastry, K.V. and Gupta, P. K. (1979). The Effect of Cadmium on the Digestive System of the Teleost Fish, *Heteropneustes fossilis*. Environmental Research, **19**: 221-230.

Sedlak, J. and Lindsay, R. H.(1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem., 24;25(1):192-205.

Schwartz, J.; Angle, C. and Pitcher, H. (1986). Relationship between Childhood Blood Lead Levels and Stature. Journal of Pediatrics, **77**: 281-288.

Taupeau, C.; Poupon, J.; Nome, F. and Lefevre, B. (2001). Lead Accumulation in the Mouse Ovary after Treatment- Induced Follicular Artesia. Reproductive Toxicology, 15: 385-391.

Tsuehiya, K. (1978). Cadmium Studies in Japan (Review). BioMedical Press, North Holland

Tyokumbur, E. and Okorie, T. (2014).Toxic trace metal contamination (Arsenic, cadmium and lead *of sarotherodon melanotheron* (Rupell, 1852) from alaro stream inIbadan J. Food Nutr. Sci., 2: 258-261

Üner, N.; Oruç, E. and Sevgiler, Y. (2005). Oxidative Stress-related and ATPase Effects of Etoxazole in Different Tissues of *Oreochromis niloticus*", Environ. Toxicol. Pharmacol., 20(1): 99–106.

Usero, J.; Gonzalez-Regalado, E. and Gracia, I. (1997). Trace metals in the bivalve molluscs (*Ruditapes decussates*) and (*Ruditapes philippinarum*) from the Atlantic coast of southern Spain. J. Environ. Int. 23 (3): 291–298.

USDA (1993). Food and drug administration, guidance document for nickel in shellfish. office of seafood, Washington, dc

Webb, M. (1975). Metallothionein and Toxicity of Cd. In: Proceedings of the NATO Scientific Conference on Ecotoxicology Research Effects of Heavy Metals and Organo halogen Compounds, Plenum Press, New York, 177-186.

WHO (2006). Evaluation of certain food contaminants. Sixty-Fourth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, 930.

Yi, Y. J. and Zhang, S. H. (2012). The relationships between fish heavy metal concentrations and fish size in the upper and middle reach of Yangtze River Procedia Environmental Sciences:1699-1707.