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Estimation of tilapia fish quality in Lake Edku through physiological analyses regarding trace element accumulation, antioxidant enzymes, proximate composition, and human health risk assessment as the ultimate consumer

Heba H. Abdel-Kader * Mohamed H. Mourad
National Institute of Oceanography and Fisheries, Alexandria, Egypt
***Corresponding Author:** hebaelalkamy3232@gmail.com

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ABSTRACT

Lake Edku is one of the most important northern lakes whose people with limited income depend on its fish as a cheap source of food. In 2016, the Egyptian government has developed a strategy to develop the historical and economic value of northern lakes included Edku Lake within the Egypt vision 2030. That is why this study was conducted to monitor the physiological state of tilapia fish caught by different fishermen around the Edku lagoon. The results reported that the highest concentration was for As in *O. aureus* (2.16±0.3µg/g), which increased the safe limits permitted by FAO/WHO, EU' and the EOSQC. *S. galilaeus* was recorded the lowest Hg level (0.35±0.1 µg/g). The results estimated that the highest mean carbohydrate (13.85±1.26), fats (22.26±1.32), and water content (77.66±0.62%) were found in *O. aureus*. Otherwise, the largest ash (10.66 ± 1.72%) and protein (65.16±1.27) concentrations were recorded in *S. galilaeus*. The current study showed that *S. galilaeus* had the highest activity of antioxidant enzymes in the liver. The present study suggested that the children should intake less than 18.51g/day or 129.62g/week *O. niloticus* muscle, 13.761g/day or 96.33 g/week *O. aureus* muscle, and 16.66g/day or 116.66 g/week *S. galilaeus* muscle. Youth, on the other hand, should consume no more than 49.38g/day or 345.67g/week *O. niloticus* muscle, 36.69g/day or 256.88g/week *O. aureus* muscles, and 44.44g/day or 311.11g/week *S. galilaeus* muscle. Finally, adults should intake fewer than 84.65g of *O. niloticus* muscle per day or 592.59g per week, 62.90g of *O. aureus* muscle per day or 440.36g per week, and 47.61g of *S. galilaeus* muscle per day or 333.33g per week.

INTRODUCTION

North Coastal Delta Lakes such as (Edku, Manzala, and Burullus) are exposed to extreme environmental contamination, as they are receiving agricultural drainage, industrial wastewater, and domestic wastewater without treatment (**Shalaby *et al*., 2017**). Edku Lake is the third largest wetland area in the Nile Delta covers an area of about 62.5 km^2 which represents less than half of its original size (120 to 130 km^2) (**Zaghloul and Hussein, 2000**). The Lake receives huge amounts of drainage water from two main drains; El-Khairy and Barsik drain (**Farouk *et al*. 2020**). These drains are responsible for increasing lake pollution related to increase levels of trace elements that have adversely affected fisheries. Many fish species are collected from Edku Lake. These fish species included tilapia species (**Farouk *et al*. 2020; Shetaia *et al*., 2020**). Tilapia species such as *Oreochromis niloticus*, *Tilapia zillii*, *Sarotherodon galilaeus*, and *Oreochromis aureus*, were ranked first followed by *Clarias gariepinus* in the total fish production of the Egyptian Lakes (**Zahran *et al*. 2015; Helal *et al*. 2020).**

Trace element accumulation has significant attention in aquatic animals because even low metal levels may threaten the health of aquatic ecosystems, and humans (**Khalil *et al*. 2017, Farouk *et al*. 2020; Abdel-Kader and Mourad, 2020).** So, the safety thresholds of trace elements in fish have been set up in collaboration with food safety agencies such as Food and Agriculture Organization (FAO), Environment Protection Agency (EPA), World Health Organization (WHO) (**Traina *et al*. 2019**). Tilapia and catfish were used as bio-indicator for the accumulation of trace elements in aquatic bodies. Trace elements such as Cadmium (Cd), Lead (Pb), and Mercury (Hg) were recorded in tilapia and *Clarias gariepinus* (**Mohamed *et al*. 2019; Ali *et al.* 2020**; **Abdel-Kader and Mourad, 2020; Abdel-Kader *et al*., 2021**). The concentrations of elements beyond permissible limits were regarded as harmful to humans and aquatic ecosystems (**Sappah *et al*., 2012**; **Khalil *et al*., 2017, Abdel-Kader and Mourad, 2019a; Abdel-Kader and Mourad, 2020; Abdel-Kader *et al*., 2021**). In addition, being aware of changes in the biochemical parameters of fish as a result of elements concentrations will aid in determining metabolic sensitivity (**Abdel-Kader and Mourad, 2019b; Ali *et al.* 2020**; **Abdel-Kader and Mourad, 2020; Abdel-Kader *et al*, 2021**).

Moreover, trace elements induce oxidative stress in the fish, the stress indicators are lower SOD superoxide dismutase, CAT catalase, and GPx glutathione peroxidase levels/ activities (**Jerome *et al*., 2017**). ROS and pro-oxidants are detoxified in a normal, healthy cell by antioxidant defenses. When the level of ROS production exceeds the rate of detoxifying exceeds, oxidative stress occurs (**Kayama *et al*., 2015**).

The production of reactive oxygen (ROS) species such as hydroxyl, superoxide radical, or hydrogen peroxide can be increased by redox-active or redox-inactive elements such as cadmium, mercury, and lead. Oxidative stress-induced dysfunction and damage of membrane lipids of cells caused lipid peroxidation reactions (**Roméo *et al*., 2000**).

In the communities surrounding Edku Lake, fishing is the way to live and earn a living, in addition, most families in these areas have low income, and fish is an alternative and inexpensive source of protein in contrast to other sources of protein. So, Awareness of the proximate composition of the various fish species, particularly when choosing consumption, is of the greatest importance. Moreover, the health risks of consuming fish that accumulate heavy metals in their bodies greatly outweigh the nutritional benefits of such fish species. Therefore, we investigated in the present study (1) the concentrations (Mean ± SE) of Hg, Al, Cd, Pb, and As concentrated in the muscle tissues of *O. aureus*, *O. niloticus*, and *S. galilaeus* from Edku Lake, Beheira Governorate- Egypt, with obtained certified safety guidelines recommended by the WHO, FAO, and EC (2) the human risk assessment, (3) proximate body composition and, (4) the stress indicators by estimated the antioxidant enzymes.

MATERIALS AND METHODS

Lake Description

Edku Lake, Egypt (30º 30` and 30º 23` E and 31º 10` and 31º 18` N), 30 kilometers northeast of Alexandria, is one of the four Mediterranean Sea's coastal delta Lakes (Fig. 1). The lake is the old Canopic branch of the Nile, is linked *via* the Al-maaddiyyah channel to the Mediterranean (Fig. 1). The position on the Edku isthmus allows both the lake and the Mediterranean to capitalize on fisheries. The Lake covers a total area of about 126 km^2. Berzik is the second drain that discharges the drainage water to the lake in the southern part. Based on the Egyptian Ministry of Water Resources and Irrigation Data, 2010, the lake got annual average drainage water of around 142×10^6 m^3 (**El-Sarraf *et al*., 2001; Badr and Hussein 2010**).

Fig. 1. A map of the study area.

Animal Collection

Different fishers caught live medium-sized tilapia fishes such as *O. aureus*, *O. niloticus*, and *S. galilaeus* from several locations surrounding Edku Lake in the spring of 2018. Fish were sent to Physiology Laboratory, the National Institute of Oceanography and Fisheries (NIOF), Fisheries division. Distilled water was used to rinse the fish. On a clean glass surface, samples are dissected with a sterile knife and separated into muscles and liver tissues. The divided pieces are kept adequately sterilized polythene bags. Labeled bags were put in a deep freezer at 25° C until different tests were completed.

Measurement of Trace Elements

Using a concentrated HNO_3 and $HClO_4$ with a ratio of 3:1, the fish tissue digested separately; the specimen 0.5 g + 10 ml of conc. HNO_3 was warmed on a hot plate for one and a half hours in a (100 ml) test tube at 100, 150, 200, and 250 ° C. Then it was treated with ($HClO_4$). Then 2 mL of (1 N HNO^3) were added onto a hot dish and the combination was warmed until it was completely digested and be completely transparent. These digested samples were transported to volumetric flasks (50 mL) plus the volume was made up by adding deionized water. Specimens were filtered using a Millipore membrane filter (0.45 µm Type HV); by using an Atomic Absorption Spectrophotometer Analyst 400-Perkin Elmer (USA), this filtrate was tested for Al, Cd, Pb, and As according to the technique of **Ayanda *et al.* (2019).**

Whereas, Hg was determined using method of **Larry *et al.* (1993)**; 0.5 g of fish tissues were then digested to reduce mercury to elemental form by adding 5 ml (stannous chloride solution), which was estimated using an MHS-Cold Vapor Technique -Atomic Absorption Spectrophotometer.

Each sample was performed in six replicates, with µg/g wet weight (ww) values (mean ± SE) being shown. Each sample was run in six replicates and the results were expressed in terms of µg/g wet weight (mean ± SE). To reduce error in estimating trace element concentrations in fish samples, both samples and blanks were treated in the same manner using the same reagents. Chemical reagents and acids (E. Merck, Germany) were analytical grades and the double-deionized water was required to produce all solutions, for calibrations, each element's standard solutions were made by diluting 1000 mg/l stock solutions. The glass items were immersed, rinsed for 24 h and cleaned in the distilled water combined with 0.5% $KMNO_4$ solution (w/v) and 10% nitric acid.

Human Risk Assessment

By means of human consumption, and according to **FAO/WHO (2004),** the estimated weekly intakes of trace elements (EWI) were compared to the current provisional tolerable weekly intakes (PTWI) for Hg, Al, Cd, Pb, and As. If EWI is less than PTWI, it indicates that food intake does not represent a substantial health risk to the consumers. According to General Authority for Fishery Resources Development, Ministry of Agriculture and Land Reclamation, Egypt, and with Central Agency for Public Mobilization and Statistics, 22.72 kg/ year was recorded as the annual share per capita/kg **(GAFRD, 2017;CAMPAS, 2017**).

Fish consumption/day/capita= 62.25 g /day= 22.72×1000/365

Estimated Daily Intake (EDI) = Conc of metal (µg/g wet weight) × Mean fish intake (g /day/capita).

Estimated Weekly Intake (EWI) per body weight (kg) = EDI × 7 days/week / reference consumer body weight

According to **Salas *et al.* (1985) and Albering *et al.* (1999**) an adult weighs 70 kg, a young person 40, and a child weighs 15 kg.

In the following equation, the percentage of the elements intake to PTWI is determined: The percent PTWI is the percentage of provisional tolerated weekly intakes and determined for each trace element using the possible safety reference dose (PTWI) in 2004 defined by the joint FAO/WHO

%PTWI = (EWI/PTWI) × 100

Maximum Daily Intake MDI (in grams) is the calculated weekly safety intake of fishes that children, youths, and adults should consume fewer than it. The estimated values should attain PTWI by FAO/WHO (2004)

MDI = PTWI × BW / Conc. of element (µg/g) × 7

The maximum weekly intake = MWI = PTWI × BW/ Conc. of element (µg/g)

Proximate Body Composition

Muscle sample (0.1 g) was homogenized for three minutes in a glass homogenizer in saline (5 ml), subsequently centrifuged for ten minutes at 3000 r.p.m. The supernatant was used to determine total protein content (**Lowry *et al*., 1951**), total lipid content was determined the method of **Henry *et al*. (1947),** and total carbohydrates in tissues were determined by the method of **Kemp *et al.* (1954).** After drying, the water content was calculated after losses one gram of sample mass at 100°C ± 2°C. The residual mass was burned to 600° C ± 10° C to measure the ash content **(AOAC, 2002)**.

Antioxidant Enzymes

According to **Nishikimi *et al.* (1972),** the enzyme's efficiency to suppress the phenazine methosulphate–mediated nitroblue tetrazolium dye reduction at 550 nm was evaluated to determine the activity of superoxide dismutase (EC1.1.1.1, SOD) for 5 minutes. SOD activity is measured in units of (U/g). Documented GSH level (EC 1.8.1.7) was estimated by using the **Beutler *et al.* (1963)** method. At 405 nm, the reduced chromogene was estimated, which was in a direct proportion to the GSH concentration expressed as (mg/g). Glutathione peroxidase activity (GPx: EC 1.11.1.9) was determined by using a substrate H_2O_2, according to **Paglia and Valentine (1967)** method. For three minutes, the process was measured indirectly at 240 nm as the oxidation rate of NADPH to $NADP^+$. Gpx activity is measured in units of mU/mL. CAT activity (EC 1.11.1.6) was determined by hydrolysis of H_2O_2 and the resulting diminution in absorbance at 510 nm was expressed as U/g (**Abei, 1984**). The activity of glutathione reductase (GR: EC 1.6.4.2) was determined using **Goldberg and Spooner's (1983)** technique, which characterized the ability of the enzyme to catalyze the reduction process of GSSG to GSH when NADPH oxidized to $NADP^+$ and was estimated as U/L at 340 nm at 37ºC.

Analysis of statistical

Data are analyzed using the Statistical Processor System Support (SPSS) 20 Armonk /USA and given as mean ± standard error, the one- way analysis ANOVA was used and the values considered statistically significant at ($P < 0.05$)

RESULTS AND DISCUSSION

Accumulation of Trace Elements

The mean levels of Hg, Al, Pb, Cd, and As in O. *aureus*, *S. galilaeus* and *O. niloticus* (µg/g ww) in Edku Lake are displayed in Fig. 2. The mean concentration of elements was at the following order in the studied tilapia species Hg< Al < Pb < Cd < As. The highest concentration was for As in *O. aureus* (2.16±0.3µg/g), but the lowest concentration was for Hg in *S. galilaeus* (0.35±0.1 µg/g).

Aluminum toxicity is mainly associated with the neurotoxicity and growth of neurodegenerative diseases in living organisms (**Verstraeten, 2008**). According to this study, the lowest and the highest Al levels observed were 0.89±0.1 for *O.aureus* and 0.56±0.2 for *S. galilaeus*, whereas, the mean concentration was 0.60±0.1 in *O.niloticus*. These average insignificant Al concentrations (F= 3.01 and *P-value* = 0.07), can be arranged as follows *O. aureus* > *O. niloticus* > *S. galilaeus*. In Egypt, **Abdel- Kader and Mourad (2020)** showed that in Burullus Lake, the average significant Al concentrations can be sorted as follows: *O.aureus*> *S.galilaeus*> *O.niloticus*. The average values from 1.96±0.3 to 1.38±0.19 µg/g this is higher than the Al content in this research. In Egypt, **Abdel- Kader *et al.* (2021)** recorded that in Edku Lake, Al concentrations in *tilapia zillii* and *C. gariepinus* muscles ranged from 0.59 ±0.12 to 1.05±0.08 µg/g on average. The values published in the literature were frequently greater than the results of this study.

Arsenic is an element found in nature that has the potential to be toxic to living organisms (**Devesa et al 2008**). In this study, the average significant As concentrations (F= 2.82 and *P-value* = 0.090), can be organized as follows *O. aureus* > *S. galilaeus* > *O. niloticus*. The lowest and highest As levels observed were 0.91±0.1mg kg^{-1} for *O. niloticus* and 2.16±0.3 mg kg^{-1} for *O. aureus*, whereas the mean concentration was 1.31±0.1 mg kg^{-1} in *S. galilaeus*. The results exceeded the FAO/WHO recommended safe limit of 2.0 µg/g. In Egypt, **Abdel- Kader and Mourad (2020)** showed that in Burullus Lake, the average significant As concentrations can be sorted as follows: *O. niloticus* > *O. aureus* > *S. galilaeus*. The average values were 1.50 ± 0.1 to

1.706 ± 0.1 µg/g and this was lower than the As content in this research. In Buriganga River and adjacent canals of Hazaribagh, Dhaka, **Islam *et al.* (2014)** recorded that As was found in the ranges of 0.62 to 0.71 mg/kg ww. In Egypt, As concentrations in *tilapia zillii* and *C. gariepinus* muscles ranged from 1.43 ± 0.17 to 1.54 ± 0.14 µg/g on average (**Abdel-Kader, *et al.* 2021**). The values published in the literature were frequently lower than the results of this study

Cadmium is a harmful trace metal found in high amounts in fish species (**Türkmen *et al.*, 2009**). In this study, the average significant Cd concentrations (F= 4.05, *P-value* = 0.03), can be sorted as follows O. *aureus* > S. *galilaeus* > O. *niloticus*, the lowest and the highest Cd levels found were 0.81±0.1 mg kg^{-1} for *O.niloticus*, and 1.09±0.2 for *O.aureus.* Mean concentrations were 0.90±0.1 mg kg^{-1} in *S. galilaeus*. In Egypt, the Cd average concentrations in the muscles of tilapia species, were sorted as follows O. *aureus* >*S. galilaeus* >*O. niloticus* collected from Burullus Lake. It ranged from 1.10 ± 0.12 1 to 1.46 ± 0.07 and µg/g. In the Nile, Egypt**, Abd El–Khalek *et al.* (2016),** recorded that Cd residues in the muscles of *S. gibbus* from Shalateen and *Sardinella sp* from Suez were 0.03 ± 0.01 to 0.38 ± 0.29 mg/g ww, `respectively. In Egypt, **Abdel-Kader *et al.* (2021)** estimated Cd concentrations in *tilapia zillii* and *C. gariepinus* muscles from Edku and recorded that the ranged values were from 0.93 ± 0.05 to 1.13 ± 0.08 µg/g. The values published in this literature were frequently higher than the results of this study.

Lead concentrations in this study were insignificant (F= 0.15 and *P-value* = 0.18), can be arranged as follows: *O. aureus* = *S. galilaeus* > *O. niloticus*. The lowest and the highest Pb levels found were 0.89±0.1 mg $kg^{-1}$for *O.niloticus* and 0.966±0.2mg/ kg^{-1} for *S. galilaeus*. Mean concentrations of Pb were 0.963± 0.2 mg /kg^{-1} in *O.aureus*, these three concentrations exceeded the permitted level as determined by the EOSQC (1993) 0.1 µg/g, **(FAO/WHO, 1999)** 0.2 µg/g, EC (2006 amended by EC 2008, EC 2011) 0.30 µg/g.

In comparison, Pb concentrations ranges were 0.20 to 1.18 mg / kg in *O. niloticus* from the Nile, Egypt (**Abdel-Khalek *et al.* 2016**). In addition, Pb levels that were recorded in tilapia from Burullus Lake in Egypt as the following ordered, *O. niloticus* > *S. galilaeus* > *O. aureus* with values 1.78 ± 0.11, 1.53 ± 0.07 and 1.33 ± 0.17 µg/g, respectively. Moreover, In Egypt, **Abdel-Kader *et al.* (2021)** recorded that the level of Pb was in significantly greater in *C. gariepinus* 1.31±0.08 than *T. zillii* 0.94 ± 0.08 µg/g. However, the values reported in the literature are generally higher than the values from this study.

Mercury pollution was caused by degassing of the earth's crust as well as anthropogenic sources. Mercury would be used in a variety of industrial applications, including fossil fuels, chlorine industries, pulp and paper, and agricultural activities. These applications result in the disposal of Hg in both dry and wet forms into the aquatic environment. According to this study, the average insignificant Hg concentrations (F= 3.01 and *P-value* = 0.07), can be arranged as follows: *O. aureus* > *O. niloticus* > *S. galilaeus*. The lowest and the highest Hg levels found were 0.35±0.1 mg kg^{-1} for *S. galilaeus* and 0.476±0.1 mg kg^{-1} for *O.aureus*. Mean concentrations were 0.39±0.1 mg kg^{-1} in *O.niloticus.* Our results in tilapia tissues were lower than the allowed records of FAO/WHO 0.5 µg/g (1992), EOSQC0.5 µg/g (1993) and EC 0.50 µg/g (2006 amended by EC 2008, EC 2011). In Edku Lake, In Egypt, **Abdel- Kader and Mourad. (2020)** estimated Hg average level of tilapia species to show *O. aureus*>*O. niloticus*> *S. galileus*, the values recorded from 0.54±0.02 to 0.78 ± 0.05µg/g. **Abdel- Kader *et al*. (2021)** recorded that the Hg concentration in *C. gariepinus* (0.59 ± 0.07 µg/g) was not significant more than *T. zillii* (0.47±0.08 µg/g). In Kafer-El-Zayat, Egypt, Elnimr **(2011)** estimated the level of elements in *Tilapia nilotica* and catfish founded that Pb and Cd were greater than the EOSQC (1993) permissible limits. However, the values reported in the literature are generally higher than the values from this study.

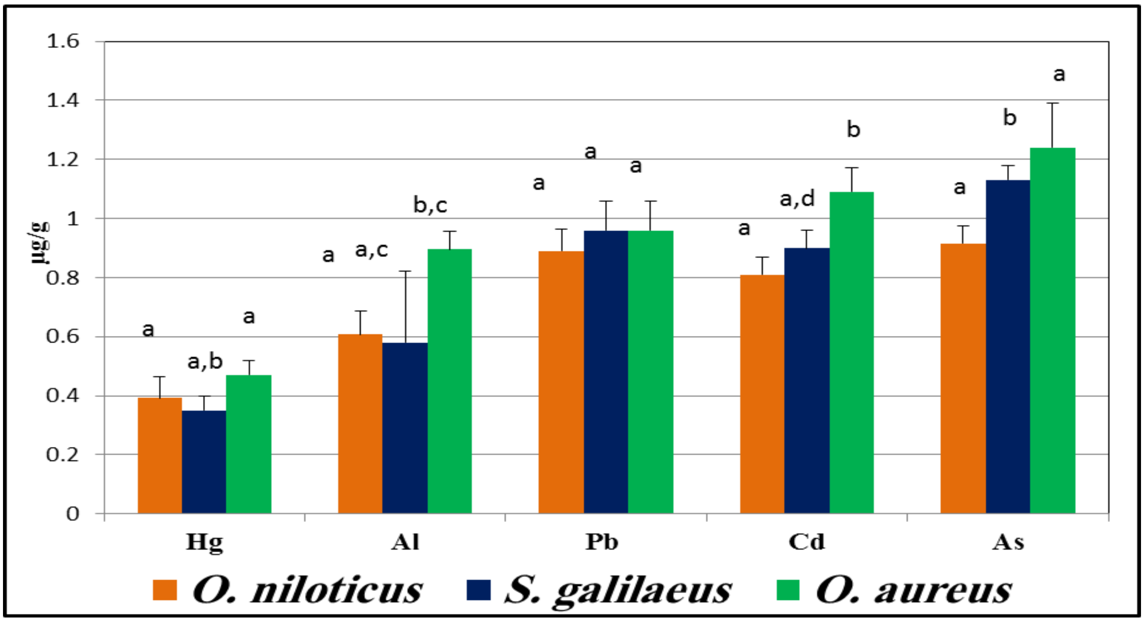


Fig. 2 Mean trace element concentration (µg/g wet weight) ± SE in the muscles of *O. aureus, S. galilaeus, and O. niloticus* (n=6). Various superscripts have statistical significance ($p < 0.05$).

Health risk assessment

Consumption of metal-polluted fish may have negative health effects on consumers; therefore, trace metal toxicity in fish should be evaluated urgently in terms of food safety for citizens. Cd, Pb, Hg, As, and Al PTWI values were 0.007, 0.025, 0.005, 0.015, and 1 mg/kg, respectively (**FAO/WHO, 2004**). Table 1 displayed EDI (µg/kg bw/d), EWI (µg/kg bw/w), PTWI %, MDI, and MWI of Cd, Pb, Hg, Al, and As in the muscles of *O. aureus*, *S. galilaeus*, and O. *niloticus* from Edku Lake that children, youth, and adults should consume.

This study found that adults' EDI, EWI, and PTWI % of elements were lower than the PTWI values established by FAO/WHO. As a result, with advice on safe levels of consumption MDI and MWI of three fish species for adults, the three fish species did not pose a risk to adult health. In Lake Geriyo, Adamawa State, Nigeria (**Bawuro et al. 2018**) demonstrated that Pb in Tilapia fish species was unhealthy for consumption, and so, they negatively affect people's health. Because there is no industrial activity near the lake, these levels could be due to anthropogenic inputs.

Table 1 of our findings revealed that the EDI and EWI of trace elements As > Pb > Cd >Al >Hg decided to rank children >youth > adults depending on consumption of *O. niloticus* and S. galilaeus muscles, While the EDI and EWI of trace elements As> Cd >Pb > Al > Hg mirrored ranking as children >youth > adults according upon *O. aureus* intake. Furthermore, Table 1 revealed that the PTWI percent followed a sequence of Cd >As> Hg>Pb >Al based on *O. niloticus* and *S. galilaeus* intake, whereas Cd >Hg >As> Pb >Al depending on *O. aureus* consumption. So, in a diet/day or week, this study calculated the EDI or EWI of muscles for three fish species. As a result, it was suggested that children consume less than 18.51g/day or 129.62g/week *O. niloticus* muscle, 13.761g/day or 96.33 g/week *O. aureus* muscle, and 16.66g/day or 116.66 g/week *S. galilaeus* muscle. Youth, on the other hand, should take no more than 49.38g/day or 345.67g/week *O. niloticus* muscle, 36.69g/day or 256.88g/week *O. aureus* muscle, and 44.44g/day or 311.11g/week *S. galilaeus* muscle. Finally, adults should consume fewer than 84.65g of *O. niloticus* muscle per day or 592.59g per week, 62.90g of *O. aureus* muscle per day or 440.36g per week, and 47.61g of *S. galilaeus* muscle per day or 333.33g per week.

Table 1 EDI (estimated daily intake) (g/day/capita), EWI (estimated weekly intake) (g/week/capita), PTWI %, MDI (maximum daily intake) , MWI (maximum weekly intake) of Hg, Al, Cd, Pb, and As in *O. niloticus, O. aureus, and S. galilaeus* muscles from Edku Lake – Egypt, consumed by a child 15 kg, a young 40 kg, and an adult 70 kg

Metals	Intake	*O. niloticus*			*O. aureus*			*S. galilaeus*		
		Child	Young	Adult	Child	Young	Adult	Child	Young	Adult
Hg	*EDI*	1.61	0.605	0.34	1.95	0.73	0.41	1.45	0.54	0.31
	EWI	11.3*	4.24	2.40	13.65*	5.11*	2.9	10.16*	3.8	2.17
	PTWI	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	PTWI%	226	84.8	48	273	102.2	58	203.2	76	43.4
	MDI	27.47	73.26	128.20	22.79	60.79	106.3	30.61	81.63	142.8
	MWI	192.30	512.82	897.43	159.57	425.53	744.6	214.28	571.42	1000
Al	*EDI*	2.48	0.93	0.53	3.69	1.38	0.79	2.33	0.87	0.49
	EWI	17.42	6.53	3.73	25.85	9.69	5.53	16.32	6.12	3.49
	PTWI	1000	1000	1000	1000	1000	1000	1000	1000	1000
	PTWI%	1.742	0.653	0.373	2.585	0.969	0.553	1.632	0.612	0.349
	MDI	3571.42	9523.80	16666.66	2407.70	6420.5	11235.9	3571.42	9523.80	16666.6
	MWI	25000	66666.66	116666.6	16853.93	44943.82	78651.68	26785.7	14285.7	125000
Cd	*EDI*	3.36	1.26	0.72	4.52	1.695	0.968	3.734	1.4	0.8
	EWI	23.52*	8.82*	5.04	31.66*	11.87*	6.78	26.14*	9.80*	5.60
	PTWI	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
	PTWI%	336	126	72	452.2857	169.571	96.85	373.42	140	80
	MDI	18.51	49.38	84.65	13.761	36.69	62.90	16.66	44.44	47.61
	MWI	129.62	345.67	592.59	96.33	256.88	440.36	116.66	311.11	333.33
Pb	*EDI*	3.69	1.38	0.79	3.98	1.49	0.85	3.98	1.49	0.85
	EWI	25.85*	9.69	5.53	27.88*	10.45	5.97	27.88*	10.45	5.97
	PTWI	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
	PTWI%	103.4	38.76	22.12	111.52	41.8	23.88	111.52	41.8	23.88
	MDI	60.19	160.5	280.88	55.8	148.8	260.28	55.8	148.8	260.28
	MWI	421.34	1123.5	1966.2	390.6	1041.6	1822.0	390.6	1041.6	1822.0
As	*EDI*	3.77	1.41	0.80	8.92	3.34	1.91	5.42	2.10	1.16
	EWI	26.43*	9.91	5.66	62.45*	23.42*	13.38	38.0*	14.72	8.15
	PTWI	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
	PTWI%	176.2	66.06	37.73	416.33	156.13	89.2	253.33	98.13	54.33
	MDI	35.32	94.19	164.82	14.88	39.68	68.58	24.53	65.42	114.46
	MWI	247.25	659.34	1153.8	104.16*	277.77	480.11	171.75	458.0	801.23

Proximate composition

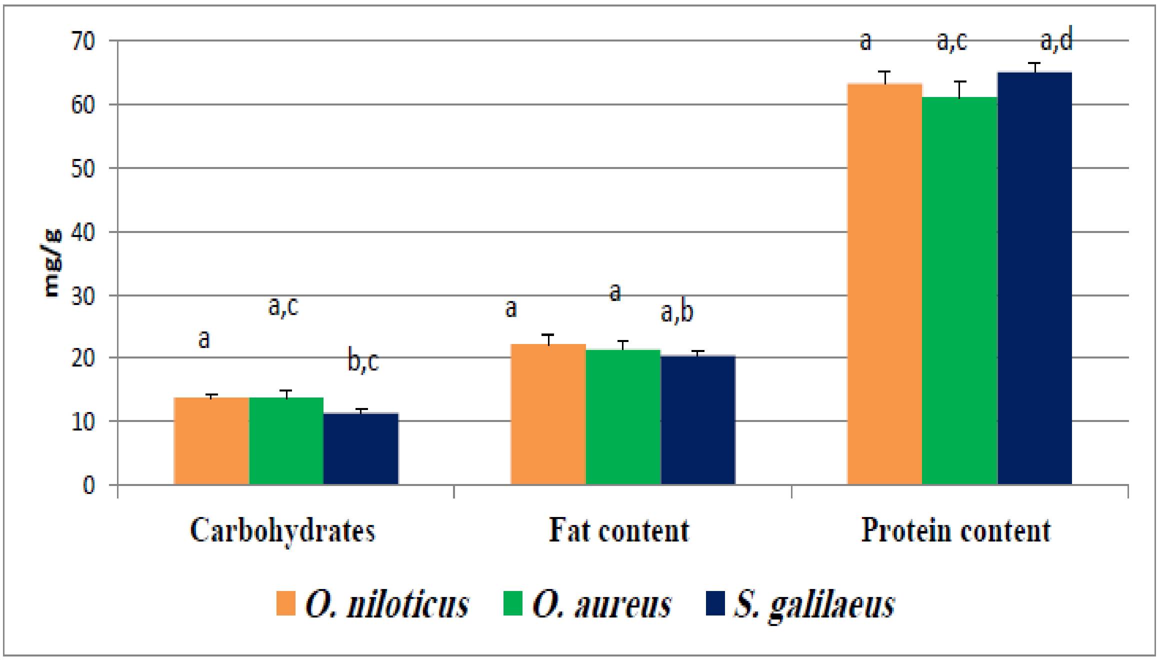
The metabolic status of most fish's proximate composition, which includes proteins, water, and lipids, typically indicates the animal's physiological state, constituting approximately 98 % mass of fish flesh (**WHO/FAO, 2011**). Carbohydrates are minor ingredients as well as vitamins and minerals. In this study, mean values for proximate body composition at *O.*

niloticus, O. aureus and *S. galilaeus* muscle tissue are displayed in (Fig. 3). The comparison of mean carbohydrate levels (mg/g) for all fish species revealed a significant difference ($P<0.05$). The highest mean carbohydrate value was found in O. *aureus* (13.85±1.26), while the lowest carbohydrate value was found in *S. galilaeus* (11.4±0.5). The mean was 13.75±0.7 for *O. niloticus*. In contrast, in Burullus Lake the *O. niloticus* was (18.66 ±.88) mg/g with the largest mean carbohydrate levels, with (16.33±0.9 and 14.5±0.9 mg/g) for *O. aureus* and S. *galilaeus,* respectively **(Abdel-Kader and Mourad, 2020).** In Edku Lake, Egypt, *T. zillii* had the highest mean carbohydrate value of 16.11 ±0.67 mg/g, whereas *C. gariepinus* had the minimum carbohydrate value of 11.28 ±0.68 mg/g (**Abdel-Kader *et al*. 2021).**
In this study, lipid content was found a significant difference in fish species, *O. niloticus* 22.26±1.32 (mg/g), had the highest lipid content, whereas, *galilaeus* 20.48±0.67 mg/g had the lowest. Mean value for *O. aureus* was 21.38±1.47 mg/g. In line with our findings, *O. niloticus* had the highest mean lipid content 15.83 0.9 mg/g, whereas *O. aureus* and *S. galilaeus* had 13.83 ±0.9 and 13.33±0.4mg/g, respectively, from Burullus Lake, Egypt (**Abdel-Kader and Mourad, 2020**). The lipid content of two fish species differed insignificantly. *T. zillii* had the highest lipid content (mg/g) (24.21±0.93), followed by *C. gariepinus* (21.96±0.95 mg/g) for the lowest result. The fat content was reduced from 1.60 to 4.30 in carp, *Cirrhinus mrigala,* after cadmium chloride and lead acetate exposure (**Bhilave *et al*. 2008**).

In this survey, a study of mean protein (mg/g) for fish species revealed a non-significant difference (P> 0.05). *S. galilaeus* had the highest mean protein value (65.16 ±1.27), followed by *O. aureus* (61.16±2.50), and *O. niloticus* (63.33±1.32). It might potentially be induced by heat shock protein synthesis or harmful free radicals, or it might be induced by elements. In comparison, *O.aureus* from Burullus Lake had the highest mean protein value of (64.83± 4.73 mg/g), whereas *O. niloticus* and *S. galilaeus* had values of 58.16±3.2 and 53.75±1.6 mg/g, respectively (**Abdel-Kader and Mourad, 2020**). Protein content may have reduced due to protein breakdown in the production of some amount of energy for the organism; the metabolic stability of proteins typically indicates an animal's physiological condition (**Stancheva *et al*., 2013**). In Egypt, Edku lake, **Abdel Kader *et al.* (2021)** demonstrated the highest mean protein value of *C. gariepinus* at 64.33±1.01, followed by *T. zillii* with a value of 55.66±1.95.

In this survey, there was an insignificant difference in the mean water content of three studied species of fish (P > 0.05). *O. niloticus* showed the highest mean moisture 77.66 ± 0.62% followed by the mean value for *S. galilaeus* 77.33 ± 0.42 % while the least mean moisture concentration was recorded in *O. aureus* 77 ± 0.87%. In comparison with **Abdel–Kader and Mourad (2020),** Burullus Lake in Egypt, the *O. aureus* indicated the greatest average water level at a value of 67.33±1.5 %, while the lowest average water level was obtained in *S. galilaeus* 60.75 ± 2.2%, which is much lower than our results. In Edku Lake, Egypt, **Abdel Kader *et al*. (2021)** recorded that the average water content of *T. zillii* was 78.33±1.58%, but the least average water content for *C. gariepinus* was 77±0.45%.

Fish species exhibited an insignificant reduction in the ash level in our data. The largest ash concentration was in *S. galilaeus* (10.66 ± 1.72%), *O. aureus* average value recorded 7±0.87%. Whereas, *O. niloticus* was the lower (5.66 ±0.62%). In comparison with **Abdel-Kader and Mourad (2020)** *O. niloticus* had the highest ash concentration 16.41±1.0 followed by *S. galilaeus,* 14.25±1.7 and O. aureus 3.76 ± 0.4 % . In Edku lake, Egypt **Abdel-Kader *et al.* (2021)** the *T. zillii* had the highest ash content (7.33±1.00%), while the *C. gariepinus* had the lowest ash content (6.66±0.85%).



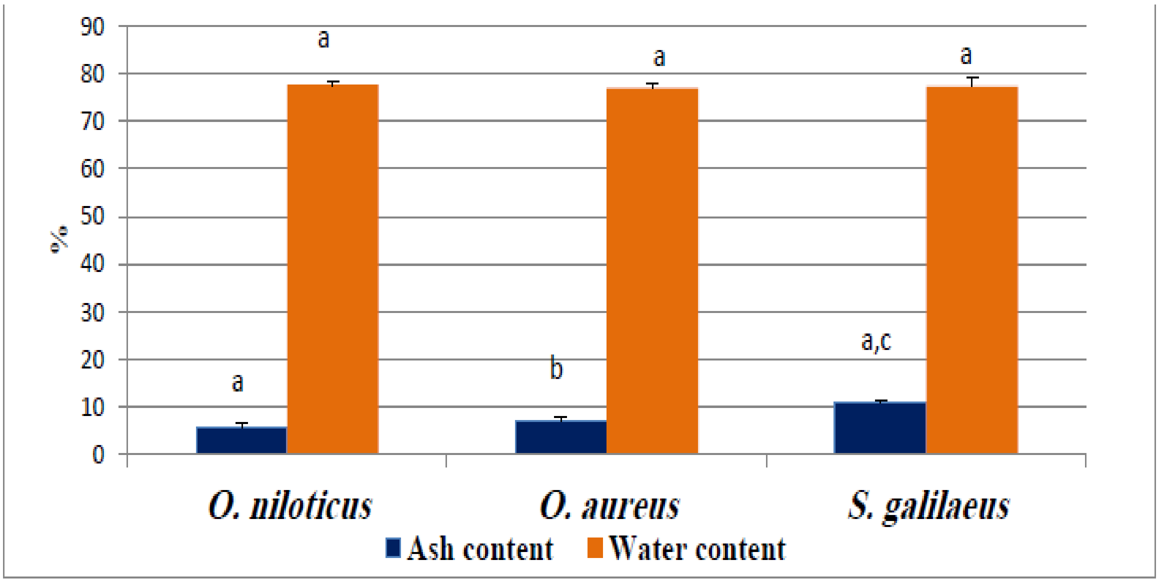


Fig. 3 Proximate body composition in muscle tissue of *O. niloticus, O. aureus* and *S. galilaeus* (n = 6). Various superscripts have statistical significance ($p < 0.05$).

Antioxidant enzymes

Enzymes can be used as biomarkers in bio-monitoring systems to identify pollution early (**Mohamed *et al*. 2019, Abdel–Kader and Mourad, 2019b**). Toxic agents in the aquatic ecosystem can interfere with the production of detoxifying enzymes including SOD, CAT, and GPx, as well as cause reactive oxygen species (ROS) (**Sreejai and Jaya, 2010**). The antioxidant is the body's first line of defense against oxidative stress (**Qu *et al*., 2014**). SOD plays a vital function in the antioxidant system of the body by interfering in the first process by turning free superoxide (O_2^-) radicals into most reagent forms of oxygen (H_2O_2). This CAT is situated largely within peroxisomes and reduces hydrogen peroxide from the metabolism of long-chain fatty acids to water and oxygen in peroxisomes. The GSH antioxidant molecule is one of the most investigated in fish. Heavy metal cations have a very strong affinity to –SH residues this leads to a GSH level decrease.

The findings from this investigation revealed that the liver activity of antioxidants CAT, GR, GSH, SOD, and GPx showed a significant reduction in mean values ± SE (Fig. 4). In this study, SOD calculated a significant difference P < 0.05 in tilapia fish species. There was a reduction in SOD (U/g) in *O.aureus* average value (49.16 ± 3.58), *O. niloticus* (60 ± 3.21U/g) and *S. galilaeus* (66.16 ±2.08) for the highest value. CAT (U/g) for fish species revealed a significant difference (*P* < 0.05). *O. niloticus* had the lowest CAT score of (35.83 ±1.99), followed by *O. aureus* (38.66 ± 2.24). *S. galilaeus* mean value (42.66 ± 1.33) for the maximum. Gpx was demonstrated to have a significant difference P < 0.05. In the *O. aureus*, there was a reduction in Gpx (mU/mL) mean value (51.5 ± 3.58) followed by *O. niloticus* mean value (60 ± 3.21 mU/mL) and the greatest value for *S. galilaeus* (66.16± 2.08 mU/mL). The difference in GR (U/L) for the species of fish (*P*>0.05) was insignificant. A minimum GR value was (48.33 ± 3.35) referred to *O. niloticus* followed by (52 ± 2.80) average value for O*. aureus*. *S. galilaeus* mean value for the highest value (54.33 ± 2.7). GSH was found to be an insignificant difference (P> 0.05) in tilapia fish species in this study. *O. niloticus* (40 ± 3.51) had the lowest GSH (mg/g.tissue) levels, followed by *O. aureus* (47± 2.6 mg/g.tissue). *O. niloticus* (51.16 ± 3.6 mg/g.tissue) was found to have the highest maximum value. According to **Khalil *et al.* (2017),** SOD, CAT, GPX, GST, and GR antioxidant enzyme activities were increased in white muscles of *O. niloticus* obtained from the Rosetta Branch of the Nile. In comparison with **Abdel-Kader and Mourad (2020)**, *S. galilaeus> O.aureus> O.niloticus* in CAT with ranges of 22.33±3.1 to 19.34 ±3.4 (U/g.tissue). *S. galilaeus> O.aureus >O.niloticus* in GR with range, 22.33 ±1.8 to 17.53 ± 2.7 U/L. *S. galilaeus* > *O.aureus* > *O. niloticus* in GSH with range 22.16±1.4 to 18.01±2.1 (mg/g.tissue). *S. galilaeus* > *O. aureus*> *O. niloticus* in SOD with range 31.38 ± 3.0 to 28.96±2.7, (U/g.tissue). *S.galilaeus* > *O.aureus*> *O.niloticus*. GPx activity was ranged from 30.66±2.9 to 24.65±3.55 mU/mL from Burullus Lake, Egypt. According to **Atli and Canli (2007),** *O. niloticus* treated to Cd, Co, Zn, and Pb for 14 days showed an increase in CAT and SOD, which might be attributed to toxicity. **Carvalho *et al.* (2012)** reported that fish adaptive mechanisms to prevent the oxidative damage of produced ROS or to fight the toxicity of water contaminants against damage produced by oxidative stress.

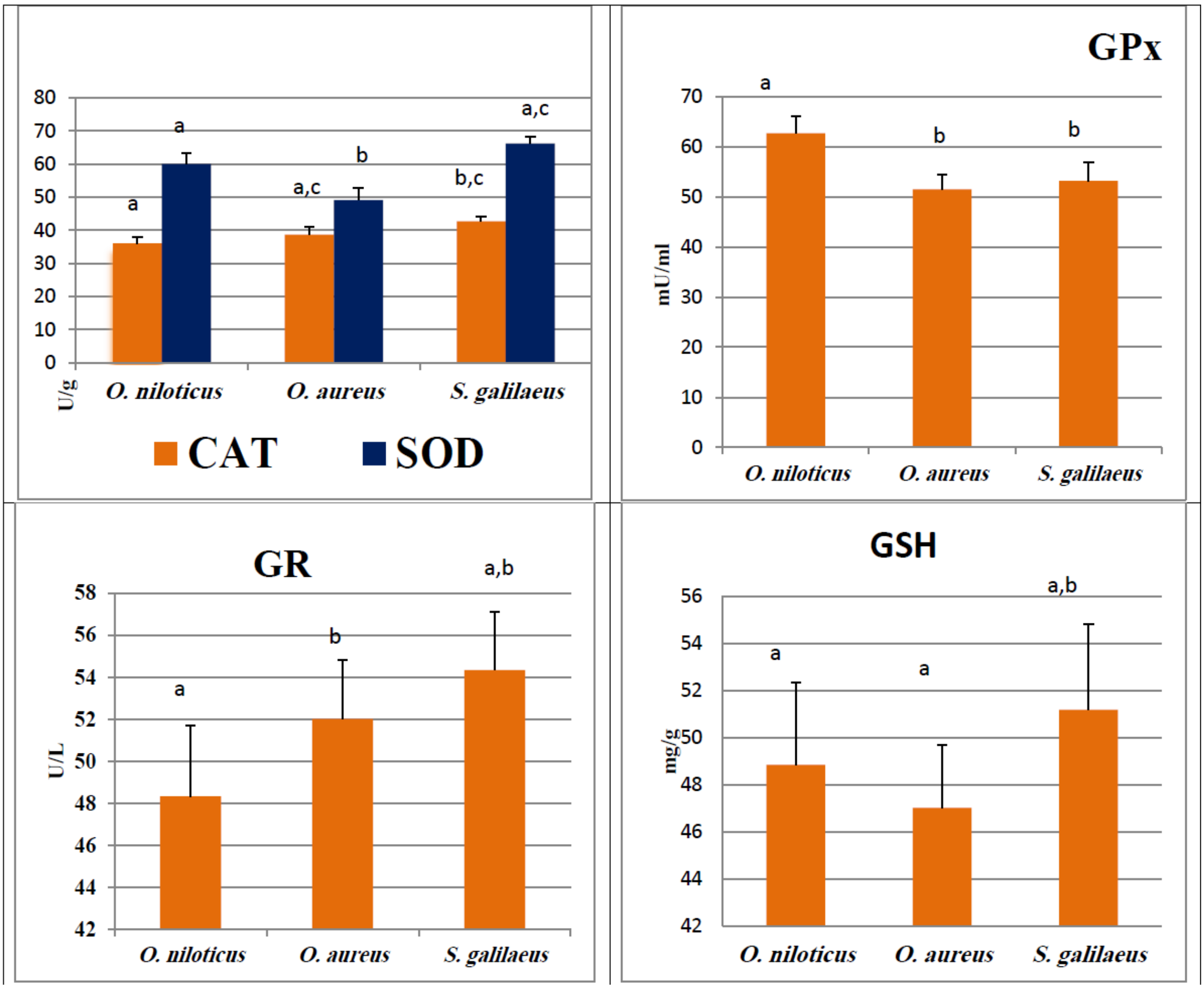


Fig. 4 Antioxidant activities in the tissues of liver of *O. niloticus, O. aureus* and *S. galilaeus* (n = 6). Different superscripts are statistically significant *p < 0.05.* Various superscripts have statistical significance (p < 0.05).

CONCLUSION

In this survey, the highest mean carbohydrate value was found in *O. aureus* > *S. galilaeus* >*O. niloticus*. Lipid content was found in fish species, *O. niloticus* > *O. aureus* >*galilaeus*. The mean protein revealed a non-significant difference (P> 0.05) in S. *galilaeus* > *O. aureus* > *O. niloticus*. The mean water content of three studied species of fish recorded *O. niloticus* > *S. galilaeus* >*O. aureus*. The largest ash concentration was in *S. galilaeus* >*O. aureus* > *O. niloticus*. In this study, *S. galilaeus* recorded the highest levels of antioxidant enzymes in the liver. This study recorded that all trace elements in *O. aureus* were consistently high, the results recorded that the highest concentration was for As in *O. aureus* that increased the safety values allowed by FAO/WHO, EOSQC, and the EU'. *S. galilaeus* was estimated the lowest Hg level. the results suggested that in a diet/day or week, the children should consume less than 18.51g/day or 129.62g/week *O. niloticus* muscle, 13.761g/day or 96.33 g/week *O. aureus* muscle, and 16.66g/day or 116.66 g/week *S. galilaeus* muscle. Youth, should take no more than 49.38g/day or 345.67g/week *O. niloticus* muscle, 36.69g/day or 256.88g/week *O. aureus* muscle, and 44.44g/day or 311.11g/week *S. galilaeus* muscle. The adults should consume less than 84.65g of *O. niloticus* muscle per day or 592.59g per week, 62.90g of *O. aureus* muscle per day or 440.36g per week, and 47.61g of *S. galilaeus* muscle per day or 333.33g per week.

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