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Research article

The health risk assessment of heavy metals to human health through the consumption of *Tilapia* spp and catfish caught from Lake Mariut, Egypt

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HIGHLIGHTS

- The levels of As, Cd, Pb, Hg and Al were measured in the flesh of *C. gariepinus, O. niloticus, O. aureus, and T. zillii* fish.
- Association between the potential risk of HMs exposure and the consumption of fish by adults, youth, and children was studied.
- Estimated dietary intakes, target hazard quotient, and the carcinogenic risk were used to assess the human health risk.

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ABSTRACT

This work assesses the concentration of As, Cd, Pb, Hg, and Al in three of Tilapia species and catfish caught from Lake Mariut. As well as the human health risk and muscle biochemical composition. Besides, the antioxidant responses of different species. The average metal concentration order was Pb> As> Cd> Al> Hg. The Cd and Pb levels in all species; besides, the Hg levels in *Oreochromis niloticus* and *Oreochromis aureus* exceeded the maximum limits set by FAO, European legislation, and FAO/WHO. The human health risk was assessed using estimated dietary intakes, the target hazard quotient (THQ), and the carcinogenic risk (CR). THQ_{As} was >1 in all examined fishes and closer to 1 in $THQ_{Cd,\ Hg}$. As well, CR level for As was higher than the permissible value. *Tilapia zillii* showed significant decreases in carbohydrates compared to *Clarias gariepinus*; also, ash content compared to *C. gariepinus, O. niloticus,* and *O. aureus*. Furthermore, water content compared to *O. aureus*. In contrast, significant increases of SOD in *O. niloticus* compared to *C. gariepinus*. In addition, CAT and GPx in *O. aureus* compared to *C. gariepinus*. Also, GR in both *O. niloticus* and *O. aureus* compared to *C. gariepinus,* and GSH in all of *Tilapia* spp. compared to *C. gariepinus*. Data obtained provide evidence of health risks to the consumers. Therefore, more caution is required.

1. Introduction

The aquatic systems' contamination with heavy metals as a result of natural anthropogenic sources has become a global issue (Ibemenuga, 2013). Because of the toxicity, persistence, and bioaccumulation of heavy metals through the food chains, they pose a potential risk to the ecological system and the human health (Salem et al. 2014). The study of heavy metals is momentous in three main aspects. Firstly, from the point of view of public health, whereas the attention was drawn to measuring the heavy metal accumulation that has serious health hazards to humans, such as As, Cd, Pb, and Hg. Such metals are toxic elements that possess no biological function and can possess carcinogenic effects. In addition, they may result in decreased mental health and cognitive development in children while in adults they can result in the rise of cardiovascular diseases; in addition, the renal and reproductive malfunction. Secondly, from the perspective of the aquatic environment to stop the biological deterioration and to determine the sources that endanger the ecological balance (Ashraf et al., 2012; Salam et al., 2019). Thirdly, from the economic point of view; whereas, this affects the fish quality, productivity, and fish marketability. Consequently, on the economy.

Fish represent the top of the aquatic food chain. In addition to their essential protein source fish have a rich content of vitamins, essential mineral compounds, and unsaturated fatty acids. Fish can normally accumulate heavy metals from water, food, and sediment (Sarah et al., 2019). The toxic heavy metal content in fish can counteract their valuable effects. Several adverse impacts of heavy metals have been known

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on human health for a long time, such as kidney failure, cardiovascular disease, liver damage, and even death (El-Moselhy et al., 2014).

Lake Mariut is one of Egypt's vital lakes, especially for the Alexandrian people. Besides, its significance for the nesting of wild birds and playing a crucial role in the Delta's western region water balance (Shreadah et al., 2020). Its mean production of fish species is about 4700 tonnes per annum. Catfish and the popular Tilapia fish represent a vital source of food for Egyptian people's consumption, and they constitute most of the Lake production (Talab et al., 2016; EEAA, 2021). The objective of the present research work is to (1) measure the concentrations of Arsenic, Cadmium, Lead, Mercury, and Aluminum in the muscle tissues of *C. gariepinus*, *O. niloticus*, *O. aureus,* and *T. zillii* compared to the maximum levels defined by FAO (1983), the regulation of the European Commission (EC, 2006), and FAO/WHO (1989); (2) to estimate the possible population health risk caused by the consumption of these fish species through the estimation of dietary daily and weekly intakes (EDI, EWI) of these metals compared to the standard provisional tolerable weekly intakes (PTWIs) and the tolerable weekly intakes (TWIs), along with the estimation of the target hazard quotient (THQ) and the carcinogenic risk (CR) for these metals; (3) to estimate the flesh biochemical composition of these fish species caught from polluted Lake Mariut and the physiological responses of the antioxidants as biomarkers of pollution in Lake Mariut.

2. Materials and methods

2.1. Study area

Lake Mariut is sited at the southern of Alexandria city, and it extends for almost 20 km between 31° 01′ 48″ and 31° 10′ 30″ N, 29° 49′ 48, and 29° 57′ 00″ E. It is considered as one of Egypt's main Delta Lakes fisheries, and it has been suffered for decades from the continuous discharge of different pollutants from the city of Alexandria and nearby lands; especially, heavy metals. Consequently, these circumstances resulted in an ample decline in its water quality, especially in the Lake Mariut main basin (LMMB). There were three main water resources used to feed the Lake. Firstly, Qalaa drain (agricultural drain mixed with the effluents of the east wastewater treatment plant (EWWTP), which is estimated with 700,000 m3/day). Secondly, the Umum drain (the main agricultural drain lining LMMB at the west side). Thirdly, the west wastewater treatment plant (WWWTP) with a load of raw industrial wastewater estimated with 300,000 m^3/day. However, recently the Qalaa drain was

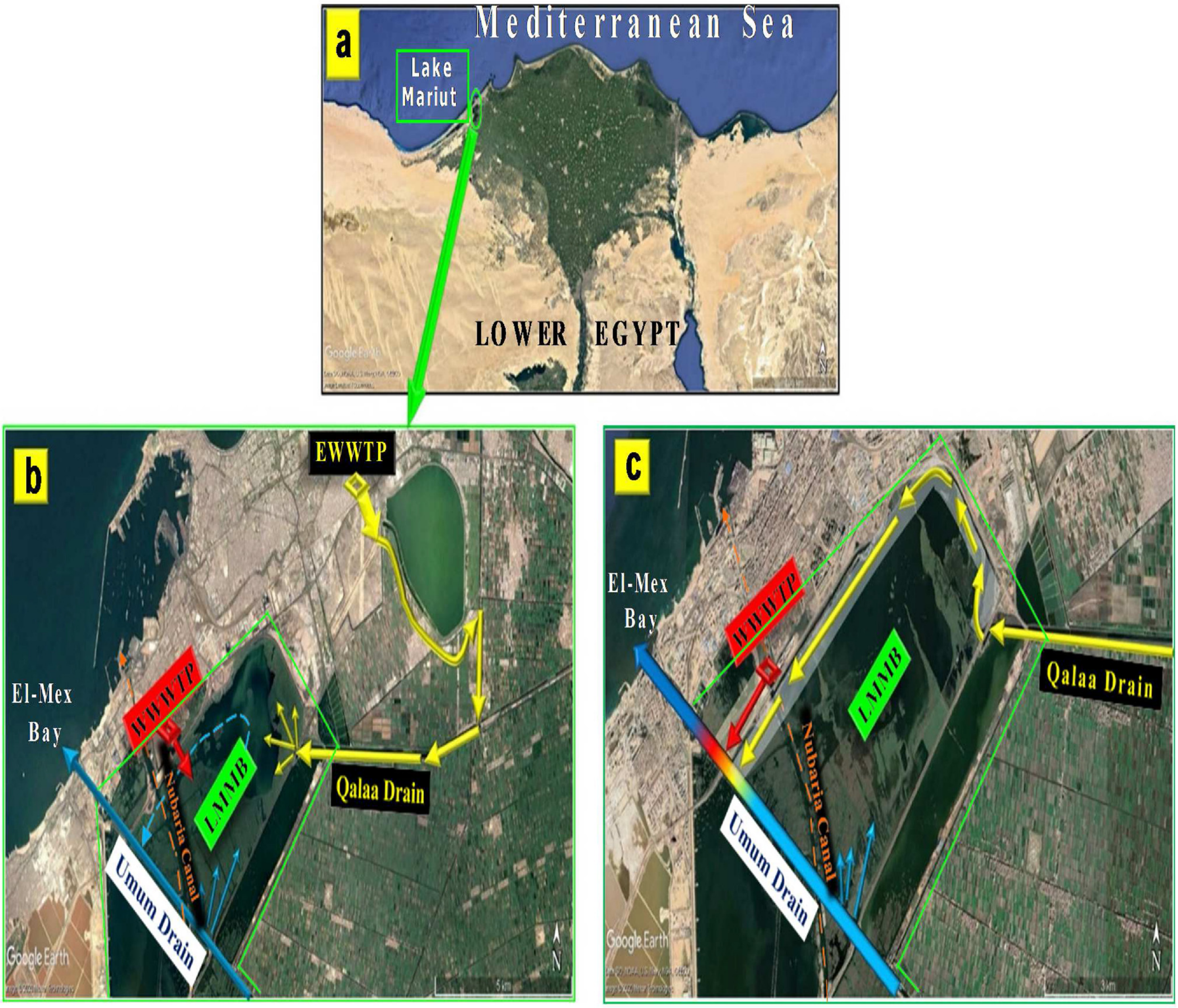

Figure 1. Lake Mariut showing the sampling site (the main fishery basin LMMB) and the sources of effluents; b before and c after the diversion of the Qalaa Drain and WWWTP effluents (After Shreadah et al., 2020).

diverted from the LMMB water body to the north and east coast of Lake Mariut. Moreover, the WWWTP effluents were diverted to be drained into a new canal north of Lake Mariut (Shreadah et al., 2020). The Lake was suffering from intense pollution resulting in the Lake's conversion from the best fertile and productive aquatic habitat in Egypt during the 1960s to the most polluted one. Besides, the remarkable extreme reduction in the quality and the quantity of the fish catchment. Accordingly, this led to great social and economic problems (El-Rayis et al., 2019). Lake Mariut is considered the Mediterranean Sea's principal source of pollution via the pumping station of El Max (Gaballah et al., 2020).

2.2. Collection of fish samples and tissue preparation

Oreochromis niloticus, *Oreochromis aureus*, *Tilapia zillii*, and African catfish *Clarias gariepinus* species were caught from the main basin of Lake Mariut (Figure 1) during (2017–2018) years. Samples were taken to the laboratory in an ice-cold box, frozen at -20 °C, and stored until analysis. The frozen samples were left to defrost, then rinsed in deionized water, and the skin was carefully and quickly peeled off. After that, fish were dissected on ice, the white dorsal muscles, liver parts were then excised, rinsed in isotonic saline of NaCl, and weighed. 10% w/v homogenate of white dorsal muscle and liver tissue was prepared by weighing 1g of the tissue, then homogenized in 9 volumes of ice-cold (0.1M) phosphate buffer (pH 7.4) using an electrical homogenizer (USA). The homogenate was then centrifuged at 6,000 rpm for 15 min at 4 °C using a cooling centrifuge (Hettich, Germany). The resultant supernatant was used for the determination of muscle biochemical composition and the antioxidant activities using a UV/vis spectrophotometer (JENWAY 6505, UK).

2.3. Determination of muscle biochemical composition

The total protein concentration was determined in the muscle supernatant following (Gornall et al., 1949) method. 20 μl of tissue supernatant or standard albumin were added to 1ml of Biuret reagent (Sodium hydroxide 0.2 N, $EDTA_2$ 18 mmol/l and Cupric sulfate 12 mmol/l). The mixture was gently mixed and incubated at 20–25 °C for 5 min. The blank was prepared as 1 ml of Biuret reagent. The absorbance of both sample and standard was measured spectrophotometrically at 546 nm against the blank. Total lipids were determined by a colorimetric method described by Zollner et al. (1962). 25 μl of the supernatant or standard were added to 1 ml sulfuric acid conc. The mixture was gently mixed well, the tubes covered with glass beads, and left in a boiling water bath for 10 min. Then cooled and pipetted into dry test tubes where 50 μl of sample or standard were added to 1.5 ml of color reagent (phosphoric acid 14 mol/l, vaniline 10 mmol/l and sulfuric acid conc). The blank was prepared as 50 μl of sulfuric acid conc added to 1.5 ml color reagent. The mixture was gently mixed and left at room temp for 30 min in the dark then poured into dry cuvettes. The absorbance of sample and standard was measured spectrophotometrically against a reagent blank at 545nm. Total carbohydrates were determined following the method described by Dubois et al. (1956). 2 ml of a carbohydrate solution were mixed with 1 ml of 5% aqueous solution of phenol. Subsequently, 5 ml of concentrated sulfuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and left for 20 min in a water bath at room temperature for color development. Then, the absorption was recorded on a spectrophotometer at 490 nm.

Water (moisture) and ash contents were determined according to AOAC (1995). The moisture was determined based on drying the samples in an oven at 135 °C for two hours, then cooling the samples in a desiccator, and weighing them again. The weight loss was divided by the original weight of wet samples and then converted to the percentage. Ash content was determined based on igniting of the pre-dried samples used for moisture analysis in a muffle furnace at 610 °C for 3 h until white ash is obtained then the ultimate weight was divided by the initial one and converted to a percentage.

2.4. Determination of antioxidant activities in the liver tissue

The SOD activity in the liver tissue supernatant was assayed according to Paoletti and Mocali (1990) by measuring the inhibition of NADH oxidation by β- mercaptoethanol in the existence of EDTA and Mn as a substrate. NADH solution was made fresh daily, and the assays were run by adding sequentially to the cuvette: 0.8 ml of 50 mM phosphate buffer (pH 7.4), 55 μl EDTA/Mn solution of 100/50 mM 40μ NADH solution of 7.5 mM, and a different volume of homogenate. The reaction is then initiated by adding 100 μl β-mercaptoethanol solution. The changes in ΔE of NADH per minute were measured spectrophotometrically at 340 nm following (Ex = 6.22/mM/1cm). One unit of SOD activity is defined as the amount of cell extracted required to inhibit the rate of NADH oxidation of the control by 50%. The specific activity was expressed as unit per gram of wet weight tissue. Catalase activity was estimated in the liver supernatant following the method of Aebi (1984) whereas it reacts with a known quantity of hydrogen peroxide (H_2O_2) in a reaction which is stopped after 1 min via catalase inhibitor. In the peroxidase existence and the remaining H_2O_2 it reacts with 4-aminophenazone and 3,5-Dichloro-2-hydroxybenzene sulfonic acid to form a chromophore with a color intensity which is inversely proportional to the catalase amount in the sample. The absorbance was then measured at 510 nm. The activity of GPx was estimated according to Paglia and Valentine (1967). To 1 ml of reaction mixture of 50 Mm sodium phosphate buffer (pH 7.4), 4 mM sodium azide, 2 units of GR, 1 mM GSH, and 0.25 mM NADPH, add 10 μl sample and 10 μl H_2O_2 (0.2 Mm H_2O_2 in phosphate buffer (PH 7.4). The activity is then measured following the ΔE changes per minute (Ex = 6.22/mM/1 cm at 340 nm). Glutathione reductase (GR) was assayed according to Smith et al. (1988) method by adding 10 μl of sample to 1 ml of a reaction mixture containing 50 mM phosphate buffer (pH 7.4), 0.15 mM NADPH, and 2 mM EDTA. The reaction starts with the addition of 10 μl of 1 mM oxidized glutathione (GSSG). The specific activities were determined following the ΔE changes per minute at 340 nm (Ex = 6.22/mM/1cm). Reduced glutathione (GSH) was assayed using the spectrophotometric/microplate reader method involved GSH oxidation by the sulfhydryl reagent 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) to the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB) at 412 nm according to Rahman et al. (2007).

2.5. Determination of heavy metal concentration and method validation

Heavy metals' total concentrations were estimated in fish white dorsal muscles. 1 g of the tissue samples was digested with a mixture of concentrated HNO_3 and $HClO_4$ in the ratio of 2:1 in a 100 ml tube. The samples were allowed to stay overnight in a water bath at 53 °C until the complete digestion, then cooled at room temperature, diluted with deionized water, and filtered into 50 mL flask. Finally, further deionized water was added to make up the volume and the filtrate was used for the analysis of As, Cd, Pb, Hg, and Al using (PerkinElmer Atomic Absorption Spectrometer AAS Perkin Elma, Pinnacle 900T, USA). All determinations were carried out in triplicate. Concentrations are expressed in μg/g and reported based on the wet weight to provide a more accurate measure of metals. Additionally, blank and standard solutions were also prepared for the calibration curves according to the concentrations recommended by AOAC Official Method 2015.01. The calibration curve was used to quantify the metal contents in the targeted fish species. A certified reference material CRM (DORM-2) for trace metals was analyzed in order to check the precision, accuracy, and uncertainty of the metal results. The results of CRM indicated that the determined levels of heavy metals are within the range of certified values and the recovery of studied metals was over 97%. All reagents used were of analytical grade, and to avoid

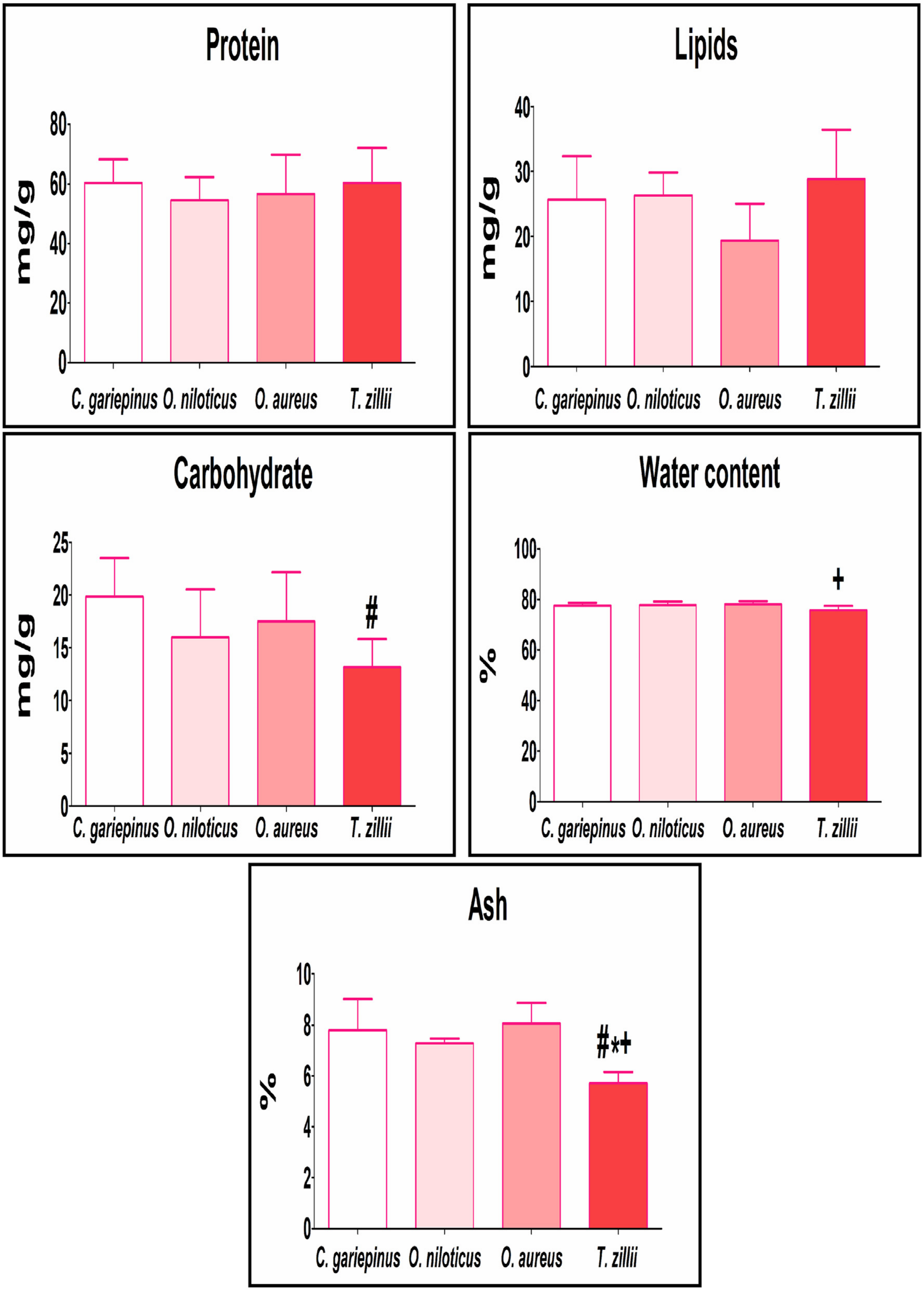

Figure 2. Changes in the muscle biochemical composition of *C. gariepinus* and Tilapia species (*O. niloticus*, *O. aureus, T. zillii*) caught from Lake Mariut. Data represent mean ± SD. Significant at ($p < 0.05$). $n = 6$. # represents a significant difference between *C. gariepinus* vs *T. zillii*, * represents a significant difference between *O. niloticus* vs *T. zillii*, and + represents a significant difference between *O. aureus* vs *T. zillii.*

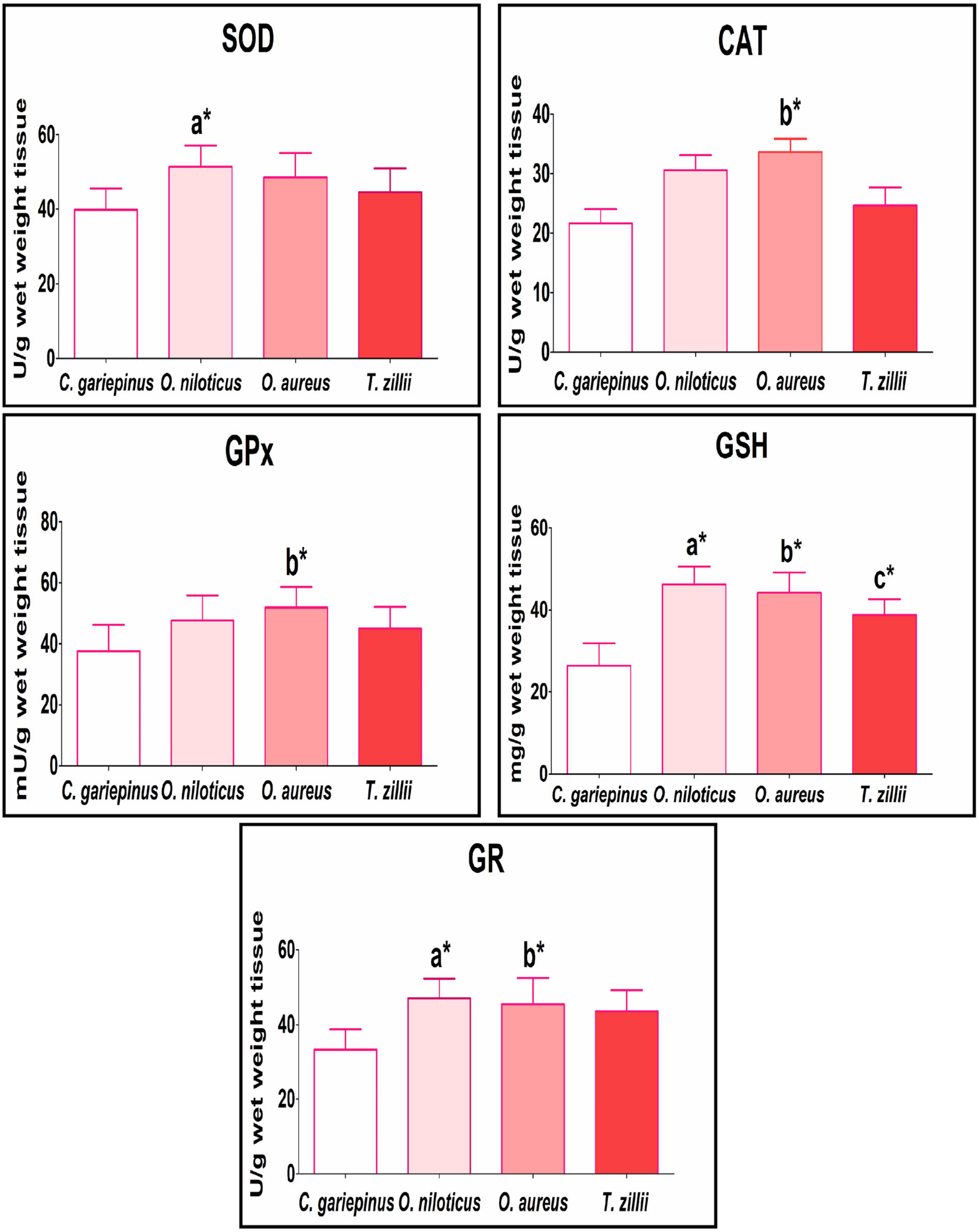

Figure 3. Antioxidant activities in the liver of *C. gariepinus* and Tilapia species (*O. niloticus, O. aureus, T. zillii*) caught from Lake Mariut. Data represent mean ± SD. * Significant at ($p < 0.05$). $n = 5$. a represents a significant difference between *C. gariepinus* vs *O. niloticus*, b represents a significant difference between *C. gariepinus* vs *O. aureus*, and c represents a significant difference between *C. gariepinus vs T. zillii*.

Table 1. Mean heavy metal concentrations (μg/g wet weight) in the muscle tissue of fish species in comparison with the international maximum permissible levels (MPLs), and the MPI.

Fish species	As	Cd	Pb	Hg	Al	(MPI)
C. gariepinus	1.59 ± 0.39	1.14 ± 0.18	1.38 ± 0.23	0.46 ± 0.13	0.70 ± 0.18	0.958
O. niloticus	1.09 ± 0.16	1.00 ± 0.24	1.74 ± 0.34	0.54 ± 0.13	0.64 ± 0.23	0.919
O. aureus	1.20 ± 0.39	0.93 ± 0.32	1.36 ± 0.27	0.57 ± 0.17	0.52 ± 0.16	0.852
T. zillii	1.36 ± 0.27	1.04 ± 0.17	1.33 ± 0.13	0.39 ± 0.15	0.70 ± 0.13	0.875
FAO MLs (1983)	-	0.05	0. 5	0.5	-	
EC MLs (2006)	-	0.05	0.3	0.5	-	
FAO/WHO MLs (1989)	-	0.1	0.5	0.5	-	

Values represent mean ± SD.

metal contamination, all glassware was soaked in 10% HNO_3 then rinsed with distilled water before usage.

2.6. Metal pollution index (MPI)

MPI was used to compare the total level of metal accumulation in various fish tissues as described by Usero et al. (1997) using the next equation:

$$\mathbf{MPI} = (M_1 \times M_2 \times M_3 \times \ldots\ldots\ldots \times Mn)^{1/n} \quad (1)$$

Mn is the metal concentration (μg/g w wt), and n is the number of studied metals.

2.7. Health risk assessment for fish consumption

The estimated dietary intakes (EDI and EWI) for daily and weekly intakes, the target hazard quotient (THQ), and the carcinogenic risk (CR) were used to evaluate the risk to the health of people consuming fish from Lake Mariut. The estimated dietary intakes were compared with the standard provisional tolerable weekly intakes (PTWIs) and the tolerable weekly intakes (TWIs) previously recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) respectively.

2.7.1. Determination of metal estimated dietary intakes (EDI and EWI)

The calculation of estimated daily intake (EDI) (μg/kg bw/day) for Egyptian people followed the next equation:-

$$(\mathbf{EDI}) = (MC \text{ x } DI) \text{ x } BW^{-1} \quad (2)$$

Where MC is the metal concentration in fish muscle (μg/g wet weight), DI is the daily average intake of fish (59.29 g/person/day) for the Egyptian population (GAFRD, 2016), and BW is the average body weight (70 kg for an adult, approximately 40 kg for the young (youth), and children approximately 15 kg). **The calculation of estimated weekly intake (EWI)** (μg/kg bw/week) followed the next equation:-

$$(\mathbf{EWI}) = EDI \times 7 \text{ days} \quad (3)$$

2.7.2. Determination of the non-carcinogenic risk

The Target Hazard Quotient (THQ) is a type of non-carcinogenic potential health risk assessment method. It was determined following the next equation (USEPA, 1989).

$$\mathbf{THQ} = (EF \times ED \times FIR \times MC) / (RFD \times BW \times AT) \times 10^{-3} \quad (4)$$

Where, EF is the exposure frequency of the population (365 day/year); ED is the exposure duration (30 years); FIR is the fish ingestion rate (59.29 g/day) (GAFRD, 2016). MC is the heavy metal concentration in the muscle tissue (μg/g), RFD represents the oral reference dose (μg/g/day) ($Pb = 4 \times 10^{-3}$, $Cd = 1 \times 10^{-3}$, $As = 0.3 \times 10^{-3}$, $Hg = 0.5 \times 10^{-3}$ and $Al = 1$) (USEPA, 2010; Jooste et al., 2014; Silva et al., 2020). BW is the average body weight (an adult: 70 kg); AT is the average time of non-carcinogenic exposure (365 day/year×ED). A THQ value of less than 1 implies that no evident risk will arise from consuming fish (Salam et al., 2019).

2.7.3. Carcinogenic risk assessment

The carcinogenic risk (CR) is an indicative of potential growth in the incidence of cancer for an individual over a lifetime resulted from potential carcinogenic exposure. The carcinogenic risk was estimated using the cancer slope factors (CSF) of 1.5 $(mg/kg/day)^{-1}$ for As and 0.0085 $(mg/kg/day)^{-1}$ for Pb, set by USEPA (Ullah et al., 2017) as the following:

$$\mathbf{CR} = CSF \times EDI \quad (5)$$

2.8. Statistical analysis

Data analysis was carried out by (one-way ANOVA), then the multiple comparisons of Tukey's test using (GraphPad Prism5.01 program, San Diego, CA, USA).

3. Results

3.1. Muscle biochemical composition

Biochemical composition (major nutrient composition) of the white muscles was presented in Figure 2. As shown, non-significant differences ($P > 0.05$) were revealed in the total protein and total lipid values among Tilapia species and catfish, and each other. In contrast, values of total carbohydrate demonstrated a significant decrease ($P < 0.05$) in *T. zillii* compared to *C. gariepinus*. Also, there was a significant decline ($P < 0.05$) of the ash content in *T. zillii* compared to *C. gariepinus, O. niloticus,* and *O. aureus*. Also, a significant decline ($P < 0.05$) of water content was noticed in *T. zillii* compared to *O. aureus*. Results from the current study indicated that the body composition varied significantly among the various fish species in Lake Mariut. Moreover, the body composition even of the same species may change in response to differences in water quality, maturity state, feeding conditions, size, environmental conditions, sex, and the period during which the organism was captured.

3.2. Antioxidant activities

Enzymatic and non-enzymatic antioxidants were presented in Figure 3. The present study revealed that there was a significant rise ($P < 0.05$) of SOD level in *O. niloticus* compared to *C. gariepinus*. Also, a significant increase ($P < 0.05$) of CAT and GPx levels in *O. aureus* compared to *C. gariepinus* was noticed. In addition, the hepatic GR showed a significant rise ($P < 0.05$) in both *O. niloticus* and *O. aureus* compared to *C. gariepinus*. On the other hand, the GSH levels exhibited a significant rise ($P < 0.05$) in the three Tilapia species compared to the catfish

Table 2. The amount of EDI, EWI (μg/kg/ bw/d/w), CR, and the Maximum Permissible Levels of PTWIs and TWIs for As, Cd, Pb, Hg, and Al set by JECFA, EFSA, and THQ.

Metal	Species	Average daily intake (μg/person/day)	Consumption for Adult			Consumption for Youth			Consumption for Child			PTWIs set by JECFA μg/kg/bw/w	TWIs set by EFSA μg/kg/bw/w	THQ
			EDI	EWI	CR	EDI	EWI	CR	EDI	EWI	CR			
AS	*C. gariepinus*	94.27	1.35	9.43	2.0×10^{-3}	2.36	16.50*	3.5×10^{-3}	6.28	43.99*	9.4×10^{-3}	15 (As inorganic) Withdrawn (2010)	-	4.49
	O. niloticus	64.63	0.92	6.46	1.4×10^{-3}	1.62	11.31	2.4×10^{-3}	4.31	30.16*	6.5×10^{-3}			3.08
	O. aureus	71.15	1.02	7.11	1.5×10^{-3}	1.78	12.45	2.7×10^{-3}	4.74	33.20*	7.1×10^{-3}			3.39
	T. zillii	80.63	1.15	8.06	1.7×10^{-3}	2.02	14.11	3.0×10^{-3}	5.38	37.63*	8.1×10^{-3}			3.84
Cd	*C. gariepinus*	67.59	0.97	6.76**	-	1.69	11.83$^{\#}$	-	4.51	31.54$^{\#}$	-	7 withdrawn (2010)	2.5 (2011)	0.97
	O. niloticus	59.29	0.85	5.93**	-	1.48	10.38$^{\#}$	-	3.95	27.67$^{\#}$	-			0.85
	O. aureus	55.14	0.79	5.51**	-	1.38	9.65$^{\#}$	-	3.68	25.73$^{\#}$	-			0.79
	T. zillii	61.66	0.88	6.17**	-	1.54	10.79$^{\#}$	-	4.11	28.78$^{\#}$	-			0.88
Pb	*C. gariepinus*	81.82	1.17	8.18	9.9×10^{-6}	2.05	14.32	1.7×10^{-5}	5.45	38.18*	4.6×10^{-5}	25 Withdrawn (2010)	-	0.29
	O. niloticus	103.16	1.47	10.32	1.2×10^{-5}	2.58	18.05	2.2×10^{-5}	6.88	48.14*	5.8×10^{-5}			0.37
	O. aureus	80.63	1.15	8.06	9.8×10^{-6}	2.02	14.11	1.7×10^{-5}	5.38	37.63*	4.6×10^{-5}			0.29
	T. zillii	78.86	1.13	7.89	9.6×10^{-6}	1.97	13.80	1.7×10^{-5}	5.26	36.80*	4.5×10^{-5}			0.28
Hg	*C. gariepinus*	27.27	0.39	2.73$^{\#}$	-	0.68	4.77$^{\#}$	-	1.82	12.73$^{\#}$	-	1.6 (MeHg) (2007)	1.3 (2012)	0.78
	O. niloticus	32.02	0.46	3.20$^{\#}$	-	0.80	5.60$^{\#}$	-	2.13	14.94$^{\#}$	-			0.91
	O. aureus	33.80	0.48	3.38$^{\#}$	-	0.84	5.91$^{\#}$	-	2.25	15.77$^{\#}$	-			0.97
	T. zillii	23.12	0.33	2.31$^{\#}$	-	0.58	4.05$^{\#}$	-	1.54	10.79$^{\#}$	-			0.66
Al	*C. gariepinus*	41.50	0.59	4.15	-	1.04	7.26	-	2.77	19.37	-	2000 (2011)	1000 (2008)	0.0006
	O. uniloticus	37.95	0.54	3.79	-	0.95	6.64	-	2.53	17.71	-			0.0005
	O. aureus	30.83	0.44	3.08	-	0.77	5.40	-	2.06	14.39	-			0.0004
	T. zillii	41.50	0.59	4.15	-	1.04	7.26	-	2.77	19.37	-			0.0005

*Values exceeding JECFA; **Values exceeding EFSA values; #Values exceeding JECFA and EFSA.

C. gariepinus. Therefore, the level of antioxidant enzymes has been extensively used as an early warning indicator of Lake Mariut pollution.

3.3. Heavy metal concentrations and metal pollution index

As listed in Table (1), non-significant differences were found ($P > 0.05$) among the different species of Tilapia and catfish *C. gariepinus* in the accumulation of As, Cd, Pb, Hg, and Al in the fish muscle tissues. In this study, the mean concentration of As in the muscle of different species of fish was in the range of (1.09–1.59) μg/g w wt. As concentration recorded in the four fish species was in the following order: *C. gariepinus* > *T. zillii* > *O. aureus* > *O. niloticus*. Concerning Cd, the mean concentration of its level recorded in the four studied fish species was within the range of (0.93–1.14). The Cd level of the different species of fish was in the following order: *C. gariepinus* > *T. zillii* > *O. niloticus* > *O. aureus*. The highest mean concentration of Pb appeared in *O. niloticus* > *C. gariepinus* > *O. aureus* > *T. zillii* with values were within the range of (1.33–1.74) μg/g w wt. Besides, the average concentration of Hg recorded in the four studied fish species was within the range of **(**0.39–0.57) μg/g w wt following this order: *O. aureus* > *O. niloticus* > *C. gariepinus* > *T. zillii.* On the other hand, the level of Al was within the range of (0.52–0.70) μg/g w wt and followed this order: *C. gariepinus* >*T. zillii* > *O. niloticus* > *O. aureus*. Consequently, the order of the average metal concentrations recorded in the studied fish muscles was as the following: Pb> As> Cd> Al> Hg. The highest concentration of these five metals appeared in *C. gariepinus* while the lowest accumulation of them appeared in *O. aureus.* This means that *C. gariepinus* has a relatively high heavy metal accumulation ability. The metal pollution index (MPI) revealed that *C. gariepinus* > *O. niloticus* > *T. zillii* > *O. aureus.*

3.4. Comparison of metal concentration in the targeted fish muscles with the international maximum permissible limits

As listed in (Table 1), the mean heavy metal concentrations in the muscle tissues of all studied fish species were compared with the maximum permissible limits (MPLs) for human consumption set by some international organizations. When we compared our results with these MPLs, we found that the mean concentrations of Cd and Pb in all fish species; besides, the Hg levels recorded in the muscle of both *O. niloticus* and *O. aureus* were higher than the MPLs (0.05 for Cd, 0.5 for Pb, and 0.5 for Hg) recommended by FAO (1983) and higher than the MPLs (0.05 for Cd, 0.3 for Pb, and 0.5 for Hg) set by regulation of the European Commission (EC, 2006). Furthermore, they were higher than the MPLs (0.1 for Cd, 0.5 for Pb, and 0.5 for Hg) as recommended by FAO/WHO (1989).

3.5. Health risk assessment for fish consumption

3.5.1. Estimated dietary intakes (μg/kg/body weight) from metals in comparison to standard PTWIs and TWIs proposed for these metals

As shown in Table (2). For As, the obtained EWIs values of this metal through *C. gariepinus* consumption by youth and children were higher than the standard PTWIs values set by JECFA. Also, regarding the three Tilapia species consumption by children, the EWIs values of As exceed the standard PTWIs values set by JECFA (15 μg/kg/bw/w). For Cd, EWIs values through consuming all fish species by adults exceed the TWIs proposed; however, EWIs of Cd through consuming the all fish species by both youth and children exceed the PTWIs and TWIs established by JECFA and EFSA (7 μg/kg/bw/w and 2.5 μg/kg/bw/w) respectively. Regarding Pb, EWIs values through all fish species consumption by children exceed the PTWIs values set by JECFA (25 μg/kg/bw/w). Furthermore, EWIs of Hg through all fish species consumption by all consumers exceed the standard PTWIs and TWIs established by JECFA and EFSA (1.6 μg/kg/bw/w, and 1.3 μg/kg/bw/w) respectively. While the EWIs of Al through all fish species consumption by all consumers do not exceed the standard PTWIs and TWIs established by JECFA (2000 μg/kg/bw/w) and EFSA (1000 μg/kg/bw/w).

3.5.2. Target hazard quotient (THQ)

The values of (THQ) for the five studied metals are presented in Table (2), calculation results of THQ for Cd, Pb, Hg, and Al are less than 1, which means that there are no health risks for people if they only intake a single heavy metal via consumption of the three Tilapia species and catfish. In contrast, the THQ value for As ranged between (3.08 and 4.49) which reveals that the consumption of these species may have health risks associated with As.

3.5.3. Carcinogenic risk (CR)

The CR levels for As and Pb are presented in Table (2). In the present study, the CR level for As in the targeted four fish species is higher than 10^{-4} (Table 2).

4. Discussion

4.1. Biochemical composition

The evaluation of fish flesh biochemical composition is momentous to confirm that fish tissues have hygienic safe qualities, in line with national, and international standards. Also, it is considered a good index for quality, physiological state, and the habitat of fish (Haredi et al., 2020). As observed in Figure 2. Non-significant differences were noted in the muscle protein and lipid content among all studied four species. Also, there was no significant change in the water content of the three Tilapia species compared to *C. gariepinus* while a significant decline ($P < 0.05$) in its level was observed in *T. zillii* compared to *O. aureus*. The water content of the fish white muscles was in the range of (75.67–78.00) % on a wet basis. These values were closer to the levels (75.34–76.74%) reported by Ajeeshkumar et al. (2015) in five selected marine fish from Mannar Gulf, India. The current research work revealed a significant decrease ($P < 0.05$) in the carbohydrate level of *T. zillii* compared to *C. gariepinus*. The carbohydrate level differs from species to species mainly because of the food availability, species feeding habitat, and the seasonal distribution (Vijayakumar et al., 2014). The results of the current work revealed that there was a significant decline ($P < 0.05$) in the ash level of *T. zillii* compared to the catfish *C. gariepinus*, and both of Nile Tilapia *O. niloticus,* and *O. aureus*. The lowest level of ash content recorded in *T. zillii* fish refers to its low mineral content, while the high levels recorded in the other species refer to its high mineral content (Ayanda et al., 2019). The total ash content in the studied fish muscles was in the range of (5.72–8.05%). These levels were closer to the levels (2.91–7.56%) reported by Ayanda et al. (2019). On the other hand, these values were lower than the values (1.46–1.93%) reported by Ajeeshkumar et al. (2015).

4.2. Enzymatic and non- enzymatic antioxidants

SOD, CAT, GPx, GR, and GSH are used as reactive oxygen species (ROS) biomarkers mediating contaminant exposure. When fish exposed to contaminants, they elicit ROS production like the superoxide anion, hydrogen peroxide, and hydroxyl radical. As the levels of ROS increase, the biological system establishes the first line of defence mechanisms through modulating antioxidant activities. These antioxidants act cooperatively or synergistically to defend organisms against the toxic influences of reactive oxygen species, tissue-specific damage, and help to keep the cellular homeostasis by removing ROS (EL-Gazzar et al., 2014; Haredi, 2018). As shown in Figure 3, the present work revealed a significant increase ($P < 0.05$) in the activity of SOD in *O. niloticus* compared to *C. gariepinus* while there was an insignificant rise ($P > 0.05$) in its level in the two other species of Tilapia when compared to the catfish *C. gariepinus.* The rise in the level of SOD could be a physiological adaptation to eradicate the generation of ROS (Haredi, 2018). Mahboob et al. (2014) observed a significant elevation in the SOD level in the liver and kidney of *O. niloticus* caught from Wadi Namar compared to a control site in Saudi Arabia.

The CAT level demonstrated a significant elevation ($P < 0.05$) in *O. aureus* compared to *C. gariepinus* while there was an insignificant rise ($P > 0.05$) in its level in the two other species of Tilapia, when compared to *C. gariepinus*. This rise in the CAT level may be a result of an efficient antioxidant defence mechanism that works against the oxidative stress caused by the exposure to metals and/or compensating for the reduction in the other antioxidant enzymes such as GPx. EL-Gazzar et al. (2014) reported an increase in the CAT activity in Cd exposed Nile Tilapia fish at 21 and 42 days of exposure.

The present research demonstrated a significant elevation ($P < 0.05$) of hepatic GPx level in *O. aureus* compared to *C. gariepinus* while there was an insignificant rise ($P > 0.05$) in its level in the two other species of Tilapia, when compared to *C. gariepinus*. This rise in its level indicates the high hydroperoxides levels in the fish liver. Also, GPx activities may be induced to withstand the toxicity of water pollutants, such as domestic, industrial, and agricultural sewages entering the Lake. This result agrees with Haredi (2018) who reported a significant increase in the hepatic GPx level of Nile Tilapia caught from Edku drain compared to fish of recovery group and El-Maadyah region of Lake Edku, Egypt. On the other hand, Haredi (2018) reported an insignificant increase in its level in El-Maadyah fish compared to fish of recovery group.

GR level showed a significant increase in both *O. niloticus* and *O. aureus* compared to *C. gariepinus* while showed an insignificant increase in *T. zilli* compared to *C. gariepinus*. This result disagrees with Alkaladi et al. (2013) who observed the decrease of GR level after 12 days of fish exposure to Zn.

The hepatic GSH activity demonstrated a significant increase in its level in the three Tilapia species compared to *C. gariepinus*. The high level of GSH protects the cellular proteins from oxidation through the redox cycle of GSH or by detoxifying the ROS directly produced by pollutant exposure (Haredi, 2018). This result agrees with Abdel-Moneim et al. (2013) who found a significant rise in the hepatic GSH of the Nile Tilapia caught from the northwest basin of Lake Mariut when compared to the reference site.

4.3. Heavy metal concentrations in the flesh of fish

In this study, no significant differences were found for each of As, Cd, Pb, Hg, and Al concentrations among all the fish species studied. The order of species relating to As accumulation is as follows: *C. gariepinus* > *T. zillii* > *O. aureus* > *O. niloticus*. For Cd and Al, they were in the following order: *C. gariepinus* > *T. zillii* > *O. niloticus* > *O. aureus.* For Pb, it follows the next order: *O. niloticus* > *C. gariepinus* > *O. aureus* > *T. zillii.* Concerning the Hg level, it follows the next order: *O. aureus* > *O. niloticus* > *C. gariepinus* > *T. zillii*. The highest accumulation level of As, Cd, and Al appeared in *C. gariepinus* **(**1.59 ± 0.39) μg/g, (1.14 ± 0.18) μg/g, and (0.70 ± 0.18) μg/g respectively. The highest concentration of Pb (1.74 ± 0.34) μg/g was found in *O. niloticus,* while the highest concentration of Hg (0.57 ± 0.17) μg/g was noticed in *O. aureus*. **MPI** revealed that *C. gariepinus* > *O. niloticus* > *T. zillii* > *O. aureus*. The metal accumulation in fish relies upon their intake and their elimination from the body (Abubakar and Adeshina, 2019).

In our study, the As accumulation ranges from (1.09–1.59) μg/g. Sallam et al. (2019) found that As values in the muscles of various species of fish collected from Lake Manzala, Egypt, ranged from (0.511–0.621) μg/g for As which seems to be lower than our As values. Lower As levels of (0.012–0.029) μg/g were also recorded in various species of fish from the Yangtze River, China (Sallam et al., 2019). This variation of As concentrations in the fish muscles depends on the place of capture that is widely affected by human activities (Zuluaga Rodríguez et al., 2015). Acute exposures of fish to As can lead to immediate death, while chronic exposure can lead to the accumulation of metalloid to toxic levels and various disease conditions. It may interfere with the fish's immune system by suppressing antibodies. Fish exposure to non-lethal arsenic levels in the short term can result in time-dependent and tissue-specific changes in T and B lymphocytes, making them vulnerable to infections. Besides, arsenic in zebrafish inhibited the synthesis of cytokines, such as TNFα, and IFN-γ; hence, compromising antiviral responses. Also, arsenicals induce several major families of stress proteins, including the heat shock proteins (hsps) both in vivo and in vitro in various organs and systems (Authman et al., 2015). The accumulation level of Cd detected in the present study ranges from (0.93–1.14) μg/g. This finding is very close to those recorded by Mensoor and Said (2018) in the muscles of *B. sharpeyi* and *B. xanthopterus* caught from different stations of the Tigris River in Baghdad, which ranged from (0.80–1.20) μg/g. In contrast, Sallam et al., (2019) reported lower Cd levels (0.006–0.024) μg/g wet weight. Also, the current values were higher than those recorded by Salam et al. (2019) in the flesh of four fish species gathered from Tok Bali Port, Kelantan, Malaysia, which ranged from (0.003–0.007) μg/g. Bilandžić et al. (2011) reported that acute exposure to high doses of cadmium can lead to severe respiratory irritation while prolonged exposure to cadmium may cause chronic pulmonary disease and testicular degeneration. Also, it may cause hyperactivity and learning difficulties in children (Wang and Du, 2013). In addition, fish exposed to cadmium showed blood constituent disorders as well as a differential blood count. Also, it had negative impacts on the Nile Tilapia meat quality, growth rate, and blood physiology. Moreover, Cd can alter the essential trace element metabolism by influencing their normal tissue distribution, such as Cu and Zn (Authman et al., 2015). Water pollution can result in fish contamination with heavy metals from anthropogenic, domestic, and industrial activities (Mensoor and Said, 2018). Our findings of Pb ranges from (1.33–1.74) μg/g are close to those reported by Mensoor and Said (2018), who estimated Pb levels of (1.05–1.10) μg/g. While our results were higher than those levels recorded in the flesh of fish caught from Lake Manzala, Egypt, which ranged from (0.635–0.704) μg/g (Sallam et al., 2019). Also, they are higher than those levels (0.001–0.024) μg/g observed by Salam et al. (2019). Exposure to Pb can lead to a broad range of health issues, such as coma, convulsions, and kidney failure (Bilandžić et al., 2011). Low levels of Pb contamination could result in some adverse effects on the health and reproduction of fish (Authman et al., 2015). The accumulation of Hg in this study ranges from (0.39–0.57) μg/g. These values are higher than those mentioned by Sallam et al. (2019) who found the levels of Hg ranged from (0.0145–0.045) μg/g and those Hg levels (0.0011–0.048) μg/g recorded in the fish collected from the River of Yangtze, China. In contrast, a significantly higher mean value of (3,154) μg/g of Hg was recorded in the fish collected from the Persian Gulf, Iran. Several studies elicit that high mercury exposure stimulates alterations in the central nervous system resulting in behavioural changes, irritability, fatigue, tremors, hearing and cognitive loss, headaches, hallucinations, and death. Mercury exposure, even at low doses, influences the endothelial and cardiovascular function such as inducting the hypertension in humans and animals (Azevedo et al., 2012). The toxicity of methylmercury (MeHg) is linked to nervous system damage in adults, the neurological development impairment in newborns, and children (Rice et al. 2014). The variation in heavy metal contents in the muscle tissues between various fish species may be due to the difference in fish habitats, metabolic capacity, environmental needs, fish age, and size. Besides, the variance in geography, feeding level and habits, and the metal capacity to bioaccumulate in the food chain. As well as the efficiency in the uptake of heavy metals by fish from contaminated water (Salam et al., 2019). Finally, the accumulation level of Al ranges from (0.52–0.70) μg/g. These values are lesser than those values (28.578–58.153) μg/g recorded by El-Sayed et al. (2011) in the muscle tissues of various fish species collected from Sharkia province, Egypt. The frequent and long-term consumption of fishes containing high Al levels represents a risk to human health. Al is toxic, especially in patients who have chronic renal failure. Also, Al accumulation is related to the neurodegenerative process in Alzheimer's disease. The physiological changes that are frequently noticed in various species of fish exposed to Al are primarily related to hematological, cardiovascular, iono-regulatory, respiratory, reproductive, endocrine, and metabolic disturbances; In addition, the gill damage (Authman, 2011).

4.4. Health risk assessment

Fish and fishery product consumption may constitute a significant route of human exposure to chemical pollutants like heavy metals (Micheline et al., 2019). Consequently, health risk to human health posed by heavy metals via the consumption of aquatic species is very essential to be assessed (Salam et al. 2019). The metal toxicity risk is defined by the metal concentration in the source and the amount of the source consumed (Gbogbo et al., 2018). Therefore, besides comparing the mean heavy metal concentrations with the maximum standards set by FAO, European legislation (EC), and FAO/WHO the daily and weekly intakes of metals through fish consumption were also determined and compared with the standard provisional tolerable weekly intakes (PTWIs), the tolerable weekly intakes (TWIs) recommended by (JECFA), and (EFSA) respectively. Also, the target hazard quotient and the carcinogenic risk were determined. Acceptable values of cadmium, lead, and mercury in the freshwater fish were established by FAO as the following: (Cd 0.05 μg/g, Pb 0.5 μg/g, and Hg 0.5 μg/g), regulation of the European Commission (EC) No. 1881/2006 (Cd 0.05 μg/g, Pb 0.3 μg/g, and Hg 0.5 μg/g); besides, FAO/WHO (Cd 0.1 μg/g, Pb 0.5 μg/g, and Hg 0.5 μg/g) as shown in Table (1). The current study showed that the average concentrations of cadmium and lead in the muscles of all fish species; besides, the levels of mercury in both *O. niloticus* and *O. aureus* exceed the maximum allowable limits (MLs) specified by FAO, EC, and FAO/WHO. On the other hand, the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA), and the European Food Safety Authority (EFSA) stipulated the (PTWIs), and (TWIs) respectively for As, Cd, Pb, Hg, and Al. For inorganic arsenic, the (PTWI) previously stipulated by JECFA (1988) (15 μg/kg body weight/week) was withdrawn later by JECFA (2010). Concerning cadmium, the EFSA stipulated and reconfirmed in (2009) and (2011), respectively a (TWI) of (2.5 μg/kg body weight/week), which substituted the previous (PTWI) of (7 μg/kg body weight/week) stipulated by JECFA (1988). A (PTWI) of (25μg/kg body weight/week) for the lead previously stipulated by (JECFA). Recently, EFSA (2010) concluded that this value was no longer appropriate and health-protective, and JECFA also confirmed this conclusion in 2010. A (PTWI) of (1.6 μg/kg body weight/week) previously established and reconfirmed by JECFA in 2003 and 2007, respectively, but the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) established a (TWI) of (1.3 μg/kg body weight/week) for (MeHg) (EFSA, 2012). JECFA (2011) stipulated a (PTWI) of (2000 μg/kg body weight/week) for Aluminum while EFSA (2008) stipulated a (TWI) of (1000 μg/kg body weight/week) for Al. The chemical form of As in fish is the key factor in defining the threats to human health. Arsenic forms differ greatly depending on the organism, geographic location, and the environment. The inorganic As form is more poisonous than the organic one (Silva et al., 2020). In fish As is generally occurs in non-toxic organic forms, and the toxic inorganic form of fish is generally less than 10% (Gbogbo et al., 2018). The current study revealed that the EWI of As through *C. gariepinus* consumption by youth and children was higher than the standard (PTWIs) of (JECFA). Also, the EWI of As through the consumption of the three Tilapia species by children was higher than the (PTWIs) of (JECFA) Table (2). When considering this result together with the THQ_{As} value, which is greater than 1, there is a probability of the occurrence of As-related adverse effects from the regular consumption of these four species from Lake Mariut. This result agrees with Gbogbo et al. (2018) who found that the EWI value of As in *C. nigrodigitatus* was higher than the (PTWIs). Regarding Cd and Hg, the health risks of exposure to cadmium are compounded by the relative incapacity of humans to excrete it. Also, several studies revealed that MeHg constitutes the major Hg form present in fish and that MeHg is the most poisonous form of Hg (Micheline et al., 2019; Silva et al., 2020). In the present study, assuming that Hg is in the form of MeHg the EWIs values for both Cd and Hg through all fish species consumption by all consumers are exceeding the (PTWIs) and (TWIs) established by JECFA and EFSA. For Pb (EWIs) values through all fish species consumption by children are exceeding the (PTWIs) values set by JECFA. Our results disagree with Zaza et al. (2015) found that the (EWIs) of Cd, Hg, and Pb through the consumption of fish and seafood products by the Italian consumers do not exceed the standard (PTWIs) and (TWIs) proposed by JEFCA and EFSA. Also, Salam et al. (2019) reported that the (EWIs) of Cd, Hg, and Pb via the consumption of Nile Tilapia, flathead grey mullet, and *C. gariepinus* fish caught from Manzala Lake were lower than the standard (PTWIs) and (TWIs) proposed by JEFCA and EFSA. Aluminium is a toxic metal and is widely exist in the diet. Al levels in seafood, vegetables, and/or fruit groups are higher than in other groups (Hardisson et al., 2017). Regarding Al EWIs values, through all fish species consumption by all of the consumers do not exceed the standard (PTWIs) and (TWIs) established by both JECFA and EFSA. The highest levels of Al were registered in both *C. gariepinus* and *T. zillii* with the value of 19.37 μg/kg body weight/week when comparing with the (PTWIs) of 2000 μg/kg body weight/week set by JECFA and the (TWIs) of 1000 μg/kg body weight/week set by EFSA for Al this suggests that the aluminium content in the flesh of the fish species tested in our study does not constitute a significant proportion of the human weekly intake. This result agreed with Rivas et al. (2014).

The calculation results in Table 2 revealed that the THQ for Cd, Pb, Hg, and Al were less than 1, indicating that there are no health risks for people if they only intake a single heavy metal via the consumption of the three Tilapia species and catfish. In contrast, the THQ_{As} value is greater than 1, indicating that the regular consumption of these four species from Lake Mariut is possible to result in the occurrence of As-related health risks to Egyptian people who consume these fish species. The THQ results in this study agree with Mehouel et al. (2019) for Cd and Pb which are less than 1, while contradicting with the THQ_{As} which is greater than 1 in our results. Bhupander and Mukherjee (2011) estimated THQ_{Hg} value of less than 1, which agreed with our results, and on the contrary to our results, they estimated THQ_{As} less than 1. Sallam et al. (2019) estimated the value of THQ_{As} was greater than 1 in Nile Tilapia, Grey mullet, and *C. gariepinus* fish caught from Manzala Lake. If the CR level is below 10^{-6}, it is considered as negligible, the CR value more than 10^{-4} is unacceptable, and the values lying in between 10^{-4} and 10^{-6} are considered as acceptable (Ullah et al., 2017). Since the CR level for As is higher than the permissible levels; therefore, there is a potential health risk of cancer for those people who are consuming the targeted four fish species. The CR results in this study disagree with Majlesi et al. (2018) for Pb which is higher than the permissible levels, but our results for As complying with Ullah et al. (2017).

5. Conclusion

Even though some fish species revealed significant differences in the body composition, all the four species were found relatively well adapted to the environment of Lake Mariut; in addition, the level of antioxidants has been extensively used as an early warning indicator of Lake pollution. According to the present findings the concentrations of Cd and Pb in all species of fish; besides, the levels of Hg measured in *O. niloticus* and *O. aureus* were higher than the MLs set by FAO, EC, and FAO/WHO. Moreover, we can deduce that the consumption of these four fish species caught from Lake Mariut may constitute a possible health risk for consumers. The children's group is the most exposed to health risk because the EWIs of As and Pb through the consumption of all fish species by children were higher than the PTWIs; as well, the EWI of As through *C. gariepinus* consumption by the youth group. Also, except for Al EWI, the EWIs of Cd and Hg via the consumption of all fish species by all consumers were higher than the standard PTWIs and TWIs set by (JECFA) and (EFSA). Additionally, the estimated THQ_{As} is greater than 1 in all examined fish species, and it is close to 1 in $THQ_{Cd,\ Hg}$. Also, the estimated CR levels for As in all fish species and for all consumers were higher than the permissible limits, raising concerns and indicating potential health risks. Therefore, more caution is required for the consumption of these fish species.

Declarations

Author contribution statement

Soha S. Hasanein: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed H. Mourad: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Afaf Mohamed M. Haredi: Performed the experiments; Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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