

## Is the consumption of *Tilapia zillii* and *Clarias gariepinus* from Edku Lake (Egypt) safe for humans: a physiological and biochemical study

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### ARTICLE INFO

#### Article History:

Received: April 5, 2021

Accepted: April 21, 2021

Online: April 30, 2021

#### Keywords:

Trace elements

*Tilapia zillii*

*Clarias gariepinus*

Edku Lake

Proximate body

Human risk assessment

### ABSTRACT

The Egyptian government seeks to develop the northern wetlands, including Edku Lake in El Beheira Governorate, to enhance the physiological functions of fish, and thus, improve their quality and promote their products. Therefore, this study was performed on the two cheapest fish species *Tilapia zillii* and *Clarias gariepinus* in the Egyptian market, by studying proximate body composition, antioxidant enzyme activity, and bioaccumulation of five trace elements. In this study, *T. zillii* showed the highest mean of carbohydrates ( $16.11 \pm 0.67$  mg/g), fats ( $24.21 \pm 0.93$  mg/g), ash ( $7.33 \pm 1.00$  %), and water content ( $78.33 \pm 1.85$  %) whereas, the highest mean of protein content was recorded in *C. gariepinus* ( $64.33 \pm 1.01$  mg/g). Antioxidants glutathione reduced level (GSH) ( $41.66 \pm 2.01$  mg/g) and catalase activity (CAT) ( $21.66 \pm 2.6$  U/g) demonstrated a significant decrease ( $p < 0.05$ ) in the liver of *C. gariepinus* than *T. zillii* ( $47.83 \pm 3.6$  mg/g and  $38.16 \pm 4.2$  U/g, respectively). Concerning trace element concentrations, the highest arsenic (As) concentration was recorded in tissues of *C. gariepinus* ( $1.54 \pm 0.08$  µg/g), whereas, the lowest mercury (Hg) concentration was recorded in the tissues of *T. zillii* ( $0.47 \pm 0.08$  µg/g). The concentrations of mercury (Hg), cadmium (Cd), and lead (Pb) in the two studied fish species exceeded the limits permitted by EOSQC, FAO/WHO, and the EU. This study recorded that the children should consume less than 16.12857 g/day or 112.90 g/week of *T. zillii* muscle and 13.27 g/day or 92.92 g/week of *C. gariepinus* muscle. Moreover, youth should consume less than 43.01 g/day or 301.7g/ week of *T. zillii* muscle and 35.39714 g/day or 247.78 g/week of *C. gariepinus* muscle. Finally, adults should consume less than 23.1 g/day and 161.7 g/week of *T. zillii* muscle, and 61.94286 g/day or 433.6 g/week of *C. gariepinus* muscle to maintain their safety based on PTWI recommended by FAO/WHO.

### INTRODUCTION

Unnatural quantities of trace elements such as Cd, Pb, Hg, As, and Al have been released to the aquatic environment via storm water and wastewater discharges following industrialization (Qin *et al.*, 2015). They are a global concern due to their potential hazard effect and concentrated capacity in the aquatic environment. Most coastal lagoons are in danger, suffering from excessive commercial, agricultural, and domestic wastewater stream discharge (UNEP 1999; Batvari *et al.*, 2013).

Edku Lake has the smallest area compared to Burullus and Manzalah Lakes, linked to the Mediterranean Sea via Bughaz El Meadia lake-sea connection (Fig. 1) (Shakweer 2006). According to EEAA (2017), the lake received vast amounts of drainage reached to  $1.738 \times 10^{12}$  m<sup>3</sup>/year, the primary source of toxic elements in aquatic ecosystems (Renieri *et al.*, 2014; EEAA 2017).

Fish considered a significant component of a balanced, healthy diet containing high-quality proteins, vitamins, and several other essential nutrients (**Pieniak *et al.*, 2010**). Moreover, fish is a major source of polyunsaturated omega 3 fatty acids (PUFAs) whose benefits reduce inflammatory diseases, coronary heart disease, cancer, arterial hypertension, and contributes to normal neurodevelopment in children (**Mozaffarian and Wu, 2011; Swanson *et al.*, 2012**). Furthermore, the American Heart Association has recommended fish consumption at least twice a week to achieve daily consumption of omega-3 fatty acids (**Kris-Etherton *et al.*, 2002**).

Tilapia and African catfish are commercially valuable fish because, in several African countries, including Egypt, they are considered a cheap food source (**Olmedo *et al.*, 2013**). Catfish and tilapia species contribute to over 80% of Lake Edku production (**GAFRD 2012**). These fish species seem to have been acclimatized to the prevailing conditions and to be able to survive, forming the main fish population in the lake.

In living organisms, the trace elements such as (Cd, Pb, Hg, As, and Al) have no known significant role; cause extreme toxic effects even at deficient concentrations to all living organisms in particular human health (**Sarmiento *et al.*, 2011**). Toxic effects occur when the mechanisms of metabolic, storage, detoxification, and excretory can no longer help counter uptake (**Obasohan *et al.*, 2008**). Eventually, lead to physiological and biochemical changes (**Olabanji and Oluyemi, 2014; Abdel-Kader and Mourad, 2020**).

Several researchers in the literature showed the toxicity of trace elements such as (Cd, Pb, Hg, Al, and As) on various fish species in the freshwater lakes such as *Oreochromis niloticus* fish from Lake Burullus, Egypt (Olabanji and Oluyemi 2014); freshwater fish *Labeo rohita* from Yellamallappa Chetty Karnataka (**Noor and Zutshi, 2016**); *O. niloticus* collected from different locations from Egypt such as Mansoura and Abassa (**El-Sappah *et al.*, 2012**); *C. gariepinus* fish from two basins at Lake Maryout, Egypt (**Abdel-Kader and Mourad, 2019a**), tilapia species and *C. gariepinus* from Burullus Lake-Egypt (**Abdel-Kader and Mourad, 2020**).

Proximate body composition is the analysis of water, fat, protein, and ash contents of fish (**Love 1970**). The biochemical profile in fish is a sensitive index of fish metabolism assessment under metallic stress. Several studies demonstrated that fish exposed to toxic elements such as Cd, Pb, Hg, As, and Al recorded low levels of proteins and lipids (**Sobha *et al.*, 2007; Selvam *et al.*, 2014; Ayanda *et al.*, 2018; Abdel-Kader and Mourad 2019b; Abdel-Kader and Mourad 2020**).

Toxic elements such as Cd, Pb, Hg, Al, and As reduce the activity of antioxidants, particularly which having the thiol group ( $\text{—SH}$ ). Toxic elements generate reactive oxygen species (ROS) such as superoxide radical ( $\text{O}_2^-$ ), hydroxyl radical ( $\text{HO}^-$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (**Romeo *et al.*, 2000; Atli and Canli, 2010**). Increased ROS production can destroy the defenses of the antioxidant of cells and result in a condition is known as “oxidative stress” and, eventually, death of cells (**Ercal *et al.*, 2001**). The antioxidant defense systems of fish tissues, particularly the liver, protect them against oxidative stress (**Basha and Rani, 2003; Atli and Canli, 2007**). Several researchers estimated the activity of the antioxidant enzymes after exposure to toxic elements such as Cd, Pb, Hg, Al, and As in the different fish species collected from different freshwater lakes (**Barata *et al.*, 2005; Monteiro *et al.*, 2010; Abdelazim *et al.*, 2018; Elarabany and Bahnasawy, 2019; Abdel-Kader and Mourad, 2019b; Abdel-Kader and Mourad, 2020**), they recorded a reduction in the activity of the antioxidant enzymes after Cd, Pb, Hg, As and Al exposure.



**Fig. 1** Location map of the study area

The objectives of this study were: (1) to determine the concentrations of five trace elements Cd, Pb, Hg, As, and Al accumulated in the muscles of *T. zillii* and *C. gariepinus* collected alive by the fishermen from Edku Lake, Beheira Governorate, Egypt, these concentrations were compared with available certified safety guidelines published by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO), European Community (EC), (2) to calculate the human risk assessment of intake of heavy metal at human health through consumption of the *T. zillii* and *C. gariepinus* muscles, (3) to check the proximate body composition in tissues samples *T. zillii* and *C. gariepinus* and, (4) finally, to determine liver antioxidants of *T. zillii* and *C. gariepinus*.



## MATERIALS AND METHODS

### Study site

Edku Lake is located 30 km northeast of Alexandria, one of the four coastal delta lakes linked to the Mediterranean Sea (Fig. 1), 30° 30' and 30° 23' E and 31° 10' and 31° 18' N. The area of the lake is 126 km<sup>2</sup>, a depth is 50-150 cm. via primary drains, El-Khairy plus Barseek. It receives huge quantities of drainage water. El-Khairy Drain's water sources come from three drains known as El-Bousely, Edku and Damanhour sub drains, residential, agricultural, as well as industrial waste transportation plus irrigation water from over 300 fish farms. Barseek drain carries farm drainage water to the lake (**Badr and Hussein, 2010**). The area has diminished from  $28.5 \times 10^3$  to approximately  $12 \times 10^3$  Fadden (**Okbah and El-Gohary, 2002**). Due to land reclamation, large areas of approximately 150 km<sup>2</sup> have vanished (**Badr and Hussein, 2010**).

### Fish sample collection

The medium-sized fish from different sites from the Edku Lake — Egypt of *T. zillii* and catfish *C. gariepinus* (six from each) were bought alive from different fishermen from different sites along the lake in spring 2018. Fish were collected in large aerated tanks filled with water from the lake and transported to the National Institute of Oceanography and Fisheries (NIOF), Physiology Laboratory. The fish rinsed with distilled water. Samples are dissected by a sterile knife on a clean glass surface and then divided into muscle and liver tissues. Within properly sterilized polythene bags, the split portions of each body are preserved. Bags were labeled and placed in the deep freezer at -25° C until various tests were carried out.

### Estimation of trace elements

Fish tissue was separated and digested separately with a concentrated HNO<sub>3</sub> and HClO<sub>4</sub> by the ratio of 3:1. In a (100 ml) test tube, the specimen 0.5 g plus 10 ml of conc. HNO<sub>3</sub> was warmed at 100, 150, 200 and 250 ° C on a hot dish for one and a half hours. Then added (HClO<sub>4</sub>) to it. Then poured (2) mL of (1 N nitric acid) and put it on a hot dish and heated the mixture to fully digest and be transparent. These digested samples were transported to volumetric flasks (50 mL) plus the volume was made up by adding deionized water. Specimens were filtered via a membrane filter (Type HV) of (0.45 µm) Millipore. This filtrate was examined for trace elements (Al, Cd, Pb, and As) following the method of **Andaleeb *et al.* (2008)** by using Atomic Absorption Spectrophotometer Analyst 400-Perkin Elmer (USA ). Hg was measured following the method of **Larry *et al.* (1993)**. Fish tissue (0.5 g) was digested then added 5 ml stannous chloride solution to reduce mercury to elemental form and then measured using Flameless Atomic Absorption Spectrophotometer equipped with mercury hydride system MHS -Cold Vapor Technique. Each sample was run in six replicates and the results were expressed in terms of µg/g wet weight (mean ± SE).

In determining trace element concentrations in fish samples, both samples and blanks were processed in the same way using the same reagents to minimize the error. The chemical reagents and acids were an analytical grade (E. Merck, Germany) and the double deionized water also used for the preparation of all solutions. The standard solutions for calibrations of each element were prepared by diluting stock solutions of 1000 mg/l. The glass objects were soaked and rinsed for 24h, together with a 0.5% KMNO<sub>4</sub> solution (w/v) and 10% of the nitric acid and washed into distilled water.

### Estimation of risk assessment

The estimated weekly intake (EWI) of trace element through human consumption was compared with the current provisional tolerable weekly intakes (PTWI) for Hg, Al, Cd, Pb, and As (FAO/WHO 2004).

If the estimated value of EWI was lower than PTWI, this means that food consumption does not pose a significant health risk to the exposed population

According to General Authority for Fishery Resources Development, Ministry of Agriculture and Land Reclamation, Egypt, **GAFRD (2017)**, and Central Agency for Public Mobilization and Statistics (**CAMPAS, 2017**), the annual share per capita/Kg was 22.72 Kg/ year.

$$\text{Estimated daily intake (EDI)} = C \times \text{mean fish intake (g /day/capita)}.$$

Where C is the concentration of trace element in fish sample (µg/g wet weight)

$$\text{Fish consumption / day/capita} = 22.72 \times 1000 \div 365 = 62.25 \text{ g /day}$$

$$\text{Estimated weekly intake (EWI)} = \text{EDI} \times 7 \text{ days/week}$$

$$\text{EWI per body weight (kg)} = \text{EWI} \div \text{reference consumer body weight}$$

(Assuming the body weight of an adult is 70 kg, the young person is 40 kg and the child is 15 kg (Salas *et al.*, 1985; Albering *et al.*, 1999).

The percentage of trace elements intake to PTWI is calculated according to the following equation:

$$\% \text{PTWI} = (\text{EWI} \div \text{PTWI}) \times 100$$

Where, %PTWI is the percentage of provisional tolerable weekly intakes and can be calculated for each trace elements by the well-known weekly safety reference dose (PTWI) set by the joint (FAO/WHO 2004)

Maximum daily intake MDI (in grams) = is the estimated safety weekly intake of fishes (in grams) that children, young people and adult should consume less than it to attain the PTWI according to **FAO/WHO (2004)**.

$$\text{MDI} = \text{PTWI} \times \text{BW} \div C \times 7$$

Where MDI is maximum daily intake; C is the element concentration (µg/g)

$$\text{MWI} = \text{PTWI} \times \text{BW} / C$$

Where MWI is maximum weekly intake; C is the element concentration (µg/g)

### Estimation of proximate body composition

In a glass homogenizer in 5ml saline, a specimen of 0.1 g of muscle was homogenized for 3 minutes, and then centrifuged for 10 minutes at 3000 rpm. The supernatant used to assess total protein content (**Lowry *et al.*, 1951**); total lipid content calculated by **Henry *et al.* (1974)**, and the method of **Kemp *et al.* (1954)** was used to assess total carbohydrates in tissues. The moisture content after drying at  $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$  was considered as losses in sample mass (1 g). To assess the ash content, the residual mass was heated to  $600^{\circ}\text{C} \pm 10^{\circ}\text{C}$  (**AOAC, 2002**).

### Estimation of activity of antioxidant enzyme

The superoxide dismutase (SOD) (EC 1.15.1.1) activity was calculated by the enzyme's ability to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye at 550 nm for 5 minutes (**Nishikimi *et al.*, 1972**). The SOD activity is expressed as (U/g). GSH level (EC 1.8.1.7) was documented using the methods of **Beutler *et al.* (1963)**. The reduced chromogene can be estimated at 405 nm in direct proportion to the concentration of GSH, expressed as (mg/g). Glutathione peroxidase (GPx) (EC 1.11.1.9) activity measured using a substrate  $\text{H}_2\text{O}_2$  via the method of **Paglia and Valentine (1967)**, the reaction monitored indirect way at 240 nm as the rate of oxidation of NADPH to  $\text{NADP}^+$  for 3 minutes. Enzyme activity was expressed as (mU/mL). CAT activity (EC 1.11.1.6) was measured using the method of **Abei (1984)** by hydrolysis of  $\text{H}_2\text{O}_2$  and the resulting decrease in absorbance at 510 nm. The results were expressed as (U/g). The glutathione reductase (GR) (EC 1.6.4.2) activity was analyzed using the method of **Goldberg and Spooner (1983)** described the ability of the enzyme catalyzes the reduction process of GSSG to GSH when NADPH is oxidized to  $\text{NADP}^+$  and calculated as (U/L) at 340 nm at  $37^{\circ}\text{C}$ .

### Statistical analyses

Using the Statistical Processor System Support (SPSS 20, Armonk USA), data are analyzed by the one way ANOVA, presented as mean  $\pm$  standard error, values considered statistically at ( $P < 0.05$ ) significant. Different superscripts are statistically significant ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

### Trace element concentrations

The average concentrations of Hg, Al, Cd, Pb, and As in *T. zillii* and *C. gariepinus* muscles collected from lake Edku are presented in Fig. (2) by mean  $\pm$  SE expressed as ( $\mu\text{g/g w/w}$ ). The elements reported the descending order  $\text{Hg} < \text{Al} < \text{Cd} < \text{Pb} < \text{As}$  in *T. zillii* and *C. gariepinus*. The highest concentration was for As in *C. gariepinus* ( $1.54 \pm 0.08 \mu\text{g/g}$ ), but the lowest concentration was for Hg in *T. zillii* ( $0.47 \pm 0.08 \mu\text{g/g}$ ).

This study recorded an average Hg concentration in *C. gariepinus* ( $0.59 \pm 0.07 \mu\text{g/g}$ ) that was insignificantly greater than *T. zillii* ( $0.47 \pm 0.08 \mu\text{g/g}$ ). The *f*-ratio's

value was 1.20085; the *p*-value was 0.298842. In our results Hg in *T. zillii* and *C. gariepinus* exceeded the permissible limit **EOSQC (1993)** (0.50 ppm wt), **FAO/WHO (1992)** (0.50 ppm wt), **EC (2006; 2008 amended 2011)** 0.50 µg/g. Also, the results exceed the concentration of Hg (0.48 ppm) in *Oreochromis niloticus* from ten Egyptian governorates was recorded by **Abdel-Mohsien and Mahmoud (2015)**. **Abdel-Kader and Mourad (2019a)** demonstrated that Hg at *C. gariepinus* from Maryout lake ranged from 0.54 to 2.50 µg/g were much higher than our results. Whereas, the level of Hg was recorded in *T. zillii* 0.60±0.01 and *C. gariepinus* 0.73±0.07 µg/g from Burullus Lake, Egypt, was lower than our results (**Abdel-Kader and Mourad, 2020**). Symptoms of organic Hg toxicity include depression, memory problems, tremors, fatigue, hair loss and headache (**Martin and Griswold, 2009**).

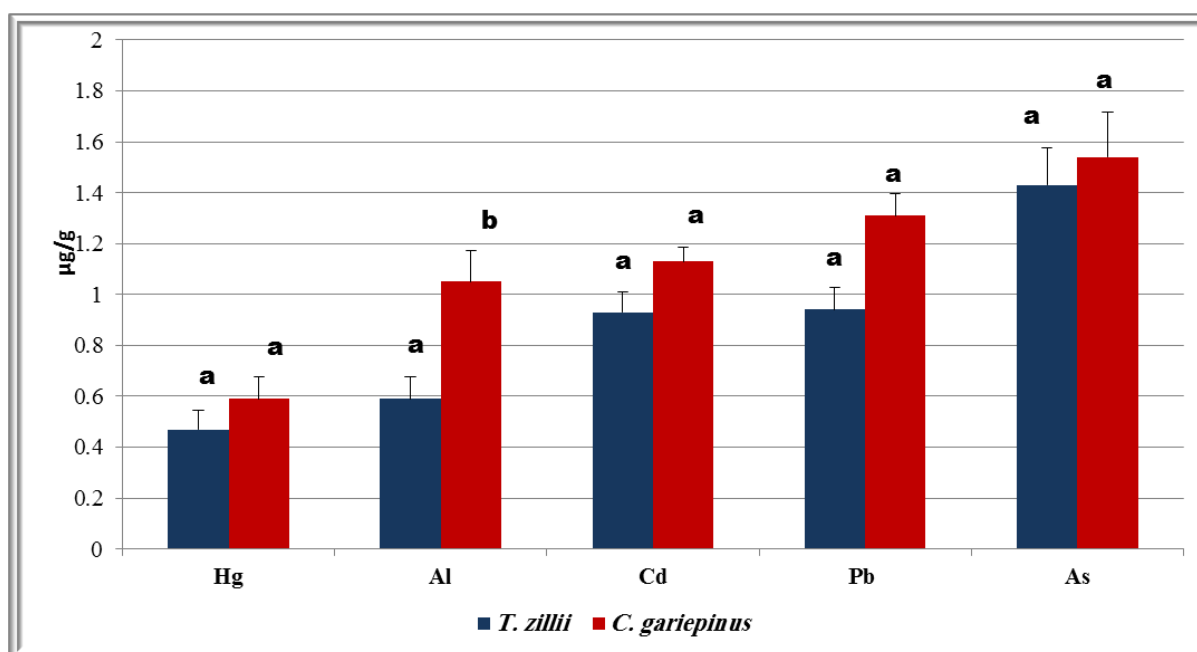
In this study, the average Al concentrations in the muscles of *C. gariepinus* was 1.05 ± 0.08 µg/g significantly (*p* < 0.05) higher than *T. zillii* 0.59 ± 0.12 µg/g (The *f*-ratio value is 9.4512. The *p*-value is 0.011755). *C. gariepinus* and *T. zillii* from Burullus Lake – Egypt with average 2.07± 0.1 and 1.85±0.1 µg/g, respectively were much higher than our results (**Abdel-Kader and Mourad, 2020**). Al in freshwater sources involves domestic waste, metal-related production processes, and discarded sewage sludge in freshwater sources (**James, 1991**) caused physiological disorders.

This study showed that the mean concentration of Cd in *C. gariepinus* 1.13±0.08 µg/g was insignificantly higher than *T. zillii* 0.93 ± 0.05, (the *f*-ratio value = 3.74752 and the *p*-value = 0.08164). So, the concentrations of Cd were reported in our results were largely above the permissible level of **EOSQC (1993)** (0.1 ppm), **WHO (2006)** (0.5 mg/kg), **FAO (2010)** (0.1 mg/kg), and **EC (2006; 2008 amended 2011)** 0.050 µg/g. The high load of Cd is due to different industrial and domestic channels induced in the Lake. Our results were greater than **Saeed and Shaker (2008)** who recorded that Cd (0.014 µg/g) in *O. niloticus* from Lake Burullus. **Olabanji and Oluyemi (2014)** recorded that Cd concentrations were 0.60 to 0.70 µg/g in *T. zillii* obtained from Opa Reservoir at Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. **Noor and Zutshi (2016)** reported Cd muscle tissue accumulation 0.41 ± 0.50 µg/g of freshwater fish *Labeo rohita* from Bangalore, Karnataka. Cd at Maryout Lake ranged from 0.7 to 1.90 µg/g which much greater than our results (**Abdel-Kader and Mourad, 2019a**). **Abdel-Kader and Mourad (2020)** recorded that Cd in *C. gariepinus* (1.66 µg/g) was insignificantly higher than *T. zillii* from Burullus Lake – Egypt. Cadmium (Cd) can cause anemia, osteoporosis, emphysema, non-hypertrophic, renal injury, and chronic rhinitis (**Flora et al., 2008**).

In this research, the concentration of Pb was insignificantly higher in *C. gariepinus* 1.31±0.08 with (*f*-ratio value = 2.19383, the *p*-value = 0.169371) than *T. zillii* 0.94 ± 0.08 µg/g. So, the Pb level in the muscles exceeded the permissible limit recommended by **EOSQC (1993)** (0.1 ppm w wt), **FAO/WHO (1999)** (0.214 µg/g wet weight) **EC (2006; 2008 amended 2011)** 0.30 µg/g. This study observed that Pb concentrations were much higher than those reported by **Saeed and Shaker (2008)**, where Pb concentrations in *O. niloticus* collected from Lake Borollus was 0.016 µg/g. **Olabanji and Oluyefmi (2014)** reported that Pb levels were 1.80 ± 2.20

to  $2.00 \pm 0.17$  mg/ kg at *T. zillii* from Opa Reservoir at Obafemi Awolowo University (OAU), Ile-Ife, **Abdel-Kader and Mourad (2019a)** showed that Pb concentration of *C. gariepinus* from Maryout Lake-Egypt was lower (0.2 to 0.8  $\mu\text{g/g}$ ) than our results. **Abdel-Kader and Mourad (2020)** reported that Pb levels in *C. gariepinus* and *T. zillii* were 2.29 and  $1.20 \pm 0.09$   $\mu\text{g/g}$ , respectively, from Burullus Lake – Egypt exceeded our the levels of our results. Tayel *et al.* (2007) suggested that high levels of lead may be due to motorboat traffic, industrial discharges, mine and smelting operations. After exposure to Pb, a significant increase in immunological metrics, indicating that Pb may decrease the respiratory system, leading to increase susceptibility to disease (**Flora *et al.*, 2008**).

The average concentration of As was insignificantly higher in *C. gariepinus*  $1.54 \pm 0.14$   $\mu\text{g/g}$  with (*f-ratio* value = 0.21817, the *p-value* = 0.650449) at  $p < 0.05$  than *T.zillii*  $1.43 \pm 0.17$   $\mu\text{g/g}$ . The concentrations of As in tissues of two studied species were in the range of the permissible limit recommended by FAO/WHO (2004) (2.0 ppm) w wt. **Abdel-Kader and Mourad (2019a)** showed that As concentration of *C. gariepinus* from Maryout Lake-Egypt, 0.40 to 1.00  $\mu\text{g/g}$  lower than our results. As levels in *C. gariepinus* and *T. zillii* from Burullus Lake – Egypt with average values of  $2.03 \pm 0.1$  and  $1.703 \pm 0.1$   $\mu\text{g/g}$ , respectively, were much higher than our results (**Abdel-Kader and Mourad, 2020**). Chronic exposures to trace elements result in the accumulation of the toxic elements lead to disease conditions (**Authman *et al.*, 2015**).



**Fig. 2** The average of trace element concentrations ( $\mu\text{g/g}$  wet weight)  $\pm$  standard error in the muscles of *T.zillii* and *C. gariepinus*, (n=6). Different superscripts are statistically significant  $p < 0.05$ .



### Human risk estimation

Trace element levels in consumed fish can cause health hazards to humans (**Castro-gonzález and Méndez-armenta, 2008**). It is, therefore, essential to determine the concentration of elements in fish to indicate that it does not expose any danger to humans and keep the elements concentration constant below acceptable levels (**Palaniappan and Karthikeyan, 2009**). As shown by PTWI for Cd, Pb, Hg, As, and Al were 0.007, 0.025, 0.005, 0.015, and 1mg/kg, respectively (**FAO/WHO, 2004**).

Table 1 showed the calculated values for the estimated daily intake (EDI) ( $\mu\text{g/kg}$  body weight/day), estimated weekly intake (EWI) ( $\mu\text{g/kg}$  body weight/week), PTWI%, maximum daily intake (MDI), and maximum weekly intake (MWI) of Cd, Pb, Hg, Al, and As in the muscles of *T.zillii* and *C. gariepinus* from Edku Lake what should consume by children, youth, and adult.

This study showed that the EDI, EWI and PTWI % of elements by adults were lower than that was reported by the PTWI values established by FAO/WHO except for Cd residue in *C. gariepinus* recorded 100.42 PTWI %. So, the two fish species did not present a risk to adult health with advice on safe levels of consumption of *C. gariepinus* for adults. Table 1 showed that the EDI, EWI and PTWI% of two fish species muscles by the children and young people pose a health risk, especially intake of Hg, Cd and As in young people, plus Pb for children. In China, **Yi et al. (2017)** confirmed that the daily fish consumption recorded in the upper Yangtze River, has shown no adverse health effects on humans. In Maryout Lake—Egypt, **Abdel-Kader and Mourad (2019a)** showed that EWI of trace elements (Cd, Hg, Pb, and As) in *C. gariepinus* was safe for adults regards to the PTWI. Moreover, in Burullus Lake—Egypt, **Abdel-Kader and Mourad (2020)** demonstrated that EWI of trace elements (Cd, Hg, Pb, As, and Al) in fish species tilapia and catfish were accepted for adults and youth, unsafe for children.

In our results, Table 1 showed that the EDI and EWI of trace elements  $\text{As} > \text{Pb} > \text{Cd} > \text{Al} > \text{Hg}$  followed the ranking order of children > youth > adults based on consumption of *T.zillii* and *C. gariepinus* muscles. Besides, Table 1 showed that the PTWI % followed a ranking order of  $\text{Cd} > \text{As} > \text{Hg} > \text{Pb} > \text{Al}$  based on consumption of *T. zillii* whereas  $\text{Cd} > \text{Hg} > \text{As} > \text{Pb} > \text{Al}$  based on consumption of *C. gariepinus*. So, our study calculated the maximum safety grams of muscles for two fish species in a diet/ day or week. Thus, it recommended that the children should consume less than 16.12857 g/day or 112.90 g/week *T. zillii* muscle, and 13.27 g/day or 92.92 g/week *C. gariepinus* muscle. Whereas, youth should consume less than 43.01 g/day or 301.7g/ week *T. zillii* and 35.39714 g/day or 247.78 g/week *C. gariepinus* muscle. Finally, adults should consume less than 23.1 g/day or 161.7 g/week *T. zillii* and 61.94286 g/day or 433.6 g/week *C. gariepinus* muscle.

**Table 1** Estimated daily intake (EDI) ( $\mu\text{g}$  /day/capita), estimated weekly intake (EWI) ( $\mu\text{g}/\text{week}/\text{capita}$ ), PTWI%, maximum daily intake (MDI), maximum weekly intake (MWI) of Hg , Al ,Cd, Pb, and As in muscles of *T. zillii* and *C. gariepinus* muscles consumed by a child 15 kg, young 40 kg, and an adult70 kg from Edku Lake - Egypt.

| Fish species   | Intake | Trace elements |          |          |          |          |
|--|--------|----------------|----------|----------|----------|----------|
|  |        | Hg             | Al       | Cd       | Pb       | As       |
| <i>T. zillii</i><br>consumed by<br>(a child 15 kg)     | EDI    | 1.9505         | 2.44     | 3.858    | 3.9      | 5.93     |
|  | EWI    | 13.65*         | 17.08    | 27.01*   | 27.3*    | 41.51*   |
|  | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
|  | PTWI%  | 273            | 1.708    | 385.8571 | 109.2    | 276.7333 |
|  | MDI    | 22.79571       | 3631.957 | 16.12857 | 56.99    | 22.79571 |
|  | MWI    | 159.57         | 25423.7  | 112.90   | 398.93   | 157.3    |
|  | EDI    | 2.44           | 4.357143 | 4.688571 | 5.545714 | 6.39     |
| <i>C. gariepinus</i><br>consumed by<br>(a child15 kg)  | EWI    | 17.08          | 30.50    | 32.82*   | 38.82*   | 44.73*   |
|  | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
|  | PTWI%  | 341.6          | 3.05     | 468.8571 | 155.28   | 298.2    |
|  | MDI    | 18.15857       | 2040.814 | 13.27429 | 40.89286 | 20.87143 |
|  | MWI    | 127.11         | 14285.7  | 92.92    | 286.25   | 146.10   |
|  | EDI    | 0.73           | 0.917143 | 1.447143 | 1.461429 | 2.224286 |
|  | EWI    | 5.11*          | 6.42     | 10.13*   | 10.23    | 15.57*   |
| <i>T. zillii</i><br>consumed by<br>(young 40 kg)       | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
|  | PTWI%  | 102.2          | 0.642    | 144.7143 | 40.92    | 103.8    |
|  | MDI    | 60.79          | 9685.229 | 43.01    | 151.9714 | 60.79    |
|  | MWI    | 425.53         | 67796.6  | 301.07   | 1063.8   | 419.5    |
|  | EDI    | 0.917143       | 1.632857 | 1.757143 | 2.037143 | 2.395714 |
|  | EWI    | 6.42           | 11.43    | 12.30*   | 14.26    | 16.77*   |
|  | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
| <i>C. gariepinus</i><br>consumed by<br>(young40 kg)    | PTWI%  | 128.4          | 1.143    | 175.7143 | 57.04    | 111.8    |
|  | MDI    | 48.42571       | 5442.171 | 35.39714 | 109.05   | 55.65857 |
|  | MWI    | 338.98         | 38095.2  | 247.78   | 763.35   | 389.61   |
|  | EDI    | 0.41           | 0.524286 | 0.825714 | 0.835714 | 1.271429 |
|  | EWI    | 2.87           | 3.67     | 5.78     | 5.85     | 8.90     |
|  | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
|  | PTWI%  | 57.4           | 0.367    | 82.57143 | 23.4     | 59.33333 |
| <i>T. zillii</i><br>consumed by<br>(an adult 70kg)     | MDI    | 106.3829       | 16949.14 | 75.26857 | 23.1     | 104.8943 |
|  | MWI    | 744.68         | 118644.0 | 526.88   | 161.7    | 734.26   |
|  | EDI    | 0.524286       | 0.932857 | 1.004286 | 1.164286 | 1.368571 |
|  | EWI    | 3.67           | 6.53     | 7.03*    | 8.15     | 9.58     |
|  | PTWI   | 5.0            | 1000     | 7.0      | 25.0     | 15.0     |
|  | PTWI%  | 73.4           | 0.653    | 100.4286 | 32.6     | 63.86667 |
|  | MDI    | 84.74571       | 9523.714 | 61.94286 | 190.7143 | 97.40143 |
| <i>C. gariepinus</i><br>consumed by<br>(an adult 70kg) | MWI    | 593.22         | 66666    | 433.6    | 1335     | 681.81   |

### Proximate body composition

Fish muscle included moisture, proteins, carbohydrates, and fats considers as main components (Love , 1980). In this analysis, mean values  $\pm$  SE are shown proximate body composition in (Fig. 3) at *T. zillii* and *C. gariepinus* muscle tissue.

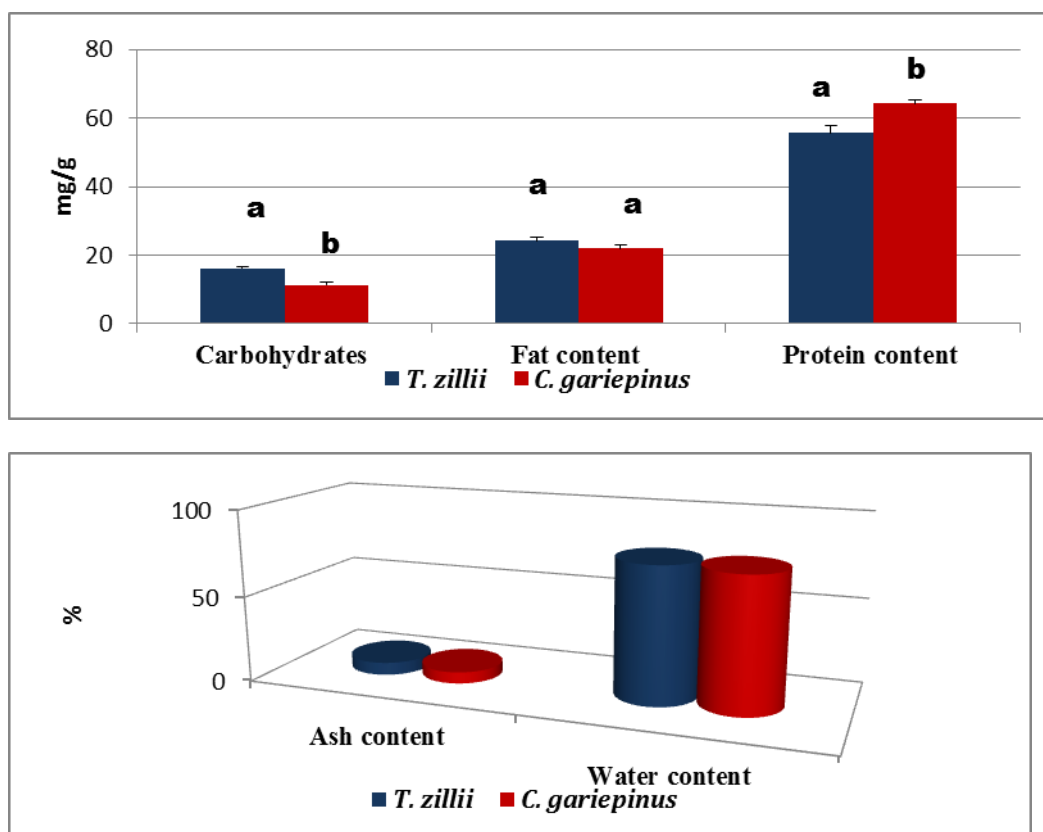
This analysis showed a significant difference ( $P < 0.05$ ) in the comparison of mean carbohydrate values (mg/g) for two fish species. *T. zillii* recorded the maximum mean carbohydrate value  $16.11 \pm 0.67$ , while *C. gariepinus* recorded the lowest amount of carbohydrate  $11.28 \pm 0.68$ . In contrast, carbohydrates of *T. zillii*  $14.1 \pm 0.7$  mg/g, was higher than *C. gariepinus*  $14.66 \pm 0.6$  from Burullus Lake (**Abdel-Kader and Mourad 2020**). Carbohydrate values in tilapia are insignificant higher than crab values. In the lobster, carbohydrate is significantly lower than the other two species (**Ayanda et al. 2018**).

In this study, lipid content recorded an insignificant difference in two fish species. There was an increase in lipid content (mg/g) observed in the *T. zillii* ( $24.21 \pm 0.93$ ) followed by *C. gariepinus* ( $21.96 \pm 0.95$  mg/g) for the minimum value. **Sobha et al. (2007)** observed a reduction in lipid content following the exposure of *Catla catla* to Cd; *Perca flavescens* (**Levesque et al., 2002**) and *Anguilla anguilla* (**Pierron et al., 2007**). The lipid content may be affected by different factors such as feed structure, geographic origin, age difference, reproductive stage, and catching season (**Murillo et al., 2014**).

A comparison of the means of protein (mg/g) showed a significant difference ( $P < 0.05$ ) for two fish species. *C. gariepinus* showed the maximum mean protein value  $64.33 \pm 1.01$  followed by *T. zillii*  $55.66 \pm 1.95$ . **Selvam et al. (2014)** reported a significant difference ( $P < 0.05$ ) in the protein content in Swat Khyber Pakhtunkhwa Pakistan between five different commercial *Cyprinida* fish species. **Palaniappan and Vijayasundaram, (2009)** noted a significant decrease in protein and lipid content in *Labeo rohita* upon As exposure. Depletion of the lipid and protein content of Pb-exposed *Catla catla* muscles (**Palaniappan et al., 2008**), can be caused by tissue organization and its use in lipoprotein cell repair, essential cellular elements of cytoplasmic cell membranes and organelles (**Filipovic and Raspor, 2003**). **Abdel-Kader and Mourad (2019b)** showed an increase in protein content from 56 to 27 mg/g and 26 to 32 mg/g in lipid content from two basins at Maryout Lake. **Abdel-Kader and Mourad (2020)** showed that the protein  $57.16 \pm 2.6$  mg/g and lipid content  $11.98 \pm 1.1$  mg/g of *T. Zillii* were greater than of *C. gariepinus*  $56.16 \pm 3.0$  mg/g and  $11.65 \pm 0.7$  mg/g, respectively, from Burullus Lake, Egypt

In this study, moisture content's mean recorded an insignificant difference ( $P > 0.05$ ) for the two fish species. *T. zillii* showed the highest mean moisture content  $78.33 \pm 1.58\%$  while the least mean moisture content was recorded in the *C. gariepinus*  $77 \pm 0.45\%$ . Water content in *C. gariepinus* ranged from  $75 \pm 4.40$  to  $53.00 \pm 7.70\%$  from two basins at Maryout Lake (**Abdel-Kader and Mourad 2019b**) which was lower than moisture content of this study. **Abdel-Kader and Mourad (2020)** showed that the water content in *T. zillii*  $64 \pm 1.9\%$  and *C. gariepinus*  $64 \pm 2.1\%$  from Burullus Lake, are much lower than that of the present study. **Ayanda et al. (2018)** recorded a significant rise in water content in tilapia from Nigeria at Makoko River may be rendering the fish sensitive to microbial spoilage, damage to fatty acids, and consequently decreasing fish quality thereby reducing its survival time (**Omolara and Omotayo 2009**)

In the present results, fish species showed an insignificant decrease in the ash content. Maximum ash content was observed in the *T. zillii* ( $7.33 \pm 1.00\%$ ) while the minimum value ( $6.66 \pm 0.85\%$ ) was recorded in the *C. gariepinus*. In contrast, the ash content was significantly reduced in tilapia from Nigeria at Makoko River (Ayanda *et al.*, 2018). Finally, Abdel-Kader and Mourad (2020) recorded that the ash content of *T. zillii*  $14.25 \pm 1.7\%$  and  $3.37 \pm 0.1\%$  of *C. gariepinus* from Burullus Lake, Egypt.



**Fig. 3** Proximate body composition in muscle tissue of *T.zillii* and *C. gariepinus*. (n = 6). Different superscripts are statistically significant  $p < 0.05$

### Antioxidant enzyme activity

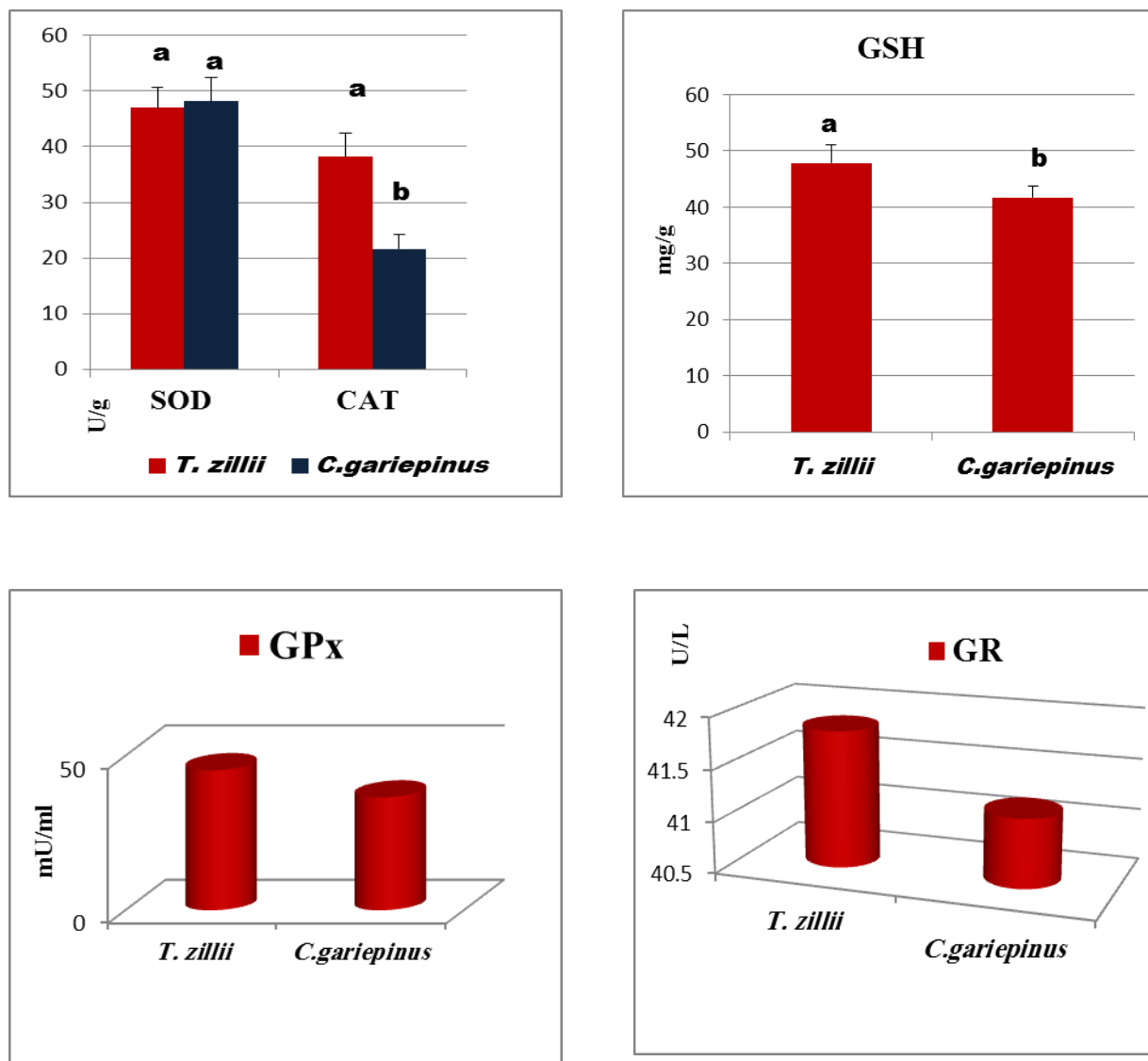
Trace elements, such as Cd, Pb, Hg, As, and Al can inhibit cellular antioxidant activity, particularly thiol-containing antioxidants and enzymes (Sevcikova *et al.*, 2011). Trace elements can promote oxidative damage by directly increasing the cellular concentration of ROS and altering the cellular antioxidant capacity in fish (Sevcikova *et al.*, 2011).

The present study recorded a non-significant decrease in antioxidant SOD, GPx, and GR activity demonstrated in the liver, whereas, CAT activity and GSH level reported significant ( $P < 0.05$ ) decrease ( $p < 0.05$ ) in the liver of *C.gariepinus* than *T. zillii* (Fig. 4) by mean values  $\pm$  SEM. GSH level and CAT, GPX, and GR activities in *C. gariepinus* ( $41.66 \pm 2.01$ mg/g,  $21.66 \pm 2.6$  U/g,  $36.5 \pm 3.01$  mU/L and  $41.16 \pm 1.0$  U/L, respectively), were less than *T. zillii* ( $47.83 \pm 3.6$  mg/g,  $38.16 \pm 4.2$  U/g,  $45.33 \pm 4.5$  mU/L, and  $41.83 \pm 3.4$  U/L, respectively). SOD activity in *T. zillii*  $47 \pm 3.5$  U/g was less than *C. gariepinus*  $48.16 \pm 4.23$  U/g (Fig. 4).

After exposure to Pb, a significant reduction in CAT activity was observed in *O. niloticus* (Elarabany and Bahnasawy, 2019). CAT activity reduced due to the generation of superoxide radicals that decrease CAT activity sharply (Ahmad 2000). When hydrogen peroxide changing to O<sub>2</sub> and H<sub>2</sub>O, CAT protects fish from oxidative stress (Atli and Canli, 2007). Decreased GR activity may deplete of GSH if additional GSH synthesis cannot occur to protect its redox status, while an increase in activity may be related to the re-establishment of oxidized GSH (Atli and Canli, 2007). An insignificant difference in GR activity was found in *Mugil* sp. (Rodriguez-Ariza *et al.*, 1993) which was sampled from contaminated pesticide areas. Abdelazim *et al.* (2018) recorded the recognized decrease in GR following exposure of the Nile tilapia to zinc oxide nanoparticles. GSH is a potent antioxidant capable of binding freely to radicals and trace elements, such as Cd and Pb, and defending cells against their adverse effects (Jan *et al.*, 2011). GSH induction is the primary step in the defense system to keep against oxidative stress; it showed that metal induces increased hepatic GSH synthesis (EL-Gazzar *et al.*, 2014). Trace elements respond to GSH thiol groups that can cause depletion of GSH then oxidative tissue stress in the tissue (Stohs and Bagchi, 1995). Monteiro *et al.* (2010) observed oxidative stress followed exposure to Hg rely on that the metal-binding GSH reduce toxicity (Elia *et al.*, 2003). GSH levels in zebrafish decreased after dimethoate exposure (Ansari and Ansari 2014), in juveniles after Pb exposure (Saliu and Bawa-Allah, 2012). The liver is the main target organ of GPx production for metabolized oxidants indicates the sensitivity to the oxidative stress to which the fish has been exposed (Lenartova *et al.*, 1997). GPx detoxifies H<sub>2</sub>O<sub>2</sub> or organic hydroperoxides produced in the LPO (Halliwell and Gutteridge, 1999). Cd caused a significant reduction in a hepatic GPx activity in *niloticus* after 42 days of exposure suggested that GPx had a tissue-specific response to Cd exposure and depended on tissue-specific alternations of CAT and SOD documented in *O. niloticus* exposed to diazinon (Durmaz *et al.*, 2006). Cd induced deactivated GPx activity with a great affinity to bind with –SH groups, and so, initializes the activity of the enzyme (Giguère *et al.*, 2005). Orun *et al.* (2008) showed significant alterations of GPx activity in fish tissues *Onchorhynchus mykiss* after Cd exposure. The direct effects of the element on enzyme molecules at the active site could be due to a reduction in GPx, although an increase in GPx activity may be attributed to the fish's stress. A substantial increase in SOD production in the liver, when exposed to a mixture of both elements may be attributable to the summation effect of both elements resulting in the accumulation of free radicals where SOD acts as an antioxidant enzyme protective (Asagba and Obi, 2000). Barata *et al.* (2005) also observed diminished SOD and CAT activity after Cd exposure in *Daphnia magna* suggested that toxicants could produce several antioxidant/prooxidant reactions because they are capable of ROS. Vinodhini and Narayanan (2009) recorded decreases in GPX, SOD, and CAT after 32 days of exposure to Pb, Cd, Cr, and Ni in *Cyprinus carpio* L. Abdel-Kader and Mourad (2019b) recorded decreases in the activity of CAT, GPX, and SOD in *C. gariepinus* from the main basin at Lake Maryout. Abdel-Kader and Mourad (2020) recorded decreases in CAT, GR, GSH, GPX, and SOD activity of *C. gariepinus* followed by *T. zillii*.



**Fig. 4** Antioxidant activities in the liver tissues of *T. zillii* species and *C. gariepinus* (n = 6). Different superscripts are statistically significant  $p < 0.05$ .



## CONCLUSION

In summary, high protein content was recorded in *C. gariepinus* followed by *T. zillii*. Whereas the high means of carbohydrates, fats, ash, and water content were recorded in *T. zillii* followed by *C. gariepinus*. GSH level and CAT activity demonstrated a significant decrease ( $p < 0.05$ ) in the liver of *C. gariepinus* than *T. zillii*. This study revealed that all toxic elements in *C. gariepinus* were consistently high, the highest element was As recorded in *C. gariepinus*, while, the lowest element was Hg in *T. zillii*. Although the trace element level concentrations exceeded the permissible levels suggested by Egypt, FAO, WHO, and EC in two fish species, the estimated weekly intake of Pb, Hg, As, and Al was below the PTWI except for Cd of *C. gariepinus* for

adults. So, consumption of collected fish from Edku lake was safe for adults (70 kg) with advice on safe levels of consumption for *C. gariepinus* for adults, whereas unsafe for young people (40 Kg) and children (15 Kg) concerning the PTWI. The EDI and EWI of As > Pb > Cd > Al > Hg followed the ranking order of children > youth > adults based on consumption of *T. zillii* and *C. gariepinus* muscles. In addition, the PTWI% followed a ranking order of Cd > As > Hg > Pb > Al based on consumption of *T. zillii* whereas Cd > Hg > As > Pb > Al based on consumption of *C. gariepinus*. So, we recommended that the children should consume less than 16.12857 g/day or 112.90 g/week *T. zillii* muscle, and 13.27 g/day or 92.92 g/week *C. gariepinus* muscle. Youth should consume less than 43.01 g/day or 301.7g/ week *T. zillii* and 35.39714 g/day or 247.78 g/week *C. gariepinus* muscle. Finally, adults should consume less than 23.1 g/day or 161.7 g/week *T. zillii* and 61.94286 g/day or 433.6 g/week *C. gariepinus* muscle. Also, the consumers should consider that the intake of two types of fish species in one meal increases the quantities of trace elements consumption.

Accordingly, this study reply to the question in the title research, that, Egyptians will be at risk if they consume more than the maximum daily or weekly intake of Cd, Pb, Hg, As, and Al through consumption of *T. zillii* and *C. gariepinus* in respective to the PTWI

#### AUTHOR CONTRIBUTIONS

Mohamed H. Mourad was responsible for the general supervision of the research and contributed to the final review of the research before sending the manuscript to the journal for publication.

Heba H. Abdel-Kader dissected the fish and kept them in sterile bags, and marked and placed them in a deep freezer until analysis. She did the analyzes including the statistical analyzes, collected literature, wrote the manuscript, linguistically reviewed it, including the grammar, and sending the manuscript to the journal for publication.

Afaf Mohamed M. Haredi collected fish samples from the fishermen and assisting in dissecting and keeping them in sterile bags in a deep freezer.

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