Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 1 – 24 (2022)
www.ejabf.journals.ekb.eg

Carcinogenic and Human Risk Assessments, Trace Element Concentrations, Proximate Composition, and Liver Antioxidants of Three Commercial Tilapia Fish Species from Manzalah Wetland, Egypt

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ARTICLE INFO
Article History:
Received: June 17, 2022
Accepted: June 24, 2022
Online: July 9, 2022

Keywords:
Target carcinogenic risk,
Trace elements bioaccumulation,
Human risk assessment,
Manzalah Lake,
Proximate composition,
Antioxidants

ABSTRACT

Manzala Wetland is Egypt's largest ecosystem and the country's second-largest lake overall. In 2016, in line with Egypt's Vision 2030, the Egyptian government developed a strategy to boost the economic potential of northern lakes, which includes Manzalah Wetland. The focus of this research was to test for trace elements including lead (Pb), mercury (Hg), arsenic (As), and aluminum (Al) in the muscle tissue of three commercially significant tilapia fish species gathered with the help of Manzalah Lake fishermen in 2018. Pb and Hg were largely above the permissible level of Food Agricultural Organization FAO, World Health Organization WHO, and Commission Regulation EU. The proximate chemical composition and antioxidants of the liver were estimated. The results showed that the highest mean carbohydrate, and fats, found in *O. aureus*, the water content and ash in T. *zillii*, and protein concentrations was in *S. galilaeus*. The results of the current investigation showed that, *S. galilaeus* had the highest levels of activity of liver antioxidants CAT, Gpx and GR, SOD in *T. zillii*, as well as GSH level in *O. aureus*. The metal with the highest concentration was Pb in *O.aureus* (2.85±0.2), whereas the least amount was Hg in *S. galilaeus* (0.55±0.04). Hg levels in *O. aureus*, *S. galilaeus*, and *T. zillii* ingested by children, youth, and adults showed the lowest of all metals, but they were higher than the permissible levels, indicating a risk to people's health from consuming the examined hazardous element through the diet of the three fish species. The target hazard quotient (THQ) value of As in *T. zillii* intake by a child demonstrated the highest value ranging from (21 a day to 147 a week), while the THQ value of Pb in *S. galilaeus* intake by an adult showed the least value of THQ (0.73 a day or 5.11 a week). Based on the values of the total target hazard quotient (TTHQ) of the three fish samples for children, youth and adults, ingesting these fish poses a high non-carcinogenic risk. *Tilapia zillii* ingested by children recorded the highest degree of carcinogenic risk (CR) (9.45E-03 a day or 6.62E-02 a week); whereas, *S. galilaeus* in adults recorded the lowest level of CR (2.19E-05 a day or 1.54E-04 a week). The THQs and CRs values of Pb, Al, As, and Hg in the three examined species were greater than one, surpassing the Environmental Protection Agency recommendation (US EPA) acceptable limits. This finding demonstrates that, daily or weekly eating of these species could pose a high risk of non-carcinogenic and carcinogenic consequences to humans; thus, the fishes of the lagoon may be dangerous for human consumption in general.

INTRODUCTION

Anthropogenic activities plus natural processes resulted in a regressive aquatic ecosystem due to the accumulation of levels of trace elements that are beyond tolerance. These toxins may have toxicological effects on aquatic ecosystem components, making them a deathtrap for aquatic life. Fishes, in particular, suffer from regressive physiological and metabolic functions **(Bichi & Anyata, 1999; Jaishankar *et al*., 2014; Ezemonye *et al.*, 2019)**. These toxins not only pose a health risk to aquatic biota, but also degrade water quality for household, agricultural, and industrial purposes. These components are hazardous to the aquatic system because they cannot be biologically decomposed and can concentrate in the environment, passing on to people who depend on aquatic foods for nutrition **(Sary *et al*., 2010)**. As a result, trace elements can concentrate in aquatic species' tissues, providing a public health risk to both living creatures **(Squadrone *et al*., 2013)**. Fish are regularly studied to evaluate the health of the aquatic system, and as a consequence, they can serve as bio monitors of pollution. Monitoring contaminants is one of the advantages of using fish in physiological investigations, since fish are one of the most vulnerable animals to the long-term effects of pollution **(Zeng *et al.* 2012; Olusola & Festus, 2015; Malakootian *et al.*, 2016; Mortazavi *et al*., 2016)**. Moreover, fish are recognized as the best animal protein source; humans ingest fish for they include important elements, such as vitamins and fatty acids (unsaturated), and other essential minerals required for proper growth and development **(Javed *et al.*, 2020)**. *Oreochromis aureus*, blue tilapia, is a freshwater species in the Cichlidae family that has developed tolerance to brackish water, This species, with its historical roots in northern Africa and the Middle East, looks blue-gray and abdominal pink-white; it can be found in abundance in lagoons and rivers. Moreover, the redbelly tilapia (*Tilapia zillii*) and Sarotherodon (*S. galilaeus*) are freshwater fish belonging to the Cichlidae family that can be found in both freshwater and saltwater throughout northern Africa and the Middle East. Three tilapia species in most sections of Lake Manzala have been identified as the basis of large commercial fishers **(El-Bokhty, 2006)**.

The biochemical composition of fish flesh can be used to assess its quality. Varied kinds of fish provide different nutritional profiles to consumers. Protein, lipids, and moisture are the three most important components acting as sources of nutritional value in fish flesh **(Agusa & Adeosun, 2016)**. To match the requirements of food regulation and commercial specifications, the proximate composition must be quantified **(WHO/FAO, 2011)**.

Lake Manzala is situated on the Nile Delta's northeastern shore, between Damietta & Port Said. It is separated from the Mediterranean Sea by a ridge of seashores, with three open linkages between the lake and the sea. These open channels allow water to pass from the lagoon to the sea **(Khalil & El-Awamri, 1988; Hossen & Negm, 2016)**. Every year, freshwater (mostly from agricultural effluent) pours into Manzala Lake *via* nine main drains and canals. It gathers and carries most of the sewage into the lake through the eastern Delta's highly populated area, traveling through Qalubyia, Sharkia, Ismailia, and Port Said Governorates, and contributing significantly to the lake's diminishing water quality **(Hasan, 2008)**. This critical contamination of Egypt's Manzalah Lake prompted the Egyptian government to implement appropriate laws and regulations to develop this lake under the presidential initiative in the 2030 vision for environmental sustainability. Lake Manzala is a small saline lake with a surface area of 1,000km². Islands, thickly vegetated areas, and "huchas" illegal fish cages obstruct water circulation and create basins with vastly different water and sediment qualities. The lake gets millions of cubic meters of untreated municipal and industrial waste each year, as well as agricultural drainage effluent **(Abu Khatita *et al*., 2015; El-Naggar *et al.*, 2016)**.

The assessment of aquatic food's health concerns has shown a global spectacular increase, notably in the third-world developing countries. The accumulation of these components may result in diseases and a high mortality rate. Thus, the estimation of trace element values in fish was used to examine the fish's physiological and biochemical state. These toxic elements may play a role in enzymatic inhibition and decreased antioxidant metabolism; however, the mechanism by which they pose toxic effects is unknown. These substances can be ingested by humans and thus may have an impact on humans directly or indirectly through the food chain. Moreover, the intake of toxic elements reduces the beneficial effects of fish on cardiovascular protection. Furthermore, these elements are related to serious health problems in both children and adults **(Subotić 2013; Okyere *et al*. 2015)**. Trace elements' harmful effects on humans are caused by the generation of free radicals, which cause DNA damage **(Valko *et al*., 2005)**. Hg, Pb, Al, and As are trace elements that have been related to serious health problems in children and adults **(Tüzen, 2003; Shrivastava *et al*., 2011; Ahmad & Sarah, 2015)**.Consequently, fish intake is often considered one of the leading causes of element human exposure **(Milanov *et al.*, 2011; Sen *et al.*, 2011; Squadrone *et al.*, 2013**). For their biological differences, children are more susceptible to toxic elements than adults. The evaluation of aquatic food's health risks has globally demonstrated a spectacular upward growth, particularly in the third-world developing nations. This elements' accumulation may result in diseases and high mortality rate **(Ahmad & Sarah, 2015**). Toxicity from heavy metals can induce various health effects in the body. They can alter and interfere with the organs' functions, including the kidney, brain, lungs, blood and liver **(Engwa *et al.*, 2019)**. Such toxicity can be either acute or chronic. Extensive exposure to these metals could result in progressive neurological, muscular, and neurological degeneration which could be similar to diseases such as muscular dystrophy, sclerosis, Parkinson and Alzheimer's disease. In addition, longer chronic exposure to some metals may lead to cancer **(Jarup, 2003)**.

Heavy metals produce many of their hazardous effects on humans through the formation of free radicals, resulting in damage of the DNA **(Valko *et al*. 2005; Ahmad & Sarah, 2015)**. An excess of reactive oxygen species is related to increased levels of harmful elements that cause free radical generation, this could lead to high permeability & ion depletion, as well as changes in enzyme performance. The responses of the antioxidant system can disclose early indicators of toxicity caused by trace elements. Hence, environmental stress is predicted utilizing the organism's physiological responses **(Sanchez *et al*., 2005; Doherty *et al.*, 2010; Zheng *et al.*, 2016)**.

The major purpose of this study was to determine the levels of Pb, Al, As, and Hg that had accumulated in the muscles of three freshwater tilapia fish species, *Oreochromis* aureus, *Sarotherodon galilaeus*, and *Tilapia zillii* collected from Manzala Lake in Egypt; local fishermen obtained these species from various locations. After that, the findings were compared to those of other studies, FAO, WHO, and EU limits levels, biochemical composition in muscles and antioxidants in the liver were estimated to evaluate the quality of fish.

MATERIALS AND METHODS

Lake Information

Lake Manzala is the biggest saline coastal wetland , located on the northeastern shoreline of the Nile delta between (Long 31o 45' and 32o 15' East, and Lat 31o 00' and 31o 25' North) (Fig. 1). The lake has a shallow depth of about 1.0m on average; it has three connections to the Mediterranean Sea, allowing the movement of biota and water from the lagoon to the sea; El Boughdady, El Gamil, and the new El Gamil are the three outlets **(Elewa *et al.*, 2007; Ali, 2008; Arafa *et al.*, 2015)**.

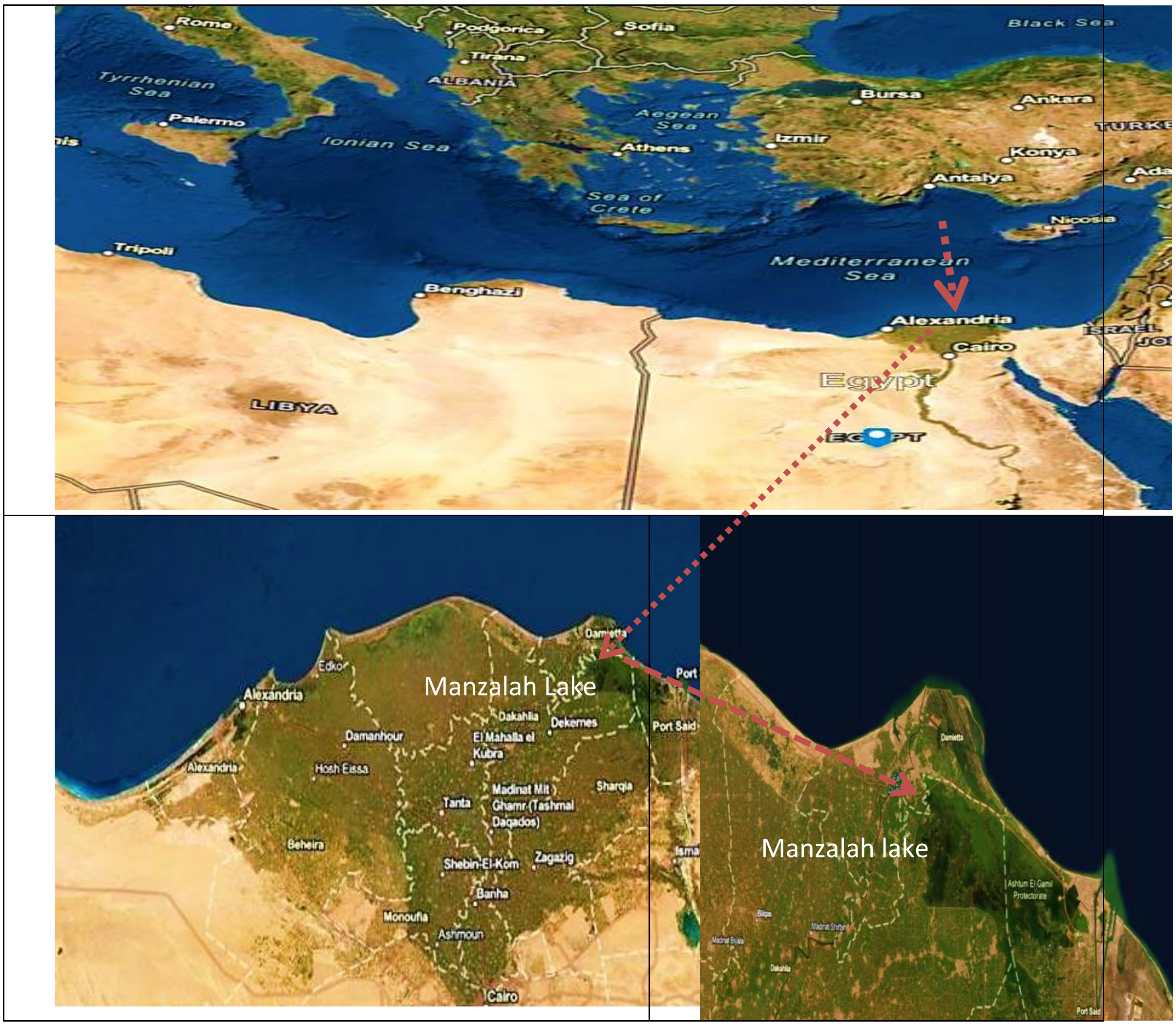

Fig. 1. A map showing the Manzalah Wetland

Analysis of fish samples

Various fishermen captured alive medium-sized tilapia fish, including *O. aureus, S. galilaeus and* Tilapia *zillii* in spring 2018 from several sites around the Manzala Lake. The samples were sent to Physiology Laboratory, the National Institute of Oceanography and Fisheries (NIOF), Fisheries division. The fish were rinsed with distilled water. On a clean glass plate, the muscles and liver tissues were detached from the samples using a sterile plastic knife. The detached parts were maintained in polythene bags that were previously sterilized. Labeled bags were placed in a deep freezer at a temperature of 25°C until the various tests were completed. The freshwater species were captured at random along the Manzalah Lake in May 2018 from Egypt when local fishermen were fishing in the lagoon. These species of fish were selected from fishermen across the lake, then delivered alive to the National

Institute of Oceanography and Fisheries (NIOF), Egypt, a physiological lab for research purposes.

All glassware was submerged in 10% (v/v) nitric acid overnight prior to rinsing in 10% (v/v) hydrochloric acid, then cleaned twice with distilled water and dried before use; the chemicals provided were Merck from Germany; reagents for the analysis of the highest grade. The calibration curve's element standard solution was made by diluting 1000mg/L Merck stock solutions. The skin of six, from each fish species, was removed after the dissection, and the musculature was gathered, wrapped in aluminium foil, and packed separately in a clean plastic bag with identifications of number & collection date. The fish were then kept frozen at -20 degrees Celsius until digestion. In Erlenmeyer flasks with a capacity of 25mL, one gram from the tissue muscle was accurately weighed, then 5mL (65%) of nitric acid was poured (Merck, Germany) into each specimen; afterwards, the samples were left to digest gently overnight. Approximately, 2.5 mL perchloric acid 72 % (Merck, Germany) was then added to each sample. Digestion was carried out in a 150°C water bath for 6 hours, or until the solutions became clear and near dry. The solutions were placed in 50mL polyethylene bottles after cooling and then filled to 25 mL with distilled water. The solution was then filtered using 0.45m Whatman no.42 filter paper into a cleaned glass beaker. By adding more deionized water up to 50mL, the filtrate was diluted. Metal measurements were completed using a Perkin-Elmer A Analyst 800- a graphite furnace (GF-AAS) atomic absorption Spectrophotometry. Concentrations of trace element in fish were measured in (µg/g) on a wet weight (ww) basis. The same method was used to digest three replicate blank samples. For Pb, Hg, As, and Al contents in samples of fish were tested. LOD was examined by the standard deviation (SD) of the three replicate blanks for elements. The quality of analyses was recorded by the certified fish muscles references material (DOLT-4 dogfish liver). The replicate analysis of the reference material indicating high accuracy with a recovery rate of 94-98 %.

Assessment of Human Risk

Daily and weekly trace element intake

The estimated daily (EDI) or weekly intake (EWI) of Pb, Hg, As, and Al by fish consumption were examined using the two equations below

$$\mathbf{EDI} = \mathbf{C} \times \mathbf{IR} \,/\, \mathbf{BW}$$

$$\mathbf{EWI} = \mathbf{EDI} \times 7$$

Where, C represents the element concentrations in fish samples (µg/g-ww); IR is the daily intake rate of fish (62.25 g/person/ day) related to the Ministry of Agriculture and Land Reclamation, Egypt (2017), and the Central Agency for Public Mobilization and Statistics (2017), and BW is the average body weight (15kg for a child, 40 kg for a youth, and 70kg for an adult) **(Salas *et al.*, 1985; Alberin *et al.*, 1999)**. The estimated weekly intake was compared to (PTWI: the provisional tolerable weekly intakes), that was determined from FAO/WHO food safety standards. EWI less than PTWI suggests that customers' food consumption does not constitute a serious health risk **(FAO/WHO, 2004)**

$$\mathbf{PTWI\ \% = EWI \,/\, PTWI \times 100}$$

The percentage **PTWI** was estimated using the FAO/WHO recommended possible health reference dose **(FAO/WHO, 2004)**.

$$\mathbf{MDI = PTWI \times BW / C \times 7}$$

$$\mathbf{MWI = MDI \times 7}$$

Maximum daily intake: MDI (in grams) on the basis of MWI of fishes that children, youth, and adults should achieve PTWI (**FAO/WHO, 2004**).

Estimation of noncancerous risks

Target hazard quotient: THQ was showed with the formula below **(US EPA, 2012)**

$$\mathbf{THQ = EF \times ED \times IR \times C \,/\, RfD \times WAB \times ATn \times 10^{-3}}$$

Where, EF: the exposure frequency- days/year; ED : the exposure duration-years; IR: the ingestion rate -g/day; C: the metal concentration in fish-µg/kg; RfD: the oral reference dose (Pb = 3.50×10^{-3}, Hg =3.0×10^{-4}, As=3×10^{-4}, and Al=3×10^{-4}) mg kg^{-1} day^{-1}; WAB: the average (kg) body weight, and ATn : the average exposure time (days/year × ED) for non-carcinogens.

Estimation of total target hazard quotient (TTHQ)

Total THQ or HI (hazard index) was estimated according to the method below **(US EPA, 2012)**

Total THQ (TTHQ) = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant n)

If the values of HQ and HI ≤ one, then humans will not suffer any obvious health consequences. If the values of HQ and HI > one, there is a risk that non-carcinogenic effects will occur, with a probability tending to rise as HQ and HI rise **(US EPA, 2019)**.

Estimation of carcinogenic risk

Target carcinogenic risk **(**TCR) was expressed by USEPA **(US EPA, 2019)** as below*:*

$$\mathbf{TCR = EF \times ED \times IR \times C \times CSF \,/\, WAB \times ATc \times 10^{-3}}$$

Where, EF : the exposure frequency -days/year; ED: the exposure duration years; IR : the ingestion rate-g/day; C : the metal concentration in fish- µg/kg; CSF:the cancer slope factor (Pb =8.5×10^{-3} As= 1.5 mg, kg^{-1} day^{-1}) **(US EPA, 2019)**;WAB: the average (kg) body weight, and ATc: the average time days/year × ED for carcinogens .

US EPA (2011) states that 10^{-6} (1 in 1,000,000) to 10^{-4} (1 in 10,000) indicate a range of allowable estimated lifetime carcinogen risks. Chemicals with risk factors less than 10^{-6} may be excluded from further evaluation as a chemical of concern. The risk involved with a target metal's carcinogenic effect is given as the increased probability of contracting cancer over a 70-year lifetime **(US EPA, 1989; NYSDOH, 2007)**.

Proximate chemical examination

In a glassware homogenizer in 5ml saline, a 0.1g muscle sample was homogenized for three minutes, After that, it was centrifuged for ten minutes at 3000rpm. The total protein content of the supernatant was determined by referring to the method of **Lowry *et al.* (1951)**. The method for determining total lipid content followed the that of **Henry *et al.* (1974)**. The method of determining total carbohydrates in tissues was used referring to **Kemp *et al.* (1954)**. After 1.0g of sample mass was lost at 100°C 2°C during drying, the water content was measured. To determine the ash content, the residual mass was burnt at 600 ±10° C **(AOAC, 2002)**.

Estimating of antioxidants

PBS (phosphate-buffered saline) solution, pH 7.4, was utilized to perfuse liver tissue before dissection. Heparin (0.16 mg/mL) was used to dissolve red blood cells and clots. The tissues were homogenized in a cold buffer of 5–10 mL per g (50 mM potassium phosphate, pH 7.5.1 Mm EDTA). Centrifuge was performed at 100,000 ×g for 15 minutes at 4°C. The supernatant was removed and kept refrigerated. If not analyzed the same day, the tissue was frozen at −80°C to preserve the sample steady for at least one month. Catalase activity (CAT) (EC 1.11.1.6) was calculated to detect the catalase reaction, which decomposes 1.0 mmol L. 1.0 of H_2O_2, was used to calculate this. At 25°C, the catalase inhibitor reaction was terminated after exactly 1 minute. In the presence of peroxidase (HRP), leftover H_2O_2 was combined with 3.5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4- aminophenazone (AAP) to generate a chromophore with color intensity; the amount of catalase in the initial sample was inversely proportionate and its absorbance at 510nm could be determined **(Abei, 1984)**. The results were reported as U/g.tissue. Glutathione reductase activity (GR) (EC 1.6.4.2) was measured by the enzyme's ability to catalyze glutathione reduction (GSSG); NADPH is converted to NADP+ when it is oxidized. At 37°C, the reduction was measured as absorbance at 340 nm. The findings were represented as U/L **(Goldberg & Spooner, 1983)**. Glutathione reduced activity (GSH) (EC 1.8.1.7) was evaluated using the method for manufacturing a yellow chemical depending on the reduction of 5.5′-dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione. Reduced chromogen may be measured directly proportional to GSH concentration at 405nm, and its absorbance can be quantified at 405nm **(Beutler *et al.* 1963)**. Enzyme activity was measured in milligrams per gram of tissue (mg/g.tissue). Superoxide dismutase activity (SOD) (EC 1.15.1.1) was measured as the ability of the enzyme to prevent phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye based on the method of **Nishikimi *et al*. (1967)**. At 25 °C, the absorbance increased for 5 minutes at 560nm. The enzyme activity was measured in units of U/g tissue. The activity of glutathione peroxidase (GPX) (EC 1.11.1.9) was an indirect measure. The assay is an indirect measure of c- GPx activity. Oxidized glutathione (GSSG), produced by c- GPx was recycled to its reduced state by the enzyme glutathione reductase (GR). The drop in absorbance at 340nm caused by NADPH oxidation to $NADP^+$ provides a spectrophotometric technique of measuring the activity of GPx enzymes. At 340nm proposing a spectrophotometric method for measuring GPx enzyme activity for molar extinction at 340nm, the NADPH coefficient was 6220 M1 cm1 A 340. A solution containing glutathione, glutathione reductase, and NADPH wa applied to a cell or tissue homogenate to test c-GPx. The enzyme activity started by introducing the substrate, hydrogen peroxide and measuring the A340. The sample GPx activity is directly proportional to the rate of reduction in the A340 **(Paglia & Valentine, 1967**). The enzyme activity was measured in micro units per milliliter (mU/mL).

Statistical estimation

The results were expressed as means ± standard errors. The data was then evaluated using a one-way analysis of variance to see if there were any differences in the amounts of elements among the different fish species under study. Tukey honestly significant difference analysis (Turkey's HSD) was used to quantify differences between means. Meanwhile, a one-tailed t-test was used to examine the significance of the differences in element means.

RESULTS AND DISCUSSION

The Concentration of toxic elements in fish muscle tissue

The assessment of element quantities is the initial process to analyze the degree of toxicity in fish. One of the most serious dangers to humans and aquatic animals is toxic element contamination in fish. Concentrations (wet weight) of elements in this study were listed in a descending order (Fig. 2) as follows: Pb (2.85) > Al (1.47) > As (1.46) > Hg (0.72) for *O.aureus*, Pb (1.87) > Al (1.85) > As (1.41) > Hg (0.55) for *S. galilaeus*, Pb (2.38) > As (1.52) > Al (1.12) > Hg (0.65) for *T. zillii*, the sum of average of the concentrations of four elements for each was maximum in *O.aureus (6.50) > S. galilaeus (5.68) > T. zillii (5.67).* Among elements, Pb was the maximum residue in *O.aureus* (2.85±0.2), while Hg was the minimum residue in *S. galilaeus* (0.55±0.04).

In this study, the three aquatic organisms assessed had insignificant Hg average levels (*P* > 0 .05, *F*= 1.51946 & *P*-value = 0.250672), which were arranged as *O.aureus* (0.72±0.04mg/kg), *T. zillii* (0.65±0.04 mg/kg), and *S. galilaeus* (0.55±0.04 mg/ kg) that almost in FAO/WHO (0.5 µg/g) is an allowable limit **(FAO/WHO, 1992**; **EC, 2006)** amended by **EC (2008)** and **EC (2011)** (0.5 µg/g). Hg average values in fish samples from the Pra River ranged between 0.40 and 0.48 mg/kg, which are less than those detected in the current study. Hg values in *O. aureus* 0.78 ,*T. zillii* 0.60 , *S. galilaeus* and 0.54 µg/g were observed in **Abdel-Kader and Mourad (2020)** indicating that practically all fish contaminated Hg levels from Burullus Lake are in the same range of our results with respect to Manzalah Lake. In comparison, Hg values in Edku Lake *S. galilaeus* (0.35±0.1) and *O.aureus* (0.476±0.1) are fairly low than our findings. The negative effect of low doses of Hg intake by children and adults can cause neurotoxicity.

In this investigation, As concentration was non- significant (*P* > 0.05), with (f-ratio value of 0.33839, and the *p*-value of 718222) in the muscle tissue observed in *T. zillii* (1.52±0.08), *O. aureus* (1.46±0.08), and *S. galilaeus* (1.41±0.08) and lower than the permissible limit suggested by **FAO/WHO (2004)** (2.0 µg/g). *T. zillii* (1.70), *O. aureus* (1.55), and *S. galilaeus* (1.50µg/g) from Burullus Lake, Egypt recorded higher average As concentrations compared to our results **(Abdel-Kader & Mourad, 2020)**. In addition, As in *O. aureus* (2.16µg/g) from Edku Lake is significantly higher than ours and recommended results and in *S. galilaeus (*1.31) **(Abdel-Kader & Mourad, 2021)**. Prolonged exposure to As may induce several effects on the liver, skin, cardiovascular and hematopoietic systems, as well as the gastrointestinal and respiratory tracts **(Mandal & Suzuki, 2002)**.

In this study, non-significant ($P > 0.05$) Al average concentration with the *f-ratio* value (0.33839), and the *P-value* 0.718222 in *S. galilaeus* was 1.85±0.1, while in *O.aureus,* it was 1.47±0.2, and 1.12±0.1 for T. *zillii*. In another study from northern lakes of Egypt, the Al levels in *O. aureus* (1.96), *T. zillii* (1.85), and *S. galilaeus* (1.93, µg/g) from Burullus Lake were greater than our values **(Abdel-Kader & Mourad, 2020)**. O. *aureus(* 0.890) and *S. galilaeus* (0.56µg/g) from Edku Lake recorded average Al residue lower than our results **(Abdel-Kader & Mourad, 2021)**. This suggests that the fish are contaminated with Al from Burullus, followed by El Manzalah, and finally Edku.

Lead (Pb) concentration was significant $P < 0.05$ with the f-ratio value (4.72118) and the *P*-value (0.025681) in the muscle tissue observed in *O.aureus* (2.85±0.2), *T. zillii* (2.38±0.1), and *S. galilaeus* (1.87±0.2). This value is higher than the recommended acceptable limit of **FAO/WHO (1993)** (0.5 µg/g), **FAO/WHO (1999)** (0.214 µg/g), and **EC (2006)** amended by **EC (2008), EC (2011)** (0.30 µg/g). In contrast, **Sarker *et al* (2022)** found Pb levels in muscle tissue in fish meal samples (0.189). In other Nortehern Lakes, *S. galilaeus* (1.53), *O. aureus* (1.33) and *T. zillii* (1.20 µg/g) recorded Pb in muscle tissue from Burullus Lake, Egypt in the study of **Abdel–Kader and Mourad (2020)**, giving results much lower than ours. Whereas, *S. galilaeus* (0.966) and *O.aureus* (0.963) which were investigated in the work of **Abdel–Kader and Mourad (2021**) estimated Pb in muscles from Edku, Egypt and recorded much lower values than our findings. This suggests that the Pb levels in the fish from Manzalah Lake were much higher than those collected from Edku and Burullus. Lead ingestion can induce renal failure and liver failure, and continued exposure can cause mental retardation, coma, and even mortality in severe cases **(Al-Busaidi *et al.*, 2011)**.

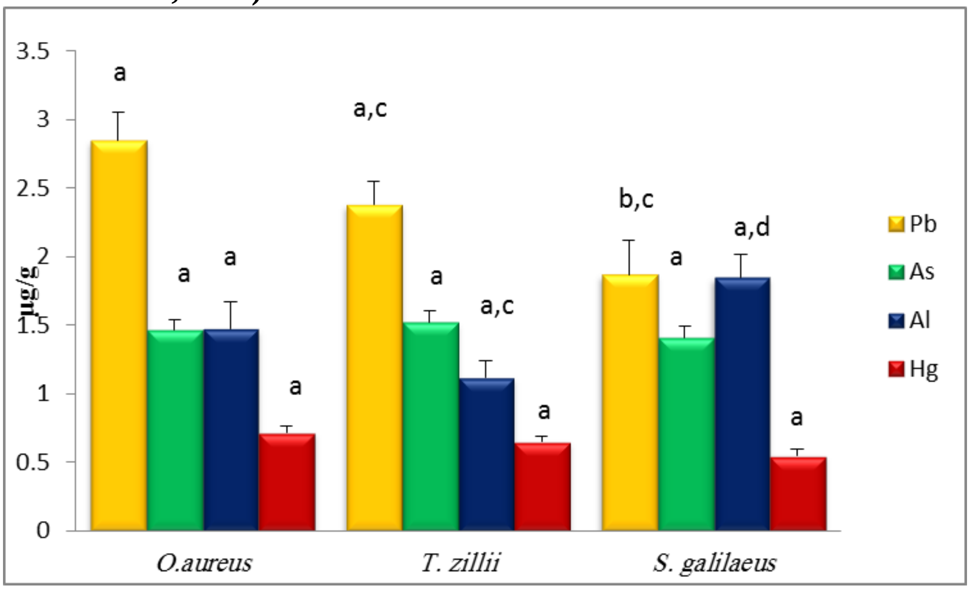

Fig. 2. Mean ±SE of concentrations (µg/g wet weight) of trace elements of *Oreochromis aureus, Tilapia zillii,* and *Sarotherodon galilaeus* from Manzalah Lake, Egypt, (n=6)
Different superscripts letters have statistical significance. P < 0.05

Human health risk assessment

Estimated daily (EDI) or weekly intake (EWI), the percent of provisional tolerable weekly intake percentage, maximum daily intake (MDI) or weekly intake (MWI)

Egypt's populations eat a lot of fish and because muscle is the most appetizing part of fish for humans, the dangers associated with its consumption must be considered.

Tables (1, 2) highlight the risk assessment results for EDI, EWI, THQ and CR. Firstly, Table (1) illustrates EDI (µg/kg bw/d), EWI (µg/kg bw/w), PTWI percent, MDI, & MWI of Pb, Hg, Al, & As in the tissue muscles of *O. aureus, S. galilaeus,* and *T. zillii* from Manzalah Lake in Egypt considering children 15kg, youth 40kg, and 70kg adult consumes.

PTWI values set by **FAO/ WHO (2004)** are as follows: for Pb (0.025), Hg (0.005), As (0.015), and Al (1) mg /kg^{-1}. The results showed that, Hg contributes the least EDI, EWI, and PTWI percent and Pb contributes the most EDI, EWI, and PTWI percent from the selected three fish species. The analysis indicates that the EDI, EWI, and PTWI percent values obtained for the Pb and As in *O. aureus*, *S. galilaeus*, and *T. zillii* eaten by a child (15kg) were greater than the FAO/WHO/ PTWI standards. Lead and As in *O. aureus* and *T. zillii* eaten by young, Pb in *O. aureus* eaten by adult, and Hg in *O. aureus, S. galilaeus,* and *T. zillii* eaten by child, young, and adult were recorded. These were over the suggested ranges, implying a risk to people's health related to the consumption of different fish species.

Table (1) provides guidance on safe levels of consumption MDI and MWI of *O. aureus*, *S. galilaeu*s, and *T. zillii* for children, youths, and adults in regards to Al >As> Pb> Hg intake. Following Table (1), our results indicate that the EDI and EWI of Pb > Al > As > Hg rank children >youth > adults based on *O. aureus, S. galilaeus,* and *T. zillii* intake.

Based on the result shown in Table (1) *O. aureus*, *S. galilaeus*, and *T. zillii* consumption, the PTWI % followed a sequence of Hg> Pb> As> Al. This indication is based on the calculated EDI & EWI of muscles for the three fish species in a diet on a day or diet in the week. This study recommended that children consume no more than 14.88g/day or 104.16g/week of *O. aureus* muscle, 19.48g/day or 136.36 g/week of *S. galilaeus* muscle, and 16.48g/day or 115.38g/week of *T. zillii* muscle. For the youth, they should consume less than 39.68g/day or 277.77g/week of *O. aureus* muscle, 51.49g/day or 363.63g/week of *S. galilaeus* muscle, and 43.95g/day or 307.69 g/week of *T. zillii* muscle. Furthermore, adults should eat less than 69.44g/day or 486.11g/week of *O. aureus* muscle, 90.90 g/day or 636.36g/week of *S. galilaeus* muscle, and 76.92g/day or 538.46g/week of *T. zillii* muscle.

Uroko *et al*. (2020) recorded that EDI of Pb from eating commercial fishes from Ore Ugba and Urbani market-Umuahia Metropolis was significant. People who ate these commercial fish eventually saturate their bodies with lead, resulting in chronic poisoning**. Iqbal *et al.* (2017)** estimated EDI values of As for *Labeo rohita* (0.016), and *Wallago attu* (0.017). **Hossain *et al.* (2022*)*** evaluated that the fewer EDI values were found for Pb by adults 0.0004 and children 0.0018 mg/day/person in some fishes at Meghna River. **Abdel-Kader and Mourad (2021)** estimated that EDI & EWI of As> Pb > Al > Hg intake by children >youth > adults regarding intake of *S. galilaeus* muscles, whereas EDI & EWI of As> Cd >Pb > Al > Hg by children >youth > adults regarding *O. aureus* intake. **Yacoup *et al*. (2012)** recorded that EDI of Pb in some fish species was greater than the allowable tolerable daily intake proposed showing a high risk to human intake fish**. Zaza *et al*. (2015)** showed that the EDI of Pb, & Hg via fish plus seafood intake by local consumers did not surpass the proposed PTWIs.

Table 1. EDI, EWI, and PTWI %, MDI and MWI for different trace elements estimated in *Oreochromis aureus, Tilapia zillii,* and *Sarotherodon galilaeus* from Manzalah Lake

Element	Intake	*O. aureus*			*T. zillii*			*S. galilaeus*		
		Child	Young	Adult	Child	Young	Adult	Child	Young	Adult
Pb	***EDI***	11.82	4.43	3.94	9.87	3.70	3.29	7.76	2.91	2.58
	EWI	82.74*	31.01*	27.58*	69.09*	25.9*	23.03	54.32*	20.37	18.06
	PTWI	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0	25.0
	PTWI%	330.96	124.04	110.32	276.36	103.6	92.12	217.28	81.48	72.24
	MDI	18.79	50.1242	87.71857	22.5085	60.0228	105.041	28.6471	76.3928	133.68
	MWI	131.57	350.87	614.03	157.56	420.16	735.29	200.53	534.75	935.82
As	***EDI***	6.05	2.27	2.02	6.30	2.36	2.10	5.85	2.19	1.95
	EWI	42.35*	15.89*	14.14	44.1*	16.52*	14.7	40.95*	15.33*	13.65
	PTWI	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
	PTWI%	282.33	105.93	94.2666	294	110.13	98	273	102.2	91
	MDI	22.014	58.7071	102.7385	21.1457	56.39	98.6828	22.7957	60.79	106.382
	MWI	154.10	410.95	719.17	148.02	394.73	690.78	159.57	425.53	744.68
Al	***EDI***	6.10	2.28	2.03	4.64	1.74	1.55	7.67	2.87	2.56
	EWI	42.7	15.96	14.21	32.48	12.18	10.85	53.69	20.09	17.92
	PTWI	1000	1000	1000	1000	1000	1000	1000	1000	1000
	PTWI%	4.27	1.596	1.421	3.248	1.218	1.085	5.369	2.009	1.792
	MDI	1457.7	3887.26 8571	6802.7214 29	1913.265 714	5102.04 1429	8928.57 1429	1158.30 1143	3088.80 2857	5405.40 5714
	MWI	10204.08	27210.88	47619.05	13392.86	35714.29	62500	8108.108	21621.62	37837.84
Hg	***EDI***	2.98	1.12	0.99	2.69	1.01	0.89	2.28	0.855	0.76
	EWI	20.86*	7.84*	6.93*	18.83*	7.07*	6.23*	15.96*	5.985*	5.32*
	PTWI	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	PTWI%	417.2	156.8	138.6	376.6	141.4	124.6	319.2	119.7	106.4
	MDI	14.88	39.68	69.44	16.482	43.955	76.922	19.48	51.947	90.908
	MWI	104.16	277.77	486.11	115.38	307.69	538.46	136.36	363.63	636.36

***Surpass PTWI regarding FAO/WHO (2004)**

Target hazard quotient (THQ) and target cancer risk (TCR)

The **USEPA** has proposed that THQ and HI (**USEPA, 2015**) are hazard analysis measures that evaluate the level of a contaminant eaten with a standard reference amount and have been broadly applied in the risk assessment of elements in contamination.

Table (2) summarizes the assumed THQs of Pb, Hg, As, and Al toxicity upon eating from the three species per a day or seven days per week from Manzalah Lake. Table (2) shows that, the estimated THQs values of Pb, Hg, and Al for the *O. aureus* > *T. zillii* > *S. galilaeus* & THQs values of As for the *T. zillii* > *O. aureus* > *S. galilaeus* more than the safe value of one except for THQ of Pb and Al in *T. zillii* for adult and *S. galilaeus* for youth and adult suggesting that, consuming these species on a daily or weekly basis may pose a health risk to humans. The THQs levels of Pb, Hg, As, and Al in children > teens > adults were given in the current results. Furthermore, the THQ value of As in *T. zillii* consumption by a child was the highest, ranging from (21 a day to 147 a week); whereas, the THQ value of Pb in *S. galilaeus* intake by an adult was the lowest (0.73 a day or 5.11 a week). From this investigation, the total target hazard quotient (TTHQ) values of 3 fish species for children, youth, and adult were determined high non-carcinogenic risk consumed by humans.

Table (2) shows the cancer risk of Pb and As from eating three types of fish from Manzalah Lake in this study. TCR of Pb levels in the permissible level of the **USEPA (2012)** allowed limits for *O. aureus* > *T. zillii* > *S. galilaeus*, but TCR of As levels for *T. zillii*> *O. aureus* > *S. galilaeus* are over the **USEPA limits (2012)**. Thus, eating these species on a daily or weekly basis poses serious danger to humans. Table (2) reported that Pb, and As levels examined the TCR level in children > teens > adults. *T. zillii* in children had the greatest level of CR (9.45E-03 a day or 6.62E-02 a week), whereas *S. galilaeus* in adults had the lowest level of CR (2.19E-05 a day or 1.54E-04 a week). As a result, the risk of cancer in consumers from eating element-contaminated fish should not be neglected. **Hossain *et al*. (2022)** recorded that both the THQ and HI values for child & adult consumers were <1, showing that the consumers did not pose a non-carcinogenic risk to humans. **Ulusoy Sühendan Mol (2022)** recorded that all THQs were less than one for the Turkish & Dutch consumers, reporting no risk for the consumption of sea bass. **Mielcarek *et al.* (2022)** recorded that THQ &HI pose for the tested studied fish species' non-carcinogenic effect. **Chukwuemeka (2018)** evaluated that the THQ, HI& CR in the fish samples was less than one and maybe slight toxicity was done. **Jia *et al.* (2017)** reported that the HI & CR values posed no non-carcinogenic risk to local inhabitants. Adversely, the CR value in *P. fulvidraco* was greater than the allowable limit, representing CR risk for consumers. **Kortei *et al*. (2020)** reported CR of Pb, Hg, and As, intake by fish eating from Rivers for both children & adults, and found no considerable non-carcinogenic harmful health risk to humans because all estimated HQ values were<1. However, the THQ values assessed for children and adults exposed to Mercury were greater than one, which suggested a possible source of negative impacts during a human lifetime. **Yi *et al.* (2017)** estimated HQ values<1 which suggested no possible health effects of metals on humans by intake fish daily from the Yangtze River, China, TTHQ exceeded 1 with possible non carcinogenic health effect. **Ezemonye *et al.* (2019)** recorded THQ < 1 which represented non carcinogenic health effects from eating shrimps and accumulated Pb.

Table 2. THQ and TCR for the different trace elements estimated in *Oreochromis aureus, Tilapia zillii,* and *Sarotherodon galilaeus* from Manzalah Lake, Egypt

Fish	Non- Carcinogenic risk THQ										Carcinogenic risk CR			
	(once a week)					(7 times a week)					(once a week)		(7 times a week)	
	Pb	As	Al	Hg	TTHQ	Pb	As	Al	Hg	TTHQ	Pb	As	Pb	As
Child														
O. aureus	3.38	20.16	15.25	9.93	48.72	23.64	141.1	106.7	69.5	341.04	1.00E-04	9.08E-03	7.03E-04	6.35E-02
T. zillii	2.82	21	11.6	8.96	44.38	19.74	147	81.2	62.7	310.66	8.39E-05	9.45E-03	5.87E-04	6.62E-02
S. galilaeus	2.21	19.5	19.17	7.6	48.48	15.52	136.5	134.2	53.2	339.36	6.60E-05	8.78E-03	4.62E-04	6.14E-02
Teens														
O. aureus	1.26	7.56	5.7	3.73	18.25	8.86	52.96	39.9	26.1	127.75	3.77E-05	3.41E-03	2.64E-04	2.38E-02
T. zillii	1.05	7.86	4.35	3.36	16.62	7.40	55.06	30.45	23.5	116.34	3.15E-05	3.54E-03	2.20E-04	2.48E-02
S. galilaeus	0.83	7.3	7.17	2.85	34.77	5.82	51.1	50.22	19.9	243.39	2.47E-05	3.29E-03	1.73E-04	2.30E-02
Adult														
O. aureus	1.12	6.73	5.07	3.3	16.22	7.88	47.13	35.52	23.1	113.54	3.35E-05	3.03E-03	2.34E-04	2.12E-02
T. zillii	0.94	7.0	3.87	2.96	14.77	6.58	49	27.12	20.7	103.39	2.80E-05	3.15E-03	1.96E-04	2.21E-02
S. galilaeus	0.73	6.5	6.4	2.53	16.16	5.11	45.5	44.8	17.7	113.12	2.19E-05	2.93E-03	1.54E-04	2.05E-02

Determination of proximate composition

Proximate composition, such as protein contents, carbohydrates, lipids, moisture contents, and ash percentage, is frequently required to verify that fish tissues have acceptable nutrition quality and meet food regulations and commercial requirements (**WHO/FAO, 2011**).

The proximate composition of three different tilapia freshwater fish was assessed (Fig. 3), with *O. aureus* recording the greatest significant mean carbohydrates (mg/g) level (16.66±1.0), *S. galilaeus* 13.66±0.6, and *T. zillii* recording the lowest 13.16±0.8**. Suganthi *et al*. (2015)** found that carbohydrate content varied from 2.87 to 4.26 mg/g, with *Lutjanus fulviflamus* having the highest carbohydrate content 4.26 mg/g, and *Stolephorus commersonii* had the lowest carbohydrate content 2.87 mg/g. **Ayanda *et al*. (2018)** reported decreasing in carbohydrates in the lobster than other studied organisms. **Abdel-Kader and Mourad (2020)** recorded that *T. zillii* from Burullus Lake had 14.1 mg/g carbohydrate content.

In fish muscle tissues, protein is the second major composition (**FAO/WHO, 2010**). In this investigation, the highest non-significant mean protein content level (mg/g) was found in *S. galilaeus* (59±4.8), followed by *O. aureus* (58.66±6.2), and *T. zillii* (56.66±4.1). **Suganthi *et al.* (2015)** reported the protein content of *Sillago sihama* was quite high (26.69 mg/g). *Plotosus canius* had the lowest concentration (14.69 mg/g). **Ayanda *et al.* (2018)** showed that protein content had the highest in crab, followed by lobster, and tilapia fish had the least. **Abdel-kader and Mourad (2020)** estimated *O. aureus* (64.83) , *T. zillii* (57.16), *S. galilaeus* (53.75) mg/g from Burullus Lake. Whereas **Abdel-Kader and Mourad (2021)** recorded *S. galilaeus* (65.16) and *O. aureus* (61.16) from Edku Lake. **Vijayan *et al.* (2015)** reported protein content of *N. orientalis* (18.6) and *C. corniger* (19.4) and *S. argus* (20.4). **Ayanda *et al.* (2019)** reported that *Chrysichthys nigrodigitatus* had the greatest protein content (19.34).

In this investigation, the greatest significant mean fat content (mg/g) level in O. *aureus* (15±0.5), S. *galilaeus* (9.9±0.3) and the lowest (8.8±0.3) in *T. zillii* . **Suganthi *et al. (*2015)** reported that the lipid content between from 1.16 to 1.91 mg/g. *Liza parsia*, had the highest lipid content in muscle tissues (1.91 mg/g) but the lowest lipid in *Hemiramphus* sp., (1.16 mg/g). **Ayanda *et al.* (2018)** recorded that significant differences in fat content were greatest in tilapia followed by lobster then lowest in crab. **Abdel-Kader and Mourad (2021)** estimates *galilaeus* (20.48) and *O. aureus* (21.38) from Edku. **Vijayan *et al.* (2016)** reported that fat content in *N. orientalis* was commensurate with spotted scat. *C. corniger* had the highest fat content (14.6). **Ayanda *et al.* (2019)** reported that the highest fat content in *Oreochromis niloticus* and the least in *Chrysichthys nigrodigitatus*. **Abdel-Kader and Mourad (2020)** evaluated *S. galilaeus* (13.830 , *O. aureus*(13.33), and *T. zillii* (11.98) from Burullus.

As a mineral, ash is an important ingredient in fish diets (**Paul and Mukhopadhyay, 2001; Paul and Giri, 2009**). In this investigation, the greatest statically significant ash content (%) level was found in *T. zillii* (14.91±1.0) whereas 14.75±0.3 was in *S. galilaeus*, and the least was in *O. aureus* (3.93±0.12). **Ayanda *et al.* (2018)** recorded that Crab had the greatest ash content then tilapia and finally lobster. **Abdel-Kader and Mourad (2020)** estimated *S. galilaeus* (14.25), *T. zillii* (12) and *O. aureus* (3.76). **Abdel-Kader and Mourad (2021)** estimated ash content in *S. galilaeus(*10.66) and *O. aureus* (7) from Edku Lake. **Ayanda *et al.* (2019)**. demonstrated that *Chrysichthys nigrodigitatus* had the highest ash content but the lowest in *Parachanna obscura*.

Water is essential for many biological molecules to function normally. It is found in tissues in two forms: linked to proteins and in a free form. In this research, the biggest non-significant mean average water content (%) level in *T. zillii* (71.16±1.3), O. *aureus* (67.25±1.5), while the less percent (65.16±2.42) in S. *galilaeus* . **Ayanda *et al.* (2018)** showed that the moisture was biggest in tilapia fish, lobster, and then crab. **Abdel-kader and Mourad (2020)** estimated *O. aureus* (67.33), *S. galilaeus* (60.75), and *T. zillii* (64) from Burullus, Egypt. **Abdel-kader and Mourad (2021)** estimated *S. galilaeus* 77.33 % and *O. aureus* 77 % from Edku Lake.

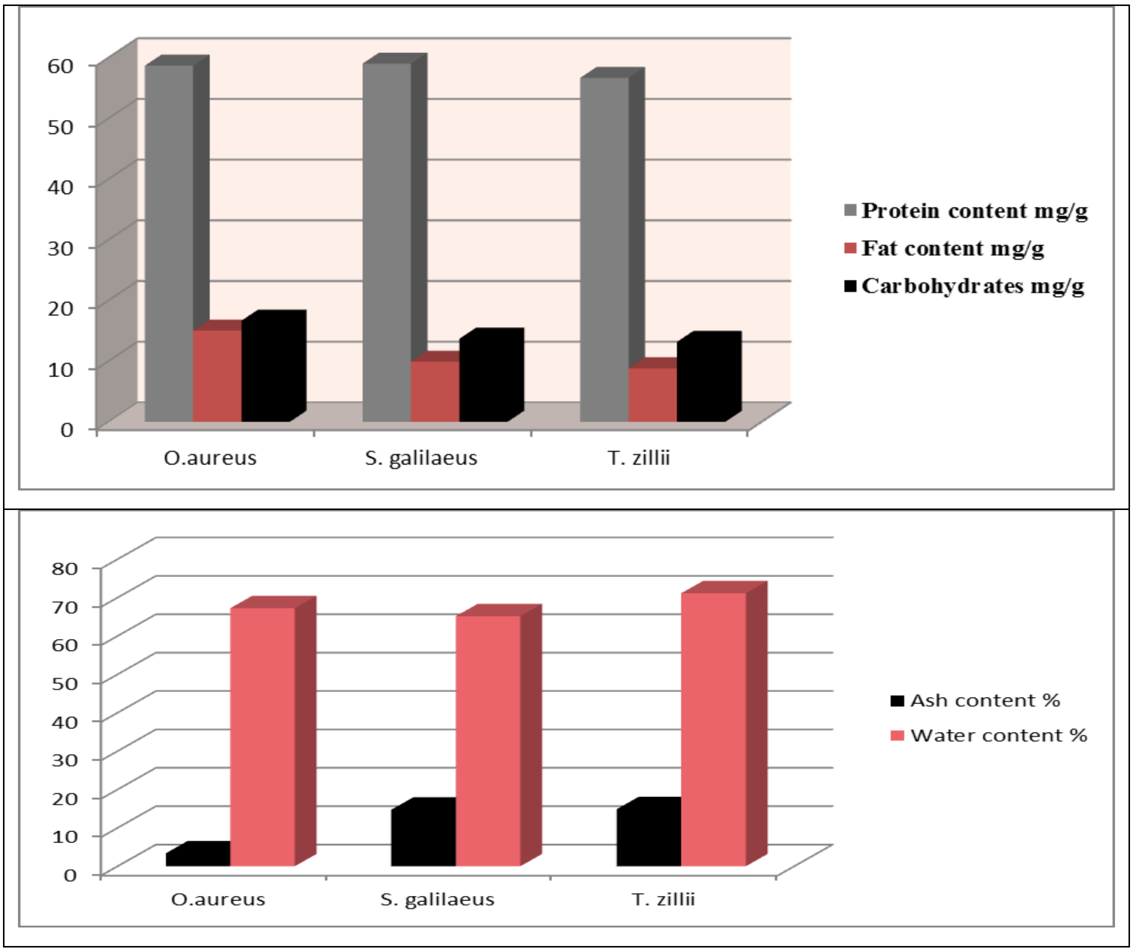

Fig. 3 Proximate composition of *Oreochromis* aureus, *Sarotherodon galilaeus*, and *Tilapia zillii* from Manzalah Lake, Egypt. (n=6).

Liver Antioxidants

Aquatic pollution by contaminants causes oxidative stress to aquatic creatures, which leads to death (**javed, 2017**). The antioxidant defense system is a critical response for reactive oxygen species (ROS) and helps protect from oxidative stress **Naz *et al.* (2019).** Numerous mechanisms in fish maintain the balance between antioxidant & prooxidant mechanisms that determine oxidative reactions **Halliwell *et al.* (1995)**.Antioxidant enzymes help cells maintain their homeostasis by scavenging highly reactive metabolites generated throughout hydrocarbon metabolism. These antioxidant enzymes interact differently with different chemical substances. SOD and CAT are the major antioxidant enzymes that aid in the neutralization of the toxicities of reactive oxygen species in fish. Superoxide dismutase is an important enzyme that acts as the first line of defense against pro-oxidants by catalyzing the conversion of superoxide radicals (O−) to H2O2 or O2 (**Abdel mageid *et al.*, 2020**). CAT (a heme-containing enzyme) that aids the elimination of Hydrogen peroxide, which is broken down to O2 and H2O (**Arojojoye and Adeosun, 2016).**

In this investigation, the liver antioxidants CAT, GR, GSH, SOD, and GPx showed an insignificant decrease in mean values ± SE (Fig. 4). SOD (U/g) calculated a non-significant difference $P > 0.05$ in tilapia fish species. SOD in *T. zillii* average value (31.33±3.2), *O. aureus* (30.66±3.5) and *S. galilaeus* (28.33±3.9) for the lowest value. CAT (U/g) in *S. galilaeus* average value (19.00±2.9), *O. aureus* (16.00±2.4) and *T.*

zillii (15.83±1.9) for the least value. Gpx (mU/mL) showed significant difference $P < 0.05$ in *S. galilaeus* (30.66±3.1), *O. aureus* (30.5±4.1) and the lowest value for *T. zillii* (23.33±1.9). The insignificant difference in GR (U/L) for the tilapia species *S. galilaeus* (18.00±0.9), *O. aureus* (16.16±3.2) and the lowest value for *T. zillii* (15.00±1.95). Insignificant difference in GSH (mg/g.tissue) was found in *O. aureus* (22.83±2.3), *S. galilaeus* (18.33±1.9) and *T. zillii* (17.66±1.7) for minimum value. **Javed *et al*. (2017)** reported that oxidative stress indicators such as SOD, and CAT, were significantly elevated in *Labeo rohita*, while GSH levels were very low. **Kumar *et al.* (2018)** discovered a significant decrease in SOD and CAT activity in Anabas testudineus from a polluted lagoon in India. **Ibrahim and Mosaad (2021)** discovered the decrease in the activity in the liver SOD, and CAT of *Crenimugil crenilabis* from Suez Canal. **Abdel-Kader and Mourad (2020)** discovered that CAT, GR, GSH, SOD, and GPx liver activity recorded a non-significant reduction in *S. galilaeus, O. aureus*, and *T. zillii* collected from Burullus Lake. **Abdel-Kader and Mourad (2021)** reported CAT, GR, GSH, SOD, and GPx reduction in the liver of *S. galilaeus*,and *O. aureus*, from Edku Lake.

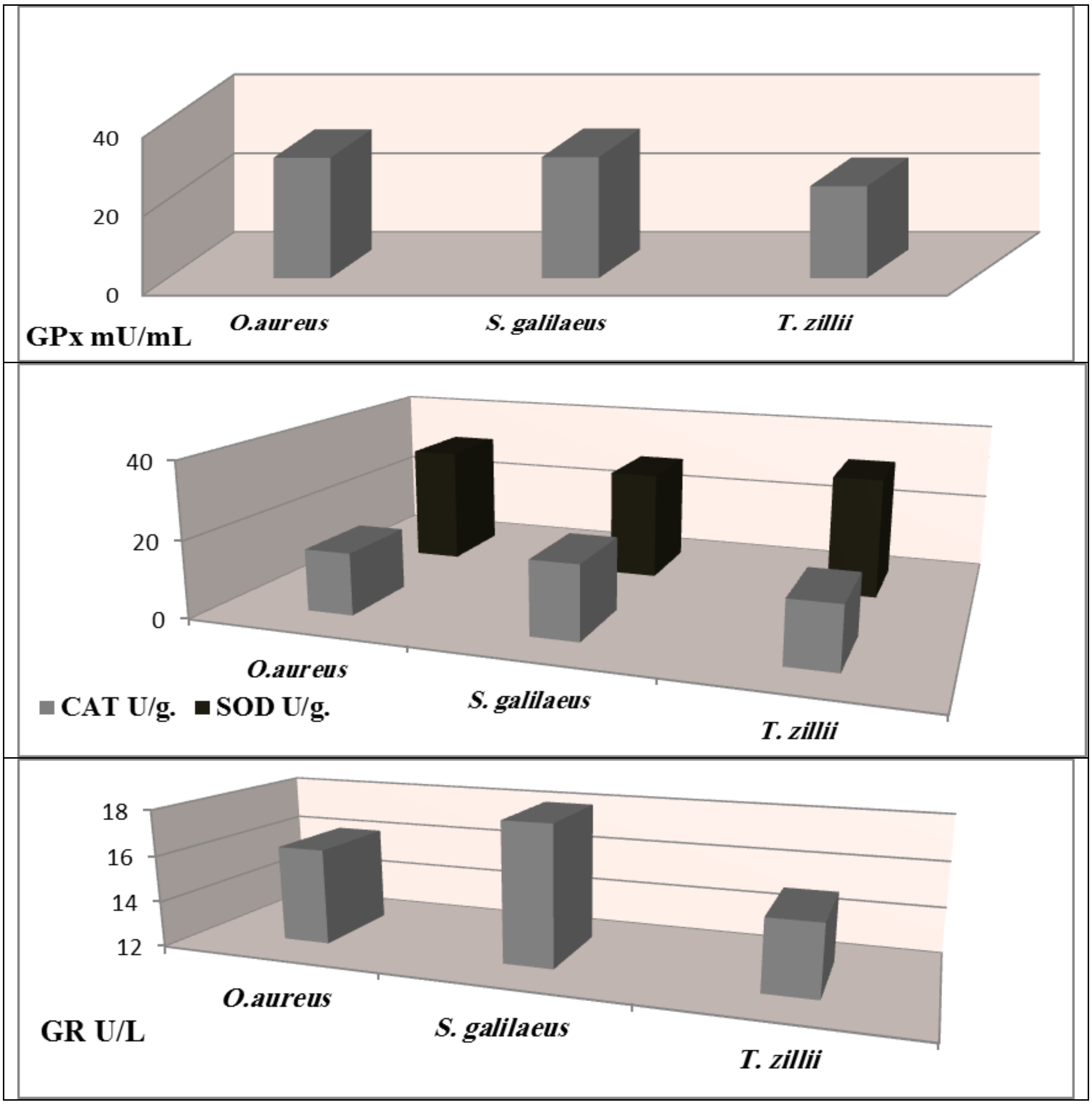

Fig. 4. Antioxidants in the liver of *Oreochromis* aureus, *Sarotherodon galilaeus*, and *Tilapia zillii* from Manzalah Lake, Egypt (n=6).

CONCLUSION

The concentrations (wet weight) of studied elements were listed in descending order as Pb > Al > As > Hg for *O.aureus*, and for *S. galilaeus*, whereas Pb > As> Al > Hg for *T. zillii*. Pb and Hg were considerably above the FAO/WHO and EC guideline levels. Based on the measured EDI or EWI of muscle for freshwater fishes, it was observed that children should eat less than 14.88 g of O. aureus muscle per day or 104.16 g per week, *S. galilaeus* muscle: 19.48 g/day (136.36 g/week) and T. zillii muscle: 16.48 g/day (115.38 g/week). Youth, should eat no more than 39.68 g/day (277.77 g/week) *O. aureus* muscle, 51.49 g/day (363.63 g/week) *S. galilaeus* muscle, and 43.95 g/day (307.69 g/week) *T. zillii* muscle. Finally, adults should eat 69.44g g/day (486.11 g/week) *O. aureus* muscle, 90.90 g/day (636.36 g/week) *S. galilaeus* muscle, and 76.92 g/day(538.46 g/week) *T. zillii* muscle. According to the total target hazard quotient (TTHQ) values of three fish samples for children, youth, and adults, consuming these fish poses a high non-carcinogenic risk. Furthermore, T. zillii had the highest level of CR in youth, while *S. galilaeus* had the lowest level of CR in adults. THQs and TCRs values of Pb, Al, As, and Hg were found in more than one in the three examined species, exceeding the USEPA permissible limits demonstrating that daily and weekly consumption of these species could pose a considerable risk of non-carcinogenic and carcinogenic effects in humans. Indicating fish were generally unhealthy for the human diet. As a result, the risk of cancer to consumers from toxic fish should not be underestimated. This study showed that the fish from Lake Manzala were unhealthy, confirming the remarkable decision of our Egyptian government to simulate them according to presidential recommendations in restoring their financial, tourism, economic and environmental importance.

Acknowledgements

The authors acknowledge the encouragement and guidance of the National Institute of Oceanography and Fisheries, NIOF, Egypt.

Funding

This study was funded by the National Institute of Oceanography and Fisheries, NIOF, Egypt.

Declaration of Competing Interest

The authors report no declarations of interest.

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