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Investigation of a Balanced Healthy Diet for Human Consumption of *Clarias gariepinus* and *Oreochromis niloticus* species from Lake Manzalah (Egypt), and Target Cancer/ Hazard Quotient (Non-Cancer) Risk Metrics

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ABSTRACT

The task of this study was to calculate a maximum daily and weekly balanced healthy diet MDI-MWI for human consumption of *Clarias gariepinus* and *Oreochromis niloticus* fish species from Lake Manzalah (Egypt), as well as determine the target cancer/hazard quotient (non–cancer), and risk assessments on human health through physiological and biochemical examinations. the Results showed that the children should not eat more than 13.39g a day or 93.75 g a week of *C. gariepinus* muscles, and 15.09g a day or 105.63g a week of *O. niloticus* muscles. The suggested daily intake for youth should be 34.01g or 238.09g a week of *C. gariepinus* muscles, and 40.24g for daily intake or 281.69g for weekly intake of *O. niloticus* muscles. Hence, adults are recommended to avoid consuming more than 59.52g a day of *C. gariepinus* muscle or 416.66g a week and 62.02g a day of *O. niloticus* muscles or 434.14g a week. This investigation also showed that *C. gariepinus* had the maximum protein and moisture contents; whereas, the maximum fat, ash, and carbohydrate contents were found in *O. niloticus*. In this research, the investigated liver antioxidants GPx activity and GSH level represented significant ($P < 0.05$) average values between both fish species. Moreover, this investigation showed that the C. *gariepinus* had much greater levels of Pb, As, Al, and Hg than *O. niloticus*, and the accumulation of elements in both species was in the following order: Pb>As>Al >Hg. Among elements, Pb and Al concentrations in the two fish species recorded analysis of variance revealed a significant difference ($P <0.05$). In addition, Pb and As concentrations in the two fish species were above the National Egyptian Organization for Standardization and Quality Control EOSQC and international standards FAO, WHO and Commission Regulation EC. Furthermore, this study investigated the estimated daily intake EDI, as well as the estimated weekly intake EWI of Pb > As >Al >Hg ingested by children>youth>adults rely on *O. niloticus* and *C. gariepinus* muscles. *C. gariepinus* in children had the greatest level of target cancer risk TCR (1.01×10^{-2} a day or 7.07×10^{-2} a week); whereas, *O. niloticus* in children showed the lowest level of TCR (6.80×10^{-6} a day or 4.76×10^{-5} a week). Remarkably, the consumers are exposed to health risks if they highly consume the fish species under study from Lake Manzalah, according to the health-risk assessment indicators.

INTRODUCTION

For fisheries, Lake Manzala is a rich natural resource region. It is the largest lake in the northern Nile Delta's shore lakes **(Hereher, 2014)**. It accounts for approximately half of the total fish production over the Delta's northern lakes and approximately one-fifth of non-marine fisheries of Egypt's yearly output. According to **El-Asmar and Hereher (2011)** based on satellite images taken in 1973, the lake's overall area is estimated to be 1100 km^2. They had severely decreased from 1052 sq.km in 1984 to 720 sq.km in 2003. They attributed the shrinkage to coastal highway development and reclamation. Besides, agricultural drainage water started from the Hadous to Faraskour drainage passing Ramsis, El-Serw, and finally to domestic and industrial Bahr El-Baqr drainage pouring in the Manzala Lake. Every year, the drains pour over 7500 million cubic meters of toxic industrial, domestic, and agricultural effluent into the lagoon **(Abu Khatita *et al*. 2015; Yacoub *et al.* 2021).**

Trace elements are natural ecosystem components occuring in various concentrations in nature. Actually, these elements were formed by natural processes such as erosion, sedimentation, and decomposition as well as human activity during industrialization and rapid population expansion. Thus, those toxic materials are introduced in large quantities into the aquatic ecosystem, which threatens its stability and impacts the aquatic environment. This, in turn, coincides with significant bioaccumulation of hazardous elements in fish, which is subsequently transferred to humans as fish consumers *via* the food chain, leading to significant public health hazards **(Okyere *et al*., 2015)**.

Since Egypt's Manzalah Lake is highly polluted, the Egyptian government has set specific laws and regulations to manage and reduce pollution in this lagoon and under the presidency administration referring to the **2030 vision** for sustainable development.

The determination of trace element concentrations in environmental biota is a significant step in establishing the health hazard that the presence of these hazardous substances poses **(Abou El-Gheit *et al.*, 2012; Wahiduzzaman *et al*., 2022)**. These chemicals are characterized as carcinogens or non-carcinogens, and the majority of them are unfortunately detected in fish **(Iqbal Yu *et al*., 2014)**. The process of estimating the impact of threats on persons is known as risk assessment. Risks are classified into two categories: non-carcinogenic and carcinogenic hazards. The non-carcinogenic risk is calculated using the hazard quotient (HQ), while the carcinogenic risk is calculated using the TCR **(Markmanuel & Horsfall Jnr, 2016)**.

The **WHO (2020)** suggested that eating fish meals 1 or 2 times each week has health benefits. Fish are high-nutrient foods that aid in the maintenance of a balanced diet and the acquisition of adequate protein, vital amino acids, as well as a significant amount of beneficial polyunsaturated fatty acids, which aid to reduce the risk of heart disease **(Chari & Abbasi, 2005; Alipour *et al*., 2015)**. In addition, fish is soft, easily digestible, and high in vitamin B complex. It's also high in omega-3 fatty acids, magnesium, potassium, cobalt, phosphorous, iron, sodium, copper, iodine, zinc and fluorine mineral **(NRC, 1998; El-Bokhty 2010; Sallam, *et al*. 2019)**.

To investigate physiological and biochemical analyses, several scientists used catfish *C. gariepinus* and *O. niloticus* as laboratory animals **(El-Sappah *et al*., 2012, 2022; Abdel-Kader & Mourad, 2019a, b; Sallam *et al.* 2019)**. *C. gariepinus* is a large, grey/black eel-like fish with a white belly **(Bruton 1979, Skelton, 2001)**. While, *O. niloticus* is amongst the most widely bred freshwater fish species in the world **(de Oliveira, 2021; FAO, 2021)**. The analysis of proximate composition should show that fish tissues are healthy and safe (**WHO/FAO, 2011)**. Additionally, toxic elements can speed up the production of reactive oxygen species (ROS) in fish tissues that can overcome antioxidants, causing oxidative stress and experience elevated levels of oxidative damage **(Storey 1996; Doherty *et al*., 2010; Zheng *et al*., 2016; Khalil *et al*., 2017; Mokhamer *et al.*, 2019; Abdel-Kader & Mourad, 2022; El-Sappah *et al*., 2022)**.

This study investigated the levels of Pb, Al, As, and Hg, concentrated in the edible muscles of *C. gariepinus* and *O. niloticus* from the Manzala Lake in Egypt. The current observations were matched with the national (EOSQC) and international standards (FAO), (WHO) and (EC). Moreover, the present study calculated MDI-MWI for the human consumption of *C. gariepinus* and *O. niloticus* fish species from the Manzalah Lake (Egypt), and determined the appropriate balanced diet for children, teens, and adults regarding the PTWI according to (FAO) and (WHO). Furthermore, this study investigated the TCRs/THQs (non–cancer) risk metrics, and human health risk assessment including the EDI, EWI, and the percentage of PTWI. Finally, this research investigated different physiological and biochemical assessments to evaluate the quality of fish.

MATERIALS AND METHODS

Lake Manzalah is Egypt's largest lagoon and the most economically significant Nile Delta Lake. It is located at the eastern edge of the Nile Delta (31o 45' and 32o 15' E longitude and Lat 31o 00' and 31o 30' N latitude) **(Khalil, 1990**; **Elewa *et al.*, 2007; Ali, 2008; Arafa *et al.*, 2015)**.

Numerous fishermen grabbed living medium-sized fish species, namely *C. gariepinus* and *O. niloticus* from various locations surrounding the Manzala Lagoon in the spring of 2018, and then specimens were transferred to the Fisheries division- Physiology Laboratory at the National Institute of Oceanography and Fisheries: NIOF, Egypt. Distilled water was used to wash the fish. A clean plastic knife was used to separate the muscles and fish liver tissues from the specimens on a sterile glass plate. Sterilized polythene bags were used to keep the detachable parts safe. Until the different tests were finished, the labeled bags were kept in a fridge or freezer at 25 degree Celsius. Throughout the night, all glassware was dipped in 10% (v/v) nitric acid then 10 percent (v/v) solution of hydrochloric acid, then it was rinsed twice with distilled water and dried until it was ready to use. The chemicals supplied were those of Merck-Germany with the highest-quality analytical reagents. The calibration curve element standard solution was generated by diluting 1000mg/l stock solutions (Merck-Germany). Necropsy was performed for six of each fish species, and the skin was discarded before the musculature was taken and stored in aluminum foil.

For trace element analysis, each item was placed in a transparent plastic bag with a unique identifier and a date for pick-up, the fish were then stored frozen at -20°C until sampled and digested. In 25 milliliter Erlenmeyer flasks, precise grams of muscle tissues were weighed. Then, to each sample, a volume of 5ml nitric acid (65 %) Merck-Germany was added. The samples were then allowed to digest slowly over the next 24 hours. Each sample was subsequently treated with 2.5ml perchloric acid (72%, Merck-Germany). Digestion took place for 6 hours in a 150°C water bath, or until the solutions were clear and nearly dry. The solutions were then transferred to 50mL PE bottles and filled with distilled water to a capacity of 25ml. After that, the mixture was filtered into a cleansed glass beaker with 0.45m Whatman no.42 membrane filter. An amount of deionized water was adjusted to 50ml. It was necessary to dilute the filtrate. Perkin Elmer Analyst 800 graphite furnace-atomic absorption spectrometer (GF-AAS) was used to perform the element measurements. The concentrations of elements in fish muscle wet weight basis were evaluated in microgram per gram (µg/g; ww).Three replicate blank samples were digested using the same procedure (prepared in just the same way as digested samples only without the sample) and prepared together for each sample set to adjust background absorption. The standard deviation of the 3 replicate blanks for trace elements was used to determine LOD. Standard reference material DOLT-4 dogfish liver was used to verify the analytical method. The recovery range was between 94-98 %.

For human health risk assessments, the equation of EDI = C× IR/BW was used to estimate the daily intake of element through the consumption of fish muscle; where, C represented the element concentrations in fish samples (µg/g-ww); IR was the daily intake rate of fish (62.25 g/person/ day) related to the Ministry of Agriculture and Land Reclamation, Egypt (2017) and Central Agency for Public Mobilization and Statistics (2017), and BW represented the average body weight (15kg for a child, 40kg for a youth, and 70kg for an adult) **(Salas *et al.,* 1985; Albering *et al.,* 1999)**. The formula of EWI= EDI× 7 was used to estimate weekly intake to set a comparison with the safety standards of FAO/WHO to PTWI. For EWI less than PTWI, customers' food consumption does not constitute a serious health risk. The percentage PTWI = EWI/ PTWI×100 was investigated by FAO/WHO recommended possible health reference dose **(FAO/WHO, 2004)**. The formula of MDI= PTWI× BW/C×7 is to investigate the advised maximum daily intake in grams, based on the maximum weekly intake formula MWI = MDI × 7 that children, teens, and adults diet should maintain PTWI to achieve balanced diet **(FAO/WHO, 2004)**. The non- carcinogenic risk was estimated by using the formula of $THQ=EF \times ED \times IR \times C / RfD \times WAB \times ATn \times 10^{-3}$ to estimate target hazard quotient **(US EPA, 2012)**. The EF shows the exposure frequency [days/year]; ED represents the exposure duration [years]; IR is the ingestion rate (g/day); C shows the metal concentration (µg/kg); RfD is the oral reference dose (Pb = 3.50×10^{-3}; Hg $=3.0\times10^{-4}$, As$=3\times10^{-4}$& Al$=3\times10^{-4}$ mg kg^{-1} day^{-1}); WAB (kg) represents the average body weight; and for non-carcinogens ATn represents the average exposure time (days/year × ED). The equation of TTHQ or HI= THQ_1 + THQ_2 +THQ_n **(US EPA 2012)** was used to estimate the hazard index (HI) as the total of the all estimated THQ (TTHQ), if THQ value >1, then it poses health risks associated with fish consumption, and hence protective measures should be taken **(US EPA, 2019)**. The TCR = EF× ED ×IR × C × CSF/WAB ×ATc × 10^{-3} (**US EPA 2019),** where CSF (mg, kg^{-1} day^{-1}) is the cancer slope factor (Pb = 8.5 × 10^{-3} and As = 1.5) (**US EPA 2019**), and ATc for carcinogens is the average time days/year × ED. If the estimated value of CR was greater than 10^{-4}, an increased risk of a carcinogenic effect is recognized; however, a CR under 10^{-4} would verify an acceptable risk **(US EPA, 1989; NYSDOH, 2007; US EPA, 2011)**.

For proximate chemical composition (moisture, fat, protein and ash) analysis, an amount of 0.1g of muscle tissue was homogenized in 5ml saline using a glass homogenizer for three minutes; it was then centrifuged for 10min at 3000rpm. Using the method of **Lowry *et al.* (1951)**, the total protein concentration of the supernatant was calculated. The study of **Henry *et al.* (1974)** was referred to in explaining the procedure for measuring total lipid content. **Kemp *et al.* (1954)** presented a technique to detect total carbohydrates. Following the loss of 1.0g of sample mass during drying at 100± 2°C, the content of water in the container was determined. The leftover material was burned at 600°C for 10 minutes to measure the ash content **(AOAC, 2002)**.

For investigating antioxidants, catalase activity (CAT, EC 1.11.1.6, U/g. tissue) was determined by the method of **Abei (1984)**. Glutathione reductase activity (GR, EC 1.6.4.2, and U/L) was estimated by the method of **Goldberg and Spooner (1983**). While, glutathione reduced activity (GSH) (C 1.8.1.7) (mg/g.tissue) was measured according to **Beutler *et al.* (1963**). Superoxide dismutase activity (SOD, EC 1.15.1.1,U/g. tissue) was investigated by the method of **Nishikimi *et al*. (1967)**. On the other hand, glutathione peroxidase (GPX, EC 1.11.1.9, mU/mL) was assessed following the study of **Paglia and Valentine (1967)**.

For investigating statistical analysis, the data were presented as means ± standard errors for the two species. A one-way ANOVA was applied to check for variations in the quantities of elements across the fish samples analyzed. Tukey's HSD analysis was used to calculate mean differences (honestly significant difference). Additionally, the significance of the variances in element means was investigated using a one-tailed t-test.

RESULTS AND DISCUSSION

Trace element concentrations

In the field of bio-monitoring and investigation of the toxicity of trace elements in the aquatic ecosystem, fishes are regarded as useful bio-indicator. Fig. (1) illustrates the element contents (Mean average ± SE μg/g ww) in the tested muscle of the two freshwater fish from the Manzala Lagoon. The results demonstrated that the *f-ratio* value (6.19241) and a *P*-value (0.016512) of the concentration of the four trace elements in the tissues of the African catfish and the Nile tilapia showed a significant difference ($P < 0.05$). The investigation revealed that the African catfish had greater levels of Pb (3.05 ±0.1) > As (1.62±0.1) > Al (1.59±0.1) >Hg (0.84±0.08) than *O.*

niloticus Pb (1.93±0.2) > As (1.27±0.1) > Al (1.06±0.09) >Hg (0.71±0.04) (Fig. 1). Among elements, Pb and Al concentrations in the two fish species recorded a significant difference ($P < 0.05$) (Fig 1) in the analysis of variance. In this study, 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), and **EC (2006; 2008)** amended **(2011)** (0.30 µg/g). The Hg level in *C. gariepinus* and the Nile tilapia exceeded the permissible limit of **EOSQC (1993)** (0.50 ppm wt), **FAO/WHO (1992)** (0.50 ppm wt), **EC (2006; 2008)** amended **(2011)** (0.50 µg/g). Whereas, the As levels in tissues of the two studied species were lower than the range of the permissible limit recommended by **FAO/WHO (2004)** (2.0 ww ppm). This study detected a significant difference between Pb levels in the two fish species the "African catfish and the Nile tilapia" (Fig. 1), recording 15.98 *f-ratio's* value and 0.00251for the *P-value*. Hg levels in the two fish species "the African catfish and the Nile tilapia" recorded insignificant differences (*f-ratio's* value = 1.851 and *P-value* = 0.203). The As levels in the African catfish and the Nile tilapia recorded insignificant *f-ratio's* value (3.1888) and the *P-value* was 0.104. Finally, a significant difference between Al levels in the African catfish and the Nile tilapia recorded *f-ratio's* value of 6.610, and a *P-value* of 0.027. The results revealed that the highest mean Pb concentration (3.05±0.4) was found in the *C. gariepinus*, while the smallest Hg concentration (0.71±0.1) was in *O. niloticus* (Fig.1).

Lead (Pb) has been listed by the WHO as one of 10 elements of severe public health hazard that necessitates the Member States to take action in order to maintain public health of workers, youth, and women of fertile age. In the body, lead has no biological purpose. In all age groups, lead exposure can have persistent and serious health implications particularly children. This is due to the fact that lead poisoning at some concentrations can have harmful impacts on the developing neurological system without displaying immediate symptoms or warning signals. Early exposure to lead can impair cognitive function and cause dyslexia, attention deficit, and sociopathy, in addition to causing toxicity to the reproductive system, kidney damage, immunotoxicity, and hypertension, and immunity weakness **(WHO, 2016; ATSDR, 2007)**. Children with kidney illness have brain and bone problems brought on by excessive amounts of aluminum in the body. Children who take some medications containing aluminum have also developed bone damage. Aluminum in the stomach prevents the absorption of phosphorus, a chemical molecule necessary for strong bones, which in these kids results in bone deterioration **(ATSDR, 2008)**. Fish contain As, a potentially harmful element, primarily as a result of its occurrence in the aquatic environment; although more frequently due to anthropogenic causes, as it enters aquatic ecosystems via the erosion of bedrock **(Rahman *et al*., 2021)**. Long term exposure to toxic metals like As has been linked to a number of health issues, including damage of the neurological, cardiovascular, haematological, cutaneous, and respiratory systems, as well as the skin, liver, and gastrointestinal tracts **(Mandal *et al.*, 2002)**. Metal poisoning caused by exposure to mercury includes symptoms such as muscle weakness, poor coordination, numbness in the hands and feet, skin rashes, anxiety, memory issues, difficulty speaking, hearing and seeing.

According to **Nzeve *et al*. (2014)**, Pb concentrations in *C. gariepinus* & *Oreochromis spirulus* niger from Kenya's Masinga reservoir ranged from 0.643 to 1.078 mg/kg and 0.55 to 0.765 mg/kg, respectively. **Abdel-Kader and Mourad (2020)** reported the following order reflecting the trace element content in *O. niloticus* from the Burullus Lake, Egypt: Pb (1.78) >As (1.50) > Al (1.38)> Hg (0.71) and *C. gariepinus* Pb concentration (2.29) > Al (2.07) >As (2.03) > Hg (0.73), which were less than the findings of the current investigation. According **to Sallam *et al.* (2019)**, the Nile tilapia and the African catfish both had Pb > As > Hg levels, and this is very close to the finding of this study. They discovered metal concentrations (μg/g wet weight) in all the investigated samples as Hg (0.045), As (0.511), and Pb (0.704) in Nile tilapia, Hg (0.0145), As (0.621), and Pb (0.635) for flathead grey mullet, as well as Hg (0.017), As (0.568), and Pb (0.64) for the African catfish. **Abdel-Kader and Mourad (2021)** recorded the residues of trace elements in the Nile tilapia from Edku Lake, Egypt as follows: As (0.91) > Pb (0.89) > Al (0.60) > Hg (0.39). **Yacoub *et al*. (2021)** recorded that the residual concentrations of Pb in Nile tilapia were 2.2±0.7 mg kg−1 from the Manzalah Lake, Egypt. **Saeed and Shaker (2008)** discovered a massively greater Pb level of 10.1μg/g in the tissues of *O. niloticus* obtained from Manzala Lagoon, Egypt in 2007, demonstrating that the lead contamination rate in Lagoon Manzala had decreased. **Mensoor and Said (2018)** found that, the mean Pb concentrations (μg/g) in the tissues of *B. sharpeyi* & *B. xanthopterus* along the Tigris River in Baghdad ranged from1.10 ±0.60 to 1.05 ± 0.40. **Alipour *et al.* (2015)** observed the amount of As (1.6) > Pb (0.67 μg g−1) in the edible muscle tissue of *Rutilus rutilus* from the Miankaleh International Lake. In addition, **Molla *et al*. (2021)** recorded that Pb > Hg in some studied species of fishes along India's west coast. In contrast to our findings, **Jovanović *et al.* (2017)** found that Hg (0.466) > As (0.333) > Pb (0.084 mg kg1) in the muscle of grocka carp, an omnivorous fish. According to **Sarkar *et al*. (2021)**, the concentration of Pb was 0.189 in the collected commercial fish from Bangladesh. **Korteia (2020)** recorded that As (0) >Pb (0.04) > Hg (0.40 mg/kg) in the mescles of *O. niloticus* and *Clarias anguillaris* from Ankobrah and Pra basins.

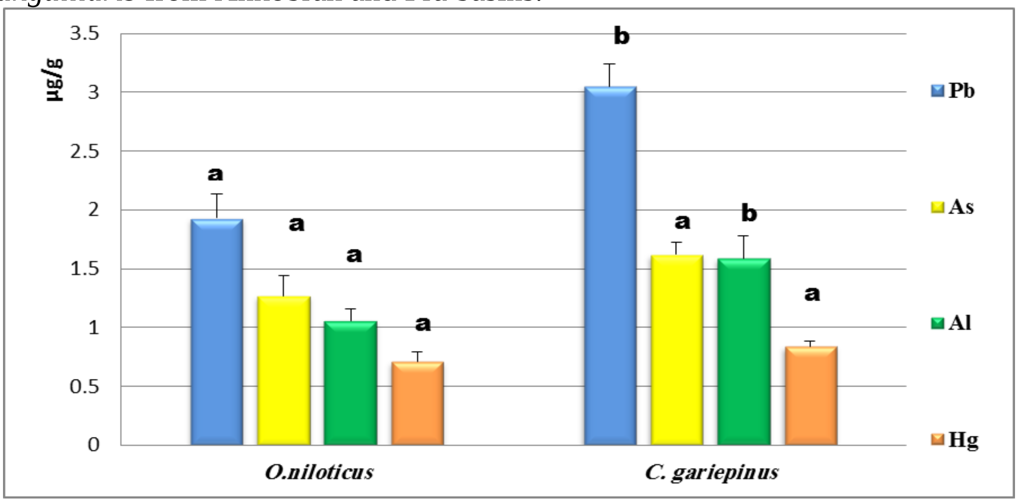

Fig. 1. Concentrations (μg/g wet weight, Mean±SE) of trace elements of *O. niloticus* and *C. gariepinus* (n=6) from Manzalah Lake, Egypt

Different superscript letters between the two fish species indicated significant differences at (*P* < 0.05)

Risk assessments

According to Environmental Protection Agency EPA regulations, determining the probability of human exposure to harmful contaminants in contaminated media is known as “Human Risk Assessment”. Risk assessment for toxicants is investigated through metrics *via* EDI, THQ, HI (TTHQ) and TCR **(US EPA, 1989; Javed & Usmani, 2016)**. Hence, this investigation showed the EDI, EWI, PTWI %, MDI as well as MWI of Pb, As, Al, and Hg residues in the muscles of *O. niloticus* and *C. gariepinus* from the Manzalah Lagoon *via* the consumption of children (15 kg), youth (40kg), an adult (70 kg) in a diet/day and week are presented in Table (1). PTWI values recommended by FAO/WHO (2004) for Pb, As, Al and Hg mg/kg of muscles are shown in Table (1).

Table (1) shows that the EDI and the EWI of Pb > As >Al >Hg ingested by children >youth > adults are related to *O. niloticus* and *C. gariepinus* muscle consumption. Table (1) reveals that the PTWI (%) followed a Pb > Hg> As> Al sequence depends on *C. gariepinus* consumption, and As> Pb > Hg> Al sequence depends on *O. niloticus* consumption of children. While, Hg > Pb > As > Al is attributed to the consumption of *C. gariepinus* and Hg > As > Pb > Al is related to the consumption of *O. niloticus* with respect to the youth. For adults, Hg > Pb> As > Al and Hg > As > Pb > Al sequences depend on the ingestion of *C. gariepinus* and *O. niloticus*, respectively.

The results in Table (1) depict that Hg showed the lowest EDI, EWI, and PTWI%; whereas, Pb recorded the highest EDI, EWI, and PTWI % through the consumption of the two selected fish species. The examination postulated that the PTWI values set by FAO/WHO exceeded the EDI, EWI, and PTWI percentages analyzed for the Pb, As, and Hg in *C. gariepinus* and Pb and As in *O. niloticus,* consumed by a child with 15kg. In addition, the EDI, EWI, and PTWI percent values analyzed for the Pb, As, and Hg in *C. gariepinus,* and Hg in *O. niloticus*, consumed by youth (40kg) were higher than the recommended PTWI of FAO/WHO. For adults 70kg, just Hg levels in *C. gariepinus* were higher than the recommended limits of FAO/WHO. The current study proved that the *C. gariepinus* and *O. niloticus* did pose human health risk. Therefore, this study calculated the safety and healthy diet (MDI and MWI) in grams to three ages for maintaining the safe diet according to FAO/WHO limits for *C. gariepinus* and *O. niloticus* fish species from the Manzalah Lake.

Our study recommended that the children should not eat more than 13.39g a day or 93.75g a week of *C. gariepinus* muscle, and 15.09g a day or 105.63g a week of *O. niloticus* muscle. The recommended daily intake for the youth is 34.01g, and the weekly intake is assumed to be 238.09g of *C. gariepinus* muscle. For *O. niloticus* muscle, 40.24g is recommended for a daily intake or 281.69g for a weekly intake. On the other hand, adults should avoid consuming more than 59.52g a day of *C. gariepinus* muscle or 416.66g a week and 62.02g a day of *O. niloticus* muscle or 434.14g a week.

Iqbal *et al.* (2017) estimated the EDI of As in *Labeo rohita* (0.016 and 0.017) for Wallago attu from the Indus River. The present observation disagrees with those of **Zaza *et al*. (2015)** who discovered that Italian consumers' EWI of Pb and Hg from fish and shellfish products did not exceed PTWIs suggested by the European Food Safety Authority EFSA & FAO/WHO. According to **Yacoub *et al*. (2021)**, the maximum tolerated daily intake (MTDI) determined by FAO and WHO for a 70 kilogram was greater than the EDI of Pb in the examined fish species from the Manzalah Lake, which indicates that eating the investigated fish poses a serious risk to human health. However, the muscles of *Oreochromis niloticus* consumed by an adult weighing 70kg were below the recommended value, which is consistent with the present findings. According to the analysis of **Sallam *et al*. (2019)**, eating *O. niloticus*, flathead grey mullet, and *C. gariepinus* from the Manzala Lake may pose a health risk to adult (70 kilogram), which forms a result coinciding with our results.

Table 1. Risk metrics for Pb, As, Al, and Hg in *C. gariepinus* and *O. niloticus* from Manzalah Lake

Fish species	Intake	Trace elements			
		Pb	**As**	**Al**	**Hg**
***C. gariepinus* consumed by a child (15kg)**	**EDI**	12.65	6.72	6.59	3.48
	EWI	88.55*	47.04*	46.18	24.36*
	PTWI	25.0	15.0	1000	5.0
	PTWI%	354.2	313.6	4.618	487.2
	MDI	17.56	19.84	1347.7	13.39
	MWI	122.95	138.88	9433.9	93.75
***O. niloticus* consumed by a child (15kg)**	**EDI**	8.00	5.27	4.39	0.71
	EWI	56*	36.89*	30.73	4.97
	PTWI	25.0	15.0	1000	5.0
	PTWI%	224.26	245.93	3.073	99.4
	MDI	27.75	25.30	2021.5	15.09
	MWI	194.30	177.16	14150.94	105.63
***C. gariepinus* consumed by young (40kg)**	**EDI**	4.74	2.52	2.47	1.30
	EWI	33.18*	17.64*	17.29	9.1*
	PTWI	25.0	15.0	1000	5.0
	PTWI%	132.72	117.6	1.729	182
	MDI	46.83	52.91	3593	34.01
	MWI	327.86	370.37	25157.23	238.09
***O. niloticus* consumed by young (40kg)**	**EDI**	3.00	1.97	1.64	1.10
	EWI	21.00	13.79	11.48	7.7*
	PTWI	25.0	15.0	1000	5.0
	PTWI%	84	91.93	1.148	154
	MDI	74.01	67.49	5390.83	40.24
	MWI	518.13	472.44	37735.84	281.69
***C. gariepinus* consumed by an adult (70kg)**	**EDI**	2.71	1.44	1.41	0.74
	EWI	18.97	10.08	9.87	5.18*
	PTWI	25.0	15.0	1000	5.0
	PTWI%	75.88	67.2	0.98	103.6
	MDI	81.96	92.59	6289.30	59.52
	MWI	573.77	648.14	44025.15	416.66
***O. niloticus* consumed by an adult (70kg)**	**EDI**	1.71	1.12	0.94	0.63
	EWI	11.97	7.84	6.58	4.41
	PTWI	25.0	15.0	1000	5.0
	PTWI%	47.88.	52.26	0.65	88.2
	MDI	62.020	118.110	9433.96	70.42
	MWI	434.14	826.77	66037.73	492.95

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

Target cancer/ hazard quotient (non-cancer) risk metrics

Table (2) provides a summary of the (non-cancer) THQs of Pb, Hg, As, and Al accumulated upon eating the two species per one day or seven days per week from the Manzalah Lake. Table (2) shows that *C. gariepinus* recorded higher estimated THQs values for Pb, Hg, and Al than *O. niloticus*, except for THQ of Pb in the youth consuming *O. niloticus* and Pb for both fishes in adults, which were both higher and above the safe level of one. This indicates that ingesting these fish species on a daily and weekly basis may be unhealthy for people. The present results indicate that the THQs values of Pb, Hg, As, and Al in children > teenagers > adults. In addition, the THQ level of As in *C. gariepinus* taken by a child was the greatest, ranging around 22.4 a day to 156.8 a week; whereas, the THQ value of Pb in *O. niloticus* intake by an adult was the lowest (0.48/ day or 3.36/week).

Based on this analysis, the TTHQ levels of two freshwater fishes for children, teenagers, and adults were proved high non-carcinogenic hazards when consumed by the three studied ages.

According to this analysis, consuming two types of fish from the Manzalah Lake increased the chance of developing cancer in regards of Pb and As. For *C. gariepinus* & *O. niloticus*, the TCR of Pb concentrations were in the US EPA authorized limits, but still the CR of As levels exceeded the USEPA limit values, confirming that the daily and/or weekly ingestion of these two species did pose cancer risk to consumers **(US EPA, 2012)**. With respect to Table (2), Pb and As considered the TCR level in children > youth > adults. *C. gariepinus* in children recorded the greatest level of TCR (1.01×10^{-2} a day or 7.07×10^{-2} a week), whereas *O. niloticus* in children showed the lowest level of CR (6.80×10^{-6} a day or 4.76×10^{-5} a week).

Talab *et al.* (2016) stated that, the level of THQ was less than the safe limits, revealing that the elements in *O. niloticus* from the Nile rayahs in Egypt did not pose risk to consumers, providing a safe healthy diet of *O. niloticus* for consumers. Additionally, the finding of **Copat *et al.* (2012)** showed that, the THQ level < 1 for Cd, Pb, and Hg for adults consuming fish species from the Mediterranean Sea.

Adversely, **Korteia (2020)** assessed that, if THQ >1 for Pb, a health risk is posed to children and adults as consumers. Additionally, **Yacoub *et al.* (2021)** confirmed that the examined fish from the Manzala Lake might have a negative impact on adults because the THQ >1 for Pb and As in all studied fish, as well as for Hg in *O. niloticus*. **Mielcarek *et al.* (2022)** recorded that the risk assessment of As, Cd, Hg and Pb intake of the studied freshwater fish did pose carcinogenic and non-carcinogenic risks for consumers.

Table 2. Target cancer/hazard quotient (non-cancer) risk metrics in *O. niloticus* and *C. gariepinus* from Manzalah Lake, Egypt

Fish	Non- cancer risk (THQ)										Target cancer 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
Children														
C. gariepinus	3.61	22.4	16.47	11.6	54.08	25.2	156.8	115.2	81.2	378.4	1.08×10^{-4}	1.01×10^{-2}	7.56×10^{-4}	7.07×10^{-2}
O. niloticus	2.28	17.5	10.97	2.36	33.11	15.9	122.9	76.79	16.5	232.0	6.80×10^{-6}	7.91×10^{-3}	4.76×10^{-5}	5.54×10^{-2}
Youth														
C. gariepinus	1.35	8.4	6.175	3.25	19.17	9.45	58.8	43.22	22.7	134.1	4.03×10^{-4}	3.78×10^{-3}	2.82×10^{-3}	2.65×10^{-2}
O. niloticus	0.85	6.56	4.1	3.66	15.17	5.95	45.92	28.7	25.6	106.1	2.55×10^{-5}	2.46×10^{-3}	1.79×10^{-4}	1.72×10^{-2}
Adult														
C. gariepinus	0.77	9.03	3.52	2.46	15.78	5.39	63.21	24.64	17.2	110.4	2.30×10^{-5}	2.16×10^{-3}	1.61×10^{-4}	1.51×10^{-2}
O. niloticus	0.48	3.73	2.35	1.57	8.13	3.36	26.11	16.45	10.9	56.82	1.45×10^{-5}	1.68×10^{-3}	1.01×10^{-4}	1.176×10^{-2}

Proximate chemical composition

Most fish are essentially composed of proteins, lipids and water. About 98 percent of the mass of fish meat is made up of these components, with the remaining small amounts being made up of carbohydrate, vitamins, and ash as a mineral **(WHO/FAO, 2011)**. Two fish species' average values for these components are displayed in Fig. (2).

With the exception of protein and ash contents, all proximate metrics in these fish species under investigation revealed significant differences (Fig. 2). This investigation showed that C. *gariepinus* had the maximum protein content (56.5±2.7 mg/g) and moisture content (61.08±2.41%); whereas, *O. niloticus* showed 55.83±1.77 mg/g and 49.33±2.97% as the minimum content for the same parameters, respectively. Furthermore, the maximum content of ash was found in *O. niloticus* (9.75±0.5%), whereas, the minimum in *C. gariepinus* (3.6±0.1%). The maximum fat and carbohydrate contents were estimated in *O. niloticus* (15.83±1.32 mg/g and

20.83±1.03 mg/g, respectively), while these parameters were at their minimum level in *C. gariepinus* (11.76±0.4 mg/g and 13.83±0.4 mg/g, respectively).

According to **Adeniyi et al. (2012)** tilapia *guineensis* had a carbohydrate content of 6.85%, which is a little higher than the value recorded for *P. senegalensis*, but lesser than that of *P. typus*, and *Clarias gariepinus* that had a carbohydrate content of 3.85%, which is relatively lesser than that of the *Pseudotolithus* samples analyzed.

Ogundiran et al. (2014) assessed that, *S. pilcladus* had the smallest moisture content (57.5%) while *G. macrocephalus* had the maximum (81.4%). Regarding the protein composition of fish, *S. pilcladus* showed the maximum values (26.3%), whereas *G. macrocephalus* recorded the minimum values (11.7%). Among the studied fish species, *G. macrocephalus* had the maximum ash content (1.48%), but *S. scombrus* had the smallest (1.11%). In terms of lipid content, S. *scombrus* had the maximum value (10.2 mg/g), while *G. macrocephalus* showed the minimum values (2.1 mg/g). *S. pilcladus* & *S. scombrus* showed the greatest and smallest carbohydrate content values (7.12 mg/g and 2.5 mg/g, respectively).

Shikha *et al.* (2019) assessed the values of the protein (23.83%), lipid (5.91%), moisture (64.44%), and ash contents (3.23%) in *C. punctatus* collected from pond water, while the corresponding values for fish captured from the open water were as follows: protein (22.21%), lipid (5.43%), moisture (62.7%), and ash content (3.67%). Moreover, **Talab *et al.* (2016)** determined the ranges of the moisture in *O. niloticus* from the Rayahs (the main irrigated canals) of the Nile Delta (78.55–80.77%), protein (16.10–17.88%), fat (1.10–1.95%), ash (0.55–1.50%) and carbohydrates (0.10–0.94%). Furthermore, **Ahmed *et al*. (2017)** assessed the range of protein content (71.46% to 89.13 %), crude fat (6.34 % to 9.66 %), moisture (75.33% to 79.33 %) and ash (3.83 % to 7.07 %) of the muscle of six commercially fish species, collected from Jebl Awlia reservoir, Sudan.

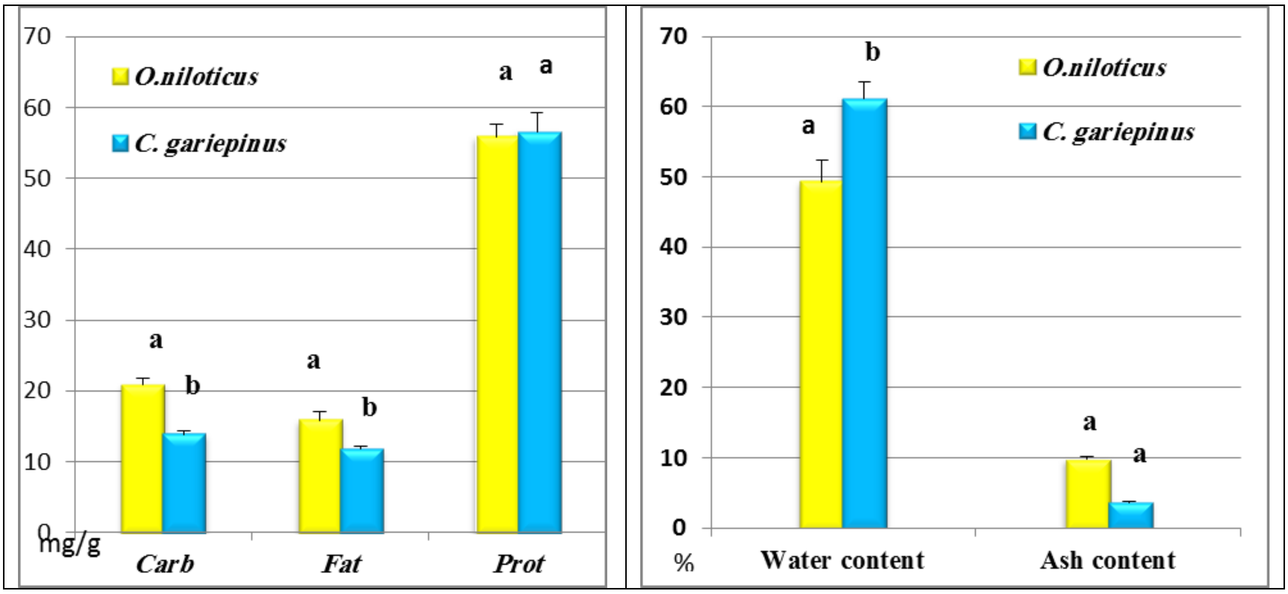

Fig. 2 Proximate composition (Mean ±SE) of *O. niloticus* and *C. gariepinus* (n=6) from Manzalah Lake, Egypt, different superscript letters between the two fish species indicated significant differences at (*P* < 0.05)

Liver Antioxidant

In this research, the investigated antioxidants SOD, CAT, and GR were not statistically significant ($P > 0.05$), except for GPx activity and GSH level, which had significant ($P < 0.05$) average values (Fig. 3). The results showed that the SOD (U/g) was diminished in *gariepinus* (26.33 ±2.4), while *O. niloticus* had the maximum (31.66 ±2.65). CAT (U/g) was in *O. niloticus* (15.5 ±2.12) but in *gariepinus* (22.13 ±1.5). GPx (mU/mL) demonstrated dimension in *gariepinus* (22.00 ±1.97), but in *O. niloticus* (27.66 ±3.13) was the greatest. GR (U/L) for *gariepinus* (15.5 ±2.2) was less than *O. niloticus* (16.83 ±2.4). Lastly, GSH (mg/g tissue) was decreased in *gariepinus* (13.5 ± 0.7), but in *O. niloticus* (17.66 ±1.4) recorded the greatest level (Fig. 3). According to **Farombi *et al.* (2007),** the flux of superoxide radicals, which were demonstrated to suppress CAT activity, could be responsible for the decrease in CAT activity. **Metwally and Fouad (2008)** recorded that the lipid peroxidation estimated analysis of CAT and SOD activity as a biomarker for trace element fish toxicity. **Abdel-Kader and Mourad (2020)** recorded a non-significant (P > 0.05) reduction in the activity of GPx, CAT, and GR in the liver of *O. niloticus*, whereas, the liver of *C. gariepinus* had the SOD and GSH with insignificant reduction from Burullus Lake. **Arojojoye and Adeosun (2016)** showed a significant reduction in SOD and CAT in the liver of *C. gariepinus* from Asejire water body. **Awoyemi *et al.* (2014)** demonstrated a significant decrease in CAT and SOD in *C. gariepinus* and *O. niloticus* after lead toxicity. **Khalil *et al*. (2017)** recorded that *O. niloticus's* liver from polluted Rosetta Branch of Nile had a significant increase in the activity of SOD, CAT, and GPx after metal exposure. According to **Saglam *et al*. (2014),** *O. niloticus*'s SOD activity was significantly increased after trace element exposure .

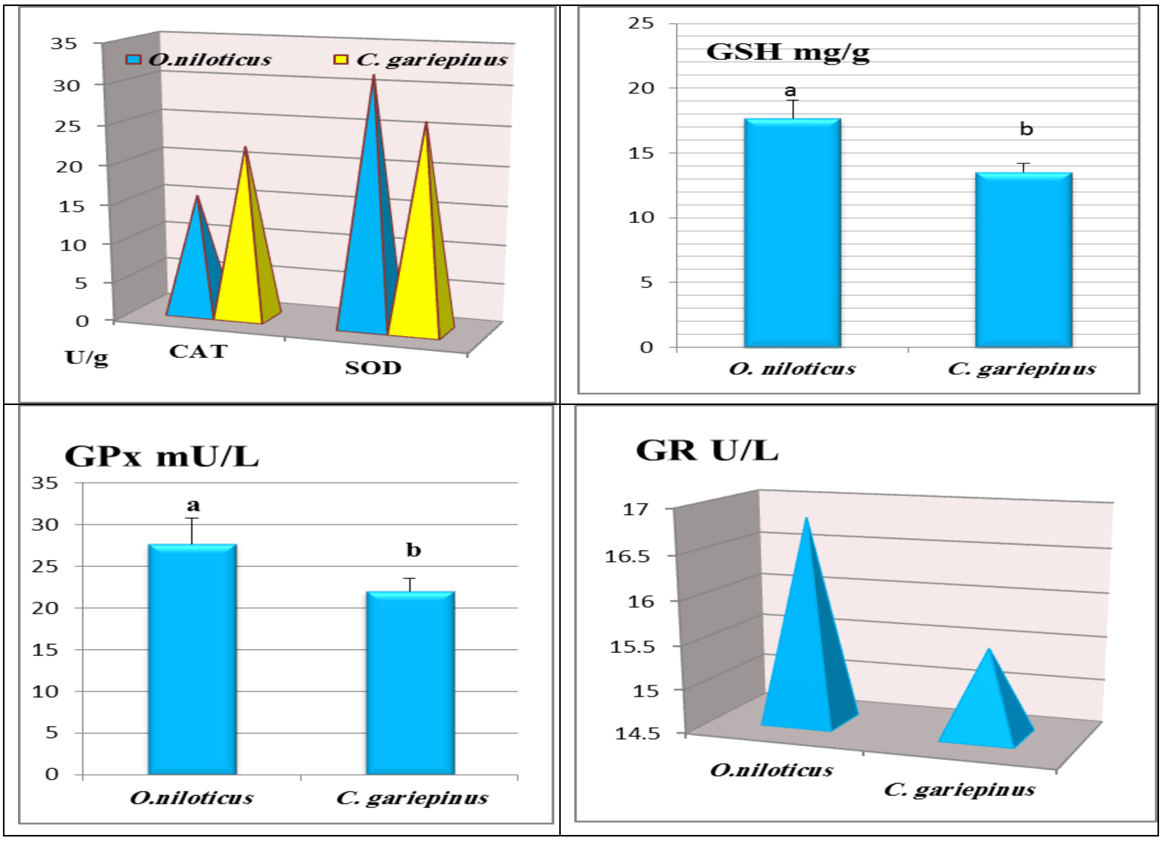

Fig. 3. *O. niloticus* and *C. gariepinus'* livers antioxidants (Mean ±SE) (n=6) from Manzalah Lake, different superscript letters between the two fish species indicated significant differences at (P < 0.05)

CONCLUSION

This investigation demonstrated that the African catfish had much greater levels of Pb > As > Al > Hg than *niloticus*. Among elements, Pb and Al concentrations in the two fish species recorded a significant difference (P < 0.05). Actually, our study proved that the *C. gariepinus* and *O. niloticus* intake did pose human health risk. Therefore, this study calculated the safety and healthy diet (MDI and MWI) by grams to three ages for maintain the safe diet according to FAO/WHO limits for C. gariepinus and O. *niloticus* fish species from Manzalah Lake. Also, this study demonstrated that C. *gariepinus* had higher estimated THQs values for Pb, Hg, and Al than *O. niloticus* except for THQ of Pb for youth in O. *niloticus* and Pb for both fish in adult, which are both higher above the safe level of one, indicating that ingesting these fish on a daily and weekly basis may be unhealthy for children > teenagers > adults. In addition, Pb and As were looked at the TCR level in children > youth > adults. *C. gariepinus* in children had the greatest level of CR, whereas *O. niloticus* in children had the lowest level of CR. In this research, the investigated antioxidants SOD, CAT, and GR were not statistically significant ($P > 0.05$), except for GPx activity and GSH level, which had significant ($P < 0.05$) average values. This investigation showed that C. *gariepinus* had the maximum protein content and moisture content, whereas *O. niloticus* had the minimum content for the same parameters, respectively. Furthermore, the maximum content of ash, fat and carbohydrate content were estimated in *O. niloticus* while these parameters were lower in *C. gariepinus*.

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