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Impact of some trace elements on the quality of edible fish species and its human health risks from Timsah Lake, Egypt

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ABSTRACT

Timsah Lake is a vital water body in Egypt, located in the Nile Delta region. It serves as a source of livelihood for local communities and provides edible fish species. Fish are often at the top of the aquatic food chain and live in marine environments, which can quickly accumulate elements in their bodies, then be transferred to consumers and put at risk. This study aimed to analyze with follow-up the quality of water and fish species to ensure that this lake, like other Egyptian lakes, is a decay in the quality of fish products. Additionally, this study aimed to estimate the human health risks in normal and high consumption. Approximately 30 fresh fish samples (*Chelon ramada, Sparus aurata Planiliza carinata, Alepes djedaba, Oreochromis niloticus*) belonging to 5 species were caught during the summer of 2019. The accumulation level of essential micronutrient (Cu, Zn) content was higher than the nonessential and toxic element (Cd). Muscles < liver in all elements in order (Zn > Cu > Cd). Target hazard quotient (THQ) and Hazard index (HI) values were < 1 in adults with normal consumption, indicating no adverse impact on human health. Target hazard quotient and hazard index (THQ and HI) for children with normal and high consumption > 1 represent possible health risks. The liver had a higher biochemical composition (moisture, protein, lipids, and glycogen) than muscle. The results suggested that consumers in the Ismailia governorate may be exposed to health hazards, and further investigation is necessary, as well as making recommendations for policymakers to establish and enforce regulations that can help protect public health and the environment from trace element pollution in Timsah Lake.

INTRODUCTION

Trace element pollution has become a worldwide issue owing to its toxicity, fundamental persistence, non-biodegradability, and accumulative tendencies (Islam *et al.*, 2018; Zhu *et al.*, 2020). Trace elements concentrations are becoming more dangerous for the health of all life on Earth. Sneddon *et al.* (2017) found trace elements negatively affect aquatic creatures and ecosystems. Essential sources of trace elements in the marine environment include industrial effluents, municipal sewage, agrochemical waste products, geological weathering, and atmospheric deposition (Rajeshkumar *et al.*, 2018). Assessment of human health risk is a potential assessment for detrimental effects on human health from exposure to chemicals in polluted environmental media (Tahiti *et al*., 2022).

Fish are at the highest of the food chain, providing humans with many health benefits: they are low in saturated fat and calories, provide a good supply of protein, and are a good source of omega-3 fatty acids. As a result, fish are frequently employed as bioindicators of pollutants to examine human activities' effects on the environment and health of humans (Kortei *et al.,* 2020; Esmaeilzade Ashini *et al.,* 2021; Łuczyńska *et al.,* 2022). However, modern industrialization has resulted in a significant environmental danger to fish habitats. Fish may collect trace elements in their tissues at greater levels than ambient amounts due to absorption along the kidney, liver, gill surface, and gastrointestinal tract wall (Esmaeilzade Ashini *et al.,* 2021; Serviere-Zaragoza *et al*., 2021; Georgieva *et al.,* 2021). Both ingested trace elements from water and food and non-dietary mechanisms like muscle and gills contribute to accumulating these contaminants in aquatic food webs. However, environmental and dietary element concentrations likely affected the observed variations in trace element accumulation (Łuczyńska *et al.,* 2019; Huang *et al.,* 2022; Tahity *et al.,* 2022). Some elements, including Cd, may cause harm to the liver, kidneys, and nervous system if they accumulate in fish tissues over the maximum allowable quantities, making fish eating a potential health concern for humans. Consuming fish with a high concentration of trace elements can potentially have severe consequences for human health (Ali & Khan, 2018; Manea *et al.*, 2020; Younis *et al.,* 2021).

Muscle is vital to human nutrition and a brilliant instrument for health risk evaluation in contamination of trace elements (Keshavarzi *et al.,* 2018; Sadeghi *et al.,* 2020). Deficits or excesses of elements like (Zn) zinc and (Cu) copper can harm human health, but they are essential for the growth of specific biochemical processes of biological systems. Nonessential element, such as cadmium (Cd), is considered the most toxic trace element for aquatic organisms and may cause cancer due to their high bioaccumulation rate (Garai *et al.,* 2021). Risk assessment is a valuable technique used for the potential impact to evaluate pollutants. In the case of trace elements, health risk assessments are commonly conducted to assess the overall exposure of individuals to these contaminants in a particular location (Prasad *et al.,* 2020).

Timsah Lake is a holding area for ships and freight, and its beaches are popular destinations for tourists and locals. Furthermore, vast quantities of effluent from agricultural, industry, freshwater, and home drains are dumped into Timsah Lake daily. In contrast, various other activities and industries contribute to this issue, including mining, phosphate fertilizer production, agricultural uses, cement manufacture, and agricultural uses (Dar *et al.,* 2020; EEAA, 2020). This study aimed to determine the levels of some trace elements in water with different organs “liver, and muscles” of some commercial fish species (Chelon ramada, Sparus aurata Planiliza carinata, Alepes djedaba, Oreochromis niloticus) collected from Timsah Lake in Ismailia City, Egypt. Furthermore, to follow up on the quality of water and fish species to ensure that this lake, like other Egyptian lakes, is not suffering a decay in the quality of fish products. Additionally, to estimate the human health risks in normal and high consumption.

MATERIALS AND METHODS

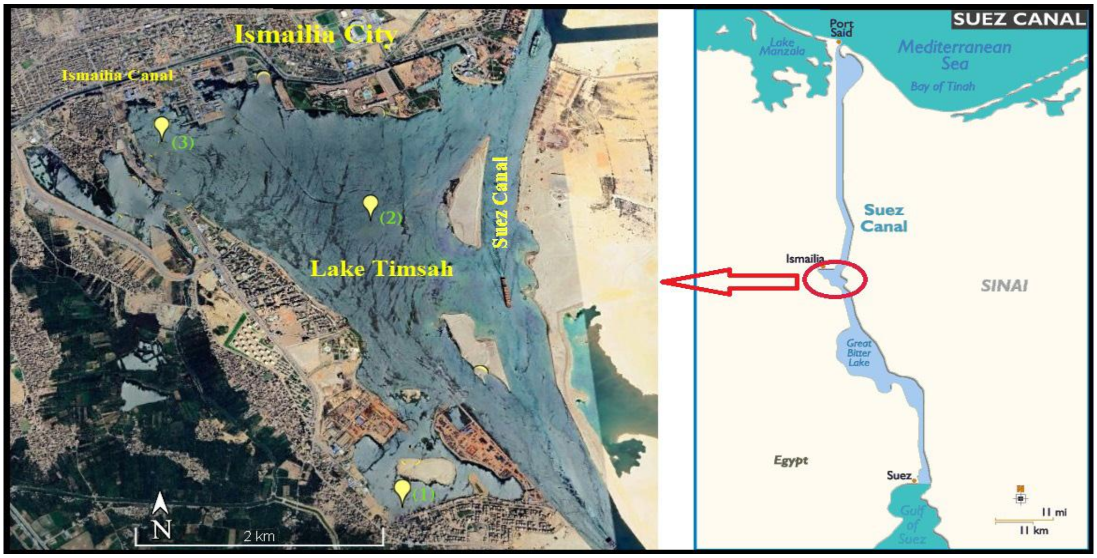
2.1. Stud Area

Timsah Lake, located in Ismailia City, is the greatest water body in the area, covering approximately 14 km^2 of surface area. It is a brackish shallow water basin strategically positioned along the Suez Canal. It lies almost equidistant between the southern city of Suez and the northern city of Port Said, at 30° 350' 46.55'N and 32° 190' 30.5' 400'E. The lake's shape is roughly triangular, with elongated sides extending about 5 km from east to west and about 4.5 km from north to south. It is particularly near the navigational passageway of the Suez Canal (Fig 1). The primary supply of salinity for Lake Timsah is the Suez Canal. At the same time, freshwater originates from various sources, such as the Ismailia Canal, sporadic seasonal streams, and effluent discharges. This influx of various water types causes salinity stratification within the lake (El-Serehy *et al.*, 2018; Dar *et al.*, 2020). The lake's health, diversity of native fish and richness, and other plant and animal populations are threatened by the increase in ship repair, sewage dumps, and agricultural runoff that have sprung up as a result of the lake's popularity (Elshobary *et al.*, 2021).

2.2. Samples Collection and Preparation:

2.2.1. Water samples:

Nine surface water samples were collected from 3 locations, representing the area along Timsah Lake (Fig 1). In the summer of 2019, water samples were collected in a 2 L plastic container. To determine trace elements (Zn, Cu, and Cd), filtered 1 L water samples in a dust-free lab were concentrated at pH 3.5 - 4.0 to applied MIBK/APDC Modified extraction process using (AAS) atomic absorption spectrophotometer (Perkin-Elmer Model (Analyst 100) (Rice *et al.*, 2012) and expressed as µg/l.



S	Late	Long
1	30 33 17.62	32 17 39.14
2	30 34 27.29	32 17 32.17
3	30 34 54.86	32 16 22.88

Fig (1): Map of Timsah Lake area of investigation showing the sampling sites of water samples during 2019

2.2.2. Fish samples:

Approximately 30 individual fish belonging to five species, 6 samples for each (*Chelon ramada*, *Sparus aurata Planiliza carinata*, *Alepes djedaba*, *Oreochromis niloticus*) commonly consumed in Timsah Lake. Samples are collected from local fishermen, put in plastic containers, and sent to the National Institute of Oceanography and Fishries (NIOF) in Suez, Egypt. Each fish species had its morphology, and its ecological and biological data were collected (Table 1) and then weighed, analyzed in the lab, and digested in Teflon jars with conc HNO_3 according to the regulations of (UNEP / IOC / IAEA / FAO 1990). Digestion was complete when the yellowish odors dissipated (Kaya & Turkoglu, 2017). They were filtered, diluted with distilled water, stored at 4°C for additional analysis (AOAC, 2005), and expressed as µg/g wet weight.

MPI is calculated to examine and indicate the total metal accumulation (metal load) in vital organs, which was calculated using Eq. (1) (Omar *et al*., 2015)

$$MPI = (M_1 \text{ x } M_2 \text{ x } M_3 \text{ x } .... M_n)^{1/n} \quad .... \quad (1)$$

Where M is the concentration of each element in water and fish samples (µg /l and µg/g wet wt.), and n is the number of elements.

2.2.2.1.Bioconcentration factor (BCF)

Aquatic toxicologists often talk about bioconcentration factors. A larger concentration in a live creature may be detected using this method. Bioconcentration factor can calculated by the ratio of heavy metal concentrations in organisms to water concentrations (Ghannam, 2021). It is given by the following relation (Kennish, 1992).

$$\mathrm{BCF} = \frac{Cbiota}{C\ water} \qquad (2)$$

C_{biota} is the concentration of the heavy metal in the muscle of the fish, and C_W is the concentration in the water.

Table (1): Biological information, morphometric measurements, and ecological characteristics of examined fish species' in Timsah Lake, Egypt, during 2019.

Lakes	Fish Name		Living Habitats	Feeding Habitats		Length	No. of Samples
	Scientific Name	English Name					
Temsah Lake	**Old name:** *Mugil capito* Cuvier, 1829 **Accepted Name:** *Chelon ramada* (Risso, 1827).	Thinlip grey mullet	pelagic-neritic	Marine; freshwater; brackish; catadromous Max. published weight: 2.9 kg	Herbivores	25 – 32 35.0 cm	6samplese for individual fish species
	Sparus aurata **Linnaeus, 1758**	Gilthead sea bream Gilthead bream	demersal;	Marine; brackish; catadromous Max. published weight: 17.2 kg	Carnivorous	33 – 40 35.0 cm	
	Old name: ***Liza carinata*** **(Valenciennes, 1836)** **Accepted Name:** *Planiliza carinata* (Valenciennes, 1836)	Keeled mullet	pelagic-neritic	Marine; brackish;	Herbivores	15.0 - 20 cm 18.0 cm	
	Alepes djedaba **(Forsskål, 1775)**	**Shrimp scad**	benthopelagic	reef-associated; amphidromous	Carnivorous	16.3 - 40.0 25 cm	
	Old name: ***Tilapia nilotica*** **(Linnaeus, 1758)** **Accepted Name:** *Oreochromis niloticus* (Linnaeus, 1758)	Nile tilapia		Freshwater; brackish; potamodromous	Herbivores	6 – 28 18.6	

2.2.3. Preparation for biochemical composition:

Each studied fish species was sampled in triplicates. Liver and muscle tissues were isolated and preserved on an ice plate before being frozen at –20°C for more biochemical composition analysis. The samples were homogenized at 0°C with 0.1 M phosphate buffer (pH 7.4) using an electric homogenizer (Wise stir Hs-30E, Germany) and then centrifuged at 4000 r.p.m. for 15 minutes in a cooling centrifuge (Bunsen) Model HEMO. The resulting supernatant was stored at –20°C for later analysis.

2.3. Fish Consumption Health Hazard Evaluation:

Human health risk assessment is the process of evaluating the dangers to human health posed by carcinogenic and non-carcinogenic substances (USEPA, 2018).

2.3.1. Target hazard quotient (THQ):

The possible non-carcinogenic risk effects on human health from exposure to trace elements are the focus of the target hazard quotient. It is used to assess the danger posed by trace element contamination and is determined by dividing the exposure dose by the reference dose (RfD) (Kamunda *et al.*, 2016; Yi *et al.*, 2017). The THQ value was determined using the following formula (Eq. 2) USEPA (2018).

$$\text{THQ}= \frac{Ef\ x\ ED\ x\ FiR\ x\ C}{RfD\ x\ BW\ x\ TA} \text{ X } 10^{-3} \qquad (3)$$

Where: $E_{F:}$ Exposure frequency, ED: Exposure Duration, FiR: Ingestion Rate of Fish, C: concentration of element studied, BW: Body weight, and TA: Average time (Table 2). Exposure parameters are used for the health risk estimations through the consumption of fish when the THQ is < 1; eating fish is helpful for your health while eating fish with a THQ >1 poses a greater danger to your health (Ahmed *et al.*, 2015; USEPA, 2015, 2018).

Table 2: Exposure parameters used for the health risk estimations through consumption of fish (USEPA, 2015, 2018)

Parameters	**Values**		
	Unit	**Adult**	**Child**
Body weight (BW.)	Kg	70	15
Exposure frequency (EF.)	Days/year	365	
Exposure Duration (ED.)	Years	30	6
Ingestion Rate of Fish (FIR) (Zaghloul *et al.*, 2022).	g/day	64.0 g/day for the normal consumer 200.0 g/day for High-consumer	
Average Time	Days/year		
For non-Carcinogenic	365 x E_D		
Reference dose mg/kg/day (RfD) (Zaghloul *et al.*, 2022)	Cd = 1x 10^{-3}, Cu= 4x 10^{-2}, Zn = 3x 10^{-1}		

2.3.2. Hazard Index (HI):

The total hazard quotient, or the hazard index (HI or TTHQ), is the summation of the target hazard quotients (THQs) for all the trace elements analyzed in a particular species. The HI estimates the growing risk of exposure (USEPA, 2011). HI value was determined using the following formula (Eq. 3) (Li *et al.*, 2013 and Akoto *et al.*, 2014) :

$$TTHQ = THQ_{Cd} + THQ_{Cu} + THQ_{Zn} \qquad (4)$$

THQs are < 1, indicating no threat to human health (Yi *et al.*, 2011). When the HI > 1, there may be a concern for potential health risks (Saha & Zaman, 2013; USEPA, 2018).

2.4. Bio-chemical composition analysis:

2.4.1. Determination of moisture content:

Three duplicates of homogenized fish samples were weighed and put in reweighed aluminum plates before drying in a hot air oven at 105°C until a consistent weight was reached, as described by (Jain & Singh, 2000). Then cooled the samples to room temperature in a desiccator, and the difference between the wet and dry weights was calculated as the water percentage, expressed as a percentage (%):

$$\% \text{ moisture} = \frac{\text{weight of the sample before drying} - \text{the weight of the sample after drying)} \times}{\text{Weight of the sample before drying}} 100.$$

2.4.2. Determination of crude protein:

The Kjeldahl method was used to determine the crude protein content; N_2 was quantified by thermal conductivity detection using a CHNS elemental analyzer (Costech ECS-4010, Valencia, USA), N_2 was converted to protein using a conversion factor of 6.38 following acid digestion (AOAC, 2016). This method was used to determine the proportion of pure protein

$$\text{Percentage (\%) of Protein} = \frac{\textit{titre volume sample - titre volume blank x 0.014 x 0.1 x 6.25}}{\textit{weight of sample used}} \times 100$$

2.4.3. Determination of glycogen:

Anthrone reagent was used according to the procedure by (Carroll et al., 1956) to measure glycogen stores in the liver and muscle. Tissue is extracted by boiling with 30% (KOH) or homogenizing with a trichloroacetic acid solution (TCA). The glycogen is precipitated from the extract by direct treatment of the precipitated glycogen with an anthrone reagent. An alternative procedure is to destroy alkali-labile carbohydrates by boiling them with KOH and then determine the glycogen in the alkali-treated mixture with anthrone reagent. The following equation was used in the Determination of glycogen concentration:

$$\frac{DU}{DS} x 0.1 x \frac{\text{volume of extract}}{\text{of tissue}} \ x\ 100\ x\ 0.9 = mg \text{ of Glycogen in } 100 \text{ gm of tissue}$$

Where:

DU indicates the unknown's optical density,

DS indicates the standard's optical density,

0.1 indicates the amount of glucose in (mg) in 2 ml of standard solution,

0.9 Which is the factor for converting glucose to glycogen.

2.4.4. Determination of total lipids:

The crude lipid content was extracted from the sample using a Soxhlet extractor with a mixture of chloroform and methanol (2:1, v/v). The crude lipid content was measured gravimetrically after oven-drying the extract at 80°C overnight. The lipid content of the sample was then calculated using the formula proposed by (Bligh & Dyer,1959):

$$\textit{Total Lipids (\%)} = \frac{\textit{weight of flask and extract fat - weight of empty flask}}{\textit{weight of dried sample}} \times 100$$

2.5. Nutritional value:

The edible parts (muscles) of the studied species' caloric value was determined by applying the following formula based on the biochemical composition analysis provided by (Falch *et al.,* 2010):

$$\text{Nutritional value (kcal/100g)} = (\text{lipid} \times 9) + (\text{protein} \times 4) + (\text{glycogen} \times 4).$$

2.6. Data analysis

Data of the elemental minerals are expressed as mean ± standard deviation (SD). Figures were plotted from mean ± standard deviation (SD) data using Microsoft Excel for Windows 10. P values of less than $\alpha = 0.05$ were considered significant. Collected results (biochemical composition and element concentration) were subjected to statistical analysis using one-way analysis of variance (ANOVA). Hierarchical clustering was performed using the R program ANOVA by R (version 4.0.3; https://www.r-project.org/). Principle component analysis was performed using the origin program 2021 (9.8).

RESULTS AND DISCUSSION

3.1. Trace element assessment

3.1.1. In Water samples

Table (3) summarizes the trace element (Cd, Cu, and Zn) concentrations in water samples from Timsah Lake during the summer of 2019. Elements concentrations in the water samples varied from 0.14 - 0.56, 0.15 - 0.50, and 3.05 - 9.80 µg/l, with an average of 0.29 < 0.33 < 7.25 for Cd, Cu, and Zn, respectively. Different copper and zinc concentrations are driven mainly by urban and industrial activity, copper discharges from sediment into the surrounding water, and cadmium levels imply the lack of a direct source (Saad *et al.,* 2016; Ghannam, 2021). None of the sampled water had Cd, Cu, and Zn contents that were higher than those recommended by WHO (2011), CCME (2007), or EDWQS (2007), which means water was safe for health and the ecosystem.

Table (3): Heavy metals concentration in water samples (µg/l) with metal pollution index from Timsah Lake, Egypt, during 2019.

Lakes	**Cd**	**Cu**	**Zn**	**MPI**
	µg/l			
Min.	0.14	0.15	3.05	0.53
Max.	0.56	0.50	9.80	1.40
Average ± S.D	**0.29 ± 0.23**	**0.33 ± 0.17**	**7.25 ± 3.66**	**0.89 ± 0.53**
WHO, 2011	3	2000	500	
CCME, 2007	1	4	50	
EDWQS, 2007	10	1000	1000	

Metal pollution index values ranged from 0.53 - 1.40, with an average mean of 0.89. MPI findings offer helpful information on the aquatic system quality in this area, which aids the decision-maker in addressing the pollution issue (Goher *et al.,* 2014).

3.1.2. In Fish samples

Trace element contamination is an important ecological component that may harm human health. Many trace elements accumulate in some fish tissues from both natural and human sources. Because of the affinity between trace elements and various fish tissues, different portions of different fish species will contain different contaminants (Hasan *et al.,* 2023). As a result, researchers analyzed economically significant fish species to learn more about trace element content and nutritional value (Huang *et al.,* 2022). Consumption of fish muscle poses considerable health concerns due to trace element bioaccumulation (Huang *et al.,* 2022). Furthermore, variables such as the fish's lifetime and physiological metabolism may have an impact (Łuczyńska *et al.,* 2020; Huang *et al.,* 2022).

The levels of (Cd, Cu, and Zn) in the liver and muscles of five fish species (*Chelon ramada, Sparus aurata Planiliza carinata, Alepes djedaba, Oreochromis niloticus*) collected from Timsah Lake, Egypt, presented in Table "4". Results showed that the highest level of elements bioaccumulation found in the liver than muscles. The amounts of elements in fish tissues followed the order Zn > Cu > Cd. The liver recorded the most significant Cd, Cu, and Zn levels in *Alepes djedaba*, *Sparus aurata*, and *Planiliza carinata*, respectively. *Alepes djedaba* recorded the highest level of all elements studies in muscles. *Chelon ramada* had the lowest levels in element studies in muscles. The element concentrations in the organs ranged from 0.19 - 1.99, 0.40 - 34.60, and 5.86 - 39.39 µg/g wet weight for Cd, Cu, and Zn, respectively.

Trace elements accumulation in fish tissues varied according to the fish's size, species, habitat, and quantity of pollution in the water. Furthermore, metabolic metabolism may be impacted (Łuczyńska *et al.,* 2020; Huang *et al.,* 2022). The greater Cu and Zn levels in fish muscles might be attributable to the organism's natural adsorption of these elements. Zn is required to function in many biological enzymes, while Cu is a component of several oxides (Huang et al., 2022). Fish muscles show that elements are within acceptable limits (FAO/WHO, 2015), while the liver is above the limits. Since muscles do not have as many binding proteins and enzymes as the liver, they do not store as many trace elements.

MPI muscle values varied from 0.78 – 1.91 in *Chelon ramada* and *Alepes djedaba*, respectively (Table 4). On the other hand, MPI in the liver is greater than in muscles. The following MPI list of fish species was reported as *Alepes djedaba* > *Oreochromis niloticus* > *Planiliza carinata* > *Sparus aurata* > *Chelon ramada*. However, the distribution of elements in fish changes depending on the route of exposure (Zaghloul *et al.,* 2022).

Bioconcentration factor in organ capacity revealed in order liver > muscles. BCF varied from 0.66 - 6.85, 1.21 - 104.85, and 0.81 – 5.43 for Cd, Cu, and Zn, respectively (Table 4). The factor of bioconcentration of heavy metal in fish muscles cannot exclusively account for the effectiveness of their accumulation. The bioconcentration and bioaccumulation factors were calculated to assess the transfer of heavy metals from water and sediments to fish. Research demonstrating high bioconcentration factors (BCF > 1) for metals in water suggests that fish may collect dangerous levels of these metals. Muscle tissue, reflecting the phenomena of bioconcentration, also has larger concentrations of these metals compared to water. In constract BCF is < 1, that mean bioconcentration is almost nonexistent. The high level of bioconcentration factor of elements studied in the liver shows that *Alepes djedaba, Sparus aurata Planiliza carinata*, and *Alepes djedaba* in muscles are a good bio-indicator for monitoring pollution with these heavy metals on fish species (Sanou *et al.*, 2021).

Table (4): The heavy metals concentration and bioaccumulation concentration of the liver and muscles in different fish species from Timsah Lake, Egypt.

Tissue	**Fish Species**	**Heavy metals concentration µg/g (wet weight) mean ± SD**			
		Cd	**Cu**	**Zn**	**MPI**
Liver	*Chelon ramada*	0.25 ± 0.15^{a}	16.20 ± 8.35^{b}	13.97 ± 4.89^{c}	**3.84**
	Sparus aurata	0.39 ± 0.10^{a}	34.60 ± 16.53^{a}	11.30 ± 3.02 c	**5.34**
	Planiliza carinata	0.45 ± 0.11^{a}	11.90 ± 4.80^{b}	39.39 ±17.72^{b}	**5.95**
	Alepes djedaba	1.99 ± 2.19^{b}	6.60 ± 4.10^{d}	17.32 ± 9.75^{c}	**6.10**
	Oreochromis niloticus	0.82 ± 0.10^{a}	3.70 ± 1.30^{c}	15.49 ± 12.22^{c}	**3.61**
Muscles	*Chelon ramada*	0.19 ± 0.14^{a}	0.40 ± 0.10^{c}	5.86 ± 2.65^{d}	**0.78**
	Sparus aurata	0.20 ± 0.10^{a}	0.60 ± 0.10^{c}	6.24 ± 2.36^{d}	**0.89**
	Planiliza carinata	0.40 ± 0.22^{a}	0.70 ± 0.10^{c}	7.90 ± 7.05^{d}	**1.30**
	Alepes djedaba	0.53 ± 0.59^{a}	1.00 ± 0.20^{c}	13.13 ± 2.23^{b}	**1.91**
	Oreochromis niloticus	0.50 ± 0.58^{a}	0.50 ± 0.20^{c}	10.38 ± 4.45^{b}	**1.37**
- Different letters following the means in each column are significantly different ($P \leq 0.05$)					
England MAFF, 2000		**0.2**	**20**	**5**	
FAO/WHO (2015)		**0.5**	**3**	**40**	
Bioconcentration factor of heavy metals concentration (BCF)					
Fish Species		**Cd**	**Cu**	**Zn**	
Liver	*Chelon ramada*	0.86	**49.09**	**1.93**	
	Sparus aurata	**1.34**	**104.85**	**1.56**	
	Planiliza carinata	**1.55**	**36.06**	**5.43**	
	Alepes djedaba	**6.86**	**20.00**	**2.39**	
	Oreochromis niloticus	**2.83**	**11.21**	**2.14**	
Muscles	*Chelon ramada*	0.69	**1.21**	0.81	
	Sparus aurata	0.66	**1.82**	0.86	
	Planiliza carinata	**1.38**	**2.12**	**1.09**	
	Alepes djedaba	**1.83**	**3.03**	**1.81**	
	Oreochromis niloticus	**1.72**	**1.52**	**1.43**	

3.3. Risk assessment:

3.3.1. Non-carcinogenic hazard (THQ)

The current study found that the THQ muscle values for normally consuming fish from Timsah Lake were < 1 in all fish species in both groups (adult and children) and high consumers, except Cd in the child in both normal and high consumers and Adults with high consumers > 1 (Table 5). Cadmium is generally weakly absorbed by the body, but once ingested, like other elements, it is slowly eliminated and accumulates in the kidney, causing renal damage (FAO/WHO, 2003). That indicated none of them was harmful to the body at the typical ingestion rate for a healthy adult. Therefore, there are no potential health concerns for people (Adults) in this group. This conclusion is similar to the findings of (Yi *et al.*, 2017), who also reported THQ values of < 1 and discovered no severe effects of trace elements on human health from ingesting fish regularly. Therefore, THQ values < 1 imply no harm to human health, according to USEPA (2018) standards. As a result, eating fish from Timsah Lake poses no health danger to the community, and the cumulative impacts of pollutants should be addressed when anticipating their potential non-carcinogenic effects on people. However, there is a risk for children in the normal and high consumption groups. Consuming fish muscles for high-consumption individuals may have adverse consequences from ingesting Cd found in certain species. Predicting the probable impacts of pollutants on people requires considering the additive effect of contaminants on the population for non-carcinogenic risk (Zaghloul *et al.*, 2022).

3.3.2. The combined risk of many elements (HI)

All elements studied had HI or (TTHQ) muscle values < 1 in (Adults with a normal consumer, while in adults and children with both normal and high consumer >1 (Table 5). All trace elements (HI) muscle value was arranged in decreasing order. *Alepes djedaba* > *Oreochromis niloticus* > *Alepes djedaba* > *Planiliza carinata* > *Chelon ramada* > *Sparus aurata* is for adult and child groups with normal and high consumers. All elements studied had HI, or (TTHQ) muscle values investigated and do not significantly threaten muscle and liver tissue at the typical ingestion rate (Pawełczyk, 2013). TTHQ < 1, as USEPA (2018) recommended, poses no threat to human health. HI values were > 1 in both the normal (adult and children) and high populations (adult and children), ranging from 0.21 to 0.55, 0.96 to 2.55, 0.65 to 1.71, and 3.01 to 7.98, respectively. This study's results corroborated those published by Ezemonye et al. (2019), indicating that eating fish has no health hazards since TTHQ was > 1.

Table (5): THQ and TTHQ (HI) non-carcinogenic heavy metals concentration in different fish species for both groups (adult and child) from Timsah Lake, Egypt.

Tissue		**Fish Species**	**Heavy metals concentration µg/g (wet weight)**							
			THQ$_{Cd}$	**THQ$_{Cd}$**	**THQ$_{Cu}$**	**THQ$_{Cu}$**	**THQ$_{Zn}$**	**THQ$_{Zn}$**	**HI**	**HI**
			Adult	**Child**	**Adult**	**Child**	**Adult**	**Child**	**Adult**	**Child**
Normal Consumer	**Liver**	*Chelon ramada*	0.23	**1.07**	0.37	**1.73**	0.04	0.20	0.64	**2.99**
		Sparus aurata	0.36	**1.66**	0.79	**3.69**	0.03	0.16	**1.18**	**5.52**
		Planiliza carinata	0.41	**1.92**	0.27	**1.27**	0.12	0.56	0.80	**3.75**
		Alepes djedaba	**1.82**	**8.49**	0.15	0.70	0.05	0.25	**2.02**	**9.44**
		Oreochromis niloticus	0.75	**3.50**	0.08	0.39	0.05	0.22	0.88	**4.11**
	Muscles	*Chelon ramada*	0.18	0.85	0.01	0.04	0.02	0.08	0.22	0.98
		Sparus aurata	0.17	0.81	0.01	0.06	0.02	0.09	0.21	0.96
		Planiliza carinata	0.37	**1.71**	0.02	0.07	0.02	0.11	0.41	**1.89**
		Alepes djedaba	0.48	**2.26**	0.02	0.11	0.04	0.19	0.55	**2.55**
		Oreochromis niloticus	0.46	**2.13**	0.01	0.05	0.03	0.15	0.50	**2.33**
High Consumer	**Liver**	*Chelon ramada*	0.71	**3.33**	**1.16**	**5.40**	0.13	0.62	**2.00**	**9.35**
		Sparus aurata	**1.11**	**5.20**	**2.47**	**11.53**	0.11	0.50	**3.69**	**17.24**
		Planiliza carinata	**1.29**	**6.00**	0.85	**3.97**	0.38	**1.75**	**2.51**	**11.72**
		Alepes djedaba	**5.69**	**26.53**	0.47	**2.20**	0.16	0.77	**6.32**	**29.50**
		Oreochromis niloticus	**2.34**	**10.93**	0.26	**1.23**	0.15	0.69	**2.75**	**12.86**
	Muscles	*Chelon ramada*	0.57	**2.67**	0.03	0.13	0.06	0.26	0.66	**3.06**
		Sparus aurata	0.54	**2.53**	0.04	0.20	0.06	0.28	0.65	**3.01**
		Planiliza carinata	**1.14**	**5.33**	0.05	0.23	0.08	0.35	**1.27**	**5.92**
		Alepes djedaba	**1.51**	**7.07**	0.07	0.33	0.13	0.58	**1.71**	**7.98**
		Oreochromis niloticus	**1.43**	**6.67**	0.04	0.17	0.10	0.46	**1.56**	**7.29**
	TDI$_s$ (µg/day)*(WHO,2003)*		58.3*		700*		8000-11000			

* Toxicological limit (µg/day) (WHO, 2003)

3.4. Biochemical composition assessment:

Researchers have found that fish types with high amounts of total protein, fats, moisture, and ash meet the nutritional needs of humans (Oliva-Teles *et al*., 2020) say that protein is the most expensive and important part of a fish's food. Protein is important for fish to grow and develop, and it is also a big part of energy balance because it is used to make ATP (Arenas *et al*., 2021).

Lipids are the fish's main energy source that does not come from protein. They also contain important fatty acids that help the fish grow and stay healthy (Arenas *et al*., 2021). By including an adequate amount of lipids in fish diets, catabolism of dietary protein can be reduced and used for energy purposes (Guo et al., 2019). Fish's livers store carbs as glycogen, which can be turned into glucose with the help of certain enzymes. Some fish's carbs are turned into fats and kept in the muscles and liver. If necessary, glucose can be made from glycogen to help fish get energy and do their biological work. The amount of glycogen in muscle, especially the liver, is believed to indicate the body's physiological state (Makarenko *et al*., 2021).

3.4.1. Biochemical composition in fish organs:

The biochemical makeup of fish muscles and liver are shown in Tables (6 and 7) and Figures (2&3). The differences in the biochemical composition of marine fish investigated in this study can be attributed to species, food availability, and geographical location (Ali *et al.,* 2013). All results demonstrate significant differences ($P< 0.05$) between various species.

Most fish species' muscles have much lower moisture content (%) than the tested fish's liver. Muscle moisture levels varied from 69.32± 2.65% to 75.62 ± 3.03% and ranged from 74.26 ± 3.04 to 89.78 ± 3.67 in the liver, respectively. The maximum moisture content in muscles was found in *Oreochromis niloticus*, whereas the highest moisture content in the liver was found in *Sparus aurata*. *Alepes djedaba* has the lowest moisture content in the muscles and the liver. Moisture content in fish muscles is influenced by factors such as season, age, and environment, which could explain the observed differences in moisture content between species (Huang *et al.,* 2022). In addition to changes in eating patterns, environmental conditions, and food availability (Mahmud *et al.,* 2019; Younis *et al.,* 2021).

Table (6): The biochemical composition of the liver and muscles in different fish species from Timsah Lake, Egypt.

Tissue	**Fish Species**	**Biochemical Composition %**			
		Protein*	**Lipid***	**Glycogen***	**Moisture****
Muscles	*Chelon ramada*	11.63 ±0.41^{a}	1.79±0.08^{b}	0.26±0.015 ^{b}	73.83±2.96^{d}
	Sparus aurata	13.96±0.49 ^{a}	**2.07±0.09** ^{b}	0.25±0.014 ^{b}	70.60±2.83 ^{d}
	Planiliza carinata	9.99 ± 0.35 ^{c}	2.04±0.09 ^{b}	**0.50±0.03** ^{b}	70.17±2.81 ^{d}
	Alepes djedaba	10.94 ±0.38 ^{ac}	1.69±0.08 ^{b}	0.39±0.02 ^{b}	69.32±2.78 ^{d}
	Oreochromis niloticus	10.38±0.36 ^{c}	0.36±0.01 ^{a}	0.17±0.004 ^{b}	75.62±3.03 ^{d}
Liver	*Chelon ramada*	15.84 ± 0.56 ^{a}	4.95 ±0.22 ^{d}	1.55±0.05 ^{d}	88.88±3.63 ^{c}
	Sparus aurata	14.51 ± 0.51 ^{a}	**5.31 ± 0.24** ^{d}	**3.38±0.16** ^{d}	89.78±3.67 ^{c}
	Planiliza carinata	14.94 ± 0.52 ^{a}	2.60±0.12 ^{b}	1.79±0.06 ^{a}	75.33±3.08 ^{d}
	Alepes djedaba	11.80 ± 0.41 ^{a}	2.68±0.12 ^{b}	1.13±0.04 ^{a}	74.26±3.04 ^{d}
	Oreochromis niloticus	8.39 ± 0.29 ^{c}	3.69±0.17 ^{db}	0.21±0.05 ^{b}	79.33±3.24 ^{dc}

- Different letters following the means in each column are significantly different ($P \leq 0.05$).

***Values are expressed as (%) of the dry weight;**

****Values are expressed as (%) of the fresh weight**

The crude protein composition of fish species varied from 9.99 ± 0.35% to 13.96 ± 0.49% for muscles and from 8.39 ± 0.29% to 15.84 ± 0.56% in liver. *Sparus aurata* and *Chelon ramada* had the most effective protein content in their muscles and livers, respectively, whereas *Planiliza carinata* and *Oreochromis niloticus* had the lowest. These results could be attributed to differences in ecological conditions, feeding habits, and food availability (Younis *et al.,* 2021). In addition, the fish liver contains a lot of total protein, which may be essential for reproductive metabolism and spawning (Guerrero-Zárate *et al.,* 2019). We found no link between protein abundance and trace element concentrations in fish muscle (Pearson correlation test, $P < 0.05$) aligned with previous studies.

Table (7): Caloric value of the liver and muscles in different fish species from Timsah Lake, Egypt.

Tissue	**Fish Species**	Total Protein	Total Lipid	Total Glycogen	Caloric value (kcal/100g
Liver	*Chelon ramada*	63.36	44.55	6.20	114.11
	Sparus aurata	58.04	47.79	13.52	119.35
	Planiliza carinata	59.76	23.4	7.16	90.32
	Alepes djedaba	47.20	24.12	4.52	75.84
	Oreochromis niloticus	33.56	33.21	0.84	67.61
Muscles	*Chelon ramada*	46.52	16.11	1.04	63.67
	Sparus aurata	55.84	18.63	1.00	75.47
	Planiliza carinata	39.96	18.36	2.00	60.32
	Alepes djedaba	43.76	15.21	1.56	60.53
	Oreochromis niloticus	41.52	3.24	0.68	45.44

Crude lipid concentration in fish species varied from 0.36 ± 0.01% to 2.07 ± 0.09% and from 2.60 ± 0.12% to 5.31 ± 0.24% in muscles and liver, respectively. *Sparus aurata* has the most lipids in both the muscles and the liver. While *Oreochromis niloticus* has the lowest in muscles and *Planiliza carinata* has the highest in the liver. The amount of lipids in marine species depends on the type of fish, the food in their territory, its life cycle, its surroundings, and how it eats. The data for fat content and trace elements in fish muscle were the same as those for protein content ($P < 0.05$). Moreover, lipid content is affected by factors such as environment, life cycle, and topographical origin. Various factors, such as the quality of water, type of feeding, time of capture, fish life cycle, and farming system, are based on the difference in the number of biochemical compounds in fish muscle (Younis *et al.*, 2021).

The glycogen level of fish species varied from 0.17±0.00% to 0.50 ± 0.03% and from 0.21± 0.05% to 3.38± 0.16% for muscles and liver respectively. *Oreochromis niloticus* has the lowest muscle and liver values, followed by *Planiliza carinata* and *Sparus aurata*, which have the most significant muscle and liver values, respectively. Fish use muscle glycogen as a source of energy to move, and changes in it are linked to yearly changes in their bodies. When fish eat less, the amount of glycogen in their muscles increases. Used glycogen stores in their muscles are constantly replaced, probably through liver reserves or a mix of both (Pyz-Łukasik *et al.*, 2020; Khomenchuk *et al.*, 2020; Tsurkan, 2021). These factors could influence the differences observed in fish, as noted by studies conducted by (Palamarchuk *et al.*, 2022; and Mushtruk *et al.*, 2021).

According to (Hernandez-Saavedra *et al.,* 2020), the metabolism of lipids, carbohydrates, and amino acids may all be negatively affected by exposure to trace elements in fish. For instance, (He *et al.*, 2023) found that cadmium exposure reduced glucose levels in fish tissues by altering carbohydrate metabolism. Changes in fish physiology and pathology may result from interactions between trace elements and fish biochemistry. Trace elements may have devastating impacts on aquatic life and human health. Therefore, their concentrations must be closely monitored and controlled.

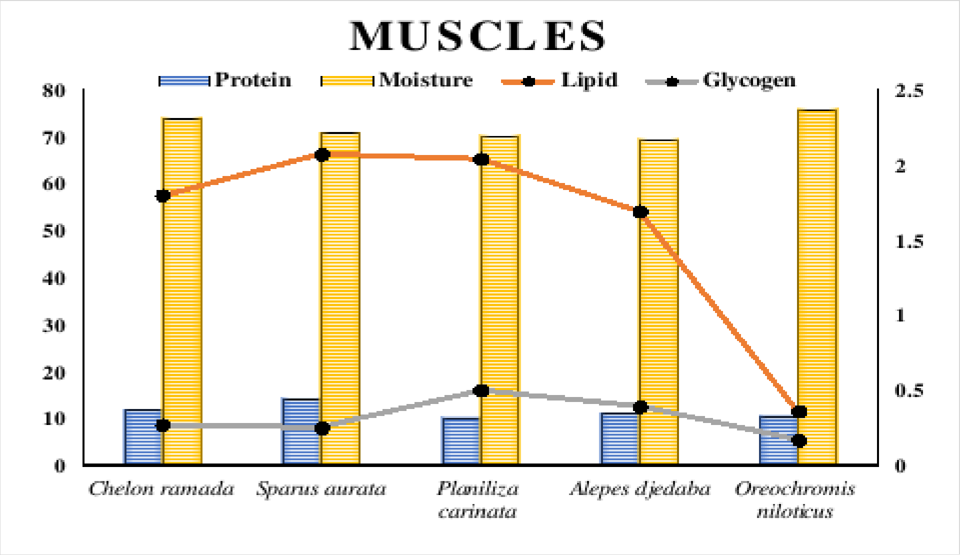


Figure (2): The muscle's biochemical composition in different fish species from the area of investigation during 2019

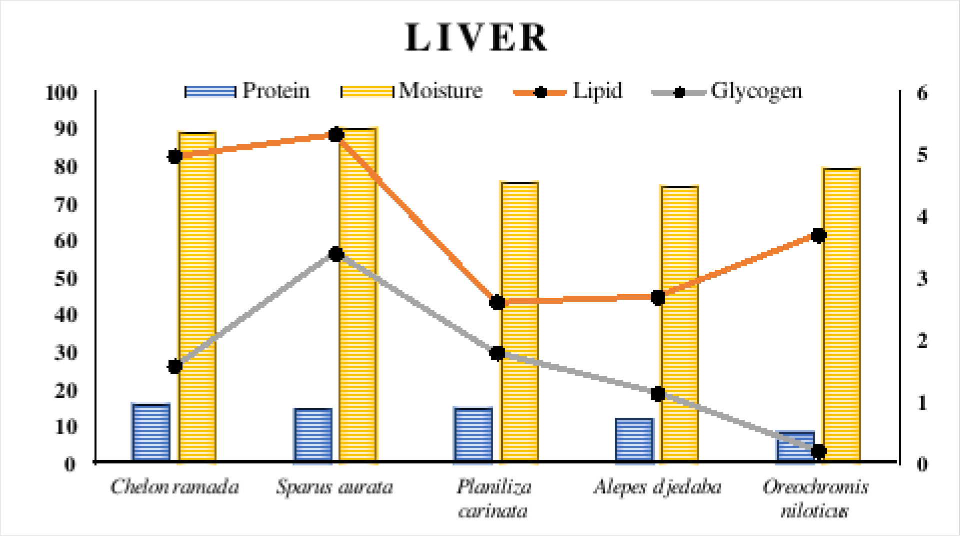


Figure (3): The liver's biochemical composition in different fish species from the area of investigation during 2019

3.5. Data analysis:

Table "4" and Figs "4 and 5" present the biochemical composition of fish muscles; the y-axis is Fish Species, but the PCA y-axis does not represent the figure. The muscle protein content of *Sparus aurata* had significantly higher protein content (13.96%), while the *Planiliza carinata* group had significantly lower protein content (9.99%) than other species. The muscle lipid of *Sparus aurata* (2.07%) was considerably higher, while that of *Oreochromis niloticus* (0.36%) was significantly lower than that of other fish species. The glycogen content of *Planiliza carinata* (0.5%) was considerably higher, while that of *Oreochromis niloticus* 0.17%) was significantly lower than that in other fish species. The moisture content in *Oreochromis niloticus* (75.62%) was considerably higher than in the other species, whereas the moisture content in *Alepes djedaba* was the lowest (69.32%). The biochemical composition of fish livers is presented in Table 9. The liver protein content of *Chelon ramada* had significantly higher protein content (15.84%), while the *Oreochromis niloticus* group had significantly lower protein content (8.39%) than other species. The liver lipid of *Sparus aurata* (5.31%) was considerably higher, while that of *Planiliza carinata* (2.60%) was significantly lower than that of other fish species. The glycogen content of *Sparus aurata* (3.38%) was considerably higher, while that of *Oreochromis niloticus* (0.21%) was significantly lower than that of other fish species. The moisture content in *Sparus aurata* (88.88%) was considerably higher than in the other species, whereas the moisture content in *Alepes djedaba* was the lowest (74. 26%).

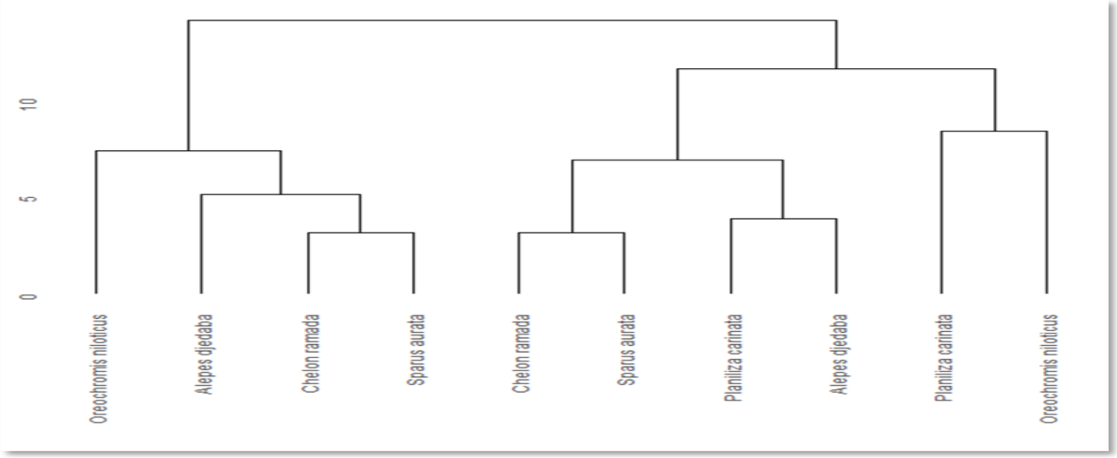


Figure (4): Hierarchical clustering analysis for the biochemical composition of different fish species from the area of investigation during 2019.

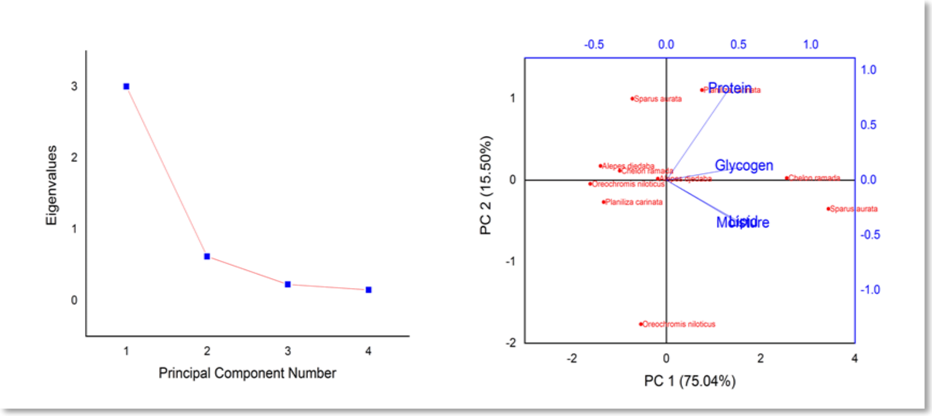


Figure (5): Principle component analysis of chemical composition for different fish species from the area of investigation during 2019.

Table "6" and Figs "6 and 7" present the element concentration of fish livers. The liver cd content of *Alepes djedaba* had significantly higher protein content (1.99%), while the *Oreochromis niloticus* group had significantly lower protein content (0.12%) than other species. The liver Cu of *Sparus aurata* (34.60%) was considerably higher, while that of *Oreochromis niloticus* (3.70%) was significantly lower than that of other fish species. The Zn of *Planiliza carinata* (39.39%) was considerably higher, while that of *Alepes djellaba* (10.32%) was significantly lower than that of other fish species. The element concentration of fish muscles is presented in Table. The muscle cd content of both Oreochromis *niloticus and Alepes* djellaba had significantly higher protein content (0.53%). The *Chelon ramada* group had significantly lower protein content (0.27%) than other species. The muscle Cu of *Alepes djedaba* (1.0%) was considerably higher, while that of *Chelon ramada* (0.40%) was significantly lower than that of other fish species. The Zn content of *Oreochromis niloticus* (53.38%) was considerably higher, while that of *Chelon ramada* (5.86%) was significantly lower than that of other fish species.

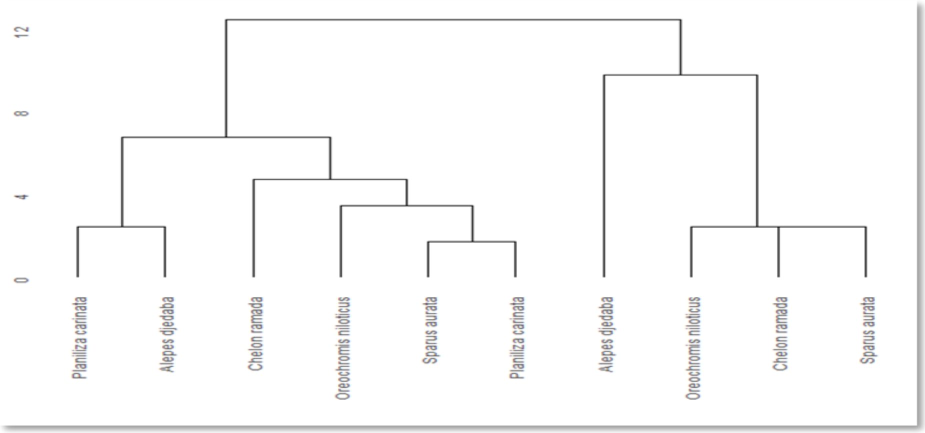


Figure (6): Hierarchical clustering analysis for the metal concentration for different fish species from the area of investigation during 2019.

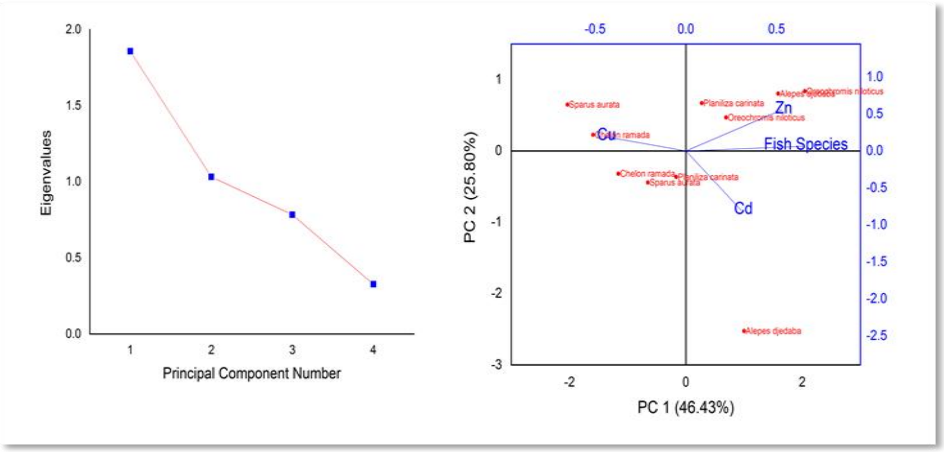


Figure (7): Principle component analysis of chemical composition for different fish species.

CONCLUSION

Even with being an excellent source of protein and fats, the fish species captured from Timsah Lake (*Chelon ramada, Sparus aurata Planiliza carinata, Alepes djedaba, Oreochromis niloticus*) may represent a possible health danger for users. Using MPI and HI values, we calculated the element load of consumable fish tissues caused by various human actions. THQ and HI levels were >1.0, particularly in the liver, but muscle fish from this lake do not pose any non-carcinogenic health risks to the community. The recommended human risk assessment considers dosage and consumption-dependent factors to more accurately predict risks to human customers. Trace elements can be toxic to humans, so it is essential to keep them below safe amounts in the shellfish we eat by applying control measures to keep them out. The recommendations for policymakers are to establish and enforce regulations that can help protect public health and the environment from trace element pollution in Timsah Lake.

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