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Original Research

Prospective Risk Assessment of some Heavy Metals on *Tilapia zillii* in Temsah Lake

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Abstract

This study was conducted to determine the pollution index of some heavy metals in Temsah Lake water to assess their transfer into *Tilapia zillii* tissues using bioaccumulation factors as well as evaluate their associated health risks. Results showed that Pb, Cd, and Cu levels increased significantly during spring, summer, and both spring and summer, respectively. The pollution index of heavy metals in the Temsah Lake revealed that Fe had a moderate effect in winter and a strong effect in the other seasons. Pb had a serious impact on aquatic life in the spring and autumn, while Cd had a serious impact in the summer and autumn. Water temperature is strongly correlated with Cu in the liver, Pb in the musculature, and Cd in the water and liver. Conversely, it negatively correlated with fish weight, length, Fe, Zn, and Cu in the musculature. Fish length showed an inverse relationship with water Cu, Cd, Pb, and Cd. The histopathological examination revealed hydropic degeneration, fatty changes, and interstitial and focal infiltration of immunocompetent cells. Muscular tissue revealed degenerative changes manifested by atrophy and fragmentation of muscular fibers in some specimens. The bioaccumulation factor of heavy metals in Tilapia zilli musculature and liver was found to be highest during winter. Fish musculature was safe for Pb, Cd, and Cu and might represent potential risks for Fe and Zn. In conclusion, warm seasons have the highest integrated biomarker response (IBRv2) scores for the detected heavy metals, also the sum of IBRv2 of the heavy metals content is the highest in musculature followed by livers then water samples. Herein, TCR results for Pb and Cd in Tilapia zilli are within the permissible range (10−4 to 10−6).

KEYWORDS
Tilapia zillii, Heavy metals, Temsah lake, Pollution.

INTRODUCTION

Water scarcity and pollution are two critical issues exacerbated by climate change. Water scarcity is a growing climatic and human-related situation that causes and interacts with other freshwater stresses such as chemical pollution (Arenas-Sánchez *et al*., 2016). Pollution from potentially toxic elements (PTEs) in the aquatic environment has become a worldwide issue due to their abundance, persistence, intrinsic toxicity, non-degradability, and ubiquity (El-Degwy *et al*., 2022).

Heavy metals are present in the environment at different stages and can enter the aquatic ecosystem through both anthropogenic and natural sources such as atmospheric deposition, geological matrix depletion, stormwater runoff, landfill draining, drilling for oil, shipments and shoreline activities, and domestic, industrial, and agricultural runoff (Abd El-Aal *et al*., 2020, Salcedo Sánchez *et al*., 2022).

Toxic heavy metals that are released into the environment include zinc, copper, nickel, mercury, cadmium, lead, and chromium (Fu and Wang, 2011). The effects of rising heavy metal levels on fish are connected to their absorption and accumulation by the organism, which causes metal-induced disruptions in the structures and functions of several tissues and organs (Pedlar *et al*., 2002). Heavy metal pollution harms various aquatic fish species by producing physiological, phenotypic, and behavioral disorders, as well as reproductive dysfunction (Bristy *et al*., 2021). Consumption of heavy metal-contaminated seafood enhances the danger to human health. Long-term exposure to heavy metals through diets may result in chronic buildup, causing harm to the human body (Adegbola *et al*., 2021).

Fish, like other aquatic animals, may store a high number of hazardous metals in their numerous organs, which can then enter the human body and cause major health problems (Mehar *et al*., 2023). The overuse of water resources, particularly freshwater resources, imposed various constraints on aquaculture development. As a result, saltwater represents an instant alternative source of raising and husbandry for a variety of marine organisms, including *T. zillii* (El-Sayed *et al*., 2019).

El Temsah Lake, located 76km from Port Said, is a key wetlands in the Suez Canal area and a significant fish source (Soliman *et al*., 2019). It is the primary brackish wetland environment in Ismailia's governorate (Almatari *et al*., 2017). The lake's tourism and fishing sector employs residents and contributes significantly to district income. However, the increasing number of permanent residents has led to increased waste quantities, including municipal sewage, agricultural runoff, and industrial effluent. Additionally, various pollution sources contribute to the lake's potential chemical pollutants (Kaiser *et al*., 2009).

Reports have recorded contamination levels of a variety of pollutants in the lake, including heavy metals (Essa *et al*., 2018), polychlorinated biphenyl, chlorinated hydrocarbons, and PAHs (Said and Agroudy, 2006). Thus, this study was conducted to measure the concertation of five heavy metals (iron, zinc, copper, lead, and cadmium) in Tilapia Zilli muscles and livers, as well as in water samples collected seasonally from Lake Temsah and evaluate their potential health risk.

MATERIALS AND METHODS

Study Area

Lake Temsah lies between latitude 30º 32′ and 30º 36′ N and longitude 32º 16′ and 32º 21′ Ethat divided into three basins: Temsah Lake, the western lagoon, and Suez Canal pathway. The sampling points (7 sites) were started at family beach (30.58′N, 32.27′E) about 300 m from the beginning of the lake then the other 6 points were located 700 m apart till reaching about 4.2 km of the lake length These sites were strategically chosen based on the physical appearance of the water and the economic activities taking place around the region.

Sampling

Samples were collected during the period from January to December 2020. One hundred and forty four *Tilapia zillii* (n= 36/ season, 12/ month) were collected seasonally for heavy metals detection at the same location of water collection. in addition to measurement of the fish 's body weights and lengths. Additionally, 96 water samples were collected seasonally (n= 24/ season, 6 samples/month) for heavy metal determination.

Twenty-four grabs were collected seasonally at depth of 50 cm at 3 different sites, 1km apart (duplicate samples/ site) then 12 composite samples were obtained by pooling equal volume of each site grabs. Samples were collected at depth of 30 cm from Temsah Lake in 2 L capacity pre-cleaned acidified polyethylene bottles. The water samples were analyzed for the presence of iron (Fe), zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) according to APHA (2017).

Clinical examination

Naturally collected fish specimens were grossly examined for determination of any clinical abnormalities and postmortem lesions according to Almacker (1970).

Heavy metals analysis

A wet digestion method was used based on the Analytical Methods for Atomic Absorption Spectrometry (Thermo Electron Corporation, type S4AA sys.). For determination of heavy metals in water and fish tissues according to the guidelines of the Analytical Methods for Atomic Absorption Spectroscopy (Perkin, 1981).

Integrated Biological Response IBRv2

A general pollution index, the IBRv2 established by Sanchez *et al*. (2013) was computed. Each biomarker's standardized value was calculated as follows:

Yi= log log Xi/X0

Yi is then normalized with the general mean (μ) and standard deviation (σ) of Yi:

Zi (Yi-μ)/σ

Th standard's biomarker responses are centered on zero, and a deviation index (A) is calculated by subtracting the mean of the standard Z0 from the mean of the heavy metals Zi.

A=Zi-Z0

Subsequently, the following function related IBRv2 values were computed:

IBRv2=∑|A|

Finally, star plots were employed to describe the results of each function. Excel software was used to compute the IBRv2 values and produce the star plots.

Pollution Index (PI)

The pollution index is based on individual metal calculations and is classified using the equation below Caeiro *et al*. (2005):

$$PI = \frac{\sqrt{[(\frac{C_i}{S_i})^2_{Max} + (\frac{C_i}{S_i})^2_{Min}]}}{2}$$

Where Ci is the concentration of each element, and Si is the standard values according to Hurley *et al*. (2012).

Bioaccumulation factor (BAF) estimation

The Bioaccumulation factor (BAF) of heavy metal level in fish musculature and livers were calculated using the following equation (Gobas *et al*., 2009):

$$BAF = \frac{concentration\ if\ fish\ \left(\frac{mg}{kg}\right)}{concentration\ in\ water\ \left(\frac{mg}{L}\right)}$$

BAF=(concentration if fish (mg/kg))/(concentration in water (mg/L))

Histopathological examination

After fishing, liver and muscle tissues were collected, dehydrated in 10% neutral buffered formalin, cleared, and embedded in paraffin wax. Sectioned at 5-7 um thick using a microtome, Harri's Hematoxylin and Eosin stain was used for histopathological examination. Slides were mounted on egg-albumin coated slides and examined using an Olympus BH-2 microscope. Images were digitized using an AMT camera system and image capture engine software, attached to an Olympus CX 41 binocular microscope, and processed in photo-editing software for improved contrast and labeling.

Human health Risk Assessments

Tolerable daily intake and estimated daily intake

The estimated daily intake (EDI) of heavy metals for adults was calculated as follows:

$$EDI\left(mg\frac{person}{day}\right) = \frac{C\ metal\ X\ D\ food\ intake}{average\ BW}$$

Where C metal is the concentration of heavy metals in fish (mg/kg wet weight), D is the average daily consumption of fish in the (0.227 kg) and Bw represents the body weight (70 kg) (Bo *et al*., 2009).

Target Hazard Quotient (THQ)

THQ were calculated according to USEPA (2015)

$$THQ = \frac{Efr\ X\ ED\ X\ FIR\ x\ C}{Rf\ Do\ X\ average\ BW\ X\ Atn\ X10-3}$$

Where, Efr is exposure frequency in 365 days/year; ED is exposure duration in 30 years, FIR is average daily consumption in Kg person/ day, C is concentration of metal in food sample in mg/kg, RfDo is refence dose in mg/kg day, and ATn is the average exposure time for non- carcinogenic in day (19,345).

Hazard Index

The metal(oid)s overall human risk, the hazard index (HI) is calculated as the sum of all THQs estimated for specific heavy metals.

$$HI = \sum_{n=1}^{i} THQn\ ; i = 1,2,3 \ldots\ldots n$$

Carcinogenic Risk

The cancer risk (CR) presented to human health by individual potential carcinogenic metals was calculated. Then, the total cancer risk (TCR), which may promote carcinogenic effects depending on exposure dose, was then calculated from ingestion of metals

$$CR = EDI\ XCfs$$

$$TCR = \sum_{n=1}^{i} CRn\ ; i = 1,2,3 \ldots\ldots n$$

Where CR = cancer risk over a lifetime by individual heavy metal ingestion, EDI = estimated daily metal intake of the population in mg/day/kg body weight, CSF = oral cancer slope factor in (mg/kg/day), and n is the number of heavy metals considered for cancer risk calculation.

For single carcinogenic metals and multi carcinogenic metals, the permissible limits are 10−6 and < 10−4, respectively (Tepanosyan *et al*., 2017).

Statistical analysis

SPSS version 22 software computer program, NY, USA (Inc., 1989-2013) was used for data processing utilizing the one-way analysis of variance test followed by pairwise comparisons using Duncan's test at p< 0.05 and Pearson correlation test.

RESULTS

Clinical examination

The collected *Tilapia zillii* had no apparent external clinical signs, but some fish showed slimy body with pale skin, liver and muscles (Figure 1).

Fig. 1. Naturally, heavy metals exposed *Tilapia zillii* showing slimness of external body with pale liver and muscle.

Heavy metals in Temsah Lake water

The results of the seasonal variations of heavy metals revealed that Pb, Cd, and Cu levels in water samples increased significantly (P≤0.0001) during spring, summer, and both spring and summer, respectively, whereas winter had the lowest levels of Pb and Cd and autumn had the lowest concentration of Cu. Fe and Zn levels did not differ significantly throughout seasons (Figure 1). Regarding seasonal effect, summer season had the highest IBRv2 scores, followed by spring, winter then autumn (Figure 2). In terms of heavy metals concentration, Zn had higher IBRv2 than Cu; Fe, Cd and finally Pb (Figure 2).

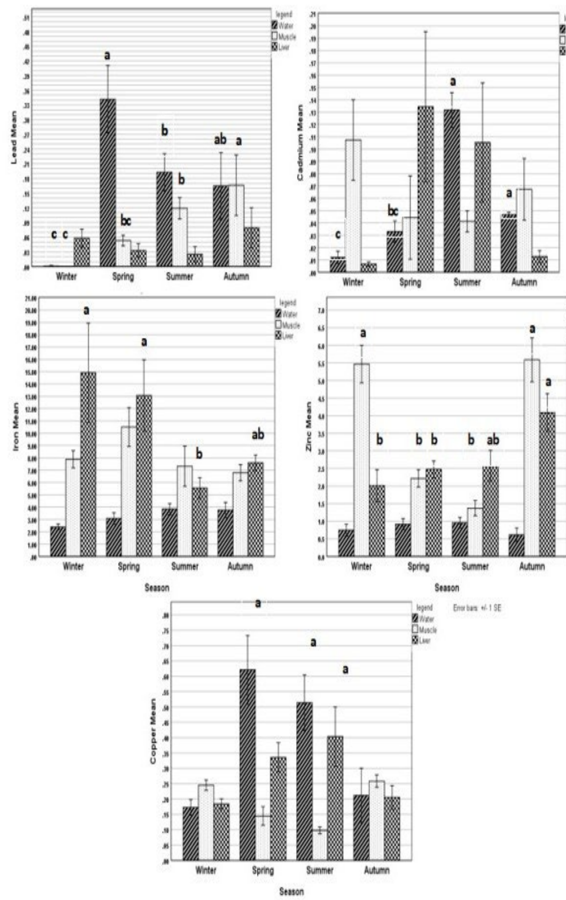

Fig. 2. Seasonal values of heavy metals (Pb, Cd, Fe, Zn, and Cu) detected in water samples and Musculature and liver tissues of *Tilapia zillii* collected from Temsah Lake. Means with different superscript letters in column are significantly different (P≤0.01).

Pollution index (PI)

The calculated PI of heavy metals in the Temsah Lake was

Pb>Cd> Fe> Zn and Cu. During the study, seasons, Zn and Cu had no impact on aquatic life, whereas Fe had a moderate effect in winter and a strong effect in the other seasons. Pb had a serious impact on aquatic life in the spring and autumn, while Cd had a serious impact in the summer and autumn (Table 1).

Table 1. Water pollution index by heavy metals in Lake Temsah during different seasons.

Heavy metal in water	Seasons	Mean ± SE	PI class	Class
Pb	Winter	0.13^{b}±0.103	1	No effect
	Spring	6.82^{a}±1.379	5	Seriously affected
	Summer	3.794^{b}±0.782	4	Strongly affected
	Autumn	3.34^{ab}±1.201	4	Strongly affected
	Average	3.177±0.488	4	Strongly affected
Cd	Winter	1.535^{b}±0.36	2	Slightly affected
	Spring	3.3^{b}±0.873	4	Strongly affected
	Summer	13.226^{a}±1.46	5	Seriously affected
	Autumn	5.063^{b}±0.504	5	Seriously affected
	Average	5.467±0.627	5	Seriously affected
Fe	Winter	2.352±0.176	3	Moderately affected
	Spring	3.079±0.464	4	Strongly affected
	Summer	3.921±0.459	4	Strongly affected
	Autumn	3.906±0.621	4	Strongly affected
	Average	3.146±0.203	4	Strongly affected
Zn	Winter	0.698±0.113	1	No effect
	Spring	0.9±0.182	1	No effect
	Summer	0.968±0.176	1	No effect
	Autumn	0.563±0.182	1	No effect
	Average	0.8±0.08	1	No effect
Cu	Winter	0.168^{b}±0.019	1	No effect
	Spring	0.62^{a}±0.112	1	No effect
	Summer	0.525^{a}±0.093	1	No effect
	Autumn	0.203^{b}±0.077	1	No effect
	Average	0.378±0.042	1	No effect

Heavy metals in Tilapia zillii

The study analyzed the seasonal variations in heavy metal concentrations in *Tilapia zillii*, specifically Pb, Cd, Fe, Zn, and Cu, in musculature and liver samples (Figure 1). Pb concentrations were highest in musculature during autumn, while Cd residues were non-significant. Fe levels were highest in winter and spring, with a trend towards increase in musculature samples. Zinc residues were highest in both tissues in autumn, with summer having the highest concentration of Cu in the liver but lowest in the examined musculature. Autumn had the most excellent IBRv2 scores, while winter had the lowest. Zn had the highest IBRv2, followed by Cu, Fe, Pb, and Cd (Figure 2).

The study examined the weight, length, and condition factor of *Tilapia zillii* collected from Lake Temsah during different seasons. The highest significant values were observed in spring, with an average mean of 38.961±2.256g and 13.424±1.175g in weight and length, respectively Table 2.

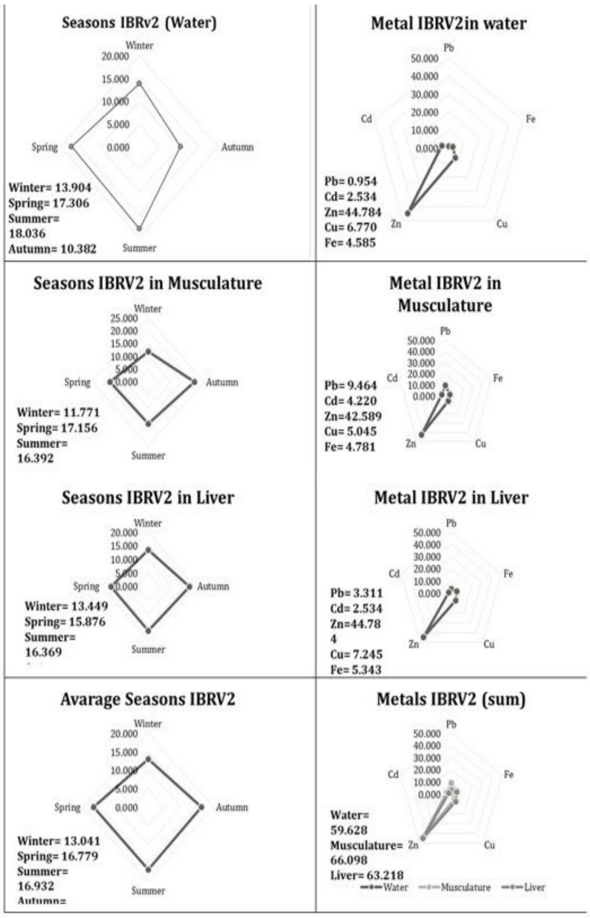

Fig. 3. Biomarkers star plots and Integrated biomarker response index (IBRv2) for seasons and heavy metals in different samples. IBRV2 in water (14.907), musculature (16.525) and liver (15.802).

The relationship patterns between the detected heavy metals in water and fish samples

There was a strong positive correlation (p≤ 0.01) between water temperature and Cu in liver (r = 0.329); Pb in musculature (r = 0.509); Cd in water and liver (0.675 and 0.375, respectively). On the other hand, water temperature was negatively correlated in a strong manner (p≤ 0.01) with fish weight (r=-0.495) and length (r= -0.734); Fe and Zn in fish musculature (r= -0.372 and -0.534, respectively) and liver (r= -0.372 and -0.305, respectively) and with Cu in musculature (r= -0.347). Moreover, fish weight showed positive correlation (p≤ 0.01) with liver Fe (r = 0.342) and Cd in musculature (r= 0.181) at p≤ 0.05; meanwhile weight was

Table 2. Weight, length and condition factor (K) of the collected *Tilapia zillii*i from lake Temsah during the different seasons.

Parameters	Weight (g)	Length (cm)	Condition factor (K)
Winter	50.402^{a}±4.74	14.984^{ab}±0.455	1.5677±0.13106
Spring	52.607^{a}±3.706	17.083^{a}±4.78	2.0236±0.11573
Summer	22.475^{b}±1.097	9.588^{b}±0.616	1.8705±0.28714
Autumn	21.686^{b}±1.876	11.75^{ab}±0.789	1.5941±0.23641
Average	38.961±2.256	13.424±1.175	1.7456±0.09836
Correlation with water Temp.	-.349-**	-.519-**	0.16

Means with different superscript letters in column are significantly different (P≤0.05). Correlation is significant at the 0.01 level (2-tailed).

negatively correlated with Pb in fish musculature and Cd in water. A direct association between fish length and Fe in musculature and liver; musculature Zn and Cu was recorded. On contrary, fish length demonstrated an inverse relationship with water Cu and Cd; liver Pb and Cd and Pb musculature (Table 3).

Histopathological examination

Microscopically, hepatic tissue revealed some pathological changes included hydropic degeneration, fatty changes with steatosis (Figure 4A). In addition to; interstitial and focal infiltration of immunocompetent cells (Figure 4A). Perivascular proliferation of melanomacrophages with their characteristic brown color were scattered among the hepatopancreas (Figure 4A). Red eosinophilic cells were distributed among the hepatopancreas cells & around the blood vessels (Figure 4B).

Microscopically, muscular tissue revealed either normal architecture of the fibers (Figure 5A) in some sections or degenerative

Table 3. Correlation coefficient between Temperature, *Tilapia zillii* weight, length and heavy metals concentrations detected in water and *Tilapia zillii* samples.

		Season	Water temp.	*Tilapia zillii*			Iron (mg/L)			Zinc (mg/L)			Copper (mg/L)			Lead (mg/L)			Cadmium (mg/L)		
					Length	K	W	M	L	W	M	L	W	M	L	W	M	L	W	M	L
Season		1	0.851**	0.349**	0.519**	0.16	0.1	0.09	0.199*	0.12	**0.562	0.06	**0.368	0.333**	0.219*	**0.363	0.229**	0.14	0.592**	0.08	0.14
Water temp.			1	0.495**	0.734**	0.18	0.06	**0.372	*0.256	0.06	0.534**	0.305**	0.19	0.347**	0.329**	0.18	0.509**	0.05	**0.675	0.05	0.375**
Weight				1	0.563**	0.19	0.06	0.01	0.342**	0.08	0.1	0.13	0.01	0.12	0.04	0.01	**0.290	0.18	0.281**	0.181*	0.18
Length					1	0.576**	0.11	0.186*	0.329**	0.1	0.29**	0.15	0.193*	0.207*	0.13	-0.06	-0.473**	**0.292	**0.279	0.1	-0.430**
K						1	0.14	0.01	0	*0.216	-0.06	0.07	*0.236	0.01	0.03	0.07	0.15	0.12	-0.13	0.04	0.07
Iron (mg/L)	W						1	0.03	0.13	0.08	0.17	0.11	0	0.06	0.04	-0.01	0.05	0.02	-0.03	0.01	0.03
	M							1	0.12	0.08	0.05	0.218*	0.02	0.1	0.05	-0.03	0.242**	0.12	0.275**	0.08	0.219*
	L								1	0.08	-0.01	**0.281	0.07	0.01	0.11	0.07	0.15	0.18	-0.14	0.08	0.19
Zinc (mg/L)	W									1	-0.06	0.08	0.186*	-0.03	0.19	0.247**	0.02	0.17	0.232*	0.02	0
	M										1	0.13	0.222*	0.52	0.279**	0.287**	0.15	0.09	0.360**	0.04	0.19
	L											1	0.05	0.06	0.02	0.02	0.08	0.1	0.06	0	0.267**
Copper (mg/L)	W												1	0.02	0.13	0.21	0.02	0.1	-0.11	0.09	0.07
	M													1	0.19	-0.15	0.14	0.01	0.230*	0.02	0.219*
	L														1	0.03	0.08	0.14	0.08	0.07	0.13
Lead (mg/L)	W															1	0.07	0.01	0.12	0.182*	0.14
	M																1	0.08	0.211*	0.05	0.1
	L																	1	*0.207	0.09	0.15
Cadmium (mg/L)	W																		1	0.06	0.02
	M																			1	0.06
	L																				1

** correlation is significant at the 0.01 level (2- tailed) * correlation is significant at the 0.05 level (2- tailed). W = water; M = musculature; L=liver changes manifested by atrophy and fragmentation of muscular fibers (Figure 5B) in other sections with leukocytic infiltration (Figure 5B).

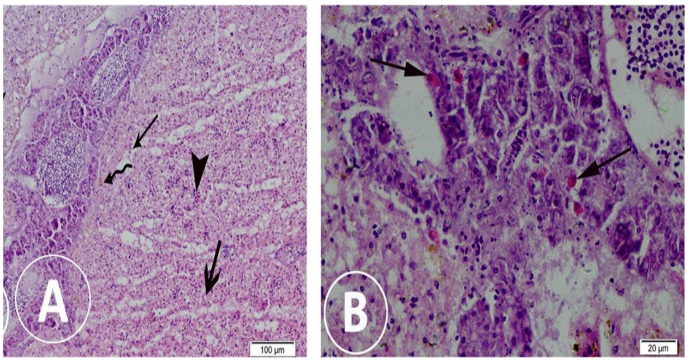

Fig.4. Photomicrographs of hepatic tissue stained with H&E Showing; (A) Hydropic degeneration (thin arrow), steatosis (thick arrow) in the hepatocytes, interstitial and focal infiltration of immunocompetent cells (arrowhead). Note the edema; perivascular and interstitial melanomacrophages (curved arrow). Scale bar 100 µm. (B) Red eosinophilic cells distributed in the hepatopancreas & around the blood vessels (arrow). Scale bar 20 µm.

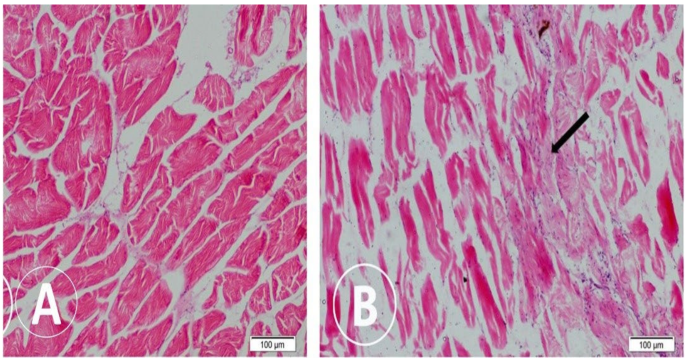

Fig. 5. Photomicrographs of muscular tissue stained with H & E Showing; (A) normal architecture of muscular fibers (B) splitting of the muscle fibers with leukocytic infiltration (arrow). Scale bar 100 µm.

Bioaccumulation factor (BAF) and Risk Assessment

Bioaccumulation factor

The findings revealed that the maximum value for Pb, Cd, Fe, and Cu in musculature was obtained during winter, while Zn was highest in autumn and winter. In terms of total BAF, the winter season had the highest values of the year, followed by autumn, spring, and summer. Except for Fe, the average values obtained by the liver were lower than those obtained by musculature. A similar seasonal influence on total BAF was observed in livers with the highest value in winter and the lowest in summer. The potential accumulation of identified heavy metals in the musculature and liver was documented in general (Table 4).

EDI stands for Estimated Daily Intake

Table 5 shows the estimated daily intakes for each metal *Tilapia zillii*. The musculature's mean EDI intakes for Pb, Cd, Fe, Zn, and Cu were 0.00021, 0.00017, 0.030014, 0.011333, and 0.000555, respectively. The mean EDI values were Zn > Fe > Cu >Pb > Cd. Furthermore, the EDI means of the liver were higher than those of the muscles (Table 4).

Hazard Index (HI) and Target Hazard Quotient (THQ)

THQ values for Pb and Cu in musculature and livers were less than unity, as were Cd in musculature and Zn in livers, accord-

Table 4. Bioaccumulation factor (BAF) and Health Risk Assessment of Heavy Metals in *Tilapia zillii* musculature and liver tissues collected from Lake Temsah.

	Tissue	Musculature					Liver				
	Season	Winter	Spring	Summer	Autumn	Average	Winter	Spring	Summer	Autumn	Average
Bioaccumulation factor (BAF)	Pb	2.54	0.16	0.52	0.78	0.41	0.06	0.03	0.03	0.08	0.04
	Cd	4.11	1.33	0.32	1.46	0.96	0.01	0.13	0.09	0.01	0.07
	Fe	4.44	3.41	2.05	1.62	2.92	14.91	13.07	6.79	7.60	10.27
	Zn	6.58	2.46	2.17	8.94	4.22	2.14	2.47	2.96	4.09	2.82
	Cu	1.25	0.23	0.23	1.24	0.45	0.18	0.34	0.37	0.21	0.30
	Total	18.91	7.60	5.29	14.04	8.96	17.30	16.05	10.24	11.99	13.51
	Tissue	Musculature					Liver				
	Risk	Estimated daily intake of metals (EDI) (mg person-1 day-1)	Tolerable daily intake TDI (mg/day/perso)	Upper tolerable daily intake UTDI (mg/day/person)	Target Hazard Quotient (THQ)	Target carcinogenic risk (TCR)	Estimated daily intake of metals (EDI) (mg person-1 day-1)	Tolerable daily intake TDI (mg/day/person)	Upper tolerable daily intake UTDI (mg/day/perso)	Target Hazard Quotient (THQ)	Target carcinogenic risk (TCR)
Health Risk Assessment	Pb	0.00	0	0.24	0.14	7.94E-7	0.18	0	0.24	0.06	9.81E-7
	Cd	0.00	0	0.64	0.45	1.04E-6	3.56	0	0.64	1.34	1.21E-6
	Fe	0.03	20.5	45	52.34	N/A	0.92	20.5	45	69.19	N/A
	Zn	0.01	8	40	1.61	N/A	0.39	8	40	0.89	N/A
	Cu	0.00			0.00	N/A	0.06			0.01	N/A
	Risk total	0.04	N/A	N/A	Hazard Index (HI): 54.54173	1.83E-6	5.11	N/A	N/A	Hazard Index (HI): 71.49426	2.19E-6

1> BAF > 100, potential accumulation; 100> BAF> 1000, significant accumulation; BAF > 1000, hazardous accumulation. Whether the THQ < 1, it is usually assumed to be secure for the risk of noncarcinogenic effects; if THQ > 1, it is assumed that there is a greater likelihood of noncarcinogenic effects as the value rises.

ing to the current investigation. Fe, on the other hand, had the greatest THQ values in both tissues. Based on THQ and single metal consumption, fish musculature was safe for Pb, Cd, and Cu (THQ< 1) and might represent potential risks for Fe and Zn (THQ > 1). However, the Hazard Index (HI) of both muscular and liver tissues was greater than one (54.54173 and 71.49426, respectively) (Table 4).

TCR (Target Cancer Risk)

TCR results for Pb and Cd in *Tilapia zillii* were 1.83E-06 and 2.19E-06 for musculature and livers, respectively (Table 4).

DISCUSSION

The study revealed that Cu, Pb, and Cd concentrations are closely associated with temperature, which might be explained by Zhao *et al*. (2013) which indicated that when the temperature rises, heavy metal ions in sediments relocate to lake water, resulting in a reduction in concentration. The mean concentrations of heavy metals in water samples were higher in the summer than in the winter, which is consistent with the findings of El-Serehy *et al*. (2012). Heavy metal levels are often observed during hot seasons, especially summer, due to higher evaporation rates, lower water levels, and metal release from bottom sediment and degradable organic matter due to microbial activity and high temperatures (Abd El-Aal *et al*., 2020). Heavy metal concentrations (Fe, Zn, Cu, Pb, Cd) in Lake Temsah water were found to be in the sequence Fe > Zn > Cu > pb > cd, which agrees with Abdel-Shafy *et al*. (1998). The study discovered that Fe and Zn were the most prevalent metals in Lake Temsah water samples, which is related to their extensive spread in the environment and natural and human processes that result in metal transportation via the atmosphere, water, and soil (Fatoki and Awofolu, 2003).

The study used the pollution index (PI) to assess the toxicity of trace metals in water samples, revealing varying levels of pollution for aquatic life in Temsah Lake. According to EPA (2021), the average concentration of heavy metals in water samples exceeded the permissible limits in Pb and Cd (0.0025 mg/L), Fe (1 mg/L), Zn (0.1 mg/L), and Cu (0.013 mg/L). According to ECS (1994), Cd and Zn were lied within the permissible limits (0.01 and 5 mg/L, respectively), but Pb, Fe, and Cu exceeded the permissible limits (0.05 mg/L Pb, 1mg/L for Fe and Cu).

The study reveals that Fe and Zn are the most abundant elements in *Tilapia zillii* tissues across all seasons, possibly due to their widespread dispersion in the environment. Both Fe and Zn in muscle tissue are linked to fish length (Karadede *et al*., 2004). Iron, a trace metal essential for fish growth, can be toxic in higher amounts to living organisms (Slaninova *et al*., 2014). Human activities such as industrial operations and waste material burning are increasing zinc pollution in the environment. The average concentration of the analyzed heavy metals in musculature, and liver samples of *Tilapia zillii* was within the permissible limits established by E.O.S.Q.C. (2010) and WHO (1992) (0.5 and 0.1 mg/ Kg, respectively) with Cd levels exceeding permissible limits of WHO (1992), E.O.S.Q.C. (2010) and FAO/WHO (2011) (0.05mg/kg). The concentration of Fe, Zn, and Cu remained within E.O.S.Q.C. (2010) 's permissible limits (30, 40 and 20 mg/kg) and FAO (1992) (30, 50 and 30 mg/kg).

The study found that the residues of heavy elements in fish in a lake were higher than their concentration in the waters, which is consistent with Abdel-Khalek *et al*. (2020), which suggested that aquatic organisms accumulate metals at higher concentrations than in water or sediment. Summertime concentrations of heavy metals in fish tissues were most outstanding in the lake, due to enhanced biological and physiological processes, food conversion rates, and drainage water outflow. Also, Authman (2008) identified seasonal changes in metal residues in fish organs due to fluctuations in drainage water flow into the canal. Metal bioavailability may also be altered by the physiological activity of fish over various seasons.

According to Mahomoud *et al*. (2011) *Tilapia zillii* females in Temsah lake have a two-year lifetime, while males have a four-year lifespan, with total lengths ranging from 7 to 16 cm and 8 to 21 cm, respectively. Consequently, the fish in this study varied in age from 2 to 4 years, and their length was highly associated negatively with Pb concentrations in both muscle and livers, as well as Cd concentrations in water and livers; on the other hand, length was directly associated with musculature Fe, Zn, and Cu concentrations. The size-specific metabolic rate of organisms influences trace metal buildup in aquatic organisms. Some metals are thought to be under homeostatic control, which means their concentrations do not increase with age or growth (Farkas *et al*., 2003; Marijić and Raspor, 2006). The positive link between particular metals and sizes might be attributed to a lack of homeostasis capacity in *Tilapia zillii* owing to prolonged metal exposure, resulting in bioaccumulation (Evans *et al*., 1993). This assumption is backed further by the fact that lipid as a proportion of body weight is normally lower in younger fish, drops during spawning, and peaks at the conclusion of the main consuming phase. The concentrations of heavy metals in fish muscle vary substantially (Weatherly and Gıll, 1987). The amounts of heavy metals in fish muscle vary substantially depending not only on fish size and age, but also on fish condition (Authman, 2008).

Moreover, Water temperature significantly influences fish metabolic rate and energetic cost, affecting nutrient digestibility and gut transit rate (Aas *et al*., 2021). The optimal temperature for tilapia growth ranges from 29°C to 31°C, with growth declining with decreasing temperatures. The lethal minimum temperature for most tilapia species is 10°C or 11°C, while 37°C to 38°C stress and diseases attack most (Nehemia *et al*., 2012). Herein, water temperature is negatively correlated with fish weight and length, making it crucial for maximizing growth, reducing feed waste, and minimizing the negative impacts of suboptimal temperatures on fish health and development.

Several tissues and organs of various fish were repeatedly used as biomarker of fish contamination due to their specific characteristics. The liver & fish musculature had important roles in life of fish. Some organs such as liver has metabolic pathways for eliminating the contaminants, researching of histopathological modifications was average easy and was largely used as biomarkers in various observations on the effects of pollutants on environmental health including on fish (Khoshnood, 2017). In this study, the hepatic tissue revealed hydrobic degeneration, fatty changes and leukocytic infiltration. Likewise, Perivascular proliferation of melanomacrophages and red eosinophilic cells were observed. Many authors recorded that exposure of the fishes to pollution by several metals increases the hepatic lesions like degeneration and leukocytic infiltration (Abdel-Moneim *et al*., 2012; Katalay *et al*., 2016; Shahid *et al*., 2020).

The evident increase in the melanomacrophages through the liver tissue was in coincidence with Steinel and Bolnick (2017) and Stosik *et al*. (2019) who reported that melanomacrophages were extremely pigmented phagocytes that were like the mammalian germinal center which might play a role in the humoral adaptive immune response. The melanin increases in melanomacrophages corresponded with the rise of ERα gene expression and reduction of testosterone concentration in goldfish after exposure to nonylphenol (Ardeshir *et al*., 2022). High melanomacrophages centers intensities might be associated to pollutant stages and parasitic diseases (Carreras-Colom *et al*., 2022).

The study investigated *Tilapia zillii* consumption in Temsah Lake as well as exposure to heavy metals (Pb, Cd, Fe, Zn, and Cu) in the lake water. The results indicated that over the research seasons, the accumulation of detected heavy metals in the musculature and liver was documented (BCF <100), with a Health Impact Ratio (HI 1) suggesting that these non-carcinogenic pollutants may not have detrimental health impacts. The allowable value for THQ according to the USEPA (2004) is <1. During the study seasons, the THQ and HI for all heavy metals in Temsah

Lake were less than one. This means that there is no non-carcinogenic health risk from consuming *Tilapia zillii*, both individually and collectively, indicating that this fish is safe to eat. Ingestion of these metals collectively through musculature and liver eating, on the other hand, poses a non-carcinogenic health concern. The US Environmental Protection Agency considers acceptable for regulatory purposes a cancer risk in the range of 1 $X10^{-6}$ to 1 $X10^{-4}$. Herein, TCR results for Pb and Cd in *Tilapia zillii* were within the permissible range (10^{-4} to 10^{-6}).

CONCLUSION

Generally, Fe and Zn are the most prevalent metals in Lake Temsah water as well as in Tilapia Zilli tissue samples. Summer has the highest integrated biomarker response (IBRv2) scores of the heavy metals in water, while autumn has the highest one in musculature and livers among the different seasons. Additionally, the highest sum of IBRv2 of the highest heavy metals content in musculature followed by livers and finally water samples. The accumulation of the detected heavy metals in the musculature and liver indicating that this fish is safe to eat, but it poses a non-carcinogenic health concern.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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