



Human Health Risks Due to Fish Consumption from Nile River at Beni-Suef-Governorate- Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. Author FM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HFN and ASH managed the analyses of the study. Author HMM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study aimed to determine the growth indices of the Nile Tilapia, detected levels of Cu, Pb, Zn, and Cr in muscles of Tilapia and expected the potential adverse human health risks according to USEPA methodologies.

Study Design: The design of the study depended on the determination of the impact of the drainage waste water on the fish quality, ecosystem health and human health.

Place and Duration of the Study: the study investigated five sampling sites, two sites in the Torrent drainage channel and three sites on the Nile River during winter and summer seasons of 2014-2015 at Beni-Suef governorate in Egypt.

Methodology: Fish tissue samples were dried at 105°C for 6 hours, burned in a muffle furnace for 6 hours at 550°C, acid-digested, and diluted with deionized water to known volume using the dry-ashing procedure. All the digested samples were analyzed by flame atomic absorption spectrophotometer (Perkin-Elmer, Model 2380). Analytical blanks were run in the same way as the

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samples, and standard solutions were prepared in the same acid matrix.

Results: Metals were found to accumulate in fish muscles at the following order Zn> Cu> Cr> Pb. Levels of Zn and Pb exceeded the permissible limits of the world health organization (WHO) especially during summer season. The calculated hazard quotients for all the detected metals did not pose unacceptable risks at both consumption rates, but the excessive and continuous consumption of fish from the current sampling sites could induce cancer for habitual consumers.

Conclusion: The present study could be considered as a beginning for further investigations for the health status of Nile River aquatic ecosystems and human populations feeding on fish produced from the study area.

Keywords: Tilapia fish; Nile river; risk assessment.

1. INTRODUCTION

Metal Pollution of world aquatic ecosystems has increased dramatically during the last few years [1-3]. The whole course of the Nile receives agricultural, domestic and industrial waste water from both pointed and non-pointed polluted sources. Such pollutants may explain the Nile water quality deterioration in Egypt [4-8]. Metals monitoring for both essential and non-essential elements in aquatic ecosystems become crucial because of their toxicity, non-degradable nature, tendency to accumulate in different biota, and their transmission throughout the food chain and biomagnification in high trophic level organisms [3,9,10,11].

Humans are exposed to metals via several routes such as direct ingestion of contaminated water or food, dermal contact, and inhalation of fume and particles [12,13]. Continuous exposure of living organisms and human beings to metals and other toxic substances may cause the decline of humans' health and aquatic ecosystem status [14,15]. Risk assessment aims to quantify the potential environmental hazards to human health into a numerical value [8,14]. The selection of a fish as a model for toxic risk assessment is because fish provide a model of exposure that is relevant to natural settings; also the safety of the aquatic products has a great concern recently [16-18]. Tilapia was identified as a potential fish model for the present study to determine the probability of inducing adverse human health effects because of its high production in the Middle East and North Africa [19], its large tissue mass, its low price in relative to other fish models, in addition to the wide acceptance of the Egyptian consumers to tilapia [7].

Several studies evaluated the concentration of metals in fish tissues in Egypt [20-23]. Based on literature review there are no studies referring to

the level of metals in muscle of tilapia collected from the Torrent drainage channel that was designed in 2007 at the eastern side of the Nile at Beni-Suef governorate and nearby Nile River sampling sites. The Torrent drainage channel receives waste water from several sources, such as the agricultural waste water, poultry and fish farms wastes, the rubbish dump leachates, and the diffused industrial and domestic waste water [24,25]. The scarcity of studies related to the human health risk assessment for fish consumption in Egypt in general and the absence of results related to the sampling sites in the eastern side of the Nile necessitated the present study. The current study aimed to (i) determine the spatial and seasonal variations of the growth indices of the Nile Tilapia, (ii) detect levels of Cu, Pb, Zn, and Cr in muscles of Tilapia and (iii) evaluate the contribution of the selected metals to the expected adverse human health risks by calculating the average daily exposure dose (ADD), hazard quotient (HQ), hazard index (HI), and cancer risk (CR) according to USEPA methodologies.

2. MATERIALS AND METHODS

2.1 The Study Area

As seen in Fig. 1, the study area was subdivided into five sampling sites (S_1 , S_2 , S_3 , S_4 and S_5), where fish (*Oreochromis niloticus*) was exposed to different metal levels and different environmental conditions.

S_1 : represented the upstream point in the Nile approximately 2.5 km before the Torrent drainage channel destination at the Nile River, it was expected to be exposed to less contamination and represented the reference site. It is located at global positioning system (GPS) coordinates of N 29°03'.726" and E 31°31'.0224".

S₂ and S₃: represented two points in the Torrent drainage channel which its entire length 2.2 km, where there are different sites for waste water discharge into it. They are located at global positioning system (GPS) coordinates of N 29°023'.1876`` and E 31°426'.8464`` for S₂; and N 29°038'.1852`` and E 31°359'.7564`` for S₃.

S₄: represented the middle stream point at the Nile where the drainage water from the Torrent drainage channel was mixed with the Nile River water. This site was exposed to different non-pointed sources of contamination through diffusion of industrial, agricultural and domestic waste water, where there is no sewer system around. It is located at global positioning system (GPS) coordinates of N 29°055'.2744`` and E 31°327'.9828``.

S₅: represented the downstream point at the Nile that is located after approximately 2.1 km from the Torrent drainage channel end at the Nile. It is located at global positioning system (GPS) coordinates of N 29°123'.2176`` and E 31°341'.6844``.

2.2. Samples Collection

Fish specimens were collected from the five sampling sites during winter season (December, January and February) and summer season (June, July and August) from 2014 to 2015. A total number of 120 *Oreochromis niloticus* were collected, where 12 fish specimen were caught from every site for each season (4 fish specimen/month). Fish were caught by the local fishermen who used local traps, webs and anglers for catching fish.

2.3 The Condition Factor (CF)

The condition factor was calculated according to the equation (1) [26,27]:

$$CF = \frac{W \times 100}{L^3} \quad (1)$$

Where,

W is the weight of the total fish (gm), and
L is the total length of the fish (cm).

2.4 Samples Preparation and Laboratory Analysis

Oreochromis niloticus specimens were freshly dissected and their dorsal muscles were

sampled, mixed, homogenized, and stored at clean polyethylene vials at -20°C until their analysis [28]. All glassware was acid-washed, rinsed in deionized water, and air-dried for 12 hours prior to usage. Fish tissue samples were dried at 105°C for 6 hours, burned in a muffle furnace for 6 hours at 550°C, acid-digested, and diluted with deionized water to known volume using the dry-ashing procedure according to Hseu [29]. All the digested samples were analyzed by flame atomic absorption spectrophotometer (Perkin-Elmer, Model 2380) according to APHA [30]. All used reagents were analytical grade (Merck, Germany). Analytical blanks were run in the same way as the samples, and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for instrument calibration were prepared on the basis of mono-element certified reference solution inductively coupled plasma standard (Merck). Standard reference material (National Institute of Standards and Technology (NIST, USA) was used to validate analysis, and the metals recoveries were between 90 and 110 %.

2.5 Metal Pollution Index (MPI)

MPI was calculated to indicate the total metal load in fish muscles of collected specimens by using equation (2) [31,32]

$$MPI = (M_1 \times M_2 \times M_3 \times \dots \times M_n)^{1/n} \quad (2)$$

Where,

M_n is the concentration of metal n (mg/kg dry wt.) in the fish muscles.

2.6. Bioaccumulation Factor (BAF)

BAF was calculated by equation (3).

$$BAF = \frac{C_f}{C_w} \quad (3)$$

Where,

C_f is the metal concentration in fish muscles (mg/kg) and
C_w is the metal concentration in water (mg/l), metals concentrations in water have been taken from the results reported by Mahmoud et al. [24].

2.7 Health Risk Assessment

The level of exposure to different metals through ingestion of fish muscles is estimated by the equation (4) [33,34]:

$$ADD = \frac{C \times IR \times EF \times ED}{BW \times AT} \quad (4)$$

Where,

ADD is the average daily exposure dose (mg/kg-day) through ingestion of metal contaminated fish muscles, C is the concentration of the measured metal in the fish muscles (mg/kg dry wt.), IR is the ingestion rate of fish muscles per day (0.0312 kg/day for normal adult, and 0.1424 kg/day for habitual or subsistent fish consumers), EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), BW is the average body weight (70 kg for normal adult), and AT is the average life time (365 days x 70 years).

The risk assessment of non-carcinogenic adverse effects was estimated by calculating the hazard quotient (HQ) from the intake of metal contaminated fish muscles, and it is expressed by the ratio of ADD to the reference dose RfD of each metal by the equation (5), when $HQ \geq 1$ the consumers may be exposed to non- carcinogenic adverse health effects [33,34]:

$$HQ = \frac{ADD}{RfD} \quad (5)$$

Where,

oral RfD (mg/kg-day) for Cu is (0.04), Pb is (0.0035), Zn is (0.3), and Cr is (0.003) [35,36].

Hazard index (HI) is introduced to evaluate the total potential for non-carcinogenic effects posed by more than one pathway, which was the sum of the HQs from all applicable pathway equations (6). $HI \geq 1$ showed a potential for adverse effects on human health:

$$HI = \sum_{i=1}^n HQ \quad (6)$$

Cancer risk (CR) was also evaluated by using equation (7) [33,37]. The range of carcinogenic risks acceptable or tolerable by USEPA [36] is $1.0E-06$.

$$CR = \frac{ADD}{SF} \quad (7)$$

Where, SF is the cancer slope factor (mg/kg-day), the SF for Pb is (0.0085) and for Cr is (0.5) the slope factor for Cu and Zn is not available [35,36].

2.8 Statistical Analysis

Statistical tests were performed by using IBM SPSS statistical package version 22. One way analysis of variances (ANOVA) was used to determine the spatial variations followed by the Tukey test for the multiple comparisons between the five sampling sites. Student t-test was used



Fig. 1. The five sampling sites in the eastern side of Beni-Suef governorate, Egypt

to determine the seasonal variations during winter and summer seasons. Pearson correlation analysis was used to evaluate the relationships between fish body indices and metals accumulation. All the results were expressed as mean and standard deviation ($M \pm SD$). $P < 0.05$ was the accepted significance level.

3. RESULTS AND DISCUSSION

In the present study 120 *Oreochromis niloticus* were collected from five sampling sites (S_1 , as a reference study site; S_2 and S_3 representing the Torrent drainage channel; S_4 where the drainage mixes with the Nile River and S_5 that lies downstream of S_4) to evaluate some growth parameters and metal levels of the studied fish. Collected data of metal concentration during winter and summer seasons were used to evaluate the hazard quotient (HQ), the hazard index (HI) and the cancer risk (CR) as indicators of human health risks and adverse health effects associated with fish consumption.

3.1 The Body Indices

The body indices assessment is one of the methods that is used to provide indication for the degree of fitness and pollution load in aquatic organisms and could be used as biomarkers for adverse effect on fish [32]. Total weight (W), total length (L), body length (BL) and body height (H) of Tilapia fish collected from S_3 have a significant increase at $P < 0.01$ when compared to their relevant values of fish collected from the other sampling sites during winter season as seen in Table 1. The condition factor (CF) values of fish collected from S_2 show significant higher values at $P < 0.05$ when compared to the other study sites during summer season. Seasonally, significant higher values are noticed for W, L, BL, and H of fish collected from the reference site (S_1) during winter season when compared to their relevant values of fish collected during the summer season. Similar seasonal variation is noticed for L, BL, and H of fish collected from S_3 during winter season that show significant higher values at $P < 0.05$ when compared to those of fish collected during summer season. CF values of fish collected from S_2 and S_3 during summer season show a seasonal significant increase at $P < 0.05$ when compared to their relevant values of fish collected during the winter season. The significant differences in the body indices of fish collected from the Torrent drainage channel could be explained by the exposure of fish to higher residual levels of livestock food stuff from

the surrounding poultry farms that is considered as a source for the fish nutrition [24,25].

3.2 Metals concentration in Fish Muscles

The concentrations of metals in the muscles of the tilapia and metal pollution index (MPI) during winter and summer seasons are summarized in table 2. Cu, Pb, Zn and Cr were detected in all the samples. Mean concentrations of Cu, Pb, Zn and Cr in the muscles of the tilapia during the two seasons followed the order: Zn > Cu > Cr > Pb except at S_1 which reveals the following order Zn > Cr > Pb > Cu during summer season.

The average Cu values range from 0.292 to 5.823 mg/kg in the fish muscles collected from the current sampling sites during winter and summer seasons. The highest mean of Cu (5.823 ± 0.77 mg/kg) is recorded at S_4 during summer season. Spatially, Cu concentrations in fish muscles collected from S_4 show a significant increase at $P < 0.05$ when compared with their relevant values at the other sites during summer season. At seasonal scale, Cu reveals a significant increase in S_3 at $P < 0.005$ and in S_4 at $P < 0.05$ during summer season. Cu concentrations in the current sampling sites during the two seasons are found to be below the permissible limits (10 mg/day) [38, 39]. Cu range in the present study is found to be almost similar to Cu level (3.9 mg/kg) that was reported by Osman and Kloas [20] along the whole course of the Nile river at Egypt for *Clarias gariepinus* muscles and less than Cu average (20 mg/kg) that was reported by Lasheen et al. [1] in the agricultural drains at the greater Cairo for *Oreochromis niloticus* muscles. The accumulation of copper in fish muscles at the current sampling sites probably due to the sewage and agricultural waste water diffusion from the surrounding area, it may be due to Cu addition as a nutrient in fertilizers and animal feeds, or it could be due to the extensive application of insecticides [40]. Cu^{2+} and the Cu hydroxyl soluble ions are found to be the more toxic forms of Cu for fish [41]. Cu is associated with the total cholesterol which induce cardiovascular disease risk factors in children and adolescents [42].

The average Pb values range from 0.071 to 1.082 mg/kg in the fish muscles collected from the present sampling sites. The highest mean of Pb (1.082 ± 0.077 mg/kg) is recorded at S_4 during summer season. At spatial scale, Pb shows a significant increase at $P < 0.05$ in S_5

when compared with its relevant value in S_1 during winter season. Seasonally, Pb increase significantly in S_1 at $P < 0.05$, S_3 at $P < 0.01$ and S_4 at $P < 0.05$. The fish muscles collected from the current sampling sites during the two seasons show Pb concentrations above the permissible limits (0.025 mg/day) of WHO [38]. Pb range in the present study is within the range reported by Abdel-Mohsen and Mahmoud [22] in the Nile River at Egypt of (0.25 - 1.05 mg/kg) and higher than Pb range reported by Bekheit [43] in the White Nile at Sudan of (0.247 - 0.307 mg/kg). Pb accumulation in fish muscles in the current sampling sites could be attributed to air-borne lead due to the fuels combustion. Moreover, the excessive application of different types of fertilizers especially in the desert reclaimed lands may be another source of Pb contamination [44]. Pb is a neurotoxic to fish; it may cause persistent behavioral defects [45].

The average Zn values range from 10.047 to 42.034 mg/kg in fish muscles collected during the current study. The highest mean of Zn (42.034 ± 0.71 mg/kg) is noticed at S_4 during summer season. Spatially, Zn shows a significant increase at $P < 0.05$ in S_4 when compared with their relevant values at the other sites during summer season. Seasonally, Zn shows a significant increase in S_1 at $P < 0.05$, S_2 at $P < 0.05$ and S_4 at $P < 0.01$ during summer season. All investigated Zn concentrations in fish muscles during summer season show higher level over the permissible limits (10-20 mg/day; 10-30

mg/day) of WHO [38] and FAO/WHO [39] respectively. During winter season, all Zn concentrations in fish muscles in the five sampling sites are below the permissible limits. The observed Zn range is higher than its relevant values (25.4 – 35.7 mg/kg) reported by Omar et al. [32] in the Nile River at the greater Cairo in *Oreochromis niloticus* muscles, and it is less than its concentration range (13.51 – 69.8 mg/kg) reported by Nacéra et al. [46] in *Cyprinus carpio* muscles from Sidi Abdeli dam at Algeria. Zn concentrations in the fish muscles in the current study may be attributed to fertilizers and pesticides application in the around agricultural lands [47]. Fish take up Zn directly from water, especially by mucous and gills, high concentration of Zn can cause growth retardation, respiratory and cardiac changes, inhibition of spawning, and a multitude additional detrimental effects which dramatically affect the fish survival [45].

The average Cr values range from 0.162 to 1.451 mg/kg in the fish muscles collected from the present sampling sites. The highest mean of Cr (1.451 ± 0.35 mg/kg) is recorded in S_3 during summer season. Cr reveals a seasonal significant increase in S_1 at $P < 0.01$, S_3 at $P < 0.05$ and S_4 at $P < 0.0001$ during summer season. All Cr concentrations in the five sampling sites during the two seasons are below FAO/WHO [39] permissible limits (10 mg/day). Cr range in the present study is higher than Cr concentration range (0.009 – 0.05 mg/kg)

Table 1. Average values \pm standard deviation of total Weight (W), Total Length (L), Body Length (BL), Body Height (H), and Condition Factor (CF) of Nile Tilapia fish collected from the sampling sites (S_1 to S_5) during winter and summer seasons

	S_1	S_2	S_3	S_4	S_5
W (gm)					
W	72.5 ± 15.6	63.1 ± 7.5	103.8 ± 22.8	71.3 ± 14.8	61.7 ± 21
S	42.7 ± 11.3	66.7 ± 8.2	72.5 ± 33.7	60.8 ± 27.5	54.8 ± 17.2
L (cm)					
W	15 ± 1.2	14.8 ± 0.65	17.3 ± 0.92	14.9 ± 1.4	14.1 ± 1.5
S	12.8 ± 1.1	14.1 ± 1.1	14.6 ± 2.7	14.2 ± 2.2	13.8 ± 1.9
BL (cm)					
W	12.3 ± 0.99	12.3 ± 0.6	14.4 ± 0.68	12.2 ± 1.1	11.7 ± 1.3
S	10.5 ± 0.97	11.5 ± 1	11.97 ± 2.3	11.5 ± 1.6	11.3 ± 1.4
H (cm)					
W	5.6 ± 0.6	4.8 ± 0.4	5.7 ± 0.6	5.4 ± 0.3	5.2 ± 0.9
S	4.2 ± 0.5	5.1 ± 0.4	4.9 ± 0.4	5.1 ± 1.1	4.8 ± 0.8
CF*					
W	2.12 ± 0.12	1.99 ± 0.29	1.97 ± 0.16	2.14 ± 0.23	2.12 ± 0.19
S	1.99 ± 0.18	2.41 ± 0.34	2.29 ± 0.31	2.05 ± 0.31	2.06 ± 0.26

*. CF (unit less)

reported by Ibrahim and Omar [21] in the Nile River at Assiut governorate in *Clarias gariepinus* muscles, higher than Cr concentration range (0.31 – 0.39 mg/kg) reported by Akan et al. [48] in River Benue at Nigeria in *Tilapia zilli* muscles and higher than Cr concentration (0.04 mg/kg) reported by Squadrone et al. [49] in the Po river at Italy in *Silurus glanis* muscles. The toxicity and associated health effects of Cr depend on its chemical speciation, where Cr^{3+} is an essential nutrient metal, while Cr^{6+} penetrates passively the gill membranes of fish and concentrates at higher levels in various organs and tissues [45, 50]. Many serious problems in fish as swimming deficits, feeding disruption, fin ray erosion, ulcerations, and death may appear due to Cr exposure [22]. Cr levels detected in the current study may be a result of the intensive use of fertilizers and manure of the livestock in the surrounding agricultural areas [51].

Metal pollution index (MPI) was used to simplify the metal load in fish muscles in one value for each site during each season. The sequence of MPI for the sampling sites follows the order: $S_5 > S_3 > S_2 > S_4 > S_1$ during winter season and $S_4 > S_3 > S_2 > S_5 > S_1$ during summer season as shown in table 2. MPI clarifies that the most contaminated fish muscles were collected from S_5 during winter season and S_4 during summer season, This could be explained by the additive effect of the wastes received from the Torrent drainage channel (S_2 and S_3) and other contaminated non-pointed sources as the fish ponds in the Nile or the sewage discharge from the surrounding human population on the water quality of the upstream site S_1 before it reaches S_4 and S_5 . Mahmoud et al. [24], [25] reported metals concentrations in the same five sampling sites during winter and summer seasons, and they found that the Torrent drainage channel (S_2 and S_3) showed higher values of salinity than the Nile River sites (S_4 and S_5). Salinity and total dissolved solids in the Torrent drainage channel may affect the metals bioavailability for fish and reduce its accumulation in their tissues. Also cations are assumed to reduce toxicity in fish by competing with toxic metal ions for binding sites on gills or other biological surfaces [52].

3.3 Bioaccumulation Factor (BAF)

BAF was calculated as the ratio between the metal concentrations in fish muscles to the metal concentrations in water. The calculated BAF results are represented in table 3. The results

show that all metals have $BAF > 1$ during winter and summer seasons, meaning that all metals are bio accumulated in the fish muscles. The highest BAF values are obtained for Cu (85.632) at S_4 , for Pb (23.773) at S_2 , for Zn (1161.914) at S_1 , and for Cr (131.909) at S_3 during summer season. All BAF values in the sampling sites during summer season show higher values than those recorded values in winter season except for Cr at S_5 and for Cu at S_1 , S_3 and S_5 . Those findings are matching with the metals accumulation increase in fish muscles at the high mountain lakes and arctic lakes due to the climate change and temperature increase [53]. Ibrahim and Omar [21] reported that the levels of metals in muscles tissues from the Nile at Assiut governorate increased significantly during summer season, due to the increase of temperature and fish metabolism. Yancheva et al. [54] in Topolnitsa reservoir of Bulgaria found that the increase in temperature lead to increase in toxicant accumulation in fish tissues, as it accelerates the cross of it through the biological membranes.

3.4 Correlation Analysis

The Pearson correlation coefficients between metals concentrations in the fish muscles and the body indices of the sampled fish during winter and summer seasons are shown in table 4. During winter season, there are positive correlations between W/L ($r = 0.982$, $P < 0.001$), BL/W ($r = 0.972$, $P < 0.01$), L/BL ($r = 0.996$, $P < 0.01$), Cu/Pb ($r = 0.910$, $P < 0.01$), Cu/Zn ($r = 0.934$, $P < 0.01$), Cu/Cr ($r = 0.918$, $P < 0.01$) and Pb/Cr ($r = 0.962$, $P < 0.01$). During summer season, there are positive correlations between W/L ($r = 0.960$, $P < 0.01$), BL/W ($r = 0.976$, $P < 0.01$), L/BL ($r = 0.994$, $P < 0.01$), H/Cu ($r = 0.898$, $P < 0.05$), and Cu/Pb ($r = 0.969$, $P < 0.01$). Pearson correlation coefficients indicate that there are no relations between metals bioaccumulation in the fish muscles from the current sampling sites and the fish body indices. There are other factors as temperature and metal bioavailability that may affect metals accumulations in the fish muscles. The positive correlation between W, L and BL during winter and summer seasons confirmed the relationship between growth and weight. The positive correlation between different metals accumulated in the fish muscles may indicate that they originate from the same sources and have the tendency to bio accumulate together.

Table 2. Average values (\pm SD) of selected metals and metal Pollution Index (MPI) for fish muscles samples (mg/kg dry weight) collected from different study sites (S_1 to S_5) during winter and summer seasons

	S_1	S_2	S_3	S_4	S_5
Cu					
W	0.991 \pm 0.47	1.529 \pm 0.34	1.625 \pm 0.1	1.034 \pm 1.36	2.138 \pm 0.22
S	0.292 \pm 0.018	3.798 \pm 1.65	3.934 \pm 0.066	5.823 \pm 0.77	2.032 \pm 0.13
Pb					
W	0.071 \pm 0.006	0.093 \pm 0.034	0.193 \pm 0.015	0.095 \pm 0.03	0.286 \pm 0.10
S	0.417 \pm 0.035	0.737 \pm 0.39	0.921 \pm 0.034	1.082 \pm 0.077	0.551 \pm 0.14
Zn					
W	10.047 \pm 6.78	14.575 \pm 2.47	12.601 \pm 8.11	11.472 \pm 2.87	17.403 \pm 6.34
S	40.667 \pm 3.42	34.831 \pm 0.13	30.982 \pm 2.66	42.034 \pm 0.71	35.678 \pm 2.656
Cr					
W	0.169 \pm 0.04	0.185 \pm 0.013	0.228 \pm 0.022	0.162 \pm 0.013	0.364 \pm 0.24
S	1.007 \pm 0.031	0.742 \pm 0.57	1.451 \pm 0.35	1.216 \pm 0.025	0.573 \pm 0.047
MPI					
W	0.588	0.787	0.974	0.654	1.403
S	1.494	2.916	3.572	4.236	2.187

Table 3. BAFs of metals concentrations in fish muscles samples collected from the sampling sites (S_1 to S_5) during winter and summer seasons

	S_1	S_2	S_3	S_4	S_5
Cu					
W	70.785	30.58	65.00	7.282	54.821
S	2.374	52.027	64.492	85.632	26.737
Pb					
W	5.071	1.1148	6.893	2.317	7.333
S	9.929	23.773	17.377	14.427	8.348
Zn					
W	197	51.502	69.236	10.307	457.974
S	1161.914	916.605	573.741	764.255	615.138
Cr					
W	18.777	5.968	14.25	5.226	24.267
S	62.938	14.84	131.909	28.279	16.371

3.5 Human Health Risk Assessment

The values of hazard quotient (HQ), hazard index (HI), and cancer risk (CR) are calculated for the five sampling sites for normal and habitual fish consumers during winter and summer seasons (table 5 and 6). The calculated HQs for the four metals during the two studied seasons are lower than 1.0 at the two ingestion rates. The highest HQs values are recorded at S_4 for the habitual consumers during summer season with values of 0.2961 for Cu, 0.6289 for Pb and 0.285 for Zn. The only exception is recorded for Cr which shows its highest HQ at S_3 0.9839. HI for the four metals was less than 1.0 at the five sampling sites for normal consumers during winter and summer seasons and for habitual

consumers during winter season. HI for all sampling sites are higher than 1.0 for the habitual consumers during summer season. CRs for Pb and Cr are higher than unity $1.0E-06$ in the five sampling sites for both normal and habitual consumers during winter and summer seasons. Health risk assessment for the four metals at the five sampling sites indicates that the habitual consumers may be exposed to non-carcinogenic adverse health effects due to the fish muscles intensive consumption during summer season (Table 5). CR calculations indicate that excessive consumption of fish over a long period of time from the studied sampling sites may induce carcinogenic adverse health effects for both normal and habitual fish consumers as shown in Table 6.

Table 4. Pearson correlation coefficients between weight (W), length (L), body length (BL), height (H), condition factor (CF), Cu, Pb, Zn, and Cr, winter season is below the diagonal and summer season is above the diagonal

	W	L	BL	H	CF	Cu	Pb	Zn	Cr
W	1	0.960**	0.976**	0.820	0.805	0.744	0.736	-0.689	0.381
L	0.982**	1	0.994**	0.876	0.623	0.832	0.826	-0.575	0.380
BL	0.972**	0.996**	1	0.832	0.647	0.770	0.773	-0.655	0.380
H	0.728	0.601	0.556	1	0.587	0.898*	0.787	-0.289	0.055
CF	-0.539	-0.669	-0.719	0.150	1	0.375	0.303	-0.728	0.047
Cu	-0.072	-0.090	-0.011	-0.326	-0.270	1	0.969**	-0.044	0.412
Pb	0.081	-0.009	0.046	0.026	-0.026	0.910*	1	-0.061	0.617
Zn	-0.384	-0.379	-0.303	-0.602	-0.127	0.934*	0.782	1	-0.075
Cr	-0.161	-0.346	-0.188	-0.126	0.096	0.918*	0.962**	0.848	1

. Correlation is significant at the 0.05 level (2-tailed).**. Correlation is significant at the 0.01 level (2-tailed).***Table 5. Hazard Quotients (HQs) and Hazard Indices (HIs) of fish consumption for both normal and habitual consumers from the current sampling sites (S₁ to S₅) during winter and summer seasons**

HQ		S1	S2	S3	S4	S5
Cu						
W	N	0.011	0.017	0.0181	0.0115	0.0238
	H	0.0504	0.0778	0.0826	0.0526	0.1087
S	N	0.0033	0.0423	0.0438	0.0644	0.0226
	H	0.0149	0.1932	0.2001	0.2961	0.1033
Pb						
W	N	0.00904	0.0118	0.0246	0.0121	0.0364
	H	0.04127	0.0541	0.1122	0.0552	0.1662
S	N	0.0531	0.0939	0.1173	0.1378	0.0701
	H	0.2424	0.4284	0.5353	0.6289	0.3203
Zn						
W	N	0.0149	0.0216	0.0187	0.017	0.0258
	H	0.0681	0.0988	0.0854	0.0778	0.118
S	N	0.0604	0.0517	0.0460	0.0625	0.053
	H	0.2758	0.2362	0.2101	0.285	0.2419
Cr						
W	N	0.0251	0.0275	0.0339	0.0241	0.0541
	H	0.1146	0.1254	0.1546	0.1099	0.2468
S	N	0.1496	0.1102	0.2156	0.1807	0.0851
	H	0.6828	0.5031	0.9839	0.8246	0.3885
HI						
W	N	0.06004	0.0779	0.0953	0.0647	0.1401
	H	0.27437	0.3561	0.4348	0.2955	0.6397
S	N	0.2664	0.2981	0.4227	0.3746	0.2308
	H	1.2159	1.3609	1.9294	2.0346	1.054

N, the normal fish consumers with ingestion rate (0.0312 kg/day); H, the habitual fish consumers with ingestion rate (0.1424 kg/day); W, winter season; S, summer season

Table 6. Cancer Risks (CRs) of the ingestion of Pb and Cr contaminated fish muscles for both normal and habitual consumers from the current sampling sites (S₁ to S₅) during winter and summer seasons

		S1	S2	S3	S4	S5
Pb						
W	N	3.8E-3	4.8E-3	1.0E-2	4.9E-3	1.5E-2
	H	1.6E-2	2.2E-2	4.6E-2	2.3E-2	6.8E-2
S	N	2.2E-2	3.9E-2	4.8E-2	5.7E-2	2.9E-2
	H	9.9E-2	1.8E-1	2.2E-1	2.6E-1	1.3E-1
Cr						
W	N	1.5E-4	1.6E-4	2.0E-4	1.4E-4	3.2E-4
	H	6.9E-4	7.5E-4	9.3E-4	6.6E-4	1.5E-3
S	N	8.9E-4	6.6E-4	1.3E-3	1.1E-3	5.1E-4
	H	4.1E-3	3.0E-3	5.9E-3	4.9E-3	2.3E-3

N, the normal fish consumers with ingestion rate (0.0312 kg/day); H, the habitual fish consumers with ingestion rate (0.1424 kg/day); W, winter season; S, summer season

4. CONCLUSION

The effect of different types of waste water on metal load of tilapia fish muscles through all the sampling sites during the two seasons was clear and proved by calculating MPI, BAF, HQ, and HI. At the current study, tilapia fish accumulated some metals as Pb and Zn at concentrations more than the permissible limits especially in summer season. MPI shows that the most contaminated fish found to be in S₅ during winter season and S₄ during summer season. Despite the low expected public health impacts caused by the consumption of each metal separately, metals concentration in fish muscles collectively may lead to human adverse health effects especially on the habitual consumers. People consumed fish collected from the sampling sites chronically found to be under the cancer risk. The cumulative risk of metals and their impacts gave an alarming sign for the importance of assessment surveys for different water bodies and for the current study area.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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