Bioaccumulation of Heavy Metals and Implications Associated with Consumption of the Thinlip Mullet (Liza ramada) Collected from Sites of Varying Salinity

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Authors’ contributions

This work was carried out in collaboration between both authors. Author MSB designed the study, performed the statistical analysis and wrote the protocol. Author FAEM managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

ABSTRACT

The bioaccumulation of heavy metals (Fe, Cu, Zn, Mn, Pb, Cd, Co, and Ni) were assessed in tissues (gills, liver, and muscles) of the thinlip mullet (Liza ramada) collected from three aquatic habitats varying in salinity in Egypt (freshwater, brackish, and offshore sites). In the freshwater site, metals accumulate in order of gills > liver > mussels with exception to Cu, Zn and Ni (liver > gills > mussels). In the brackish site, the order of metal accumulation for Fe, Cu, Zn, and Co were: liver > gills > mussels, and for Ni, Pb, and Mn were: gills > liver > mussels, while; Cd accumulated in order of mussels > gills > liver. In offshore site, Metals accumulate in order of: liver > gills > mussels except for Mn and Ni (gills > liver > mussels) and Cd (mussels > gills > liver). The overall metal concentrations for the three sites were ranked in the order of Fe > Mn > Zn > Cu > Pb > Cd > Co >
1. INTRODUCTION

Aquatic environments are vulnerable to pollution from urban development and in industrial waste [1]. Heavy metals from natural and anthropogenic sources pose particularly serious threats to both the environment and human health [2,3]. Heavy metals can be toxic to human health [e.g., As, Cd, Pb, Hg], whereas others are likely to be [e.g., Ni, V, Co] or essential [e.g., Cu, Zn, Fe, Mn] to human health [4,5]. However, even essential metals can also be toxic at high concentrations [6,7,8]. As heavy metals also have long persistence, toxicity to humans and other organisms, and bioaccumulation [9], heavy metals are recognised as one of the most important pollutant groups in the aquatic environment.

Heavy metals are absorbed from water by suspended sediments and then precipitate to the surface sediment that provides food and habitats for fish and other aquatic organisms. This cycle promotes the bioaccumulation of heavy metals in aquatic organisms [1,10-14]. Heavy metals can negatively affect fish species, causing low fertility and mortality [7,15,16].

Many fish that are caught for human consumption are located towards the end of the aquatic food chain and can accumulate metals from their environment; this can have an adverse effect on the health of consumers, causing chronic or acute disease [1,5,16-18].

Fish provide humans with an important source of proteins, minerals, vitamins, and polyunsaturated fatty acids, especially omega-3 [16,19,20]. In Egypt, sources of fish for human consumption are marine-derived [11.70%] or are obtained from inland capture [23.75%] or aquaculture [64.55%] [21]. The main species produced in capture fisheries are mullet [16%], catfish [7.72%] and Sardinella [3.1%]. In aquaculture, tilapia production [38%] is the highest, followed by that of mullet [16%], grass carp [11.71%] and common carp [2.38%] [22]. Given the presence of heavy metals in fish destined for human consumption, it is important to determine and assess the levels of heavy metals in such fish; this is also important because of the need to meet both nutritional and safety standards [1].

A significant part of the human diet in Egypt comprises fish, both farmed and wild. Thus, there is a need to understand the accumulation of heavy metals in species relevant to human consumption. The present study aims to explore the accumulation patterns of selected heavy metals [Fe, Mn, Zn, Cu, Pb, Cd, Co, and Ni] in three components [liver, gills, and muscles] of thinlip mullet [Liza ramada] collected from three different environments [freshwater, brackish, and offshore sites], to assess the public health risks associated with the consumption of the edible parts [muscles] of fish harvested from these areas, given the levels of heavy metals that they contain, and to determine the safe dietary intake of these metals.

Risks to human health caused by toxic metals that accumulate in fish can be assessed using various methods [23]. Carcinogenic and non-carcinogenic effects are represented by a comparison between exposure concentrations and thresholds for adverse effects [24]. The target hazard quotient [THQ] set by the US Environmental Protection Agency [25] is commonly used to evaluate potential non-carcinogenic health risks resulting from the ingestion of various metals through fish consumption [3,9,13]. This risk estimation method is widely used, and its validity and usefulness in assessing the human health risk resulting from the ingestion of heavy metals through fish consumption have been validated elsewhere [1,26-28].

Keywords: Target hazard quotient; risk assessment; estimated daily intake; aquatic; habitats; Egypt.
2. METHODOLOGY

2.1 Sampling and Analysis

The fish selected for this study was the thinlip mullet (*L. ramada*). This species accounts for a significant percentage of the fish farmed for human consumption in Egypt. It is farmed using various approaches, such as (e.g. wild-caught, aquaculture etc.). Fish samples with an average weight of 250–500 gm were obtained; 33 samples from the El-Serw freshwater farm (freshwater habitat), 12 samples from fisheries located in the northern section of Lake Manzala (a brackish habitat), and 9 samples from a Mediterranean offshore area (offshore habitat), (Fig. 1). All fish samples were brought to the laboratory on the same day. The gills, liver and muscles from each fish were oven-dried at 90°C for 24 h and homogenised using a mortar and pestle. The organs (0.2–0.5 mg dry weight) were digested in a flask overnight using nitric acid (10 ml), and the resulting solution was then placed on a hot plate for 2 h at 90°C until it went clear. Upon cooling, the digest was filtered into a 10-ml volumetric flask and made to volume using distilled and deionised water [29]. The levels of Fe, Mn, Zn, Cu, Pb, Cd, Co, and Ni were determined using an atomic absorption spectrophotometer [AAS] model [Shimadzu AA-6800] and expressed as mg/kg dry weight.

2.2 Target Hazard Quotient

THQ is defined as the ratio of the exposure level of a single metal over a specified period to a reference dose (RD) of the same metal for the same exposure period. The THQ approach assumes a level of exposure [i.e., RD] below which it is unlikely that even sensitive members of the population (pregnant, infants, those with compromised immune systems, etc) will experience adverse health effects [30,25]. If the exposure level (EF) exceeds this threshold, there is a human health risk associated with the consumption of the aquatic product (in this case *L. ramada*). The THQ is calculated using Equation 1:

\[
\text{THQ} = \frac{\text{EF} \times \text{ED} \times \text{MC} \times \text{FIR} \times 10^{-3}}{\text{RD} \times \text{ABW} \times \text{TA}}
\]

EF, ED, FIR, MC, RD, ABW, and TA are defined in Table (1). If the THQ is >1, there is a risk to the health of the exposed population from consuming the product; if the THQ value is <1, there is no risk. In the current study, exposure to many pollutants (metals) was assessed; therefore, the total THQ or hazard risk (HI) was also calculated as the arithmetic sum of the individual metal THQ values, following the method of [26,27,31]. [Equation 2]:

\[
\text{TTHQ (HI)} = \sum_{n=1}^{N} \text{THQ}_n
\]

where \(n\) is the particular heavy metal tested

![Fig. 1. Sampling sites representing a brackish habitat (northern section of Lake Manzala), offshore habitat (Mediterranean offshore area), and a freshwater habitat (El-serw freshwater farm)](image-url)
3.1 Iron [Fe]

The mean concentrations of Fe in gills were ranked according to site as follows: brackish (790.78±162.58 mg/kg\(^{-1}\) dry weight (dw)) > offshore (459.8±44.49 mg/kg\(^{-1}\) dw) > freshwater (10.85±5.88 mg/kg\(^{-1}\) dw). In liver, the mean concentrations were 26.94±21.88 mg/kg\(^{-1}\) dw, 20.21±7.65 mg/kg\(^{-1}\) dw, and 5.68±5.21 mg/kg\(^{-1}\) dw for offshore, brackish, and freshwater sites, respectively. In muscles, the highest mean concentrations were 69.17±24.15 mg/kg\(^{-1}\) dw in the offshore site followed by (65.53±10.62 mg/kg\(^{-1}\) dw) for the brackish site, and (6.37±13.99 mg/kg\(^{-1}\) dw) for the freshwater site.

3.2 Copper (Cu)

The levels of Cu in gills were sequenced as: freshwater (10.85±5.88 mg/kg\(^{-1}\) dw) > brackish (6.14±1.35 mg/kg\(^{-1}\) dw) > offshore (4.33±2.8 mg/kg\(^{-1}\) dw). The levels in liver were 26.94±21.88 mg/kg\(^{-1}\) dw, 20.21±7.65 mg/kg\(^{-1}\) dw, and 5.68±5.21 mg/kg\(^{-1}\) dw for freshwater, brackish, and offshore sites, respectively. In muscles, the mean concentrations were the highest in the freshwater site (2.9±2.8 mg/kg\(^{-1}\) dw) followed by (2.13±1.02 mg/kg\(^{-1}\) dw), and (1.47±0.32 mg/kg\(^{-1}\) dw) for, brackish and offshore sites, respectively.

3.3 Zinc (Zn)

Levels of Zn in gills were ranked as: freshwater (60.43±17.15 mg/kg\(^{-1}\) dw) > brackish (51.54±7.09 mg/kg\(^{-1}\) dw) > offshore (51.18±3.51 mg/kg\(^{-1}\) dw). In liver, metal concentration arranged as: brackish (144.84±20.83 mg/kg\(^{-1}\) dw) > offshore (126.3±28.11 mg/kg\(^{-1}\) dw) > freshwater (63.68±21.01 mg/kg\(^{-1}\) dw). The highest Zn concentration in muscles was measured in fish from the freshwater site (23.47±6.23 mg/kg\(^{-1}\) dw) followed by the offshore site (20.19±3.5 mg/kg\(^{-1}\) dw) and brackish site (16.62±3.02 mg/kg\(^{-1}\) dw).

3.4 Manganese (Mn)

The highest mean concentrations of Mn in gills (150.28±15.22 mg/kg\(^{-1}\) dw) and liver...
(17.45±11.68 mg/kg⁻¹ dw) were recorded in brackish site followed by the offshore site (14.01±35.59 mg/kg⁻¹ dw in gills and 9.15±7.27 mg/kg⁻¹ dw in liver) and freshwater site (58.56±29.99 mg/kg⁻¹ dw in gills and 8.06±6.3 mg/kg⁻¹ dw in liver). The maximum levels in muscles were measured in fish obtained from the offshore site (3.07±0.91 mg/kg⁻¹ dw) followed by the brackish site (3.04±1.2 mg/kg⁻¹ dw) and freshwater site (2.06±0.86 mg/kg⁻¹ dw).

3.5 Lead (Pb)

Lead concentrations in gills in the three sites were ranked as: freshwater (22.15±15.72 mg/kg⁻¹ dw) > offshore (10.82±16.37 mg/kg⁻¹ dw) > brackish (8.99±12.87 mg/kg⁻¹ dw). The highest Pb levels in liver were recorded in fish from the offshore site (17.58±15.29 mg/kg⁻¹ dw) followed by the brackish site (8.78±7.18 mg/kg⁻¹ dw) and freshwater site (4.92±6.3 mg/kg⁻¹ dw). However, in muscles, the highest Pb levels were measured in fish from the offshore site (9.39±10.06 mg/kg⁻¹ dw) followed by the freshwater site (4.97±6.17 mg/kg⁻¹ dw) and brackish site (4.55±6.19 mg/kg⁻¹ dw).

3.6 Cadmium (Cd)

Concentrations of Cd in fish from the offshore site were ranked as follow: muscles (0.82±1.23 mg/kg⁻¹ dw) > gills (0.47±0.81 mg/kg⁻¹ dw) > liver (0.27±0.25 mg/kg⁻¹ dw). The highest levels of Cd in the brackish site, were detected in muscles (0.05±0.1 mg/kg⁻¹ dw) followed by liver (0.04±0.05 mg/kg⁻¹ dw) and gills (0.03±0.05 mg/kg⁻¹ dw). Cd was not detected in any part of fish collected from the freshwater site.

3.7 Cobalt (Co)

While the levels of Co measured in fish gills were 1.88±2.04 mg/kg⁻¹ dw, 1.71±0.71 mg/kg⁻¹ dw, and 0.29±0.97 mg/kg⁻¹ dw for brackish, offshore, and freshwater sites, respectively. The levels in liver were 22.56±43.24 mg/kg⁻¹ dw and 0.29±0.37 mg/kg⁻¹ dw for offshore and brackish sites, respectively. However, this metal was not detected in the fish liver and muscles obtained from the freshwater site.

3.8 Nickel (Ni)

Nickel levels in gills were ranked as: offshore (1.9±2.19 mg/kg⁻¹ dw) > brackish (1.6±2.91 mg/kg⁻¹ dw) > freshwater (1.09±2.46 mg/kg⁻¹ dw), and for liver as: offshore (1.8±1.64 mg/kg⁻¹ dw) > freshwater (1.14±2.93 mg/kg⁻¹ dw) > brackish (0.6±1.2 mg/kg⁻¹ dw). The mean concentration of Ni in the fish muscles obtained from the offshore site was (1.6±1.17 mg/kg⁻¹ dw) however, it was not detected in the other sites.

Significant differences were detected between sites for Cu and Co, and between fish organs for Pb, Cd, Co, and Ni. exerts strong positive correlations between Fe and Zn (P<0.001), Fe and Pb (P<0.001), Fe and Mn (P<0.001), Mn and Ni (P<0.001), and Cd and Ni (r= P<0.01. Significant positive correlations (p<0.05) were also observed between Zn and Co and between Fe and Ni.

3.9 Risk Analysis

The estimated THQ of each metal is presented in Table (4). The THQ values for Fe, Cu, Zn, and Mn were less than 1, the highest mean THQ values were observed for Pb, Cd, Co, and Ni across the three habitats (Fig. 3). The EDIs of the measured metals were calculated for each site and are listed in Table (5). The highest EDI values were observed for Fe and Zn. The EDI values in this study (Fig. 4) significant differences were detected between sites for Cu and Co, and between fish organs for Pb, Cd, Co, and Ni (Table 6). Table (7) exerts strong positive correlations between Fe and Zn (P<0.001), Fe and Pb (P<0.001), Fe and Mn (P<0.001), Mn and Ni (P<0.001), and Cd and Ni (r= P<0.01. Significant positive correlations (p<0.05) were also observed between Zn and Co and between Fe and Ni.

4. DISCUSSION

The measured metals in L. ramada fish obtained from the freshwater site were accumulated higher in gills followed by liver and muscles with exception to [Cu, Zn and Ni] which accumulated higher in liver followed by gills and muscles. In fish from the brackish site, Cu, Zn, and Co were accumulated much higher in liver than gills and muscles while; Fe, Ni, Pb, and Mn were accumulated much higher in gills than liver and muscles. However, the measured metals in the offshore site were accumulated as [liver > gills > muscles] with exception to Mn and Ni [gills > liver > muscles]. Cd in the two sites [brackish and offshore] was accumulated higher in muscles followed by gills and liver.
### Table 2. Accumulation of selected heavy metals in gills, liver and muscle of *L. ramada* fish (mg/kg dry weight)

| Sites                  | Fish parts      | Fe       | Cu       | Zn        | Mn        | Pb       | Cd       | Co       | Ni       |
|------------------------|-----------------|----------|----------|-----------|-----------|----------|----------|----------|----------|
| fresh                  | gill            | 202.4±164.4 | 10.85±5.88 | 60.43±17.15 | 58.56±29.99 | 22.15±15.72 | ND       | 0.29±0.97 | 1.09±2.46 |
|                        | liver           | 39.19±64.08 | 26.94±21.88 | 72.3±28.11  | 8.06±6.3  | 4.92±6.3  | ND       | ND       | 1.14±2.93 |
|                        | muscles         | 6.37±13.99 | 2.9±2.83  | 23.47±6.23  | 2.06±0.86  | 4.97±6.17  | ND       | ND       | ND       |
| brackish               | gill            | 790.7±162.6 | 6.14±1.35  | 51.54±7.09  | 150.28±15.22 | 8.99±12.67  | 0.03±0.05 | 1.71±0.71 | 1.6±2.91  |
|                        | liver           | 459.8±44.49 | 20.21±7.65  | 144.84±20.83 | 17.45±11.88 | 8.78±7.18  | 0.04±0.05 | 22.56±43.24 | 0.6±1.2   |
|                        | muscles         | 65.53±10.62 | 1.47±0.32  | 16.62±3.02  | 3.04±1.2  | 4.55±1.9  | 0.05±0.1  | 0.29±0.37 | ND       |
| offshore               | gill            | 211.67±46.03 | 4.33±2.8  | 51.18±3.51  | 140.17±35.59 | 10.82±16.37 | 0.47±0.81 | 1.88±2.04 | 1.9±2.19  |
|                        | liver           | 722.95±365.1 | 5.68±21.01 | 63.68±21.01 | 9.15±7.27  | 17.58±15.29 | 0.27±0.25 | 2.4±2.53  | 1.8±1.64  |
|                        | muscles         | 69.17±424.15 | 2.13±1.02  | 20.19±3.5   | 3.07±0.91  | 0.39±10.06 | 0.82±1.23 | 1.11±0.96 | 1.6±1.17  |

### Table 3. Concentrations of the selected metals from the literature, (mg/kg dry weight)

| Sites                  | Sp.             | Fish parts | Fe       | Cu       | Zn        | Mn        | Pb       | Cd       | Co       | Ni       |
|------------------------|-----------------|------------|----------|----------|-----------|-----------|----------|----------|----------|----------|
| Hara biosphere of southern Iran (mg kg\(^{-1}\) ww) | Liza klunzingeri | gill | -        | -        | -        | 0.44±0.08 | 0.32±0.06 | -        | 1.78±0.09 |
|                        |                 | muscles   | -        | -        | -        | -        | 0.67±0.11 | 0.63±0.07 | -        | 2.06±0.11 |
| coastal lagoon         | Liza saliens    | gill | -        | -        | -        | 114.4     | -        | -        | -        | -        |
|                        |                 | liver     | -        | -        | -        | -        | 0.32±0.04 | 0.16±0.06a | -        | 1.52±0.1 |
| Fernandesia 2007       |                 | muscles   | -        | -        | -        | -        | -        | -        | -        | -        |
| [63]                   |                 | -         | -        | 2.64     | -        | -        | -        | -        | -        | -        |
| Ennore estuary         | *M. cephalus*   | gill | 15.14±1.26 | 5.59±0.74 | 8.05±0.96 | 9.64±0.96 | 5.25±0.59 | 2.856±0.25 | -        | -        |
|                        |                 | liver     | 14.665±1.301 | 6.068±0.94 | 7.467±0.895 | 9.789±0.979 | 4.334±0.495 | 3.146±0.398 | -        | -        |
|                        |                 | muscles   | 10.278±1 | 3.346±0.56 | 1.4,13±0.46 | 1.5,20±0.68 | 1.15±0.23 | 1.0, 0.953±0.12 | -        | -        |
| southern part of Caspian Sea | Liza aurata | gill | 371.52±222.44 | 5.53±1.01 | 60.14±26.60 | 3.61±0.70 | 0.90±0.59 | -        | 1.43±0.36 |
|                        |                 | liver     | 415.35±223.97 | 160.39±40.01 | 78.97±29.93 | -        | 2.60±0.76 | 1.07±0.68 | -        | 1.01±0.38 |
| Jelodar 2011           |                 | muscles   | 67.52±33.53 | 4.54±1.07 | 13.69±7.23 | -        | 1.50±0.53 | 0.35±0.23 | 0.73±0.32 |
| [64]                   |                 | -         | -        | 4.33±6.03 | -        | -        | 2.64 - 21.4 | 0.32 - 2.72 | 0.29 - 1.10 | 4.61 - 17.52 |
| Estuaries              | Liza klunzingeri | gill | -        | -        | -        | 0.66-5.74 | 0.44±2.03 | 0.5-2.80 | 0.48-4.91 |
|                        |                 | muscles   | -        | 0.89-4.28 | -        | -        | 0.5-2.50 | 0.08-0.44 | ND-1.63 | 0.48-2.73 |
| Tuzla lagoon 137       | *Mugil cephalus* | gill | -        | 3.43     | -        | -        | 4.54     | 1.27     | -        | -        |
| [66,67]                |                 | liver     | -        | 4.77     | -        | -        | 2.12     | 0.21     | -        | -        |
| Rosario 41 [68]        | Liza ramada     | gill | -        | 6.27     | -        | -        | -        | -        | ND      | ND       |
|                        |                 | liver     | -        | 267.45   | -        | -        | -        | -        | ND      | ND       |
| Ataturk Dam Lake 7[67] | Liza abu        | muscles   | -        | 1.36     | -        | -        | -        | -        | ND      | ND       |

ND= Not Detected
Fig. 2. Concentration of (Fe, Cu, Zn, Mn, Pb, Cd, Co, and Ni (mg/kg·dw) measured in *L. ramada* fish collected from freshwater, brackish, and offshore sites. (a) & (b) levels in gills, (c) & (d) levels in liver and (e) & (f) levels in muscles.

Fig. 3. Target hazard quotients [THQs] for the heavy metals measured in fish from each site compared with the standard for non-carcinogenic risks [Indicated by the horizontal green line].
Table 4. The estimated target hazard quotients (THQs) for the heavy metals measured in fish from each study site compared with the oral reference dose

| Heavy metal | THQ in study sites | Oral reference dose [RD/mg/kg⁻¹/day]¹ |
|-------------|--------------------|---------------------------------------|
|             | Freshwater | Brackish | Offshore |                         |
| Fe          | 0.005      | 0.048    | 0.051    | \(7 \times 10^{-1} = 0.7\) |
| Cu          | 0.037      | 0.019    | 0.027    | \(4 \times 10^{-2} = 0.04\) |
| Zn          | 0.040      | 0.028    | 0.035    | \(3 \times 10^{-1} = 0.3\) |
| Mn          | 0.008      | 0.011    | 0.011    | \(1.4 \times 10^{-1} = 0.14\) |
| Pb          | 0.639      | 0.585    | 1.208    | \(4 \times 10^{-3} = 0.004\) |
| Cd          | ND         | 0.026    | 0.422    | \(1 \times 10^{-3} = 0.001\) |
| Co          | ND         | 0.489    | 1.909    | \(3 \times 10^{-4} = 0.0003\) |
| Ni          | ND         | ND       | 0.042    | \(2 \times 10^{-2} = 0.02^a\) |
| TTHQ [HI]   | 0.73       | 1.21     | 3.70     |                         |

¹ According to [25,32,39]. ND=not detected.

Table 5. The estimated daily intake [EDI] of the heavy metals measured in fish from each study site

| Heavy metal | EDI Freshwater | EDI Brackish | EDI Offshore | Recommended EDI [mg/person/day] | EW I [EDI*7 days] |
|-------------|----------------|--------------|--------------|----------------------------------|-------------------|
| Fe          | 3.276          | 33.7         | 35.57        | \(45^a\)                         | 22.93             |
| Cu          | 1.49           | 0.76         | 1.09         | \(30^d\)                         | 10.43             |
| Zn          | 12.072         | 8.55         | 10.38        | \(60^d\)                         | 84.5              |
| Mn          | 1.057          | 1.56         | 1.58         | \(10^c\)                         | 7.4               |
| Pb          | 2.6            | 2.34         | 4.83         | \(0.21^a\)                       | 17.9              |
| Cd          | ND             | 0.03         | 0.42         | \(0.06^a\)                       | ND                |
| Co          | ND             | 0.1          | 0.57         | \(30^b\)                         | ND                |
| Ni          | ND             | ND           | 0.84         | \(0.3^b\)                        | ND                |

¹PTDI: provisional tolerable daily intake [60 kg body weight] [54,55].
²Average daily intake from food [57,58].
³ESADDI: estimated safe and adequate daily dietary intake [59].
⁴PMTDI: provisional maximum tolerable daily intake [70 kg body weight] [38,53].
⁵TULs: tolerable upper intake levels for Fe (>19 years), established by the Food and Nutrition Board [56].

Fig. 4. The estimated daily intake [EDI] of the heavy metals measured in fish from each study site compared with the recommended dietary allowance [RDA]
and reported that the two species especially in gills and liver. However, [37] high concentrations collected from Ennore estuary contained very Vasanthi et al. Tigris River and Ataturk Dam Lake. Also, metals in liver and gills in many types of fish in [36], which indicated elevated levels of heavy with the date recorded by these fish. The present results are consistent could affect the health of humans who consumed by humans, any toxins in this tissue the muscles are generally the part of the fish skin, which prevents the direct absorption of pollutants from the environment [34,35]. As, muscles are covered by this may be due to the tendency of the liver and gills to accumulate pollutants at different levels of their environment [34,35]. As, muscles are covered by skin, which prevents the direct absorption of pollutants from the environment [36]. Given that the muscles are generally the part of the fish consumed by humans, any toxins in this tissue could affect the health of humans who consume these fish. The present results are consistent with the date recorded by Karadede and Unlu [36], which indicated elevated levels of heavy metals in liver and gills in many types of fish in Tigris River and Ataturk Dam Lake. Also, Vasanthi et al. [1] found that the fish M. cephalus collected from Ennore estuary contained very high concentrations of heavy elements, especially in gills and liver. However, [37] reported that the two species (Mugil cephalus and Liza ramada) collected from five locations in Lake Manzala, contained the highest concentrations of heavy metals in gills tissue of both fish species, while the lowest concentrations were recorded in muscles tissue. Very high concentrations of Fe in liver and gills were recorded in the three sites, these results were much higher than that reported by Vasanthi et al. [1], he assumed that the very high Fe level could be attributed to haemoglobin found in highly vascularized liver tissues of M. cephalus. Fe levels in the fish muscles from the freshwater site were similar to the result obtained by Omar et al. [38] in M. cephalus collected from Lake Qaroun, and Qaroun fish farms, while the brackish and offshore sites were much higher. Cu values measured in the fish muscles in this study were similar to the result obtained by Vasanthi et al. [1], and below the result obtained by Bahnasawy et al. and Omar et al. [37,38]. It also was below the acceptable limits cited by many organisations, e.g., 30 ppm [39,40]; 20 µg/g wet weight [UK Food Standards Committee Report] [41], and 10 µg/g wet weight [Australian Food Standard Code] [3]. Cu, an essential element present in many enzymes, has an important role in haemoglobin formation [3,42]. Similar to other metals, high levels of Cu can cause toxic effects in consumers.

Although Zn is an essential element, excessive intake can lead to deficiencies in Fe and Cu, as well as nausea, vomiting, fever, headache,
tiredness, and abdominal pain. It is also a human skin irritant [3]. Zn levels were similar to data obtained by Bahnasawy et al. and Omar et al. [37,38], and much higher compared with the result obtained by Vasanthi et al. [1], and below the permissible limit for Zn is 40 ppm [39,40,43]. Mn values measured in the fish in this study were similar to the result obtained by Vasanthi et al. [1] and higher than that recorded by Omar et al. [38]. The obtained results of Mn were higher than the permissible limits for Mn range from 0.5 mg/kg\(^{-1}\) [44,45] to 1.0 mg/kg\(^{-1}\) [43,46]. Mn deficiency may cause sexual abnormalities in mammals [47]. Pb levels in fish from all sites were much higher than the data recorded by Several authors [37,38,40], and the recommended maximum limits, i.e., 0.5 mg/kg\(^{-1}\) [43] and 2.0 mg/kg\(^{-1}\) [44,48]. Pb in fish muscles should not exceed 9.6 µg/g dw according to the UK Food Standards Committee Report [10].

Cd accumulation in the human body can cause toxic effects at very low concentrations, including hepatic and reproductive effects and even cancer [3,47]. In this study, levels of Cd in fish muscles from the offshore site were below the obtained data by Bahnasawy et al. [37] in Liza ramada, and similar to the recorded data by Vasanthi et al. [1]. However, it was higher than the data recorded by Mohammadnabizadeh et al. [40] who worked on Liza klunzingeri and Sillago sihama caught from the Hara biosphere of Southern Iran, and exceed the permissible limit [0.5 mg/kg\(^{-1}\)] for fish as food according to Several authors [20,43,48]. Cd measured in muscles of fish from all sites was below specified limits, i.e., 2.0 mg/kg\(^{-1}\) [44,45], 0.1 mg/kg\(^{-1}\) [39,40], and 1.0 µg/g\(^{-1}\) [49]. There are no permissible limits cited for cobalt in fish. Ni is present in aquatic environments at a very low concentration but can lead to noxious effects, such as pneumonia, cirrhosis, and emphysema [3,50]. Ni values in this study were similar to the result obtained by Mohammadnabizadeh et al. [40], and below the threshold limit reported by Western Australian Food and Drug Regulations [51] (6.5 µg/g on a wet weight basis, which equals 26.4 µg/g on a dry weight basis assuming a 79% moisture content of fish muscles).

The estimated THQ values for Fe, Cu, Zn, and Mn were less than 1, indicating that there would be no adverse health effects associated with the consumption of L. ramada from any of the three habitats regarding these metals. The highest mean THQ values were observed for Pb, Cd, Co, and Ni across the three habitats, which suggests that consumer might experience some adverse health effects of these four metals by consuming these fish. The THQ averages were ranked as Co> Pb> Cd> Ni > Zn > Cu > Fe > Mn. The cumulative health risk [TTHQ] or HI was calculated by summing the THQs of the eight metals to assess the exposure to a mixture of metals of humans consuming L. ramada from each habitat. TTHQ was less than 1; a value of 0.73 was obtained for the freshwater site, suggesting that L. ramada harvested from this site could be safely consumed for life. However, L. ramada harvested from the brackish and offshore habitats was found to be a high health risk for consumers, with total THQ values of 1.21, and 3.70, respectively.

An important aspect of assessing the risk to human health resulting from potentially harmful metals in fish is knowledge of the dietary intake of such substances, which must remain within determined safety standards [52]. The highest EDI values were observed for Fe and Zn. The EDI values in this study were compared with the recommended dietary allowance [RDA] of individual metals set by many organizations, including the FAO/WHO Expert Committee on Food Additive [JECFA] for Cd, Cu, Pb, and Zn [53-55]; the Food and Nutrition Board FNB for Fe [56]; the World Health Organization for Co and Ni [57,58]; and the National Research Council [NRC] for Mn [59]. The EDI values for Ni and Cd were higher than the recommended levels, indicating health effects; however, the values for the remaining metals were lower than the recommended levels, suggesting that a health risk associated with exposure to the examined metals would be unlikely.

Two-way ANOVA was performed to determine any significant differences in heavy metal concentrations among the tissues and study sites. A probability level of 0.05 was considered statistically significant. Correlation-based analyses can provide an indication of the potential relationships between metals, such as common sources, related dependence, and similar behaviours [8,60,61,62,63,64]. Strong positive correlations were found at the p<0.01 level, and Significant positive correlations were found at the p<0.05. The strong correlation between the studied heavy metals indicates a similar level of contamination or release from the same pollution sources [2]. Fish muscles (edible part) comprise a considerable amount of different heavy metals that can lead to deleterious health effects on humans, the accumulated effects of these metals indicates that the health of consumers who rely on fish around the
contaminated studied sites are at risk. However, the variations in the modality of metals accumulation among fish species may be due to differences in feeding habits and lifestyle in the studied sites [38,65,66,67,68].

5. CONCLUSIONS

Metals accumulation in the gills and liver were higher than that in muscle. A high correlation between specific heavy metals indicates a similar level of contamination or release from the same pollution sources. Adverse health effects associated with the consumption of fish contaminated with Pb, Cd, Co, and Ni, on the contrary, the consumption of fish contaminated with Fe, Cu, Zn, and Mn had no adverse effects. The estimated daily intake [EDI] of the measured metals were lower than the recommended dietary allowance [RDA] of individual metals set by many organisations, except Ni and Cd. The consumption limits of the eight metals presented in this study provide important information that could be used to reduce potential health risks resulting from human consumption of *L. ramada* in the study region.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vasanthi LA, Revathi P, Mini J, Munuswamy N. Integrated use of histological and ultrastructural biomarkers in *Mugil cephalus* for assessing heavy metal pollution in Ennore estuary, Chennai. Chemosphere. 2013;91:1156–1164. Available: http://dx.doi.org/10.1016/j.chemosphere.2013.10.021
2. Beheary MS, El-Matary FA. Risk evaluation of heavy metals in sediments of the fish farming area in the Mediterranean section of lake Manzala. Scientific Journal for Damietta Faculty of Science. 2015;4(2):69–78.
3. Saha N, Mollah MZI, Alam MF, Rahman MS. Seasonal investigation of heavy metals in marine fishes captured from the Bay of Bengal and the implications for human health risk assessment. Food Control. 2016;70:110–118
4. Biswas S, Prabhu RK, Hussain KJ, Selvanayagam M, Satpathy KK. Heavy metal concentration in edible fishes from coastal region of Kalpakkam, southeastern part of India. Environmental Monitoring and Assessment. 2012;184(8):5097–5104. DOI: 10.1007/s10661-011-2325-y
5. Authman MMN, Zaki MS, Khalil AEA, Abbas HH. Use of fish as bio-indicator of the effects of heavy metals pollution. J Aquac Res Development. 2015;6(4):328. DOI: 10.4172/2155-9546.1000328
6. Tekin-Özcan S. Determination of heavy metal levels in water, sediment and tissues of tench (*Tinca tinca*, L. 1758) from Beysehir Lake (Turkey). Environmental Monitoring and Assessment. 2008;145:295–302. DOI: 10.1007/s10661-007-0038-z
7. Wei Y, Zhang J, Zhang D, Tu T, Luo L. Metal concentrations in various fish organs of different fish species from Poyang Lake, China. Ecotoxicology and Environmental Safety. 2014;104:182–188. Available: http://dx.doi.org/10.1016/j.ecoenv.2013.01.001
8. Abdel-Khalek AA, Elhaddad E, Mamdouh S, Marie MS. Assessment of metal pollution around sabal drainage in river nile and its impacts on bioaccumulation level, metals correlation and human risk hazard using *Oreochromis niloticus* as a bioindicator. Turkish Journal of Fisheries and Aquatic Sciences. 2016;16:227-239. DOI: 10.4194/1303-2712-v16_2_02
9. Saha N, Zaman M. Evaluation of possible health risks of heavy metals by consumption of foodstuffs available in the central market of Rajshahi City, Bangladesh. Environmental Monitoring and Assessment. 2013;1-12.
10. Demirak A, Yilmaz F, Tuna AL, Ozdemir N. Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. Chemosphere. 2006;63(9):1451–1458.
11. Vicente-Martorell JJ, Galindo-Riano MD, Garcia-Vargas M, Granado-Castro MD. Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted estuary. J Hazard Mater. 2009;162:823–836.
12. Naji A, Ismail A. Sediment quality assessment of Klang Estuary, Malaysia. Aquat Ecol System. 2012;15(3):287–293.
13. Copat CH, Arena G, Fiore M, Ledda C, Fallico R, Sciaccia S, Ferrante M. Heavy metals concentrations in fish and shellfish...
from eastern Mediterranean Sea: Consumption advisories. Food Chem Toxicol. 2013;53:33–37.
14. Taweel A, Shuhaimi-Othman M, Ahmad AK. Assessment of heavy metals in tilapia fish (Oreochromis niloticus) from the Langat river and engineering lake in Bangi, Malaysia, and evaluation of the health risk from tilapia consumption. Ecotoxicology and Environmental Safety. 2013;93:45–5. Available: http://dx.doi.org/10.1016/j.ecoenv.2013.03.031
15. Naji A, Khanb FR, Hashemi SH. Potential human health risk assessment of trace metals via the consumption of marine fish in Persian Gulf. Marine Pollution Bulletin 2016;109:667–671. Available: http://dx.doi.org/10.1016/j.marpolbu.2016.05.002
16. Yi Y, Tang C, Yi T, Yang Z. Health risk assessment of heavy metals in fish and accumulation patterns in food web in the upper Yangtze River, China, Shangshong Zhang. Ecotoxicology and Environmental Safety. 2017;145:295–302. Available: http://dx.doi.org/10.1016/j.ecoenv.2017.07.022
17. Varol M. Assessment of heavy metal contamination in sediments of the Tigris River (Turkey) using pollution indices and multivariate statistical techniques. J Hazard Mater. 2011;195:355–364.
18. Fang HW, Huang L, Wang JY, He GJ, Reible D. Environmental assessment of heavy metal transport and transformation in the Hangzhou Bay, China. J Hazard Mater. 2016;302:447–457.
19. Vieira C, Morais S, Ramos S, Delerue-Matos C, Oliveira MBPP. Mercury, cadmium, lead and arsenic levels in three pelagic fish species from the Atlantic Ocean: Intra- and inter-specific variability and human health risks for consumption. Food and Chemical Toxicology. 2011;49:923–932. DOI: 10.1016/j.fct.2010.12.016
20. Beheary MS, Abu-Almaaty AH, El-matary FA. Genetic polymorphism and heavy metals in catfish Clarias gariepinus from different sites as a result of environmental contamination. Adv. Environ. Biol. 2015; 9(18):20–32.
21. FACP. Fishery and aquaculture country profile report, the Arab republic of Egypt, Food and Agriculture Organization of the United Nations, FAO Fisheries and Aquaculture Department; 2010.
22. GAFRD. General authority for fish resources development: Report on fish production. Cairo, Egypt; 2001.
23. Peng Q, Nunes LM, Greenfield BK, Dang F, Zhong H. Are Chinese consumers at risk due to exposure to metals in crayfish? A bioaccessibility-adjusted probabilistic risk assessment. Environ Int. 2016;88:261–268.
24. Solomon KR, Giesy JP, LaPoint TW, Giddings JM, Richards RP. Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem. 2013;32:10–11.
25. USEPA. United states environmental protection agency, guidance for assessing chemical contaminant data for use in fish advisories. Fish Sampling and Analysis EPA, 823-B-00-007, third ed Office of Science and Technology, Washington, DC. 2000:1.
26. Chien LC, Hung TC, Choang KY, Yeh CY, Meng PJ, Shien MJ, Han BC. Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. Sci Total Environ. 2002;285:177–185.
27. Wang X, Sato T, Xing B, Tao S. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci Total Environ. 2005;350:28–37.
28. Zhang HD, Huang B, Dong LL, Hu WY, Akhtar MS, Qu MK. Accumulation, sources and health risks of trace metals in elevated geochemical background soils used for greenhouse vegetable production in southwestern China. Ecotoxicol Environ Saf. 2017;137:233
29. UNEP/IAEA. References methods for marine pollution studies. PERSGA-UNESCO University, Egypt. 1986:39.
30. USEPA. United States environmental protection agency, risk assessment guidance for superfund. Human Health Evaluation Manual EPA /540/1–89/002 Office of Emergency and Remedial Response, Washington, DC. 1989;
31. Li P, Zhang J, Xie H, Liu C, Liang S, Ren Y. Heavy metal bioaccumulation and health hazard assessment for three fish species from Nansi Lake, China. Bulletin of Environmental Contamination and Toxicology. 2015;94(4):431-436.
32. FAO. Food and Agriculture Organization, Food Security Statistics; 2005. Available: http://www.fao.org/faostat/foodsecurity/index_en.htm
33. Rao LM, Padmaja G. Bioaccumulation of heavy metals in *M. cyprinoides* from the harbor waters of Visakhapatnam. Bull Pure Appl Sci. 2000;19A(2):77–85.

34. Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM. Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. Sci. Total Environ. 2000;256:87–94.

35. Canli M, Atli G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut. 2003;121(1):129–136.

36. Karade H, Unlu E. Concentrations of some heavy metals in water, sediment and fish species from the Ataturk Dam Lake (Euphrates), Turkey. Chemosphere. 2000; 41:1371–1376.

37. Bahnasawy M, Khidr MA, and Dheina N. Seasonal variations of heavy metals concentrations in mullet, *Mugil cephalus* and *Liza ramada* (Mugilidae) from Lake Manzala, Egypt. Journal of Applied Sciences Research. 2009;5(7):845-852.

38. Omar WA, Abdel-Khalek AA, Abo-Hegab S, Zaghliouf KH. Risk assessment and toxic effects of metal pollution in two cultured and wild fish species from highly degraded aquatic habitats. Arch Environ Contam Toxicol open access at Springerlinkcom; 2013. DOI: 10.1007/s00244-013-9935-z

39. FAO/WHO. Evaluation of certain food additives and contaminants In: Thirty third Report of the Joint FAO/WHO Expert Committee on Food Additives World Health Organization, Geneva WHO Technical Report Series No 776; 1989.

40. Mohammadnabizadeh S, Pourkhazzab A, Afshari R, Nowrouzi M. Concentrations of Cd, Ni, Pb, and Cr in the two edible fish species *Liza kunzingeri* and *Sillago sihama* collected from Hara biosphere in Iran. Toxicological & Environmental Chemistry. 2012;94(6):1144-1151. DOI: 10.1080/027722482012693494

41. Cronin M, Davies IM, Newton A, Pirie JM, Topping G, Swan S. Trace metal concentrations in deep sea fish from the North Atlantic. Marine Environmental Research. 1998;45(3):225-238.

42. Sivaperumal P, Sankar T, Nair PV. Heavy metal concentrations in fishes, shellfish and fish products from internal markets of India vis-a-vis international standards. Food Chemistry. 2007;102(3):612-620.

43. FAO (Food and Agriculture Organization), "Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products," FAO Fisheries Circular. 1983;464:5-100.

44. WHO (World Health Organization). Guidelines for drinking water quality recommendation WHO: Geneva. 1985;1:130.

45. Obasohan EE. Heavy metals concentrations in the offal, gill, muscle and liver of a freshwater mudfish (*Parachanna obscura*) from Ogba River, Benin city, Nigeria. African Journal of Biotechnology. 2007;6(22):2620–7.

46. Ahmed MK, Abdul Baki M, Kundu GK, Islam MS, Islam MM, Hossain MM. Human health risks from heavy metals in fish of Buriganga river, Bangladesh. Springer Plus. 2016;5:1697. DOI: 10.1186/s40064-016-3357-0

47. Ahmed MK, Shaheen N, Islam MS, Habibullah-al-Mamun M, Islam S, Mohiduzzama M. Dietary intake of trace elements from highly consumed cultured fish (*Labeo rohita, Pangasius pangasius* and *Oreochromis mossambicus*) and human health risk implications in Bangladesh. Chemosphere. 2015;128:284-292.

48. Anim AK, Ahialye EK, Duodu GO, Ackah M, Bentil NO. Accumulation profile of heavy metals in fish samples from Nsawam, along the Densu River, Ghana. Research Journal of Environmental and Earth Sciences. 2011;3(1):56–60.

49. EU. Commission Regulation (Ed) No 466/2001 Setting Maximum Levels for Certain Contaminants in Food Stuffs. 2001;13.

50. Malik N, Biswas AK, Qureshi TA, Borana K, Virha R. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Environmental Monitoring and Assessment. 2010;160:267–76.

51. Plaskett D, Potter I. Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. Marine and Freshwater Research. 1979;30(5):607-616.

52. Storelli MM. Potential human health risks from metals (Hg, Cd, and Pb) and polychlorinated biphenyls (PCBs) via seafood consumption: Estimation of target hazard quotients (THQs) and toxic equivalents (TEQs). Food and Chemical Toxicology. 2008;46:2782–2788.

53. JECFA. Evaluation of certain food additives and contaminants Twenty-sixty report of
the joint FAO/WHO Expert Committee on Food Additives (WHO technical report series, No 683) Geneva: World Health Organization; 1982.

54. JECFA. Evaluation of certain food additives and contaminants Thirty-third report of the joint FAO/WHO Expert Committee on Food Additives [WHO technical report series, No 776] Geneva: World Health Organization; 1989.

55. JECFA. Evaluation of certain food additives and contaminants Fifty-third report of the joint FAO/WHO Expert; 2000.

56. FNB. Food and Nutrition Board, Institute of Medicine Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: a Report of the Panel on Washington, DC; National Academy Press; 2001.

57. WHO. World Health Organization, Guidelines for drinking water quality; (2nd ed, 2) Geneva: World Health Organization; 1996.

58. WHO. World Health Organization, Concise international chemical assessment document 69: cobalt and inorganic cobalt compounds Geneva: World Health Organization; 2006.

59. NRC. National Research Council recommended dietary allowances (10th ed) Washington, DC: National Academy of Sciences. 1989:241-243.

60. Diop C, Dewaelé D, Cazier F, Diouf A, Ouddane B. Assessment of trace metals contamination level, bioavailability and toxicity in sediments from Dakar coast and Saint Louis estuary in Senegal, West Africa. Chemosphere. 2015; 138:980– 987. DOI: 10.1016/j.chemosphere201412041

61. Rejomon G, Nair M, Joseph T. Trace metal dynamics in fishes from the southwest coast of India. Environ Monit Assess. 2010;167:243–255.

62. Krishna PV, Prabhavathi K, Prakasa Rao R. Potential health risk assessment of heavy metal accumulation in the selected food fishes from Krishna Esturine region of Southern Deltaic Region of India. Current Trends in Biotechnology and Pharmacy. 2018;12(3):293-301.

63. Fernandes A, Fontainhas-Fernandes A, Peixoto F, Salgado MD. Bioaccumulation of heavy metals in Liza saliens from the Esmoriz–Paramos coastal lagoon, Portugal. Ecotoxicology and Environmental Safety. 2007;66:426–431. DOI: 10.1016/j.ecoenv.2006.02.007

64. Jelodar TH, Baei SM, Najafpour SH, Fazli H. The comparison of heavy metals concentrations in different organs of Liza aurata inhabiting in southern part of Caspian Sea. World Applied Sciences Journal 14 (Special Issue of Food and Environment). 2011;96-100. [ISSN 1818-4952]

65. Safaieh A, Monikh FA, Savari A, Doraghi A. Heavy metals concentration in mullet fish, Liza abu from petrochemical waste receiving creeks, Musa estuary (Persian Gulf). Journal of Environmental Protection 2011;2:1218-1226. DOI: 10.4236/jep.2011.29140

66. Dural M, Ziya Lugal Goksu M, Ozak AA. Inves-tigation of Heavy Metal Levels in Economically Import-ant Fish Species Captured from the Tuzla Lagoon. Food Chemistry. 2007;102(1):415-421. DOI: 10.1016/j.foodchem.2006.03.001

67. Karadede Hl, Oymak SA, Unlu E. Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, from the Ataturk dam lake (Euphrates), Turkey. Environment International. 2004;30(2):183-188. DOI: 10.1016/S0160-4120(03)00169-7

68. Franca S, Vinagre C, Cacador I, Cabral HN. Heavy metal concentrations in sediment, benthic in-vertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portu-gal). Marine Pollution Bulletin. 2005;50:993-1018. DOI: 10.1016/j.marpolbul.2005.06.040.

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