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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.

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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 accumulates 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 >

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Ni. There were significant differences between sites for Cu and Co, and between fish organs for Pb, Cd, Co, and Ni. Strong positive correlations were found between Fe and Zn ($P<0.001$), Fe and Pb ($P<0.001$), Fe and Mn (r= $P<0.001$), Mn and Ni ($P<0.001$), and Cd and Ni (r= $P<0.01$). Significant positive correlations ($p<0.05$) were observed between Zn with Co and Fe with Ni. Target hazard quotient (THQ) values for Pb, Cd, Co, and Ni were higher than 1 for the three sites, which suggests that adverse health effects might arise from the consumption of these fish. The estimated daily intake (EDI) values for Ni and Cd were higher than the recommended levels, indicating health effects; however, the values for rest of the metals were lower than the recommended EDI levels, suggesting that they are unlikely to pose a health risk.

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

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 are 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].

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].

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.

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^{-1} 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:

$$\mathrm{THQ} = \frac{[\mathrm{EF} \times \mathrm{ED} \times \mathrm{M_C} \times \mathrm{FIR} \times 10^{-3}]}{[\mathrm{RD} \times \mathrm{ABW} \times \mathrm{TA}]} \quad (1)$$

EF, ED, FIR, M$_C$, 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]:

$$\mathrm{TTHQ}\ [\mathrm{HI}] = \frac{\sum \mathrm{THQ}n}{\mathrm{n}} \quad (2)$$

where *n* is the particular heavy metal tested

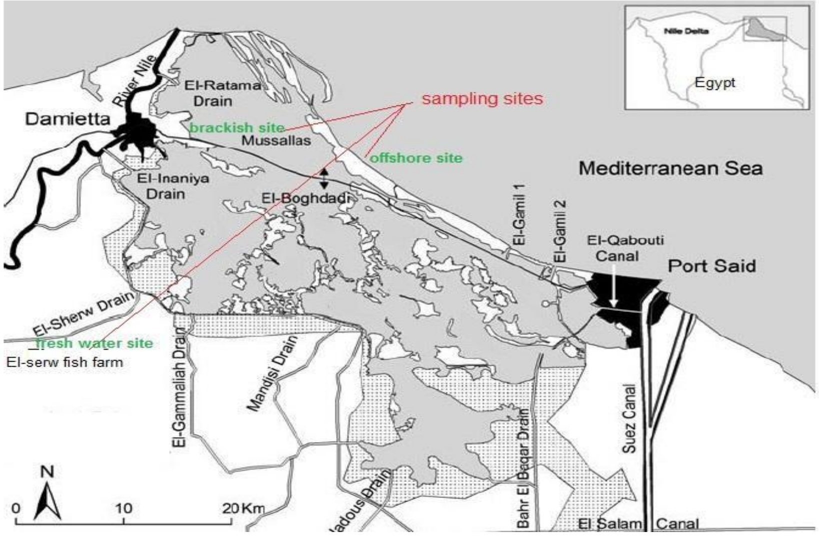


Fig. 1. Sampling sites representing a brackish habitat (northern section of Lake Manzala), offshore habitat (Mediterranean offshore area), and a freshwater habitat (El-serw freshwater farm)

Table 1. Assumptions used to calculate the target hazard quotients [THQs] for each metal

Assumption	Description	Value
FIR	Food ingestion rate for fish [g/person/day]	36[a]
EF	Exposure frequency [days/year]	365[b]
ED	Exposure duration equivalent to the average lifetime [years]	70[b]
MC	Metal concentration in fish [mg/kg. dw]	Determined by this study
ABW	Average body weight [kg]	70
TA	Average exposure time for non-carcinogens	365 days/year × ED[b]

a: According to FAO [32]. b: According to USEPA [25,30].

2.3 Estimation of Daily Intake

The estimated daily intake (EDI) depends on metal concentration, level of consumption, and consumer's body weight. To evaluate the metal risk associated with fish consumption, it was assumed that the level of the ingested dose was equal to the absorbed pollutant dose and that cooking had no effect on the level of pollutants [30]. The average adult body weight is 70 kg and the ingestion rate of fish is 36 g/person/day [32]. The EDI of Fe, Cu, Zn, Mn, Cd, Pb, Co, and Ni via consumption of the edible parts of the studied fish was calculated according to Equation 3:

$$\mathrm{EDI}\ [\mathrm{mg/kg-bw/day}] = \frac{\mathrm{PIR} \times \mathrm{M_C}}{\mathrm{WB}} \quad (3)$$

Where PIR is the consumer's ingestion rate per day, MC is the concentration of the metal in the fish, and WB is the consumer's body weight.

2.4 Data Analysis

Descriptive statistics, e.g., standard deviation, maximum, minimum, means are computed. Pearson's correlation coefficient and two-way ANOVA were performed using software package SPSS version 22.

3. RESULTS

The levels of Fe, Cu, Zn, Mn, Pb, Cd, Co, and Ni were measured in different parts of *L. ramada* fish collected from fresh, brackish, and offshore sites. The mean concentrations of these metals are represented in Table (2) and Fig. 2 (a and b) for gills, (c and d) for liver, and muscles (e and f). Heavy metals concentration in various tissues of *L. ramada* is compared with some previous studies from other locations of the world (Table 3).

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 (211.67±46.03mg/kg^{-1} dw) > freshwater (202.44±154.44 mg/kg^{-1} dw). In liver, the mean concentrations were 722.95±365.09 mg/kg^{-1} dw, 459.8±44.49 mg/kg^{-1} dw, and 39.19±64.08 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 Cupper (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±2.83 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) > freshwater (72.3±28.11 mg/kg^{-1} dw) > offshore (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 (140.17±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)

Nikel 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 (1.64±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.

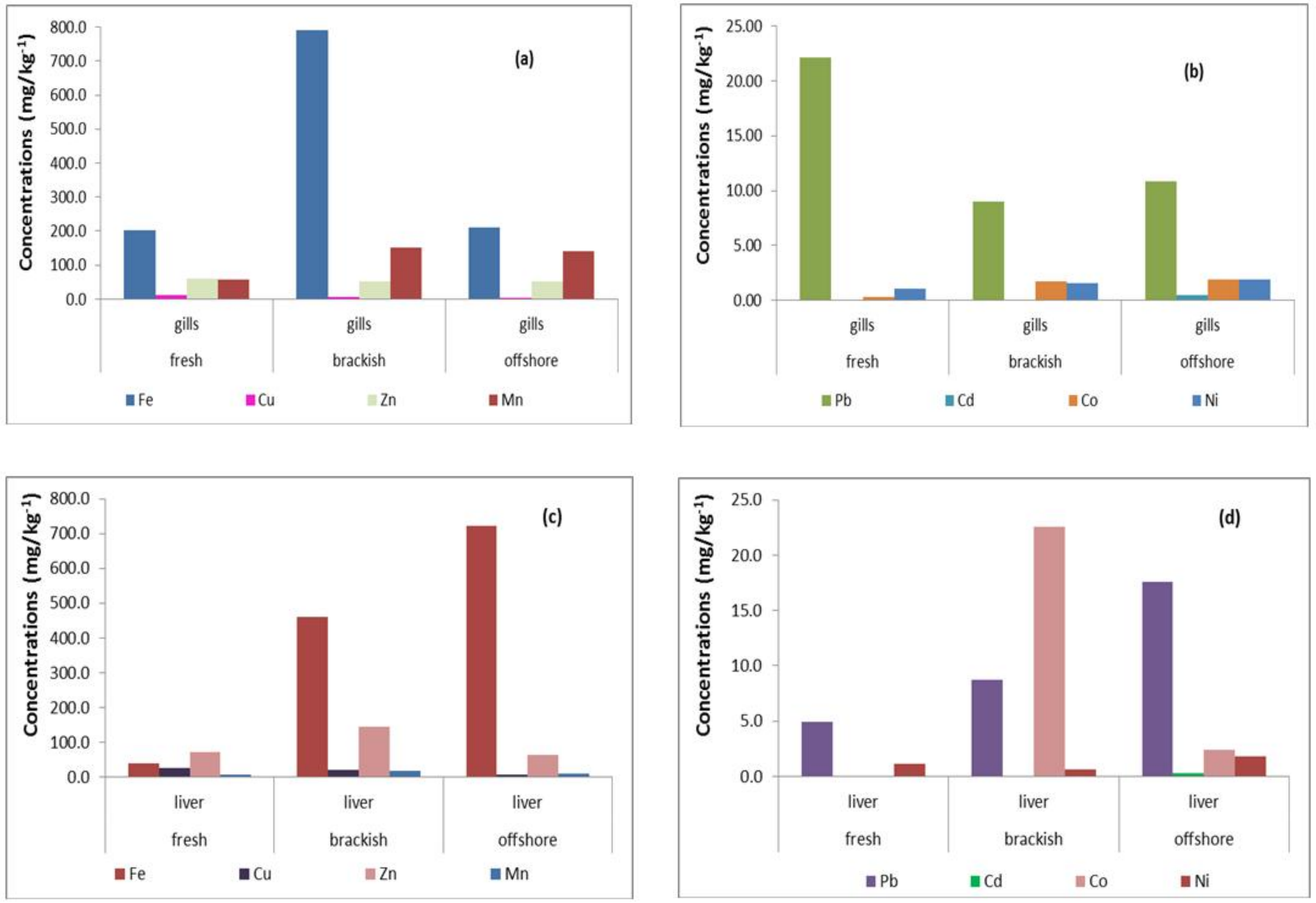
Table 2. Accumulation of selected heavy metals in gills, liver and muscle of *L. ramada* fish (mg/kg^{-1} dry weight)

Sites	**Fish parts**	**Heavy metals**							
		Fe	**Cu**	**Zn**	**Mn**	**Pb**	**Cd**	**Co**	**Ni**
fresh	gill	202.44±154.4	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	790.78±162.6	6.14±1.35	51.54±7.09	150.28±15.22	8.99±12.87	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±11.68	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±6.19	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±5.21	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±24.15	2.13±1.02	20.19±3.5	3.07±0.91	9.39±10.06	0.82±1.23	1.11±0.96	1.64±1.17

ND= Not Detected

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

Sites	**Sp.**	**Fish parts**	**Heavy metals**							
			Fe	**Cu**	**Zn**	**Mn**	**Pb**	**Cd**	**Co**	**Ni**
Hara biosphere of southern Iran	*Liza klunzingeri*	gill	-	-	-	-	0.44±0.08	0.32±0.06	-	1.78±0.09
(mg kg^{-1} ww)		liver	-	-	-	-	0.67±0.11	0.63±0.07	-	2.06±0.11
[40]		muscles	-	-	-	-	0.32±0.04	0.16±0.06a	-	1.52±0.1
coastal lagoon	*Liza saliens*	gill	-	-	114.4	-	-	-	-	-
Fernandesa 2007		liver	-	254	-	-	-	-	-	-
[63]		muscles	-	2.64	-	-	-	-	-	-
Ennore estuary	*M. cephalus*	gill	15.14 ± 1.26	5.598 ± 0.74	8.058 ± 0.96	9.648 ± 0.96	5.253 ± 0.59	2.856 ± 0.25	-	-
[1].		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, 4.132 ± 0.46	1, 5.208 ± 0.68	1.15 ± 0.23	1, 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
Jelodar 2011		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
[64]		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
Estuaries	*Liza klunzingeri*	gill	-	4.33 - 6.03	-	-	2.64 - 21.41	0.32 - 2.72	0.29 - 1.10	4.61 - 17.52
Safahieh 2011		liver	-	5.05 - 36.22	-	-	0.66 - 5.74	0.44 - 2.03	0.5 - 2.80	0.48 - 4.91
[65]		muscles	-	0.89 - 4.28	-	-	0.5 - 2.50	0.08-0.44	ND-1.63	0.48 - 2.73
Tuzla lagoon 13 7	*Mugil cephalus*	gill	-	3.43	-	-	4.54	1.27	-	-
[66,67].		liver	-	4.77	-	-	2.12	0.21	-	-
Rosario 41 [68]	*Liza ramada*	muscles	-	1.6	-	-	3.7	0.9	-	-
Ataturk Dam Lake 7[67]	*Liza abu*	gill	-	6.27	-	-	-	-	ND	ND
		liver	-	267.45	-	-	-	-	ND	ND
		muscles	-	1.36	-	-	-	-	ND	ND


(a)
Concentrations (mg/kg⁻¹)
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
gills
fresh
gills
brackish
gills
offshore
Fe
Cu
Zn
Mn
(b)
Concentrations (mg/kg⁻¹)
25.00
20.00
15.00
10.00
5.00
0.00
gills
fresh
gills
brackish
gills
offshore
Pb
Cd
Co
Ni
(c)
Concentrations (mg/kg⁻¹)
800.0
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
liver
fresh
liver
brackish
liver
offshore
Fe
Cu
Zn
Mn
(d)
Concentrations (mg/kg⁻¹)
25.0
20.0
15.0
10.0
5.0
0.0
liver
fresh
liver
brackish
liver
offshore
Pb
Cd
Co
Ni

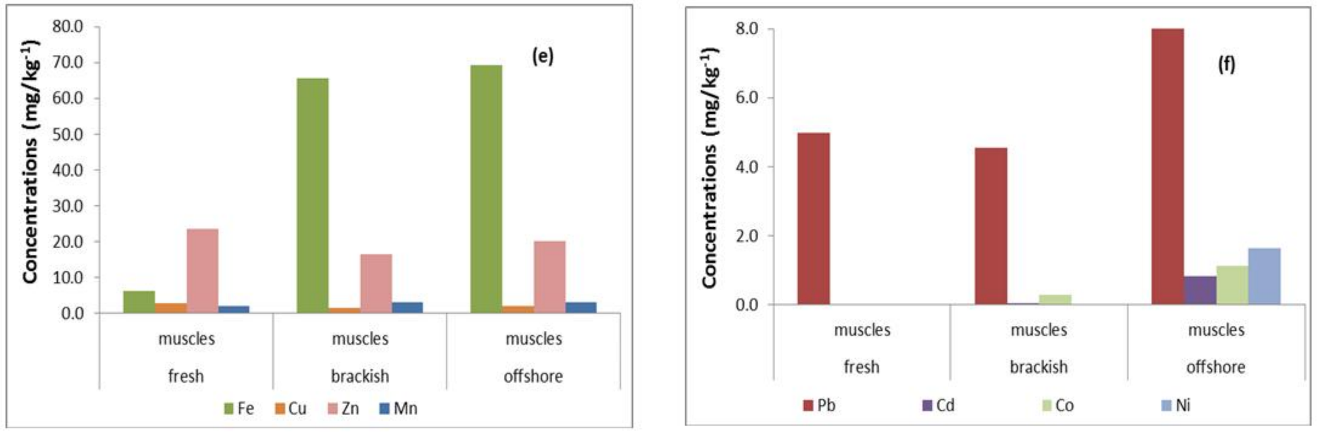


Fig. 2. Concentration of (Fe, Cu, Zn, Mn, Pb Cd, Co, and Ni (mg/kg-^{1}dw) measured in *L. ramada* fish collected from freshwater, brackish, and offshore sites. (a) & (b) levels in gills, (c) & (d) levels in liver and (e) & (f) levels in muscles

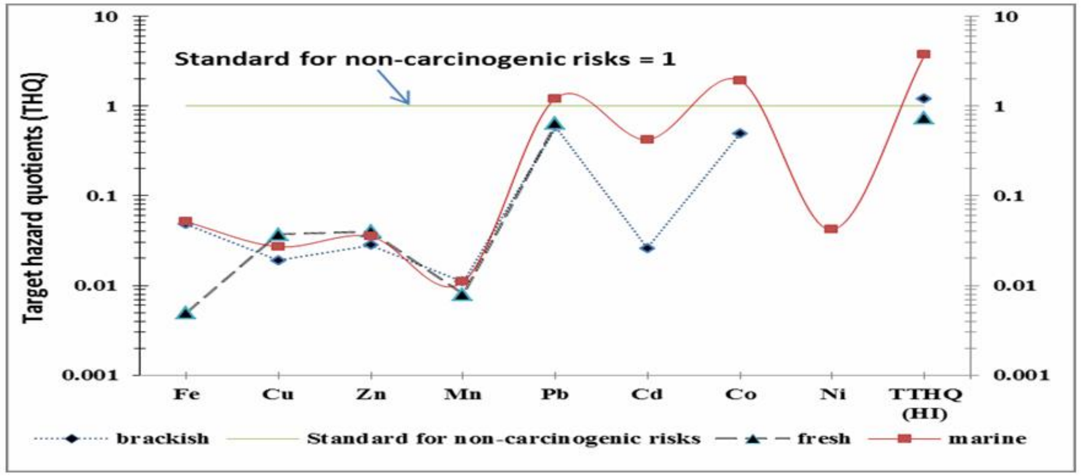


Fig. 3. Target hazard quotients [THQs] for the heavy metals measured in fish from each site compared with the standard for non-carcinogenic risks [Indicated by the horizontal green line]

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][a]
	Freshwater	Brackish	Offshore	
Fe	0.005	0.048	0.051	7×10^{-1}= 0.7
Cu	0.037	0.019	0.027	4×10^{-2} = 0.04
Zn	0.040	0.028	0.035	3×10^{-1} = 0.3
Mn	0.008	0.011	0.011	1.4×10^{-1} = 0.14
Pb	0.639	0.585	1.208	4×10^{-3} = 0.004
Cd	ND	0.026	0.422	1×10^{-3} = 0.001
Co	ND	0.489	1.909	3×10^{-4} =0.0003
Ni	ND	ND	0.042	2×10^{-2} = 0.02[a]
TTHQ [HI]	0.73	1.21	3.70	

[a] *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				EWI [EDI*7 days]		
	Freshwater	brackish	offshore	recommended EDI [mg/person/day]	Freshwater	brackish	offshore
Fe	3.276	33.7	35.57	45[e]	22.93	235.91	249.01
Cu	1.49	0.76	1.09	30[d]	10.43	5.29	7.66
Zn	12.072	8.55	10.38	60[d]	84.5	59.83	72.67
Mn	1.057	1.56	1.58	10[c]	7.4	10.93	11.04
Pb	2.6	2.34	4.83	0.21[a]	17.9	16.38	33.82
Cd	ND	0.03	0.42	0.06[a]	ND	0.18	2.95
Co	ND	0.1	0.57	30[b]	ND	1.03	4.01
Ni	ND	ND	0.84	0.3[b]	ND	ND	5.9

[a] *PTDI: provisional tolerable daily intake [60 kg body weight] [54,55].*

[b] *Average daily intake from food [57,58].*

[c] *ESADDI: estimated safe and adequate daily dietary intake [59].*

[d] *PMTDI: provisional maximum tolerable daily intake [70 kg body weight] [38,53].*

[e] *TULs: tolerable upper intake levels for Fe [>19 years], established by the Food and Nutrition Board [56].*

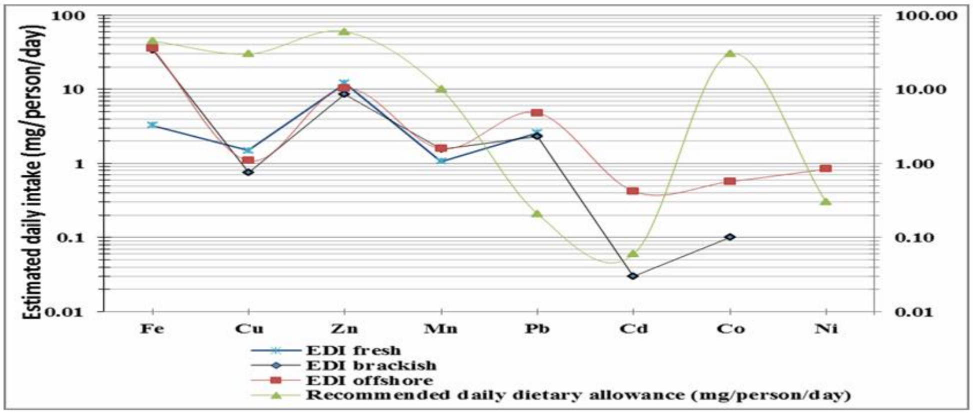


Fig. 4. The estimated daily intake [EDI] of the heavy metals measured in fish from each study site compared with the recommended dietary allowance [RDA]

Table 6. Analysis of variance results of the heavy metals measured in fish from the study sites

Metal	Site			Organ			Site * Organ		
	df	F value	Sig.	df	F value	Sig.	df	F value	Sig.
Fe	2	45.78	0.000	2	31.17	0.000	4	19.73	0.000
Cu	2	2.67	0.08	2	15.53	0.000	4	1.2	0.32
Zn	2	40.61	0.000	2	34.46	0.000	4	12.65	0.000
Mn	2	157.04	0.000	2	211.97	0.000	4	115.74	0.000
Pb	2	9.05	0.000	2	0.88	0.42	4	0.69	0.60
Cd	2	9.67	0.000	2	0.42	0.66	4	0.96	0.44
Co	2	2.35	0.11	2	1.17	0.32	4	1.9	0.13
Ni	2	8.43	0.000	2	0.89	0.42	4	0.75	0.56

Table 7. Pearson correlations between different heavy metals measured in fish from the study sites

Fe		Cu	Zn	Mn	Pb	Cd	Co	Ni
Fe	1							
Cu	-0.022	1						
Zn	0.537**	0.205	1					
Mn	0.558**	-0.123	0.247	1				
Pb	0.414**	-0.184	0.264	0.199	1			
Cd	0.085	-0.118	0.053	0.176	0.255	1		
Co	0.204	0.174	0.346*	0.036	0.072	0.015	1	
Ni	0.345*	-0.108	0.192	0.455**	0.138	0.540**	-0.005	1

**=is significant at P<0.05 level [2-tailed]. **=is significant at the P<0.01 level [2-tailed].*

Generally, the highest heavy metals accumulation in the studied fish *L. ramada* occurred in the liver and gills followed by muscles with exception to cadmium. Gills are in direct contact with the environment and represent the main target for metal uptake; thus, metal adsorption at the gill surface could have an important influence on total metal levels in fish. By contrast, the concentrations of heavy metals in the liver reflect the role of this organ in the long-term storage and detoxification of pollutants [1,33]. Therefore, gills and liver are often recommended as an index of environmental pollution than other fish organs. 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 $\mu g/g^{-1}$ [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] (5.5 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 [EDIs] 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.

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