Original Research

Levels of Particular Trace Elements in Some Tigris River's Fish Species and Evaluation of Their Health Risks

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Abstract

The concentrations of selected trace elements such as Iron (Fe), Lead (Pb), Manganese (Mn), Cadmium (Cd), Cobalt (Co), and Nickel (Ni) were assessed in the muscle, gill, and liver of three selective fish species, namely *Cyprinus carpio* (L, 1758)*, Aspius vorax* (Heckel, 1843)*, and Barbus xanthopterus* (Heckel, 1843)*.* Samples were collected between March 2022 and February 2023 from four strategically chosen sites spanning different areas of the river. The results indicate that the trace elements are clearly concentrated in the muscle and gill according to the following order: Fe>Pb>Mn>Cd>Co>Ni. However, in the liver, the results showed that these trace elements followed the concentration levels Fe>Pb>Cd>Mn>Co>Ni. This result confirms our findings that muscles, which are the edible portion of the fish for the Iraqi people, contain higher levels of Cd, which exceeded the permissible limits, especially in *Cyprinus carpio* in sites 1 and 2. *Barbus xanthopterus* has registered the highest concentration of Cd if compared to the safety limit in all sampled sites. Fe and Cd in the muscle and liver in *Cyprinus carpio* displayed significant differences between sites (ANOVA, $p<0.0001$). The canonical correspondence analysis (CCA) confirms our results that Co and Mn in site 3 were associated with the three fish species (*Cyprinus carpio* in muscle tissue*, Aspius vorax* in the liver, and *Barbus xanthopterus* in the gill). However, Cd, Co, Mn, and Ni in site 4 were associated with the liver and gill in *Cyprinus carpio* and with muscle tissue in *Aspius vorax*. The estimated daily intakes for all trace elements were lower than the tolerable daily intakes. Therefore, the consumption of fish species would not cause health problems for consumers. Whereas the total target hazard quotients (TTHQs) for *Barbus xanthopterus* from sites 2, 3, and 4 exceed one, this means that there may be a potential for adverse non–cancer health effects to occur. This proposes a potential health risk for individuals

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who consume large quantities of this fish species from these specific sites. These findings suggested that fish should be used, under a future project of biomonitoring programs, as bioindicators for trace element contamination and a relevant tool for water quality assessment in the Tigris River.

Keywords: bioindicators, fish tissues, Tigris river, trace elements, water pollution

Introduction

Trace element pollution is considered one of the most serious problems in aquatic environments due to several factors, such as domestic and agricultural activities, industrial wastewater, and mining [1, 2]. The trace elements can be characterized by their high stability, long lifetime, and ability to complexation with organic compounds [3]. Therefore, for all the reasons above, it is difficult to remove them [3]. Various kinds of contaminations have been affecting aquatic ecosystems around the world; in the recent few years, trace elements have been reported as one of the major pollutants that seriously deteriorate the aquatic ecosystems in the world [4]. Harmful trace elements, which are released from anthropogenic activities, accumulate in aquatic living organisms' beings through the trophic levels [5]. Hence, the urgent need to eliminate trace element pollutants from ecosystems emerged due to some aromatic herbs used in medicine, pharmaceutical products, and decorations. These herds are promising species for the phytoremediation of trace elements from soil and water due to their rapid growth and substantial biomass generation [6]. On the other hand, bioremediation with macro and microalgae is a potential way to minimize environmental degradation by deactivation of some dangerous compounds, making them suitable for phytoremediation as a safe way to reduce environmental pollution [7]. Furthermore, algae have an outstanding capacity to absorb, process, and sequester trace elements, reducing their contamination in aquatic and terrestrial environments. This has led to the increased use of algae in wastewater treatment, industrial effluent cleanup, and ecological restoration. Integrating algal systems with existing wastewater treatment infrastructure provides necessary protection for the sustainability of urban environments mainly polluted by trace elements [8].

Causes of freshwater pollution, like industrial and agricultural activities, pose serious risks to human health via trace elements, especially fish uptake [9]. In other words, fish tissue tends to store and accumulate trace elements [10]. There are two main routes to uptake trace elements by fish, which is considered a top of aquatic food. The first route is directly from the water through the gills, while the second is indirectly feeding through the food chain by ingestion of prey [11, 12]. The concentrations of trace elements can be significant in different parts of fish, either in organs or in tissues. This depends on the concentration in water and in the food chain of aquatic environments [13]. Because of trace elements' solubility in lipids and their resistance to several degenerative processes in tissue,

the accumulation of trace elements can be concentrated in different organs of fish more than in the surrounding water [14, 15]. In this regard, fish can be assumed to be a healthy food in the human diet due to their nutritional benefits, mainly proteins of high biological quality [16]. Furthermore, fish contain high nutritional benefits related to unsaturated omega fatty acids and proteins of high biological quality that contribute to human health. Fish are also considered a critical protein source and one of the most popular and widely consumed by people [17].

Several studies have shown that trace elements induce disastrous effects on aquatic animals, including fish, which are considered tools for the accumulation of trace element pollution in aquatic environments [13-18]. It appears that the Tigris River is exposed to trace elements pollution because of agricultural, domestic, medical, and industrial pollutants directly released into the river without industrial or biological treatments [1]. The main goal of this study is to assess the potential water pollution by analyzing the Fe, Pb, Mn, Cd, Co, and Ni trace elements and their bioaccumulation in the muscle, gill, and liver in three fish species: *Cyprinus carpio* (L, 1758*), Aspius vora* (Heckel, 1843), and *Barbus xanthopterus* (Heckel, 1843), which were collected from four sites in the Tigris River within Baghdad City. Most of the Iraqi studies conducted in the Tigris River in the last decades focused on the estimation of trace elements in fish species without assessing their potential health problems. This work highlighted the assessment of the potential health risks associated with these trace element contaminations. In order to evaluate the health risks of exposure to trace elements through fish consumption, the target hazard quotient (THQ) is measured and compared against the acceptable guideline. Accordingly, the estimated daily intake (EDI) of each element is compared against the tolerable daily intake (TDI) for the Iraqi people. Consequently, the possibility of the accreditation of fish species as bioindicators of trace elements contamination in the Tigris River is suggested.

Materials and Methods

Study Area

Fish samples were taken from four sites to analyze selective trace element contamination, including Fe, Pb, Mn, Cd, Co, and Ni in the muscle, liver, and gill tissues of fish from the Tigris River. The selected sites are distributed from the Northern side to the Southern side of Baghdad City as follows: Site 1 (S1) Al-Rashdea Area

(agricultural area), Site 2 (S2) Bab Al-Moazam Area (near medical hospitals), Site 3 (S3) Doura Area (near thermal power plant), and Site 4 (S4) Zafaraniya Area (before the confluence of Tigris with Diyala Rivers).

The details and locations of these sites are presented in Fig. 1. The vertical distance between S1 and S2 is nearly 25 km, the distance between S2 and S3 is about 9.3 km, and the distance between S3 and S4 is around 12 km (Fig. 1).

Sampling

Fish species were collected monthly from the four selected sites along the Tigris River in Baghdad City from March 2022 to February 2023 (Fig. 1). The fish samples were transferred immediately to the laboratory for dissection of the muscle, gill, and liver of each fish. All parts samples were separately packed and kept at (-20ºC) until analysis. Fish samples were identified according to the methodology used by Hump Hries et al. (2023) [19].

Samples Digestion

A part of each fish sample (muscle, gill, and liver) was isolated and placed into Falcon tubes (20 mL). Half a gram of each sample was placed in the digestion tubes. Triplicate samples were prepared from each of the specimens to reduce the error rate. A mixture of 7 mL HNO_3 (Product Number 98361, made in the United States of America) and 1 mL H_2O_2 (Product Number 141413, made in the United States of America) were added to each tube. Digestion was performed using a microwave digestion system (Milestone Start D, USA). Afterward, the tubes were allowed to cool to room temperature. Then, they were diluted with distilled water to 25 ml. The metals Pb, Cd, Fe, Co, Mn, and Ni were analyzed through flame atomic absorption spectrophotometry (AAS) (Perkin Elmer A-Analyst 200 instrument, copyright @ 2007, version 6 model Wavelength range: 189-900 nm). The procedure analyses were made in accordance with the Standard Methods of the American Public Health Association (APHA) for the examination of water and wastewater
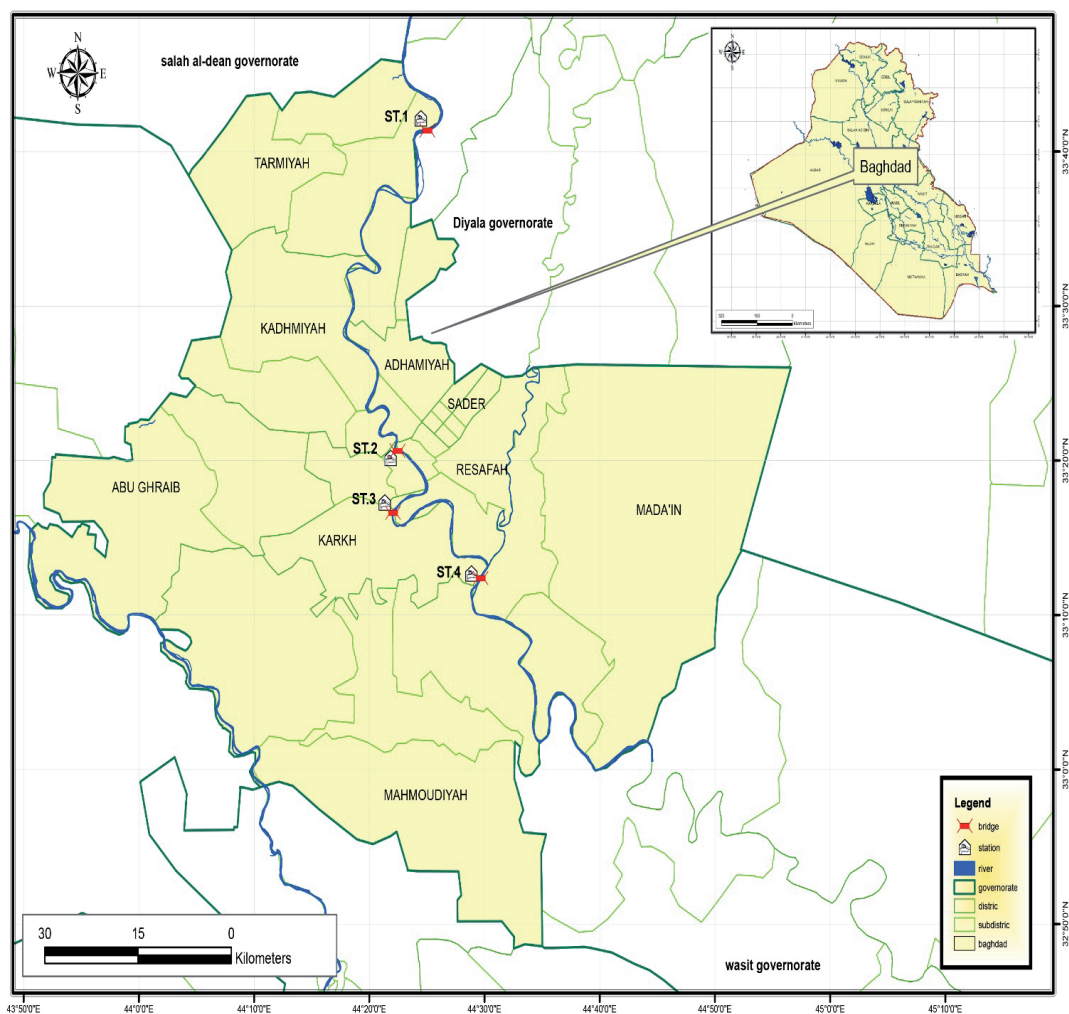

Fig. 1. Location of the studied sites in the Tigris River within Baghdad City Rashdea Area (site 1), Bab Al-Muazam Area (site 2), Al- Doura Area (Site 3), Al- Zafaraniya area (site 4) sampled during March 2022 to February 2023.

[20]. A blank solution was prepared for each type of fish that was analyzed but lacked any fish samples (muscle, gill, and liver). The aim of using a blank solution is to check for sample manipulation and contamination of the digestion procedure and to understand the pollution problem resulting from using chemicals during working conditions in the laboratory as correction values.

Data Processing and Statistical Analyses

The data processing and statistical analyses were conducted using the Analysis of Variance (ANOVA) test to find out if there is a significant difference in the trace elements of the four considered sites under investigation. The relationships between the different parameters were analyzed using a canonical correspondence analysis (CCA) achieved by the Past program. ANOVA and CCA were also carried out with the Excel (XL) Stat software using graph Pad Prism 9 Program.

Determination of Risk Factors

The estimated daily intake (EDI) and target hazard quotient (THQ) from three fish species (*Cyprinus carpio, Aspius vorax,* and *Barbus xanthopterus*) were used in this study. Specifically, the risk factors assessment methods were conducted based on estimating the concentrations of trace elements in only muscle tissues of fish species (the most commonly consumed part by the Iraqi people compared to other parts). The EDI Equation of [21, 22] is used

$$EDI = \frac{TEC \times IRd}{BW} \qquad (1)$$

TEC and IRd are the trace element concentration in the examined sample (mg kg^{-1} wet weight) and the daily fish ingestion rate (g day^{-1}), respectively. Referring to the data of the FAO [23], the average fish consumption is 2.6 g day^{-1} per adult in Iraq (all used values are based on wet weight) [23]. The BW is the average body weight of Iraqi adults (70 kg). The estimated EDI of each element was compared to the guideline of TDIs [24]. The non-cancer health risks, including the target hazard quotient (THQ) and total target hazard quotient (TTHQ) (or hazard index (HI), a dimensionless value), were also used in this study. THQ is essentially used to assess the potential for non-carcinogenic health risks associated with exposure to chemical pollutants of diverse toxicity profiles.

The standards of THQ are listed below:
THQ < 1: Exposure is unlikely to cause adverse health effects.
THQ = 1: Exposure may cause adverse health effects.
THQ > 1: Exposure is likely to cause adverse health effects.
Thus, the acceptable guideline for THQ and TTHQ is 1. Accordingly, if the THQ and TTHQ are greater than 1, this means that there may be a potential for adverse non–cancer health effects to occur. However, if the THQ and TTHQ are less than 1, non-cancer health effects are not expected [25]. The following Equations were used to calculate the THQ and TTHQ [26]:

$$THQ = \frac{EF \times ED \times IRd \times TEC}{RfD \times BW \times \times AT \, non \, cacer} \times 10^{-3} \qquad (2)$$

$$TTHQ \, (or \, HI) = THQ \, (Pb) + THQ(Cd)$$
$$+ \dots + THQ(Mn) + THQ(Ni) \qquad (3)$$

RfD is the oral reference dose for trace elements, ED is the exposure duration (26 years) [27], EF is exposure frequency (350 days year^{-1}) and AT is averaging time for non-carcinogens (365 days year^{-1} × 26 years) [25].

Results and Discussion

Trace Element Levels in Fish Species Tissues

The mean concentrations of trace elements in the muscle, liver, and gills of *Cyprinus carpio* are described in Fig. 2 and Table 1. The highest mean concentrations of trace elements revealed in these three parts of fish species showed that in the fish muscle, the trace elements of Pb, Mn, and Ni are detected with the highest mean concentrations of 10.30±7.68, 8.53±7.46, and 2.47±2.28 mg kg^{-1} on site 3, respectively.

However, the trace elements of Cd, Fe, and Co are distinguished by the highest mean concentrations of 2.31±1.38, 79.91±13.99, and 6.08±6.64 mg kg^{-1} on site 4, respectively. In the case of the liver, the highest mean values of Pb, Cd, and Mn are 26.16±21.08, 19.47±12.40, and 34.62±25.59 mg kg^{-1} on site 4, respectively, and that of Fe is 212.91±124.61 mg kg^{-1} on site 2. In the gill tissue, the highest mean concentrations of Pb, Cd, and Mn are 15.70±34.51, 2.31±5.59, and 3.80±2.66 mg kg^{-1} on site 2, respectively. The trace element levels in the muscles and gills are therefore distributed according to the following pattern: Fe>Pb>Mn>Cd>Co>Ni, while in the liver, they are distributed as follows: Fe>Pb>Cd>Mn>Co>Ni.

The highest detected mean concentrations of trace elements in three parts of the muscles, liver, and gills of *Aspius vorax* are represented in Table 2. The mean concentration of Cd in *Aspius vorax* varied among muscles, liver, and gills. The maximum mean concentration of Cd is about 20.18±28.57 mg kg^{-1}, which was detected in the gill (site 3); however, the minimum mean rate was observed in the muscle (0.97±0.89 mg kg^{-1}; site 1). The minimum mean concentration of Ni is 0.28±0.26 mg kg^{-1} in site 1 for muscle. The highest mean concentration of Fe is measured in the gill (319.83±81.01 mg kg^{-1}; site 2) (Table 2 and Fig. 3). For *Barbus xanthopterus* fish species, the highest values of Cd are 56.0, 28.3, and 32.0 mg kg^{-1} on site 2 for muscle, liver, and gill,
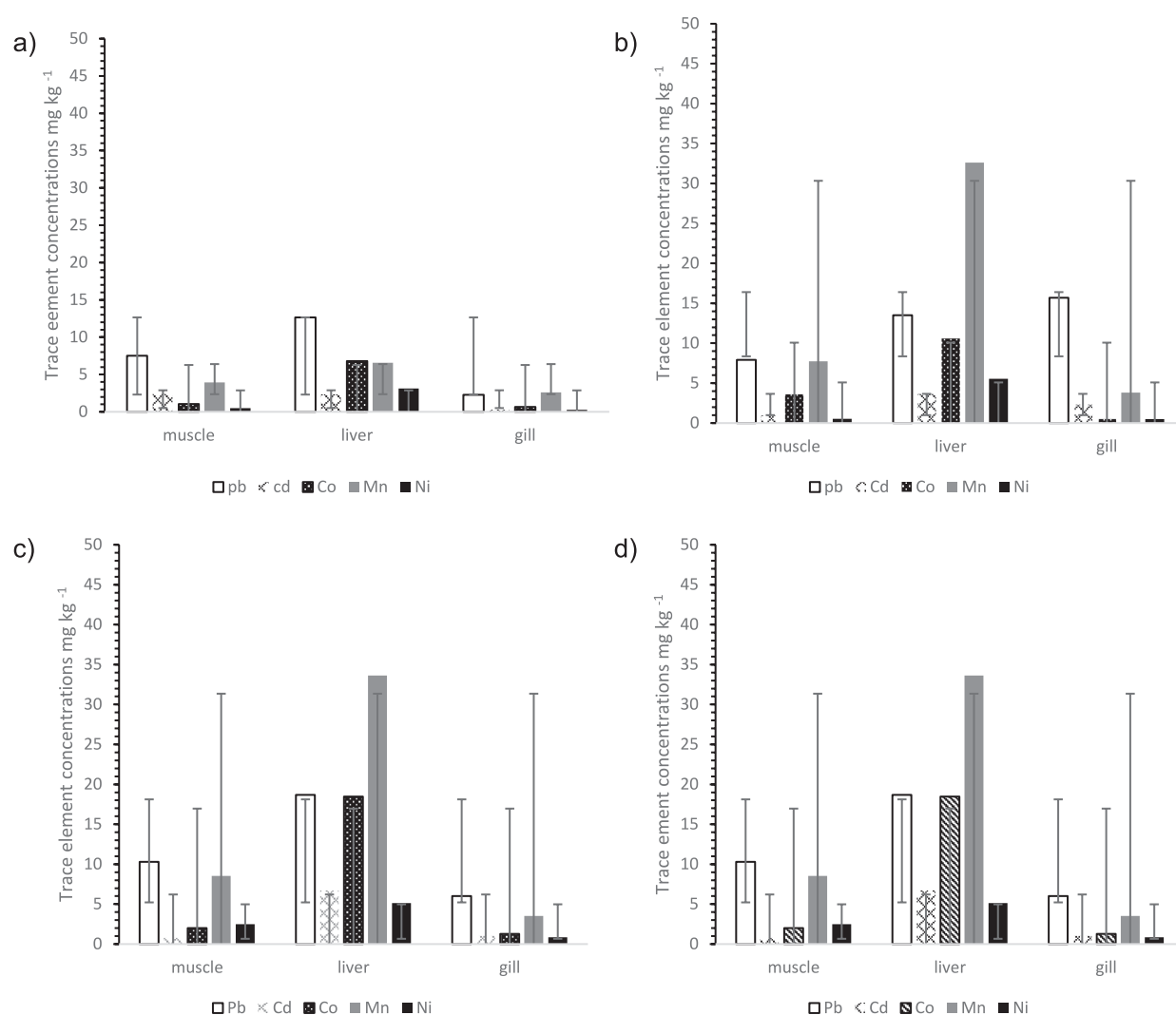
Fig. 2. Spatial variation of trace element levels in *Cyprinus carpio* fish species tissues caught in Tigris River of Baghdad City during March 2022 to February 2023: site 1 a), site 2 b), site 3 c) and site 4 d).

respectively (Table 3 and Fig. 4). The highest mean concentration of Pb was 24.81±14.87 mg kg^{-1} detected on site 4 for liver and 40.73±14.80 mg kg^{-1} on site 2 for gills, while 27.66±9.07 mg kg^{-1} on site 3 for muscles. The mean concentrations of Fe are about 107.33±27.49, 245.66±130.34, and 232.08±60.11 mg kg^{-1} observed on site 3 for muscle, liver, and gill, respectively. The highest mean concentrations of Mn are 4.00±1.75 mg kg^{-1} measured on site 4 for muscle and 14.07±9.11, 9.32±6.46 mg kg^{-1} on site 2 for liver and gill.

The trace element concentrations in the muscle, liver, and gill are therefore distributed according to the following pattern: Fe>Pb>Cd>Mn>Co>Ni (Table 3 and Fig. 4). In Iraq, muscles are considered the most edible portion of the fish; therefore, the mean concentrations of trace elements in fish muscle tissue should be compared against the international permissible limits. Our findings showed that the mean concentrations of tested trace elements detected in the muscle of fish species exceeded the maximum permissible limits for fish (Table 4). In this regard, the mean concentration of

Cd in *Cyprinus carpio* has been detected to be greater than the permissible limits by 64%. Furthermore, the highest mean concentration of Cd, which exceeds the permissible limits by 207%, was recorded in *Barbus xanthopterus* (Table 4). Moreover, the Mn trace element in the *Cyprinus carpio* is also detected to be over than the maximum permissible limits by 15.2%.

Table 1 presents the ANOVA results of trace element values of *Cyprinus carpio*. Significant differences are revealed between the tested sites. This can be stated as Fe and Cd (ANOVA, $P<0.0001$) in the muscle and liver, Mn and Co (ANOVA, $P<0.001$) in the liver and gills, and Ni (ANOVA, $P<0.05$) in the gills. In contrast, no significant differences among sites are found for Pb (Table 1). Trace element concentrations estimated in *Aspius vorax* indicate significant differences between sites, *i.e.*, Pb (ANOVA, $P<0.0001$) in muscles, Mn (ANOVA, $P<0.001$) in the liver and gills, Co and Ni (ANOVA, $P<0.05$) in muscles (Table 2). However, *Barbus xanthopterus* has significant differences between sites for most trace elements. In fact, Pb, Cd, Co, and Ni showed significant

Table 1. Mean values and standard deviation (S.D) of some trace element levels in muscle, liver, and gill of *Cyprinus carpio* fish species at sites sampled in Tigris River before and after Baghdad City during (March 2022 to February 2023), Lead (Pb), Cadmium (Cd), Iron (Fe), Cobalt (Co), Manganese (Mn), and Nickel (Ni), in the last column, results of one-way ANOVA. F values are found by dividing the between group variance by the within group variance. Asterisks denote significant difference between four sites at (12 months): * p<0.05; ** p<0.001 and ***p<0.0001. BLD: below detection limit.

Fish tissue	List of trace elements	Site 1			Site 2			Site 3			Site 4			F (values)
		Min	Max	Mean±S.D	Min	Max	Mean±S.D	Min	Max	Mean±S.D	Min	Max	Mean±S.D	
Muscle (mg kg⁻¹)	Fe	12.0	78.0	38.00±21.72	12.0	88.0	46.25±24.94	38.0	98.0	70.83±18.82	50.0	99.0	79.91±13.99	11.487 (<0.0001)***
	Pb	0.8	23.0	7.51±8.13	1.9	19.0	7.90±5.27	0.7	22.0	10.30±7.68	0.6	19.0	9.41±7.35	0.393 (0.758)
	Cd	0.4	9.0	2.30±2.39	0.23	2.40	1.00±0.65	0.09	3.00	0.82±0.83	0.6	5.3	2.31±1.38	3.865 (0.015)*
	Co	0.54	3.40	1.03±0.77	0.09	12.00	3.64±3.62	0.32	5.40	2.01±1.97	0.56	26.00	6.08±6.64	3.782 (0.017)*
	Mn	0.09	12.00	3.95±3.81	1.0	19.0	7.73±6.08	0.34	21.00	8.53±7.46	0.36	6.40	2.84±2.11	3.336 (0.028)*
	Ni	0.06	0.99	0.48±0.29	0.056	0.990	0.52±0.30	0.34	8.40	2.47±2.28	0.05	2.00	0.49±0.59	8.175 (<0.0001)***
Liver (mg kg⁻¹)	Fe	88.0	230.0	148.83±43.99	20.0	390.0	212.91±124.6	10.0	299.0	150.91±98.28	24.0	209.0	143.25±53.40	1.721 (0.177)
	Pb	1.0	27.0	12.63±9.57	0.40	29.00	13.51±10.13	1.30	37.0	18.69±14.80	2.5	67.0	26.16±21.08	2.159 (0.106)
	Cd	0.34	5.30	2.35±1.74	0.90	6.40	3.66±1.81	0.99	25.00	6.72±6.43	2.5	39.0	19.47±12.40	14.593 (<0.0001)***
	Co	0.01	19.00	6.80±5.55	0.3	29.0	10.59±8.61	10.20	25.10	18.45±3.90	0.37	30.1	11.61±8.79	5.730 (0.002)**
	Mn	0.66	18.90	6.57±6.46	17.1	72.0	32.60±16.59	0.76	89.0	33.62±23.62	0.43	85.00	34.62±25.59	5.757 (0.002)**
	Ni	0.11	22.00	3.11±6.18	0.43	15.00	5.53±4.70	0.14	15.20	5.13±5.56	1.0	20.1	6.93±6.27	0.915 (0.442)
Gill (mg kg⁻¹)	Fe	19.0	89.0	42.91±19.62	1.2	83.0	44.93±24.44	1.44	120.0	46.45±38.70	29.0	210.0	79.41±49.02	2.970 (0.042)*
	Pb	0.01	7.00	2.29±2.38	0.33	123.0	15.70±34.51	2.00	18.10	6.00±4.55	0.34	27.20	6.12±7.72	1.235 (0.309)
	Cd	0.15	0.99	0.31±0.228	0.2	20.0	2.31±5.59	0.1	4.2	1.05±1.31	0.29	3.40	0.97±1.04	0.975 (0.413)
	Co	0.09	3.50	0.65±0.93	0.09	0.98	0.55±0.31	0.43	3.10	1.28±0.77	0.09	4.20	1.77±1.23	5.085 (0.004)**
	Mn	0.04	14.20	2.60±3.92	0.25	8.50	3.80±2.66	0.23	10.0	3.51±4.20	0.24	4.30	1.84±1.11	0.923 (0.438)
	Ni	0.03	0.67	0.26±0.20	0.23	0.77	0.50±0.18	0.23	3.20	0.85±0.87	0.27	0.98	0.51±0.20	3.142 (0.035)*

Table 2. Mean values and standard deviation (S.D) of some trace element levels in muscle, liver, and gill of *Aspius vorax* fish species at sites sampled in Tigris River before and after Baghdad City during (March 2022 to February 2023), Lead (Pb), Cadmium (Cd), Cobalt (Co), Iron (Fe), Manganese (Mn), and Nickel (Ni), in the last column, results of one-way ANOVA. F values are found by dividing the between group variance by the within group variance. Asterisks denote significant difference between four sites at (12 months): * $p<0.05$; ** $p<0.001$ and ***$p<0.0001$. BLD: below detection limit.

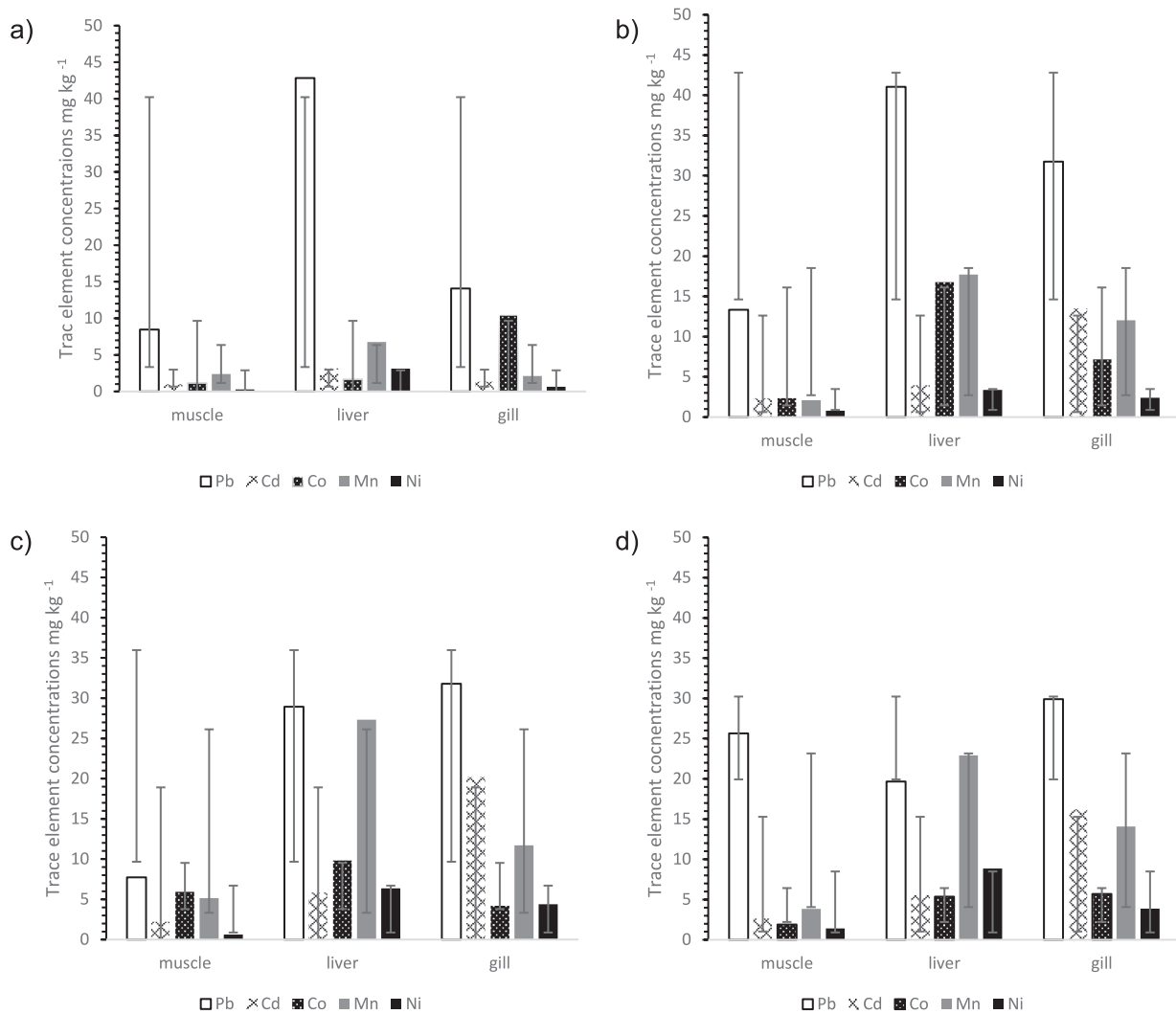| Fish tissue	List of trace elements	Site 1			Site 2			Site 3			Site 4			*F* (values)
		Min	Max	Mean ± S.D	Min	Max	Mean ± S.D	Min	Max	Mean ± S.D	Min	Max	Mean ± S.D	
Muscle (mg kg^{-1})	Fe	29.00	98.00	51.08±22.45	19.00	210.0	82.75±69.6	28.10	87.0	46.50±22.22	10.0	122.0	63.08±39.41	1.698 (0.181)
	Pb	0.44	20.00	8.45±5.87	3.20	34.00	13.35±9.39	0.30	21.1	7.72±5.02	0.05	54.00	25.65±15.00	8.843 (<0.0001)***
	Cd	0.09	3.20	0.97±0.89	0.29	5.30	2.67±2.039	0.038	8.30	2.25±2.71	0.39	7.30	2.68±2.76	1.400 (0.256)
	Co	0.009	4.200	1.15±1.49	0.09	7.30	2.38±2.14	0.07	20.0	5.95±7.57	0.29	6.20	1.89±2.15	3.169 (0.034)*
	Mn	0.09	12.30	2.38±4.18	0.09	6.20	2.10±1.72	1.10	13.2	5.14±3.77	0.29	10.20	3.83±3.53	2.005 (0.127)
	Ni	0.012	0.980	0.28±0.26	0.09	3.20	0.73±0.95	0.29	1.20	0.62±0.29	0.29	4.20	1.40±1.26	3.977 (0.014)*
Liver (mg kg^{-1})	Fe	40.0	322.0	160.83±77.63	105.0	302.0	190.58±71.9	45.0	324	202.66±07.16	109	405.0	254.41±86.04	2.432 (0.078)
	Pb	24.0	65.0	42.83±11.76	1.2	76.0	41.04±23.8	1.2	65.0	28.95±20.24	2.1	43.1	19.66±14.83	4.253 (0.010)*
	Cd	1.2	5.7	3.14±1.53	2.3	5.3	3.99±0.77	2.5	7.5	5.84±1.615	0.6	7.5	5.55±2.07	8.012 (<0.0001)***
	Co	0.09	5.80	1.64±1.65	4.3	39.0	16.77±8.24	0.99	21.0	9.81±5.38	2.40	13.10	5.35±3.42	18.221 (<0.0001)***
	Mn	0.9	19.0	6.75±5.44	1.4	52.0	17.71±17.16	11.0	62.0	27.33±14.32	3.0	43.0	22.91±11.50	5.709 (0.002)**
	Ni	0.32	8.30	3.11±2.83	0.23	7.50	3.36±2.33	0.04	13.2	6.35±4.90	0.5	64.0	8.83±17.51	1.027 (0.390)
Gill (mg kg^{-1})	Fe	10.0	107.0	74.58±31.85	209.0	497.0	319.83±81.0	20.0	294.	197.16±81.11	109	509	246.91±113.9	18.821 (<0.0001)***
	Pb	0.55	43.00	14.05±13.26	15.0	56.0	31.75±12.11	2.88	66.0	31.79±19.40	17.0	46.0	29.91±9.30	4.507 (0.008)**
	Cd	0.009	4.300	1.34±1.57	2.0	43.0	13.48±13.04	2.4	106.	20.18±28.57	2.4	35.6	16.21±10.25	2.891 (0.046)*
	Co	0.09	99.00	10.45±28.00	0.23	23.00	7.17±7.85	1.3	8.4	4.21±2.15	0.66	12.40	5.70±3.50	0.396 (0.756)
	Mn	0.09	12.00	2.12±3.37	3.2	34.0	12.04±9.41	3.0	23.0	11.70±6.15	1.3	23.3	14.07±7.75	6.923 (0.001)**
	Ni	0.09	2.00	0.64±0.60	0.39	5.30	2.41±1.61	1.4	8.5	4.40±2.22	2.4	7.8	3.85±1.63	12.777 (<0.0001)***

Fig. 3. Spatial variation of trace element levels in *Aspius vorax* fish species tissues caught in Tigris River of Baghdad City during March 2022 to February 2023 : site 1a), site 2 b), site 3 c) and site 4 d).

differences between sites (ANOVA, P<0.0001), while Fe and Ni have no significant differences (ANOVA, P>0.05) in the gills (Table 3).

Table 4 indicated that the Ni, Cd, Mn, and Pb trace elements in all fish species exceeded the safety limit. The same findings observed by Mensoor and Said (2018) proved high concentrations of these trace elements in the muscle, intestine, and gill tissues of *Barbus xanthopterus* living in the Tigris River in the Baghdad Region [28]. Jawad et al. (2022) stated that the concentrations of trace elements in the gill, liver and muscle of some fish species such as *Liza abu* and *Barbus xanthopterus* in Al-Masab Alamm River, Al Nassiriya, Iraq which exceeded the accepted levels of World Health Organization [29]. However, Al-Aboudi et al. (2022) indicated that the concentrations of Cd and Pb revealed in the muscles of *Cyprinus carpio* fish species collected from AL Gharraf River at Thi Qar city were lower than the maximum levels of the World Health Organization [30]. Furthermore, Al-Enazi and Lazim (2023) found that Fe had the highest accumulative rate among the

other studied elements in fish; the lowest accumulative rate was recorded with Pb [3].

Regarding trace element concentrations in the Tigris River within Baghdad City, several reasons can deduce this discrepancy between the current findings and the existing literature. First of all, trace element concentrations can fluctuate throughout the year due to changes in water flow, temperature, and precipitation patterns. Spatial heterogeneity is another vital reason why the trace element concentrations can significantly vary at different locations throughout the Tigris River. Indeed, different levels of pollution can be attributed to the different sampling sites. Levels of trace elements in fish can also be indirectly influenced by seasonal differences, biological differences (structure, composition, biological diversity, size of fish species, dark/white muscle, age, sex, and sexual maturity), and some environmental factors, i.e., salinity, temperature, pH, and pollution [31, 32].

Our findings show that these three fish species in sites 3 and 4 are frequently contaminated by massive

Table 3. Mean values and standard deviation (S.D) of some trace element levels in muscle, liver, and gill of (*Barbus xanthopterus*) fish species at sites sampled in Tigris River before and after Baghdad City during (March 2022 to February 2023), Lead (Pb), Cadmium (Cd), Iron (Fe), Cobalt (Co), Manganese (Mn), and Nickel (Ni), in the last column, results of one-way ANOVA. F values are found by dividing the between group variance by the within group variance. Asterisks denote significant difference between four sites at (12 months): * p<0.05; ** p<0.001 and ***p<0.0001. BLD: below detection limit.

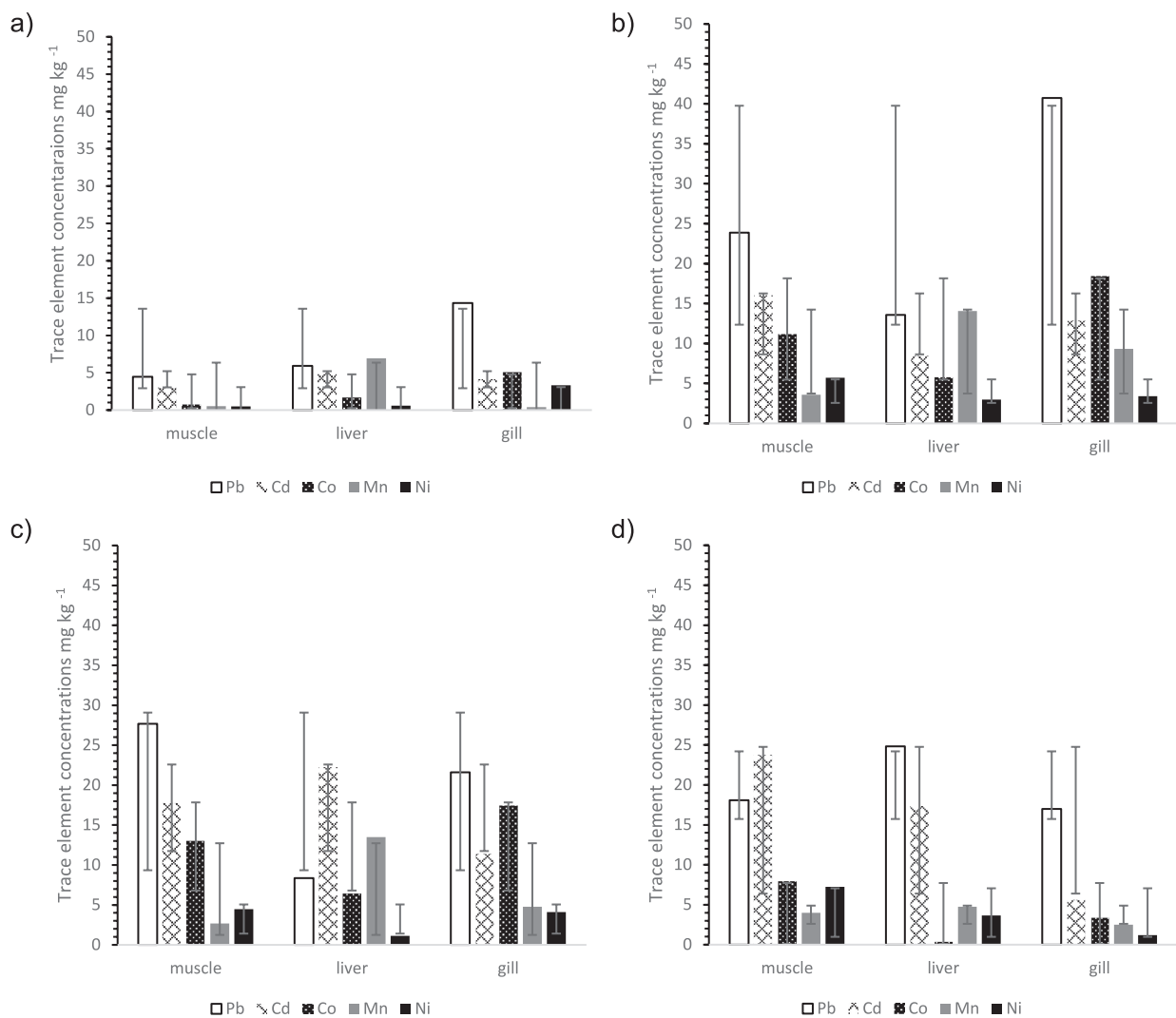| Fish tissue	List of trace elements	Site 1			Site 2			Site 3			Site 4			F (values)
		Min	Max	Mean±S.D	Min	Max	Mean±S.D	Min	Max	Mean±S.D	Min	Max	Mean±S.D	
Muscle (mg kg⁻¹)	Fe	34.0	99.0	64.83±25.13	34.0	102.0	74.16±25.49	49.0	144.0	107.33±27.49	20.0	191.0	95.66±42.59	4.717 (0.006)**
	Pb	1.9	8.6	4.47±2.06	6.4	45.0	23.8 6±13.21	14.0	45.0	27.66±9.07	2.0	38.0	18.08±9.38	14.179 (<0.0001)***
	Cd	2.3	6.3	3.06±1.17	0.88	56.00	16.04±15.1	12.0	26.0	17.77±4.41	13.0	34.0	23.75±8.20	11.506 (<0.0001)***
	Co	0.29	3.80	0.78±0.97	2.4	22.0	11.19±6.75	0.2	34.0	13.02±12.41	2.30	20.30	7.95±5.65	6.003 (0.002)**
	Mn	0.39	0.99	0.53±0.17	1.3	5.3	3.57±1.43	0.22	6.40	2.69±2.14	2.40	7.80	4.00±1.75	11.704 (<0.0001)***
	Ni	0.23	0.98	0.48±0.22	0.33	0.920	0.571±6.41	0.34	1.03	0.46±2.51	0.34	1.30	0.72±4.17	6.177 (0.001)**
Liver (mg kg⁻¹)	Fe	29.0	309.0	216.00±78.11	103.0	480.0	240.7±115.3	123.0	509.0	245.66±130.3	109.0	409.0	231.08±80.85	0.191 (0.902)
	Pb	1.7	10.4	5.93±3.22	2.6	35.0	13.58±10.82	2.4	23.3	8.36±6.61	4.3	54.0	24.81±14.87	8.607 (<0.0001)***
	Cd	0.99	9.50	5.20±2.28	0.79	28.30	8.44±7.46	0.4	54.2	22.25±15.50	1.3	43.0	17.35±11.74	6.756 (0.001)**
	Co	0.03	5.32	1.70±1.50	0.22	18.30	5.81±5.14	2.4	12.3	6.14±3.18	0.12	0.87	0.37±0.19	10.422 (<0.0001)***
	Mn	0.02	29.00	6.93±9.97	0.97	29.30	14.07±9.11	0.32	30.20	13.50±8.77	2.4	12.3	4.74±3.05	3.913 (0.015)*
	Ni	0.23	1.40	0.60±0.36	0.3	9.6	2.98±2.86	0.23	4.30	1.14±1.30	0.39	9.50	3.64±3.04	5.236 (0.004)**
Gill (mg kg⁻¹)	Fe	109.0	309.0	210.75±48.58	109.0	390.0	227.08±84.9	108.0	308.0	232.08±60.11	102.0	409.0	195.41±127.6	0. 451 (0.718)
	Pb	0.9	53.0	14.34±14.91	7.8	68.0	40.73±14.80	6.4	34.1	21.60±8.44	4.8	34.0	17.00±9.24	11.407 (<0.0001)***
	Cd	4.0	23.0	4.13±2.18	2.4	32.0	12.87±6.80	1.2	32.0	11.46±8.62	0.6	14.0	5.64±4.95	5.887 (0.002)**
	Co	2.4	8.5	5.08±2.26	2.4	45.0	18.45±10.41	0.99	43.00	17.44±13.61	0.4	11.0	3.39±.14	9.837 (<0.0001)***
	Mn	0.08	1.40	0.38±0.39	1.45	20.30	9.32±6.46	0.234	9.760	4.76±3.17	0.09	6.30	2.50±1.94	12.606 (<0.0001)***
	Ni	0.39	30.00	3.32±8.42	0.09	10.30	4.29±3.28	0.980	10.50	4.10±2.53	0.09	3.67	1.18±1.24	1.086 (0.365)

Fig. 4. Spatial variation of trace element levels *Barbus xanthopterus* fish species tissues caught in Tigris River of Baghdad City during March 2022 to February 2023: site 1 a), site 2 b), site 3 c) and site 4 d).

amounts of Cd, which derives from several external discrete sources, such as the industrial non-treated wastewater agricultural runoff and other anthropogenic inputs released directly to the Tigris River [33, 34]. The analysis of trace element concentrations showed that the greatest concentrations of Cd and Pb are revealed in muscles compared to the gill and liver. Cd binds to proteins with a high affinity for metallothionein, which is very abundant in muscle tissue. Thus, for Pb, the highest

concentration was found in muscles compared to other trace elements, whereas Mn was more concentrated in the gills than in muscles [35].

Our result indicates that the highest concentrations of Cd are observed in muscles; however, Rajeshkumar and Li (2018) showed that the greatest concentrations of trace elements are found in the gill and liver of fish species compared to the lowest concentrations in muscles [36]. According to Sow et al. (2019) [37], gills and the liver

Table 4. Mean concentrations of trace elements detected in muscle of fish species in present study and maximum permissible limits for fish with World Health Organization (mg kg^{-1}).

Fish species	Pb	Cd	Fe	Co	Mn	Ni	Reference
Cyprinus carpio,	8.79	1.64	58.75	3.19	5.76	0.98	Present study
Aspius vorax	8.45	0.97	51.08	1.15	2.39	0.28	Present study
Barbus xanthopterus	4.48	3.07	64.83	0.79	0.53	0.48	Present study
World health organization	30	1.0	-	-	5.0	1.0	(WHO, 2011)

are considered as active metabolic and target organs of trace elements because of their physiological role in fish metabolism. This is in comparison to the trace element levels of lower concentrations in muscles due to a lower level of metabolic activity in the muscles. Indeed, the gill is the first target for the entry of trace elements and the exposure organ. However, the liver plays an important role in several processes of trace elements like uptake, storage, and accumulation. The highest mean concentration of Cd in muscle of all fish species is revealed in site 4 of the Zafaraniya Area due to different effluents, which are released in a massive quantity, including wastewater from urban agglomerations and industrial activities. Site 3 of the Doura Area has reported the second highest levels of Cd because of its vicinity to high population density and discharge of untreated wastewater. In this regard, the highest concentration of trace elements is detected in gills as they stick to the lamellae and mucus [38]. However, the lowest concentrations of trace elements are accumulated in muscles because they are an inactive tissue for accumulating such metals [22]. All studies showed that Pb, Cd, Fe, Co, Mn, and Ni trace element concentrations were highly accumulated in the liver, which is the organ responsible for the detoxification of trace elements. In addition, liver and muscle tissues recorded nonessential trace elements such as Cd. This result explained that Cd is the usual bioaccumulated trace element in hepatic tissues [39]. Al-Enazi and Lazim (2023) [3] have obtained similar results with high contents of Fe, Pb, and Cd in the liver.

The concentrations of Fe in all fish species ranged between 10 to 497 mg kg^{-1} besides having the highest concentration in the muscle tissues (Table 1, 2, and 3). Due to the high concentration of iron in fish tissues, we kept it just in the tables and excluded it from the figures (Fig. 1, 2, and 3). Fish can absorb soluble iron from the water through gills or from the peritoneal cavity and store it in the liver, considering that Fe is an important trace element in the composition of vital tissues [23]. The expected reason is that iron is an essential element for many biological and physiological functions [23]. In this regard, Al-Enazi and Lazim (2023) reported similar results after conducting a specific study on three fish species: *Liza abu, Mesopotamichthys sharpeyi,* and *Carasobarbus luteus,* which are collected from the Tigris River. The study showed that Fe had the highest accumulation rate among other elements (141.145) [3]. The industrial wastewater released from oil factories can explain the high contamination of the Tigris River by Fe [3]. Manganese is highly identified in the fish samples of site 4 due to the discharge of domestic sewage and industrial effluents into the river [40]. Table 4 shows that high concentrations of Cd in muscle in *Cyprinus carpio* and *Barbus xanthopterus* can be associated with chemical fertilizers used in agricultural areas in the Tigris River [30].

Canonical Correspondence Analysis

The CCA plot, taking account of the studied trace elements for each fish species and sampling sites, explained 65.01% of total variables for the F1 axis, which selected positively Co and Mn. This result indicates that the highest mean concentrations of Co and Mn recorded in site 3 are associated with the three fish species (*Cyprinus carpio* muscle*, Aspius vorax* liver, and *Barbus xanthopterus* gill). Also, the mean concentrations of Cd, Co, Mn, and Ni in site 4 are associated with the liver and gill tissues of *Cyprinus carpio* and with the muscle of *Aspius vorax,* and are negatively selected with axis 1. The F2 axis explained 32.51% of the total tested parameters and selected the Pb trace element negatively, showing the greatest concentrations in site 2 in the liver of *Aspius vorax* and *Barbus xanthopterus* and in the gill of *Aspius vorax.* Furthermore, the 1 and 2 axes, which explained 65.01% and 32.51%, respectively, for the mean concentration of trace elements with fish tissues and study sites, selected the Pb, Fe, and Ni positively, showing the greatest concentrations in site 1 in the liver with *Barbus xanthopterus* and in the muscles with *Cyprinus carpio* (Fig. 5). The site 4, which is close to the south of Baghdad before the conjunction of Tigris with Diyala Rivers, has the highest values in Cd in water and sediment, which appeared in the liver, gill with *Cyprinus carpio* and in muscles with *Aspius vorax* (Table 1). The F1 axis has also selected positively *Aspius vorax* liver, *Barbus xanthopterus* gill, and *Cyprinus carpio* gill coupled with Co, Mn, and site 3 [41]. The high concentrations of Pb, Fe, and Ni trace elements in site 1, which is located near the northern Baghdad Gate and is known as an agricultural area with palm groves and orange trees. This explains the high amount of these trace elements in the muscle of *Cyprinus carpio* and in the liver of *Barbus xanthopterus,* due to the presence of this metal in fertilizers and pesticides [42]. Furthermore, the CCA analysis revealed that Pb and Fe trace elements reach the wastewater released from medical sources in site 2. These elements are more bioaccumulated in the liver of *Aspius vorax* and *Barbus xanthopterus* and in the gill of *Aspius vorax.* It should be noted that site 2 has several factories, such as the battery manufacturing plant, electrical industrial, and some power production plants [43].

Risk Factors Assessment

The estimated daily intake (EDI) and the tolerable daily intake (TDI) values of trace elements calculated for these three analyzed fish species are summarized in Table 5. The EDI of six trace elements ranged between 0.018 for Ni in site 4 to 2.957 for Fe in site 4 in *Cyprinus carpio.* However, the EDI of *Aspius vorax* varies from 0.01 for Ni in site 1 to 3.062 for Fe in site 2. Furthermore, the minimum EDI of *Barbus xanthopterus* is 0.018 for Ni in site 1, and the maximum value is 3.971 for Fe in site 3. These results show that the distribution of trace
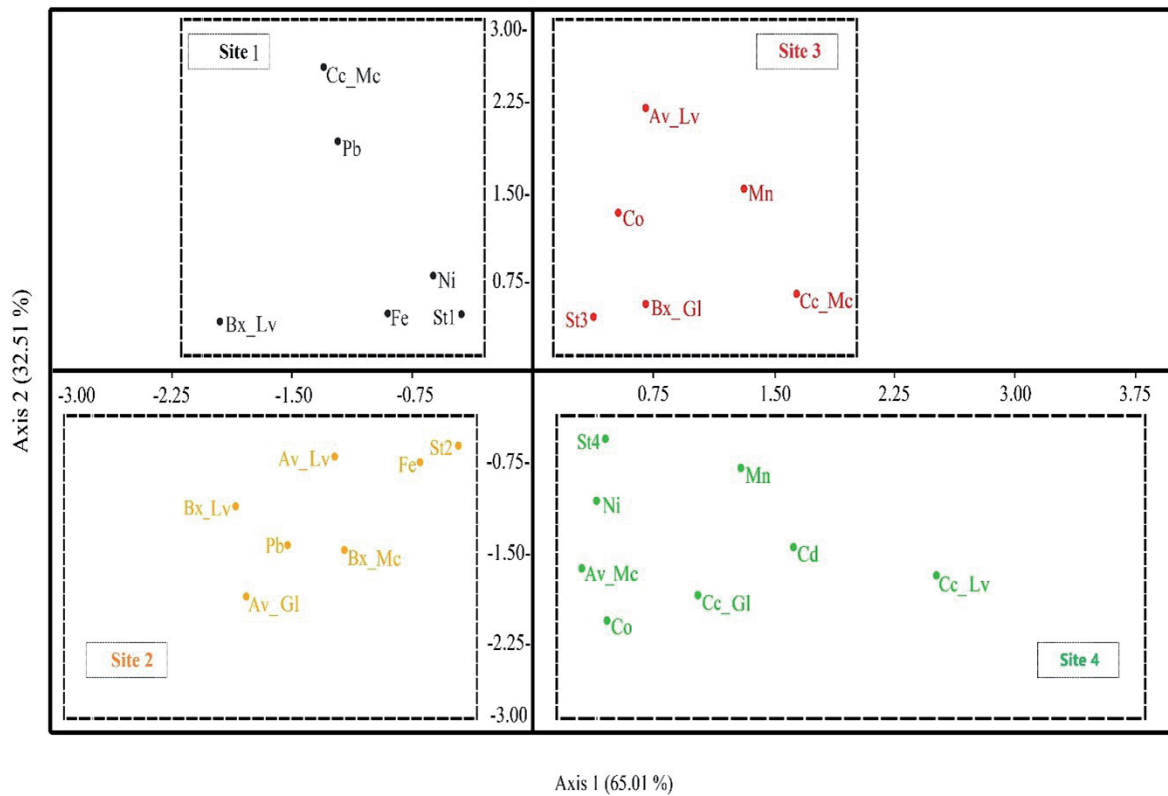
Fig. 5. Canonical correspondence analysis on mean values of several trace elements Fe (Iron), Pb (Lead), Cd (Cadmium),Co (Cobalt), Mn (Manganese) and Ni (Nickel) for three fish species Cc (*Cyprinus carpio*), Av (*Aspius vorax*), Bx (*Barbus xanthopterus*) in different fish tissues Mc (muscle), Lv (liver) and Gl (gill). Four sampled sites in the Tigris river within Baghdad City during March 2022 to February 2023 St1 (site 1), St2 (site 2), St3 (site 3) and St4 (site 4).

Table 5. Estimated daily intake (EDI) µg kg^{-1} body weight of different trace elements in muscle tissue of fish species at sites sampled in Tigris River before and after Baghdad City during (March 2022 to February 2023) comparison to the Tolerable daily intake (TDIs) µg kg^{-1} body weight.

Fish species	Locations	Pb	Cd	Fe	Co	Mn	Ni
Cyprinus carpio	Site 1	0.278	0.089	1.406	0.038	0.146	0.018
	Site 2	0.293	0.037	1.711	0.135	0.286	0.019
	Site 3	0.381	0.030	2.621	0.074	0.316	0.092
	Site 4	0.348	0.086	2.957	0.225	0.105	0.018
Aspius vorax	Site 1	0.313	0.036	1.890	0.043	0.088	0.010
	Site 2	0.494	0.099	3.062	0.088	0.078	0.027
	Site 3	0.286	0.083	1.721	0.220	0.190	0.023
	Site 4	0.949	0.099	2.334	0.070	0.142	0.052
Barbus xanthopterus	Site 1	0.166	0.113	2.399	0.029	0.020	0.018
	Site 2	0.883	0.593	2.744	0.414	0.132	0.212
	Site 3	1.024	0.658	3.971	0.482	0.100	0.165
	Site 4	0.669	0.879	3.540	0.294	0.148	0.267
TDIs (µg kg^{-1} bw^{-1} day^{-1}) WHO (2011)		3.57	1	8	1	1.8	1.53

Table 6. Target hazard quotient (THQ) and Total Hazard quotient (TTHQ) of different trace elements in fish species at sites sampled in Tigris River before and after Baghdad City during (March 2022 to February 2023). THQ is the target hazard quotient; TTHQ = HQ Pb + HQ Cd + HQ Fe+ HQ Co+ HQ Mn+ HQ Ni and RFD is the oral reference dose (mg kg^{-1} day^{-1}).

Fish species	Locations	Pb	Cd	Fe	Co	Mn	Ni	Total THQ *(TTHQ)*
Cyprinus carpio	Site 1	0.070	0.022	0.353	0.010	0.037	0.045	0.54
	Site 2	0.074	0.009	0.430	0.034	0.072	0.049	0.67
	Site 3	0.096	0.008	0.659	0.019	0.079	0.231	1.09
	Site 4	0.088	0.022	0.743	0.057	0.026	0.046	0.98
Aspius vorax	Site 1	0.079	0.009	0.475	0.011	0.022	0.026	0.10
	Site 2	0.124	0.025	0.770	0.022	0.020	0.068	0.17
	Site 3	0.072	0.021	0.433	0.055	0.048	0.058	0.11
	Site 4	0.239	0.025	0.587	0.018	0.036	0.130	0.17
Barbus xanthopterus	Site 1	0.042	0.028	0.603	0.007	0.005	0.045	0.73
	Site 2	0.222	0.149	0.690	0.104	0.033	0.532	1.73
	Site 3	0.257	0.165	0.998	0.121	0.025	0.415	1.98
	Site 4	0.168	0.221	0.890	0.074	0.037	0.672	2.06
RFD (WHO, 2011)		0.004	0.001	0.7	0.03	0.14	0.02	< 1

elements in fish species in comparison to TDI was insignificant (Table 5). Therefore, it is supposed that the daily intake of trace elements via the consumption of fish species would not cause health problems for consumers. The results of target hazard quotients (THQs) are provided in Table 6. This index was used to evaluate the individual trace elements throughout the consumption of these three fish species. The THQs of trace elements in each fish species from the Tigris River in Baghdad City have not overridden one through the consumption of fish, which theoretically demonstrates that humans do not acquire a major health hazard from individual trace element ingestion throughout the consumption of fish species. More addition, the total THQ consumption of *Barbus xanthopterus* from sites 2, 3, and 4 and *Cyprinus carpio* from site 3 are above one. This is an indication of possible health risks for all studied fish species in 2, 3, and 4 sites from the Iraqi consumption [44, 45].

Comparison Trace Element Concentrations with Other Aquatic Ecosystems

Trace element concentrations detected in the muscle, gill, and liver of the fish species in this study are compared with other investigation studies of riverine fish (Table 7). In a previous study performed in the Iraq Tigris River, Mensoor and Said (2018) recorded low mean concentrations of Cd and Pb in the muscle of *Barbus xanthopterus* [28, 31]. All fish species analyzed in this present study show high concentrations of Pb and Cd in muscle samples, whereas, Mustafa et al. (2020), recorded that high mean concentrations of

Table 7. Comparison of the trace element concentrations in present study with those of fish from other aquatic ecosystem.

Tissue	Location	Species	Pb	Cd	Fe	Co	Mn	Ni	Reference
Muscle	Tigris River, Iraq	*Cyprinus carpio*	8.79	1.64	58.75	3.19	5.76	1.00	Present study
	Tigris River, Iraq	*Aspius vorax*	8.45	0.97	51.08	1.15	2.39	0.28	Present study
	Tigris River, Iraq	*Barbus xanthopterus*	4.48	3.07	64.83	0.79	0.53	0.48	Present study
	Tigris River, Iraq	*Barbus xanthopterus*	1.10	0.80	-	-	-	-	Mensoor & Said (2018)
	Tigers River, Iraq	*Carasobarbus luteus*	44.22	22.16	-	-	-	-	Mustafa et al. (2020)
	Tigris River, Turkey	*Luciobarbus mystaceus*	-	0.083	-	-	0.856	0.657	Varol et al. (2020)
	Tigris River, Turkey	*Capoeta umbla*	-	0.065	-	-	1.146	0.795	Varol et al. (2020)
	Tigris River, Turkey	*Cyprinion macrostomus*	0.33	0.05	175	0.05	10.29	2.7	Töre et al. (2021)
	Xiang River, China	*Carassius auratus*	-	0.021	-	-	0.215	-	Jia et al. (2017)

Gill	Tigris River, Iraq	*Cyprinus carpio*	7.533	1.165	53.430	1.069	2.943	0.533	Present study
	Tigris River, Iraq	*Aspius vorax*	26.88	12.81	209.63	6.89	9.99	2.83	Present study
	Tigris River, Iraq	*Barbus xanthopterus*	23.42	8.53	216.33	11.09	4.24	3.23	Present study
	Tigers River, Iraq	(*Carasobarbus luteus*	36.50	32.42	-	-	-	-	Mustafa et al. (2020)
	Tigris River, Turkey	*Luciobarbus mystaceus*	-	-	-	-	-	-	Varol et al. (2020)
	Tigris River, Turkey	*Capoeta umbla*	-	0.102	-	-	15.464	1.520	Varol et al. (2020)
	(Wadi El- Arab, Jordan	*Cyprinus carpio*	-	0.70	-	-	-	-	Al-Weher (2008)
	Danube River, Serbia	*Cyprinus carpio*	-	0.03	-	0.0001	10.05	-	Subotic et al. (2013)
	Xiang River, China	*Carassius auratus*	-	0.078	-	-	7.241	-	Jia et al. (2017)
Liver	Tigris River, Iraq	*Cyprinus carpio*	17.75	8.05	163.98	11.87	26.86	5.18	Present study
	Tigris River, Iraq	*Aspius vorax*	33.12	4.63	202.13	8.40	18.68	5.42	Present study
	Tigris River, Iraq	*Barbus xanthopterus*	13.18	13.31	233.38	3.53	9.81	2.10	Present study
	Tigers River, Iraq	(*Carasobarbus luteus*	43.92	42.19	-	-	-	-	Mustafa et al. (2020)
	Tigris River, Turkey	*Luciobarbus mystaceus*	-	0.359	-	-	3.051	1.240	Varol et al. (2020)
	Tigris River, Turkey	*Capoeta umbla*	-	0.207	-	-	6.254	0.989	Varol et al. (2020)
	Danube River, Serbia	*Cyprinus carpio*	-	0.28	-	0.0001	2.21	-	Subotic et al. (2013)
	Xiang River, China	*Carassius auratus*	-	0.093	-	-	1.616	-	Jia et al. (2017)

Pb and Cd are observed in the muscle, gill, and liver of *Carasobarbus luteus* with comparison to *Capoeta umbla* collected from the Tigris River in Turkey [22, 41].

The *Barbus xanthopterus* in the present study has high mean concentrations of Cd in muscle, gill, and liver but low mean concentrations of Mn and Ni in muscle and liver (Table 7). Töre et al. (2021) [46] reported lower mean concentrations of Pb, Cd, and Co in the muscle of *Cyprinion macrostomus* from the Tigris River (Turkey) than those estimated in these three studied fish species, but high concentrations of Fe, Mn, and Ni (Table 7). On the other hand, Jia et al. (2017) [47] reported similar concentrations of Cd and Mn for muscle and liver tissue of *Carassius auratus* in Xiang River, China, but a high rate of Mn in gill.

Conclusions

The concentrations of Cd were found to be higher than the maximum permissible limits in *Cyprinus carpio* for s sites 1 and 2. However, the Cd concentration for *Aspius vorax* in site 1 appeared to be less than the stranded limit. The *Barbus xanthopterus* in all sites showed the most important concentration of Cd compared to the accepted limits. This finding proved that Mn of *Cyprinus carpio* in sites 2 and 3 and *Aspius vorax* in site 3 were found to be higher than the maximum permissible limits. The spatial analysis indicated significant differences of Pb, Cd, Co, Mn, Fe, and Ni in the muscle and liver of *Cyprinus carpio*. However, trace elements values analyzed in *Aspius vorax* indicated significant differences between sites, i.e., Pb in muscles. In this regard, significant differences can be distinguished between studied sites in *Barbus xanthopterus* for most trace elements i.e., Pb, Cd, Co, and Ni trace elements. The CCA proves that fish species should be considered as indicators of water quality, environmental changes of the anthropogenic kind, and origin inputs of pollutants.

Sites 1 and 4 are the most contaminated by Ni, Co, and Mn trace elements, mainly bioaccumulated in the muscle and liver of *Cyprinus carpio* and *Aspius vorax*. The Pb and Fe in muscles of *Cyprinus carpio* and in the liver of *Barbus xanthopterus* were negatively correlated with a tendency toward a greater concentration in sites 1 and 2, which are close to an agricultural area and medical hospital wastewater, respectively. The distribution of trace elements in fish species to the comparing TDI was very low. Therefore, it was supposed that the daily intake of trace elements via the consumption of fish species would not cause health problems for consumers.

The Total THQ consumption of *Barbus xanthopterus* from sites 2, 3, and 4 and *Cyprinus carpio* in site 3 were above one, reflecting a possible health risk for these two fish species in these sites to the Iraqi consumers. In conclusion, these results suggest that these three fish species can be used as bioindicators to trace elements contamination in the Tigris River. Therefore, further and urgent work research should be investigated in order to study other trace elements and their bioaccumulation in particular water, plant, phytoplankton, zooplankton, invertebrates, and some crustaceans are needed mainly in the Tigris River and in other polluted aquatic

environment of Iraq such as Euphrates River, Marshes, Lakes.

Acknowledgments

Conflict of Interest

The authors declare no conflict of interest.

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