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**Research Article**

# Determination of selected macro and microelements in muscle tissue of freshwater fish in Iraq

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**Abstract**

The concentration of four major (Na, K, Ca and Mg), seven essential (Cu, Zn, Fe, Mn, Cr, Ni and Co) and three toxic (Cd, Pb and Hg) elements were determined in muscle of nine freshwater fish species (*Mesopotamichthys sharpeyi*, *Luciobarbus xanthopterus*, *Luciobarbus grypus*, *Cyprinus carpio*, *Silurus triostegus*, *Planiliza abu*, *Leuciscus vorax*, *Luciobarbus schejch* and *Carasobarbus luteus*), that were purchased from local fish markets in central Iraqi cities (Baghdad, Hillah and Karbala) during April and May 2017. Atomic spectroscopy and stripping voltammetric technique were used to analyze the samples after microwave digestion. The highest concentration found in mg $kg^{-1}$dry weight was that of potassium (9014-10879) followed by sodium (999-2039), calcium (797-3081) and magnesium (1206-1819). The essential elements, Cu, Zn, Fe, Mn, Cr, Ni and Co were found at comparatively lower concentrations of less than 60 mg $kg^{-1}$ d.w., whereas average levels of the toxic elements Cd, Pb and Hg were generally very low ranging between 0.019 and 5.387 mg $kg^{-1}$ d.w. The average daily contribution of major and essential elements from fish to the Iraqi requirements were found to be 0.7-3.4% and 0.5-12.0% of the internationally recommended standards, respectively. Levels of the toxic elements Cd and Pb stayed behind permissible levels, while the level of mercury was above that level. Controlling agriculture and industrial effluents into the Euphrates–Tigris Basin of Iraq and proper sitting of pond waters to minimize the risk of contamination by heavy metals is highly recommended.

**Keywords:** Euphrates–Tigris Basin, Iraqi fish, Macro and microelements, Atomic spectroscopy, Stripping voltammetry

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## Introduction

Euphrates–Tigris Basin is the largest and most important river system between Nile and Indus rivers. The basin is shared mainly by four countries: Iraq, Syria, Turkey and Iran. The total area of Iraq is 438 320 $km^2$, of which more than 50 percent is comprised of the Euphrates and Tigris River Basin. More than 70 fish species are living in this Basin, which is a fairly rich range when compared with other freshwater ecoregions in the Near East and North Africa. In the upper Euphrates–Tigris basin, more than half of these species are of the family *Cyprinidae*, while the same family accounts for about 75 percent of all the species found in the lower Euphrates–Tigris Basin (FAO, 2014). The main species cultured in Iraq is common carp and to a lesser extent grass and silver carp. Khishni or Abukhraiza (*Planiliza abu*), forms about 16% of the total fish species marketed in 2003-2011. Barbus species are also cultured in small quantities (FAO, 2004; FAO, 2014).

Fish is well known to be a good nutritious with high protein content, low saturated fatty acids and high omega fatty acids content. Fish also has remarkable concentration of major elements such as calcium, magnesium, sodium, and potassium which are vital to human health and essential trace elements like Cu, Zn, Fe, Mn, Cr, Ni, Co, and Ni, which play key roles in proper functioning of enzyme systems, hemoglobin formation, and vitamin synthesis. Any drastic excess or deficit of these elements can disturb the biological functions (Łuczyñska *et al*., 2009).

Due to the rapid development of industry and agriculture activities, fish also have non-essential metals including cadmium, lead and mercury (Naz and Chatha, 2022) , which are taken up by fish and accumulate in their tissues (Amankwaa, *et al*., 2021) causing serious harm to fish consumers. The harmful effect of heavy metals can be neurotoxic, carcinogenic, mutagenic or teratogenic (Bosch *et al.*, 2016**)**.

In Iraq freshwater fishes are usually consumed without any information about their metallic contents. Wars, sanctions and general turmoil within Iraq over the past 30 years have led to a dramatic rise in contamination of aquatic systems by heavy metals. In spite of these challenges, little information has been published concerning the status of macro and microelements in freshwater fish in the Iraqi region of the Euphrates–Tigris Basin (Abaychi and Al-Saad, 1988). This study was undertaken to determine the concentration of Na, K, Ca, Mg, Cu, Zn, Fe, Mn, Cr, Ni, Co, Cd, Pb and Hg in the muscles of nine freshwater fish species mostly consumed in Iraq. Based on these levels, the total daily intake of these elements were evaluated and compared to the internationally permissible values. The information can be used to enhance the agricultural productivity and environmental protection in Iraq.

## Materials and methods

A total of 90 specimens belonging to nine mostly consumed freshwater fish species (Table 1), were purchased during April-May 2017 from local fish markets in central Iraqi cities (Baghdad, Hillah and Karbala). The chosen fish species are Bunni (*Mesopotamichthys sharpeyi*), Gattan (*Luciobarbus xanthopterus*), Shabbout (*Luciobarbus grypus*), Carp (*Cyprinus carpio*), Jerry (*Silurus triostegus*), Abu Khraiza (*Planiliza abu*), Shilik (*Leuciscus vorax*), Nebbash (*Luciobarbus schejch*) and Himri (*Carasobarbus luteus*). Similar sized fish were collected (0.90-1.10 kg in weight and 36.4–44.6 cm in total length) to minimize any difference in trace element concentration resulting from size. Muscle portions were selected for analysis, representing the most likely consumed tissue. For *Planiliza abu*, the entire edible part of each individual was included in the analysis.

**Table 1: Some biological features of fish in the Euphrates-Tigris Basin in Iraq (FAO, 2014).**

| Scientific name | Family name | FAO/English | Iraqi name | Sexual maturity/ Length/weight | Spawn | Sex ratio |
|---|---|---|---|---|---|---|
| *Cyprinus carpio* | Cyprinidae | Common carp | Carp | <1 year 15 cm/40 gm | Spring and Autumn | 1:1 |
| *Mesopotamichthys sharpeyi* | Cyprinidae | Weed barbel | Bunni | 3-4 years 40 cm/750 gm | May | 1:1 |
| *Luciobarbus xanthopterus* | Cyprinidae | Kersin | Gattan | 4 years 50 cm/1500 gm | April-May | 1:0.67 |
| *Luciobarbus grypus* | Cyprinidae | Rumi | Shabbout | 4 years 42 cm/2000 gm | April-May | 1.0:0.87 |
| *Silurus triostegus* | Siluridae | Euphrates wels | Jerry | 3 years 5 cm/1000 gm | May and continues for few months | 1:1.1 |
| *Planiliza abu* | Mugilidae | Euphrates mullet | Khishni, Abu Khraiza | 2 years 12 cm | April-May and Autumn | 1:0.93 |
| *Leuciscus vorax* | Cyprinidae | Oriental asp | Shilik | 4 years 40 cm/1000 gm | Spring and Autumn | 1:0.69 |
| *Luciobarbus schejch** | Cyprinidae | Yellow Kersin | Shabbout Orontis (Nebbash) | | | |
| *Carasobarbus luteus* | Cyprinidae | Brown barbel, bynny | Himri | 2.5 years 15 cm/45 gm | April-May and October | 1:1.5 |

*****This species is considered to be a junior synonym of *Luciobarbus pectoralis*, but that status needs confirmation (Freyhof, 2014).

The samples were transported to the laboratory in an ice box and washed thoroughly with distilled water. Bone and skin were separated and muscle was minced and kept in freezer at -20°C until taken for analysis.

From each individual fish, approximately 5 g was dried to constant weight at 80°C for 24 hours in acid-washed petri dish. The selected fishes had moisture content of 74.05 to 80.40% close to the range usually reported in literature (Lilly *et al.*, 2017). One gram of dried sample was placed in a Teflon vessel with 10 ml $HNO_3$ and 2 mL $H_2O_2$ and subjected to microwave digestion using CEM MARS-6 Closed Vessel Microwave Digestion System. Completely digested samples were filtered and diluted to 25 ml with double distilled water. A blank digest was carried out in the same way.

Calcium and magnesium were analyzed by flame atomic absorption spectrometry (Shimadzu, 1650) with deuterium background correction. The absorption wavelengths were 285.2 nm for magnesium and 422.7 nm for calcium. Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. In order to eliminate the influence of phosphorus during determination of calcium, $LaCl_3$ solution was added to all the samples and standards. Sodium and potassium were determined by emission flame photometry (Jenway, PFP7/ Camlab UK) at 589 nm and 766 nm, respectively.

The micro elements copper, zinc, iron, manganese, chrome, nickel, cobalt, cadmium, lead and mercury, were determined by stripping voltammetry using Metrohm 797 VA Trace Analyzer. Stripping voltammetry is superior to other trace analysis techniques as it works in presence of high salt concentrations. The details of instrument control, data acquisition and voltammetric data analysis were described elsewhere (Matloob, 2016) with certain modifications. The detection limits for Na, K, Ca, Mg, Cu, Zn, Fe, Mn, Cr, Ni, Co, Cd, Pb and Hg were 20, 20, 4.0, 8.0, 6.0, 6.0, 12.0, 10.0, 9.5, 4.0, 6.5, 0.6, 0.5 and 0.4 µg $L^{-1}$, respectively (AOAC, 2002; Matloob, 2016).

Accuracy and precision of the results were checked as described elsewhere (Matloob, 2016), by making use of the standard reference material of Oyster tissue 1566a (National Institute of Standards and Technology, Gaithersburg, MD, USA) for the 14 elements. Percent coefficient of variation (CV%) obtained for all the elements were ranging from 1.18 to 5.12%. The results of percentage recoveries for the studied elements were within the range of 87–114%. Both accuracy and precision data were acceptable by AOAC international guidelines (AOAC, 2002).

Concentrations of macro and microelements were reported on a dry weight basis (mg $kg^{-1}$ d.w.). Descriptive statistic (mean, standard deviation, median and interval) and analysis of variance (ANOVA) were conducted using SPSS 17 statistical package program. Logarithmic transformation was done on the data to improve normality. In order to verify if means obtained from macro and microelements of different fish species were statistically different at *p* level <0.05, the Tukey's multiple comparison test was applied. To determine correlation between the element pairs in tissues, Pearson's correlation coefficient matrix for the elements was applied.

To evaluate the average quantity of freshwater fish consumed by ordinary Iraqis, fifty undergraduate female students were requested to monitor the amount of fish consumed by their families during a six-month period. The average quantity of fish consumed after six months was found to be 32.5±8.9 g daily. The average daily intake of trace elements from fishes was calculated by multiplying the mean concentration of each element by the average consumption rate of ordinary Iraqis. The outcome was compared with internationally recommended levels.

## Results

The concentrations of Na, K, Ca, Mg, Cu, Zn, Fe, Mn, Cr, Ni, Co, Cd, Pb and Hg in muscle of the analyzed fish species are presented in Tables 2, 3 and 4. The median was slightly lower than mean concentration for all studied elements, reflecting asymmetry in distribution of concentrations toward values lower than the mean.

**Table 2: Major elements concentration* ($mg\ kg^{-1}$ dry weight) in muscle of 9 freshwater fish species widely consumed in Iraq.**

| Scientific name | Na | K | Ca | Mg |
|---|---|---|---|---|
| *Cyprinus carpio* | 999±271[a]<br>644-1370 | 8552±2242[a]<br>5286-11498 | 836±239[a, b]<br>499-1246 | 1206±151.7[a]<br>988-1414 |
| *Mesopotamichthys sharpeyi* | 1467±675[a]<br>950-2230 | 10470±1184[a]<br>9633-11307 | 797±69.8[b]<br>748-847 | 1604±15.56[a]<br>1593-1615 |
| *Luciobarbus xanthopterus* | 1578±182[a]<br>1380-1738 | 10158±2256[a]<br>8691-12756 | 1380±488[a, b]<br>882-1858 | 1588±472[a]<br>1043-1862 |
| *Luciobarbus grypus* | 1762±116[a]<br>1762-1991 | 10879±1250[a]<br>9440-11697 | 1924±259.9[a, b]<br>1767-2224 | 1819±109.1[a]<br>1700-1914 |
| *Silurus triostegus* | 2039±692[a]<br>1331-2713 | 8333±4465[a]<br>4605-13284 | 1110±65.6[a, b]<br>1036-1160 | 1520±384[a]<br>1220-1953 |
| *Planiliza abu* | 1691±329[a]<br>1390-2042 | 7960±2218[a]<br>5416-9496 | 1969±692.4[a, b]<br>1215-2576 | 1720±52.42[a]<br>1662-1764 |
| *Leuciscus vorax* | 1208±118[a]<br>1122-1342 | 9014±3227[a]<br>7011-12737 | 1845±291.3[a]<br>1639-2051 | 1795±281[a]<br>1511-2073 |
| *Luciobarbus schejch* | 1388±234[a]<br>1122-1560 | 8763±1137[a]<br>7746-9990 | 3081±560.7[a, b]<br>2684-3477 | 1563-221.6[a]<br>1417-1818 |
| *Carasobarbus luteus* | 1557±118[a]<br>1423-1645 | 10346±1455[a]<br>8226-11503 | 1495±1126[a, b]<br>526.2-2731 | 1692±154.9[a]<br>1552-1821 |
| Average/ $mg\ kg^{-1}$ d.w. | 1521±395 | 9386±2544 | 1604±561 | 1612±265 |
| Median/ $mg\ kg^{-1}$ d.w. | 1557 | 9014 | 1495 | 1604 |
| Range / $mg\ kg^{-1}$ d.w. | 999-2039 | 9014-10879 | 797-3081 | 1206-1819 |

*Values are expressed as mean ± standard deviation, number of samples analyzed for each species = 5. Equal letters in the same column are not significantly different at $p$ level<0.05.

The average macro element content of the nine fish species was identified to have the following decreasing sequence: K>Mg>Ca≈Na, whereas the concentrations of essential and toxic trace elements were noted in the following order: Fe≈Zn>Mn>Cu>Cr>Ni>Co and Hg>Pb>Cd, respectively ($p<0.05$).

The only statistically significant differences ($p\leq0.05$) were noted in the level of: Ca in Nabash and Binni, Zn in Shaboot and Gittan and in Himri and Gittan, Fe in Carp and Nabash and Mn in Shaboot and Carp (Tables 3 and 4).

**Table 3: Essential trace elements concentration* (mg $kg^{-1}$ dry weight) in muscle of 9 freshwater fish species widely consumed in Iraq.**

| Scientific name | Cu | Zn | Fe | Mn | Cr | Ni | Co |
|---|---|---|---|---|---|---|---|
| *Cyprinus carpio* | 2.76±0.48[a]<br>2.18-3.34 | 33.04±5.73[a. c]<br>25.45-42.09 | 40.90±10.19[b]<br>29.21-57.71 | 0.82±0.3[b]<br>0.53-1.26 | 0.135±0.069[a]<br>0.033-0.252 | 0.440±0.314[a]<br>0.174-0.878 | 0.014±0.004[a]<br>0.010-0.019 |
| *Mesopotamichthys sharpeyi* | 1.86±0.95[a]<br>0.33-3.29 | 23.17±4.55[a, c]<br>19.94-26.37 | 32.08±0.88[a, b]<br>31.45-32.70 | 2.54±1.00[a,b]<br>1.84-3.24 | 0.501±0.133[a]<br>0.407-0.595 | 0.405±0.230[a]<br>0.141-0.559 | 0.169±0.095[a]<br>0.102-0.236 |
| *Luciobarbus xanthopterus* | 2.91±1.58[a]<br>1.91-5.23 | 20.60±5.93[c]<br>13.85-26.67 | 43.02±13.04[a, b]<br>26.49-59.41 | 2.33±0.53[a,b]<br>1.59-2.81 | 0.251±0.098[a]<br>0.139-0.320 | 0.576±0.221[a]<br>0.319-0.705 | 0.050±0.049[a]<br>0.022-0.107 |
| *Luciobarbus grypus* | 1.69±0.60[a]<br>1.01-2.13 | 56.12±5.45[a]<br>50.36-61.52 | 26.69±7.50[a, b]<br>21.73-35.32 | 5.50±1.90[a]<br>3.63-7.42 | 0.840±0.581[a]<br>0.240-1.400 | 1.865±1.503[a]<br>0.802-2.927 | 0.057±0.002[a]<br>0.055-0.058 |
| *Silurus triostegus* | 2.07±0.70[a]<br>1.39-2.79 | 30.55±2.90[a, c]<br>27.27-32.77 | 46.69±12.19[a, b]<br>28.48-62.49 | 1.33±0.60[a,b]<br>0.77-1.88 | 0.534±0.383[a]<br>0.193-0.934 | 1.005±0.551[a]<br>0.615-1.394 | 0.075±0.011[a]<br>0.067-0.083 |
| *Planiliza abu* | 1.64±0.91[a]<br>0.97-1.98 | 35.83±6.52[a, c]<br>29.01-44.70 | 46.42±9.52[a, b]<br>38.68-60.31 | 2.47±1.61[a,b]<br>1.17-4.27 | 0.680±0.299[a]<br>0.397-0.992 | 0.576±0.042[a]<br>0.546-0.605 | 0.050±0.004[a]<br>0.047-0.053 |
| *Leuciscus vorax* | 1.70±0.47[a]<br>1.18-2.09 | 26.06±4.97[a. c]<br>20.06-30.83 | 28.67±6.36[a, b]<br>26.14-35.90 | 2.18±0.46[a,b]<br>1.78-2.81 | 0.315±0.098[a]<br>0.225-0.420 | 0.372±0.058[a]<br>0.335-0.438 | 0.015±0.004[a]<br>0.012-0.018 |
| *Luciobarbus schejch* | 1.86±0.63[a]<br>1.27-2.74 | 26.24±1.36[a, c]<br>22.00-34.28 | 20.42±5.99[a]<br>16.51-27.31 | 2.68±2.14[a,b]<br>1.05-4.07 | 0.325±0.286[a]<br>0.154-0.523 | 0.560±0.211[a]<br>0.334-0.753 | 0.038±0.025[a]<br>0.020-0.055 |
| *Carasobarbus luteus* | 3.73±1.37[a]<br>2.77-5.30 | 51.80±11.86[a, b]<br>38.76-61.72 | 47.33±11.95[a, b]<br>33.68-55.92 | 2.48±1.32[a,b]<br>1.13-3.80 | 0.205±0.096[a]<br>0.115-0.357 | 1.029±0.978[a]<br>0.401-2.156 | 0.054±0.019[a]<br>0.041-0.076 |
| Average/mg $kg^{-1}$ d.w. | 2.25±0.99 | 33.71±6.49 | 36.91±9.93 | 2.48±1.35 | 0.421±0.297 | 0.759±0.686 | 0.058±0.040 |
| Median/ mg $kg^{-1}$ d.w. | 1.86 | 30.55 | 40.90 | 2.47 | 0.325 | 0.576 | 0.050 |
| Range / mg $kg^{-1}$ d.w. | 1.64-3.73 | 20.60-56.12 | 20.42-47.33 | 0.82-5.50 | 0.135-0.840 | 0.372-1.865 | 0.014-0.169 |

*Values are expressed as mean ± standard deviation, number of samples analyzed for each species=5. Equal letters in the same column are not significantly different at *p* level<0.05.

**Table 4: Toxic trace element concentration* (mg/$kg^{-1}$ dry weight) in muscle of 9 freshwater fish species widely consumed in Iraq.**

| Scientific name | Cd | Pb | Hg |
|---|---|---|---|
| *Cyprinus carpio* Linnaeus | 0.031±0.028[a]<br>0.012-0.077 | 0.794±0.309[a]<br>0.330-2.215 | 1.681±0.772[a]<br>1.203-2.572 |
| *Mesopotamichthys sharpeyi* | 0.067±0.044[a]<br>0.014-0.119 | 0.493±0.209[a]<br>0.203-0.782 | 2.964±1.250[a]<br>2.080-3.848 |
| *Luciobarbus xanthopterus* | 0.019±0.007[a]<br>0.013-0.027 | 0.777±0.272[a]<br>0.472-0.995 | 1.753±0.670[a]<br>1.133-2.464 |
| *Luciobarbus grypus* | 0.050±0.042[a]<br>0.022-0.112 | 1.235±0.507[a]<br>0.785-1.880 | 4.231±1.351[a]<br>2.684-5.180 |

**Table 4 (continued):**

| Scientific name | Cd | Pb | Hg |
|---|---|---|---|
| *Silurus triostegus* | 0.080±0.035$^{a}$<br>0.056-0.120 | 0.800±0.521$^{a}$<br>0.435-1.396 | 2.571±1.571$^{a}$<br>1.080-4.212 |
| *Planiliza abu* | 0.032±0.013$^{a}$<br>0.024-0.051 | 1.148±0.590$^{a}$<br>0.334-2.098 | 5.387±2.029$^{a}$<br>3.165-7.142 |
| **Scientific name** | **Cd** | **Pb** | **Hg** |
| *Leuciscus vorax* | 0.058±0.035$^{a}$<br>0.018-0.191 | 1.152±0.428$^{a}$<br>0.845-1.455 | 2.743±1.774$^{a}$<br>0.648-4.168 |
| *Luciobarbus schejch* | 0.023±0.009$^{a}$<br>0.014-0.032 | 0.445±0.205$^{a}$<br>0.233-0.688 | 2.122±1.829$^{a}$<br>0.507-0.988 |
| *Carasobarbus luteus* | 0.032±0.012$^{a}$<br>0.023-0.048 | 1.198±0.665$^{a}$<br>0.181-2.324 | 3.162±0.855$^{a}$<br>2.262-3.964 |
| Average/ mg kg$^{-1}$ | 0.044±0.030 | 0.894±0.469 | 2.957±1.510 |
| Median/ mg kg$^{-1}$ | 0.032 | 0.800 | 2.743 |
| Range / mg kg$^{-1}$ | 0.019-0.080 | 0.445-1.235 | 1.681-5.387 |

*Values are expressed as mean ± standard deviation, number of samples analyzed for each species=5. Equal letters in the same column are not significantly different at *p* level<0.05.

Values of correlation coefficients between the metal contents in all tested fish species are given in Table 5. Various degrees of significant correlations (mostly at $p<0.001$) were found between the 14 analyzed elements. High positive correlations (≥0.500) were found between Ca-Fe, Cu-Fe, Mn-K, Mn-Zn, Cr-Na, Cr-Mg, Cr-Cu, Cr-Mn, Ni-Na, Ni-Zn, Ni-Mn, Ni-Cr, Pb-Mg, Pb-Zn, Hg-Mg, Hg-Zn, Hg-Mn, Hg-Cr and Hg-Pb.

**Table 5: Pearson correlation coefficient matrix and level of significance* for element concentrations in 9 fish species consumed in Iraq.**

| | Na | K | Ca | Mg | Cu | Zn | Fe | Mn | Cr | Ni | Co | Cd | Pb | Hg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Na | 1.00 | | | | | | | | | | | | | |
| K | 0.09$^{c}$ | 1.00 | | | | | | | | | | | | |
| Ca | 0.02 | -0.17$^{c}$ | 1.00 | | | | | | | | | | | |
| Mg | 0.41 | 0.38$^{c}$ | 0.42 | 1.00 | | | | | | | | | | |
| Cu | -0.15$^{c}$ | 0.28$^{c}$ | -0.34$^{c}$ | -0.33$^{c}$ | 1.00 | | | | | | | | | |
| Zn | 0.25$^{c}$ | 0.35$^{c}$ | 0.09$^{c}$ | 0.34$^{c}$ | 0.22$^{c}$ | 1.00 | | | | | | | | |
| Fe | 0.32$^{c}$ | -0.23$^{c}$ | -0.57$^{c}$ | -0.23$^{c}$ | 0.56$^{c}$ | 0.10 | 1.00 | | | | | | | |
| Mn | 0.33$^{c}$ | 0.63$^{c}$ | 0.41$^{c}$ | 0.71$^{c}$ | -0.32 | 0.58$^{c}$ | -0.49$^{c}$ | 1.00 | | | | | | |
| Cr | 0.66$^{c}$ | 0.11$^{c}$ | 0.17$^{c}$ | 0.56$^{c}$ | 0.68$^{c}$ | 0.38$^{c}$ | -0.16$^{c}$ | 0.68$^{b}$ | 1.00 | | | | | |
| Ni | 0.59$^{c}$ | 0.47$^{c}$ | 0.12$^{c}$ | 0.40$^{c}$ | -0.01$^{c}$ | 0.82$^{c}$ | -0.05$^{c}$ | 0.74$^{b}$ | 0.60$^{a}$ | 1.00 | | | | |
| Co | 0.35$^{c}$ | 0.42$^{c}$ | -0.40$^{c}$ | 0.11$^{c}$ | -0.16$^{c}$ | -0.16$^{c}$ | -0.01$^{c}$ | 0.14$^{c}$ | 0.34$^{b}$ | 0.00$^{b}$ | 1.00 | | | |
| Cd | 0.39$^{c}$ | 0.01$^{c}$ | -0.41$^{c}$ | 0.16$^{c}$ | -0.42$^{c}$ | 0.04$^{c}$ | -0.02$^{c}$ | -0.02$^{c}$ | 0.41$^{b}$ | 0.17$^{b}$ | 0.50 | 1.00 | | |
| Pb | 0.18$^{c}$ | 0.08$^{c}$ | 0.02$^{c}$ | 0.55$^{c}$ | 0.10$^{c}$ | 0.70$^{c}$ | 0.30$^{c}$ | 0.37$^{c}$ | 0.29$^{b}$ | 0.49 | -0.42$^{c}$ | 0.00$^{c}$ | 1.00 | |
| Hg | 0.44$^{c}$ | -0.02$^{c}$ | 0.21$^{c}$ | 0.64$^{c}$ | -0.41 | 0.53$^{c}$ | 0.12$^{c}$ | 0.52 | 0.78$^{c}$ | 0.39$^{c}$ | 0.15$^{c}$ | 0.11$^{c}$ | 0.60$^{c}$ | 1.00 |

*Level of significance: a $p<0.05$; b $p<0.01$; c $p<0.001$.

## Discussion

Considerable variations in the elemental concentration (Tables 2 to 4) among different fish species are usually attributed to interactions between physiological factors (absorption, accumulation and growth), chemical factors (metal concentration, bioavailability and speciation) and environmental factors (temperature and food concentration) (Casas and Bacher, 2006). The macro and micro essential elements K, Na, Ca, Mg, Zn, and Fe were the most abundant elements in the studied species, whereas concentrations of non-essential elements, like Cd and Pb were the lowest. These results are consistent with almost all published studies (except in limited cases) that the accumulation levels of essential metals in fish are generally higher and more homeostatic than non-essential metals (Wong *et al*., 2001; Pourang *et al*., 2004).

*Comparison of macro and microelements level with published data*

A notable number of surveys are carried out in different countries to determine and compare concentrations of metals in various fish species and to estimate human exposure by their consumption (Bosch *et al*., 2016). Unfortunately, there is not enough data concerning level of macro and microelements in freshwater fishes in Iraq for comparative purpose. For this reason, the current results were compared with recently published data for freshwater species only. The results are not compared with marine fishes (saltwater fish) because most marine organisms have evolved different mechanisms to maintain osmoregulation in their tissues in presence of variable concentrations of ions in the ambient water, sediments, and food. These mechanisms may lead to eliminate some ions with intensified absorption of other ions by the organism (Niemiec *et al.*, 2016).

The concentration of each macro and microelement in freshwater fishes are described below and compared to the open literature listed in Tables 6 and 7. Many published articles have expressed element concentrations on a wet weight basis. For easy and direct comparison, a dry/wet weight conversion factor of 4.8 was used to convert all the wet into dry weight basis (Lilly *et al.*, 2017).

**Table 6: Comparison of average macro element contents (mg kg$^{-1}$ dry weight) in muscle of river fish consumed in Iraq with freshwater fish of other areas.**

| **Country: Fishing site**<br>**Fish species**<br>**(Reference)** | **Na** | **K** | **Ca** | **Mg** |
|---|---|---|---|---|
| **Iraq:** Euphrates–Tigris basin<br>*M. sharpeyi, L. xanthopterus, L. grypus, C. carpio, S. triostegus, P. abu, L. vorax, L. schejch, C. luteus.*<br>(Present work) | 999-2039 | 9014-10879 | 797-3081 | 988-1414 |
| **Poland:** Mazurian Great Lakes<br>*Rutilus rutilus, Abramis brama, Coregonus albula, Esox lucius, Perca fluviatilis, Lota lota.*<br>(Łuczyñska *et al.*, 2009) | 1483-4613 | 14298-23870 | 437-1048 | 827-1430 |
| **Poland:** commercial fish farms<br>*Onchorhynchus mykiss, C. carpio, Acipneser baerii.*<br>(Brucka-Jastrzębska *et al.*, 2009)* | - | - | 956-1331 | 586-1229 |

| Country: Fishing site<br>Fish species<br>(Reference) | Na | K | Ca | Mg |
|---|---|---|---|---|
| **Turkey:** Beyşehir Lake<br>*Pseudophoxinus anatolicus, Alburnus akili, Squalius lepidus, Gobio microlepidotus, Carassius gibelio, C. carpio, Scardinius erythrophthalmus, Tinca tinca, Sander lucioperca.*<br>(Özparlak *et al.*, 2012) | 221-424 | 263-514 | 66.33-538.83 | 142.76-283.06 |
| **Russia:** Bugach pond<br>*C. gibelio, P. fluviatilis.*<br>(Gladyshev *et al.*, 2001) | 2740-2840 | 15890-17030 | 1910-2020 | 1290-1360 |

*a dry/wet weight conversion factor of 4.8 was used.

**Table 7: Comparison of average microelement contents (mg kg$^{-1}$d.w.) in muscles of freshwater fish consumed in Iraq with other different areas.**

| Country: Fishing site<br>Fish species<br>(Reference) | Cu | Zn | Fe | Mn | Cr | Ni | Co | Cd | Pb | Hg |
|---|---|---|---|---|---|---|---|---|---|---|
| **Iraq:** Euphrates–Tigris basin<br>*M. sharpeyi, L. xanthopterus, L. grypus, C. carpio, S. triostegus, P. abu, L. vorax, L. schejch, C. luteus.*<br>(Present Work, 2017) | 1.64-3.73 | 20.16-56.12 | 20.42-47.33 | 0.82-5.50 | 0.135-0.840 | 0.28-5.90 | 0.014-0.169 | 0.019-0.080 | 0.445-1.235 | 1.681-5.387 |
| **Iraq:** Different water sources in south of Iraq<br>*M. sharpeyi, L. xanthopterus, L. grypus, C. carpio, L. vorax, L. schejch.*<br>(Abaychi and Al-Saad, 1988) | 3.9-10.3 | 9.3-13.8 | 46.7-68.8 | 1.2-7.3 | 4.2-7.4 | 1.5-4.0 | 0.5-0.8 | nd-0.02 | 0.16-0.82 | - |
| **Iran:** Wetland<br>*L. grypus, C. luteus, M. sharpeyi, C. carpio, P. abu, S. triostegus.*<br>(Hosseini Alhashemi *et al.*, 2012) | 0.77-8.22 | 10.06-90.14 | - | 0.49-11.21 | 0.1-2.6 | 0.35-5.70 | 0.20-5.31 | 0.001-0.57 | 0.81-14.55 | - |
| **Poland:** Mazurian Great Lakes<br>*R. rutilus, A. brama, C. albula, E. lucius, P. fluviatilis, L. lota.*<br>(Łuczyñska *et al.*, 2009) | 0.6-2.7 | 17-58.5 | 4-11.3 | 0.4-1.2 | - | - | - | - | - | - |
| **Poland:** commercial fish farms*<br>*O. mykiss, C. carpio, A. baerii.*<br>(Brucka-Jastrzębska *et al.*, 2009) | 1.92-4.32 | 29.18- 38.88 | 22.08-43.68 | - | - | - | - | - | - | - |

| **Country: Fishing site Fish species (Reference)** | **Cu** | **Zn** | **Fe** | **Mn** | **Cr** | **Ni** | **Co** | **Cd** | **Pb** | **Hg** |
|---|---|---|---|---|---|---|---|---|---|---|
| **USA:** River Creek Missouri* *Moxostoma duquesnei, Hypentelium nigricans, Moxostoma erythrurum, Lepomis megalotis, Micropterus salmoides, Micropterus. dolomieu.* (Gale *et al.*, 2004) | 1.15-2.23 | 71.52-237.6 | - | - | - | - | - | 0.029-1.147 | 0.192-62.64 | - |
| **Country: Fishing site Fish species (Reference)** | Cu | Zn | Fe | Mn | Cr | Ni | Co | Cd | Pb | Hg |
| **Malaysia:** Kelantan River *Barbonymus gonionotus, B. schwanenfeldii, Chitala chitala, Clarias gariepinus, Cyclocheilichthys apogon, Hampala macrolepidota, Hemibagrus nemurus, H. wyckii, Notopterus notopterus, Osteochilus vittatus, Phalacronotus micronemus, Puntioplites bulu, T. maculatus.* (Hashim *et al.*, 2014) | - | - | - | - | - | 0.024-0.183 | - | 0.013-0.053 | 0.022-0.156 | - |
| **India:** ponds in Tamil Nadu *C. carpio.* (Vinodhini and Narayanan, 2008) | - | - | - | - | 1.083 | 0.633 | - | 0.646 | 1.46 | - |
| **India:** Cauvery River *Channa striata, C. catla, Oreochromis mossambicus, Etroplus suratensis, Mystus vittatus, Cirrhinus mrigala.* (Dhanakumar *et al.*, 2015) | BDL-0.04 | 11.57-47.23 | 9.42-108.10 | BDL-6.12 | 0.5-1.56 | 0.20-0.42 | - | 0.29-2.92 | - | - |
| **China**[**]**:** Yangtze River basin (Yi *et al.*, 2011) | 1.73-90.05 | 3.81-243 | - | - | nd-3.864 | - | - | 0.005-9.60 | 0.053- 48.5 | nd-0.259 |
| **Turkey:** Ataturk Dam Lake *S. triostegus, Acanthobrama marmid, Leuciscus vorax, Capoeta trutta, Carasobarbus. luteus, Alburnus mossulensis, C. carpio.* (Mol *et al.*, 2010) | 0.101-2.785 | 10.27-19.74 | - | - | - | - | - | nd | nd-0.236 | nd-0.649 |

| **Country: Fishing site Fish species (Reference)** | **Cu** | **Zn** | **Fe** | **Mn** | **Cr** | **Ni** | **Co** | **Cd** | **Pb** | **Hg** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Turkey:** Ataturk Dam Lake* *P. abu, S. triostegus.* (Karadede *et al.*, 2004) | 3.94-31.15 | 8.93-97.97 | 2.35-99.12 | 0.34-3.79 | - | nd-5.14 | nd | - | - | - |
| **Turkey:** Saricay stream, Milas *Squalius cephalus, Lepomis gibbosus.* (Yılmaz *et al.*, 2007) | 0.10-5.28 | 16.56-72.34 | 5.93-7.39 | 0.269-7.49 | - | Nd | nd-0.965 | 0.005-0.187 | 0.144-4.205 | - |
| **Country: Fishing site Fish species (Reference)** | Cu | Zn | Fe | Mn | Cr | Ni | Co | Cd | Pb | Hg |
| **Turkey:** Keban Dam* *C. carpio, Luciobarbus esocinus, Capoeta trutta, C. umbla, Luciobarbus mystaceus, O. mykiss.* (Varol and Sünbül, 2017) | 1.39-44.45 | 19.30-171.4 | - | - | - | - | - | 0.001-0.010 | 0.014-0.538 | - |
| **Nigeria:** Asa River* *Coptodon zilli*, *Synodontis membranaceus.* (Eletta *et al.*, 2003) | - | 26.98-72.35 | 18.19-89.3 | 2.69-12.91 | 0.43-1.68 | Nd | - | nd | 3.74-7.54 | nd |
| **Spain:** water reservoirs* *M. salmoides, T. tinca.* (Belo *et al.*, 2007) | 7.30-13.82 | 32.88-34.99 | 22.32-24.19 | 1.58-2.59 | - | - | - | 0.14-0.19 | 2.40-2.54 | - |
| **France:** River Seine R. rutilus, *P. fluviatilis*. (Chevreuil *et al.*, 1995) | 1.4-1.9 | 48-120 | 64-90 | 4.8-5.6 | 0.3-1.3 | - | - | 0.05-0.46 | 0.1-4.8 | - |
| **Hungary:** Lake Balaton *Abramis brama.* (Farkas *et al.*, 2003) | 1.77-2.22 | 10.9-14.5 | - | - | - | - | - | 0.42-0.61 | 0.44-1.63 | 0.10-0.19 |
| **Russia:** Bugach pond *C. gibelio, P. fluviatilis.* (Gladyshev *et al.*, 2001) | 4.34-5.1 | 50.84-104.1 | 44.81-68.86 | 2.38-7.83 | 0.36-1.13 | 0.46-2.17 | - | <0.1 | 0.12-0.21 | - |

| **Country: Fishing site**<br>**Fish species**<br>**(Reference)** | **Cu** | **Zn** | **Fe** | **Mn** | **Cr** | **Ni** | **Co** | **Cd** | **Pb** | **Hg** |
|---|---|---|---|---|---|---|---|---|---|---|
| **Korea:** Freshwater aquaculture<br>*Anguilla japonica, C. carpio, O. mykiss, Tachysurus fulvidraco, Silurus asotus.*<br>(Choi *et al*., 2016)* | - | - | - | - | - | - | - | 0.000-0.672 | 0.000- .243 | 0.192-2.290 |
| **Bangladesh:** Buriganga River<br>*Heteropneustes fossilis*<br>(Begum *et al*., 2013) | 7.80-8.50 | 24.47-28.82 | - | - | 1.40-1.70 | - | - | 0.3-0.4 | 1.79-2.20 | - |
| **Country: Fishing site**<br>**Fish species**<br>**(Reference)** | Cu | Zn | Fe | Mn | Cr | Ni | Co | Cd | Pb | Hg |
| **Bosnia:** Neretva River*<br>*Salmo trutta fario, C. carpio, Squalis cephalus, C. gibelio, S. erythrophthalmus, Mugil cephalus.*<br>(Djedjibegovic *et al*., 2012) | 0.326-77.08 | - | - | - | - | - | - | 0.062-0.264 | 0.264-3.374 | 0.230-1.925 |

BDL: Below detection limit; nd: not determined; LOD: Limit of detection;
*a dry/wet weight conversion factor of 4.8 was used.
** Fish species studied by Yi *et al.*(2011) were: *Mylopharingodon piceus, Ctenopharyngodon idella, Squaliobarbus curriculus, Hypophthalmichthys molitrix, H. nobilis, C. carpio, C. auratus, Sinibrama macrops, Chandichthys erythropterus, C. mongolicus, Sarcocheilichthys nigripinnis, Coreius heterodon, C. guichenoti, Rhinogobio cylindricus, R. ventralis, R. typus, Platysmacheilus longibarbatus, Leptobotia elongata, Misgurnus anguillicaudatus, Tachysurus fulvidraco, T. dumerili, Hemibagrus macropterus, Silurus asotus, Chondrostoma nasus, Coilia brachygnathus, C. mystus, Anguilla japonica, Siniperca scherzeri, Odontobutis obscurus, Channa argus, Hyporhamphus intermedius, Collichthys lucidus, Pampus argenteus, Cynoglossus robustus, and crustaceans Palaemon carinicauda and Macrobrachium nipponense.*

*Major elements*

Fish and fish products are important sources of macronutrients, such as sodium, potassium, phosphorus, calcium and magnesium. Potassium is directly connected with sodium in intercellular and intracellular fluids, respectively. They play important physiological roles, including regulation of fluid balance, membrane potential, and muscle contraction (Whelton and He, 2014). Among the nine investigated fishes, the most abundant major element in fishes was potassium. As shown in Table 3 potassium was found within the range of 9014-10879 mg $kg^{-1}$ d.w. in all fish species compared to sodium which ranged from 999 to 2039 mg $kg^{-1}$ d.w. These levels are remarkably higher than those reported in Table 6 by Özparlak *et al.* (2012) for Turkish Beyşehir Lake fish, but within or slightly lower than the values determined by Łuczyñska *et al.* (2009) for Polish commercial fish farms and Gladyshev *et al.* (2001) for Russian Bugach pond.

Calcium is known to be an essential element required for numerous functions in human body including strengthening of skeleton, nerve function, hormonal effects, and many enzymatic reactions that require calcium as a cofactor (FAO/WHO, 2004). The range of calcium content in all samples was between 797 and 3081 mg $kg^{-1}$ d.w. Amount of calcium varied from one fish species to another with a maximum value of 3081±560.7 mg $kg^{-1}$ d.w. found in *L. schejch* and minimum value of 797±69.8 mg $kg^{-1}$ d.w. observed in *M. sharpeyi*. The average Ca level recorded in this study (1604±561 mg $kg^{-1}$ d.w.) lied within or close to the ranges reported in Table 6 by Gladyshev *et al.* (2001), Brucka-Jastrzębska *et al.* (2009) and Łuczyñska *et al.* (2009), while much higher than those determined by Özparlak *et al.* (2012) for Turkish Beyşehir Lake fish.

Magnesium is another essential element the body requires; it is a cofactor of many enzymes involved in energy metabolism, protein synthesis, DNA and RNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance is its role in regulating potassium fluxes and its involvement in metabolism of calcium (FAO/WHO, 2004). In the present work magnesium varied within a relatively narrow range of 1206-1819 mg $kg^{-1}$ d.w. in all fish species. Similar to calcium, the average Mg levels recorded in this study (1612±265 mg $kg^{-1}$ d.w.) lied within or close to the ranges reported by Gladyshev *et al.* (2001), Brucka- Jastrzębska *et al.* (2009) and Łuczyñska *et al.* (2009), whereas much higher than the levels (142.76-283.06 mg $kg^{-1}$ d.w.) reported by Özparlak *et al.* (2012) for Turkish Beyşehir Lake fish.

*Essential trace elements*

The essential trace elements have high affinities for ligands containing sulphur and nitrogen, and hence are bound easily to organic molecules, such as proteins and enzymes. The amount of these elements in the organism does not exceed the level which allows the enzyme system to function properly without interference.

Iron (Fe) is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes, including oxygen transport, electron transport and deoxyribonucleic acid (DNA) synthesis (Abbaspour *et al.*, 2014). As shown in Table 3 the concentrations of Fe in fish species ranged from 20.42 mg $kg^{-1}$ d.w. in *L. schejch* to 47.33 mg $kg^{-1}$ d.w. in *C. luteus*. These levels were close to those of 6 out of 11 published values (Table 7) that ranged from 2.03 to 108.10 mg $kg^{-1}$ d.w. As it is usually suggested these so different Fe contents may reflect different fish species, their quality and difference in locations of sampling area as well as difference in sampling depth.

Zinc (Zn) concentrations in fish muscle samples ranged in this work from 20.60 mg $kg^{-1}$ d.w. in *L. zanthopterus* and 56.12 mg $kg^{-1}$ d.w. in *L. grypus*. These levels were higher than the concentration range (9.3-13.8 mg $kg^{-1}$) recorded by Abaychi and Al-Saad in 1988 for the same type of fish species in Iraq. Similar to Fe the published values of zinc reported in Table 7 varied considerably from 8.2 to 243 mg $kg^{-1}$ d.w. It was found to be more than 100 mg $kg^{-1}$ d.w. in 4 out of 18 published articles. In this study the average zinc content was within the range reported in 9 out of 18 articles.

Adequate zinc nutrition is essential for human health because of zinc's critical structural and functional roles in 300 enzyme systems that are involved in gene expression, cell division and growth and immunologic and reproductive functions (Hess *et al.*, 2009).

Copper **(**Cu**)** concentrations in fish muscle samples ranged from 1.64 mg $kg^{-1}$ d.w. in *P. abu* to 3.73 mg $kg^{-1}$ d.w. in *C. luteus*. The copper levels recorded in this study were close to the range reported in Table 7 by 13 out of 18 published articles and far lower than 4 out of 18. Copper is an essential trace element in both humans and animals. It is a cofactor of many redox enzymes and involved in a numerous biological processes, including antioxidant defense, neuropeptide synthesis and immune function. Dietary Cu deficiency can result in adverse consequences throughout life period (Bost *et al.*, 2016).

Manganese (Mn) is an essential metal for the human diet, as it is required for proper immune function, regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, blood coagulation and hemostasis, and defense against reactive oxygen species. The beneficial effects of Mn are due to the incorporation of the metal into metalloproteins. Although necessary for life, Mn is toxic when it is in excess. Thus, maintaining appropriate levels of intracellular Mn is critical (Horning *et al.*, 2015). Compared to Fe, manganese recorded lower concentrations in all the fish samples. The concentration ranged from 0.82 to 5.50 mg $kg^{-1}$ d.w.; the lowest concentration of 0.82 mg $kg^{-1}$ d.w.; was measured in *C. carpio* while the highest concentration; 5.50 mg $kg^{-1}$ d.w. was measured in *L. grypus*. For comparison, the concentration range of Mn in this work was narrow and close to the published values in Table 7.

Chromium (Cr) is an essential nutrient involved in the metabolism of glucose, insulin and blood lipids. Long term deficiency of chromium may cause an increase in risk factors associated with diabetes and cardiovascular diseases (Anderson, 2000). The average concentrations of Cr in the nine fish samples were 0.421±0.297 mg $kg^{-1}$ d.w. The lowest detectable concentration; 0.135 mg $kg^{-1}$ d.w. was measured in *C. carpio* while the highest concentration; 0.840 mg $kg^{-1}$ d.w. was measured in *L. grypus*. These levels were within the range (nd-3.864 mg $kg^{-1}$ d.w.) given by 8 out of 10 published articles while lower than the range (4.2-7.4 mg $kg^{-1}$ d.w.) recorded by Abaychi and Al-Saad (1988) for fish species from Iraq and (9.85-24.45 mg $kg^{-1}$ d.w.) by Özparlak *et al*. (2012) for fish species from Turkey.

Nickel (Ni) was recorded in very low concentrations in the samples relative to other essential heavy metals. The average concentration of Ni in the samples was 0.759±0.686 mg $kg^{-1}$ d.w. The highest concentration, 1.865 mg $kg^{-1}$ d.w., was measured in *L. grypus* with the lowest detectable concentration of 0.372 mg $kg^{-1}$ d.w. measured in *L. vorax.* These values were generally within the range (nd-5.70 mg $kg^{-1}$ d.w.) given in the literature and slightly lower than those reported (1.50-4.00 mg $kg^{-1}$ d.w.) earlier by Abaychi and Al-Saad in 1988 for freshwater fishes from the same basin (Table 7).

Nickel's essentiality in higher organisms is questionable. No enzymes or cofactors that include nickel are known in higher organisms. This led to the uncertainty in deciding whether nickel is essential to humans. Despite the uncertainty nickel is believed to play a role in physiological processes as a co-factor in the absorption of iron from the intestine (Das *et al*., 2008). However, Ni accumulation in the environment may represent a serious hazard to human health. Among the known health related effects of nickel are skin allergies, lung fibrosis, variable degrees of kidney and cardiovascular system poisoning and stimulation of neoplastic transformation (Denkhaus and Salnikow, 2002).

Cobalt (Co) was detected in narrow range of 0.014– 0.169 mg $kg^{-1}$ d.w. in all the analyzed samples. The highest concentration (0.169 mg $kg^{-1}$ d.w.) was found in Binni (*M. sharpeyi*) while other samples had very low Co concentrations. Few articles in the literature were interested in determining the cobalt levels in freshwater fishes. The concentration range recorded in Table 7 was nd-5.9 mg $kg^{-1}$ d.w., which was higher than the levels in the present work. The physiological role of cobalt in humans is based on its role in cobalamin (vitamin $B_{12}$). Cobalamin acts as a cofactor for two enzymes in humans: methylmalonyl-CoA mutase and methionine synthase. Both enzymes are important for normal formation of red blood cells and for nerve tissue health (Yamada, 2013).

*Toxic elements*

Fish are important aquatic organisms that are used as bio-indicators of aquatic ecosystems for estimation of heavy metal pollution and risk potential for human consumption. Accumulation of heavy metals in fish results primarily from surface contact with the water, by breathing, and via the food chain. Uptake by these three routes depends on environmental levels of heavy metals in the habitat of the fish. Liver is the tissue accumulating highest level of essential and non-essential metal concentrations. It is a storage and detoxification organ for the metals and an organ where enzyme-catalyzed reaction related to these metals take place (Dhanakumar *et al*., 2015). On the other hand, the muscle tended to accumulate less metal. This is generally attributed to the presence of mucous layer (coating the fish skin surface) that serves as a barrier protecting the integrity of fish muscle tissues by forming complexes with heavy metals (Schlenk and Benson, 2001).

Yi *et al.* (2011) reported that concentration of heavy metals in the sediment and fish were 1000-100,000 and 10-1000 times higher than those in the water respectively. Due to this high concentration gradient, heavy metals of the sediment may be absorbed strongly by fish and accumulated in multiple organs (Choi *et al.*, 2016). Toxicity is implied when the concentration of trace elements available for metabolic activity may aggravate potential risk to the organism if not controlled or detoxified (Rainbow, 1997). Most organisms maintain an ideal concentration for a specific element essential for regular biological functioning, but when there is an excess of this element within the internal environment it can cause adverse effects to the organism and require regulation. The same approach may be applied to elements not required for necessary biological functions. Rainbow (1997) describes two mechanisms by which organisms are able to detoxify extra metals. One involves metallothionein binding with trace elements, thereby making the trace metal unavailable to tissues within the organism. The second approach is to encapsulate the elements in a bond through calcium granules, which make them unavailable to the internal environment and the organism is able to excrete them. However, controlling the sources of contamination of water and sediments in the aquatic system is the key method for protection of the fish resource.

Lead (Pb) has a serious effect on brain and intellectual progress in children, while long term exposure in both adults and children may also cause damage to kidneys and reproductive and immune systems (FSAI, 2009). Table 4 shows that Pb concentration in muscle ranged from 0.445 to 1.235 mg $kg^{-1}$ d.w. These values were within the range reported by 15 out of 18 published articles presented in Table 7. Lead values found in this study were much lower than those reported for freshwater fish species from China (0.053-48.5 mg $kg^{-1}$d.w., Yi *et al*., 2011), USA (0.192-62.64 mg $kg^{-1}$d.w., Gale *et al.*, 2004), and Iran (0.81-14.53 mg $kg^{-1}$d.w., Hosseini Alhashemi *et al.*, 2012). In general, concentration of Pb in Iraqi fish is relatively low reflecting uncontaminated freshwater ecosystems. High Pb contents recorded in the literature can be found in freshwater fishing areas receiving discharges of untreated industrial waste water or areas close to traffic polluted sites.

Cadmium (Cd) is considered as one of the most ecotoxic metals that exhibit highly adverse effects on mitochondrial structure and function on DNA, and on gene expression. The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage and skeletal changes in occupationally exposed populations (Pinot *et al.*, 2000). The lowest concentration of Cd (0.019±0.007 mg $kg^{-1}$d.w.) was measured in *L. xanthopterus* while the highest concentration (0.080±0.035 mg $kg^{-1}$) was measured in *S. triostegus*. The

Cd values recorded in this study were relatively very low. It is close to the range reported in 15 out of 19 published articles in Table 7, but significantly lowers than the other four. Actually, cadmium content in fish muscle tissue is generally very low, being preferentially accumulated in kidney and liver, which should not be consumed. However, comparing with previous data reported by Abaychi and Al-Saad (1988), cadmium and lead concentrations in fish from the Euphrates–Tigris basin were slightly increased from 1988 to 2017, indicating that heavy metal concentrations in the basin increased during that period.

Mercury (Hg) and its compounds (organic and inorganic) are highly toxic, the most common being methyl mercury. Excessive exposure to mercury is associated with a wide spectrum of adverse health effects, including damage to the central nervous system and the kidney (Dufault *et al*., 2009; Bosch *et al*., 2016). The primary source of mercury contamination in man are through eating fish. In the present work, the concentration range of mercury in freshwater fishes was (1.681-5.387 mg $kg^{-1}$ d.w.). The highest was in *P. abu* (5.387 mg $kg^{-1}$d.w.), followed by *L. grypus* (4.231 mg $kg^{-1}$ d.w.) and *C. luteus* (3.162 mg $kg^{-1}$ d.w.) and the lowest was in *C. carpio* (1.681 mg $kg^{-1}$d.w.). These levels are relatively higher than the published data in Table 7 for different freshwater fish species. A good control by local authorities should be applied with a certain frequency, to prevent future contamination of fishes and the natural consequences to human health.

*Correlations*

Table 5 shows a surprisingly large number of inter-elemental correlations in Iraqi freshwater fish species. 82 of the examined 91 possible inter-elemental correlations were statistically significant (74 at $p$<0.001, 7 at $p$<0.01 and 1 at $p$<0.05). Generally, 61 of these significant correlations were positive and only 21 were negative. Positive correlations may support the idea that some elements have similar sources and therefore can be related to geological structure of the area. Negative relationships between some elements could be due to increased uptake of an element as a consequence of low levels of another element or due to antagonism. Table 5 also shows high positive correlations between transition metals; Mn-Zn (r=0.58), Fe-Cu (r=0.55), Cu-Cr (r=0.68), Cr-Mn (r=0.68), Ni-Zn (r=0.82), Ni-Mn (r=0.74), and Ni-Cr (r=0.6). A possible explanation of the present findings could be that transition metals may form (simultaneously) stable covalent complexes with large molecules like proteins, enzymes, and hormones according to their similar chemical characteristics, including oxidation state (Da Silva *et al*., 2005).

Strong correlation between the toxic metals Pb and Hg with Mg and Zn; Mg-Pb (r=0.55), Mg-Hg (r=0.64), Zn-Pb (r=0.70) and Zn-Hg (r=0.53) (Table 5), reflect inhibition role of these elements on biomolecular activities. At present, there is no biological or mechanistic explanation to many of the reported correlations and more studies are needed in this area.

*Estimation of dietary exposure to macro and microelements*

Average quantity of fish consumed after six months was 32.5±8.9 g daily. This figure is twice (16.0 g) the level given by Iraqi Household Socio-Economic Survey, IHSES established in 2007 (FAO, 2010). The large difference may be emerged from the fact that the survey of this study was conducted exclusively in urban society. However, in other communities of the world much higher fish consumption levels were recorded, 71 g $day^{-1}$ (Cambodia), 57 (Indonesia), 160 (Malaysia) and 85 (Thailand, Agusa *et al.*, 2007). The average daily intake of minerals from fish was calculated by multiplying mean concentration of each element by average consumption rate (on a dry weight basis) of ordinary Iraqis.

Dietary Reference Intakes (DRIs) were used to determine nutritional adequacy. DRIs consist of four reference intakes: recommended daily allowances (RDA), adequate intake (AI), estimated average requirement (EAR) and tolerable upper intake level (TUL). Table 8 collects (in mg) estimated daily intake of Iraqi consumers from fish only: Na (10.3), K (63.8), Ca (10.9), Mg (11.0), Cu (0.02), Zn (0.23), Fe (0.25), Mn (0.02), Cr (0.003), Ni (0.005) and Co (<0.001); together with [RDA] for Ca (1000), Mg (320-420), Cu (0.9), Zn (8-11), and Fe (8-18); [AI] for Na (1500), K (4700), Mn (1.8-2.3) and Cr (0.025-0.035); and [TUL] for Ni (1.0) (Otten *et al.,* 2006). None of Dietary Reference Intakes (DRIs) were internationally determined for Co.

Because freshwater fish are consumed in small quantities in Iraq (32.5 g of raw fish daily), the concentration of Na, K, Ca, Mg, Cu, Zn, Fe, Mn, Cr, and Ni presented in Table 8 slightly contribute (0.7, 1.4, 1.1, 2.6-3.4, 2.9, 2.1-2.9, 1.0-1.4, 0.9-1.1, 8.6-12.0, 0.5%, respectively) to the recommended dietary allowance. Chromium was the only essential element that contributed significantly (8.6-12.0%) to the recommended allowance. However, the average daily consumption of Na, Ca, Mg, Cu, Zn, Fe, Mn, and Ni by Iraqi adults from fish stayed far below specified tolerable upper level (TUL) of these metals (2300, 2500, 350, 10, 40, 45, 11, and 1.0 mg $kg^{-1}$, respectively). This means that any adverse health effects from consumption of these elements cannot be expected under normal condition. Moreover, living organisms have evolved transport mechanisms for active uptake, enabling cells to regulate their intercellular concentrations. Accordingly, essential elements practically do not produce toxic effects due to this homeostatic mechanism. But, if a heavy metal level is too high, the homeostasis mechanism may cease to function and the metal may compete with the essential ones for protein-binding sites and cause toxicity effects.

Table 4 shows the average concentrations (in mg kg$^{-1}$ d.w.) of Cd (0.044), Pb (0.894), and Hg (2.957) in Iraqi freshwater fishes. Using the average quantity of fish consumed by Iraqis, these quantities contribute, <1, 6, and 20 µg daily of cadmium, lead, and mercury, respectively. FAO/WHO Joint Expert Committee on Food Additives (JECFA) has recommended a provisional maximum tolerable daily intake (PTDI) of Cd and Pb from all sources of 50 and 215-µg, respectively, assuming an average Iraqi weight of 60 kg (WHO, 2004). Based on these references, the average daily intake of Cd and Pb by Iraqi consumers from fish alone were <2.0 and 2.8% of the PTDI values, respectively, which would not cause any adverse health concerns to consumers.

**Table 8: Estimated daily intake of heavy metals (mg kg$^{-1}$ d.w.) by Iraqi adults (19-50 years old and 60 kg body weight) from freshwater fish muscles relative to the recommended standards.**

| M | Average metal content mg kg$^{-1}$ dry weight | Quantity of fishes Consumed g day$^{-1}$ wet | dry | Estimated daily intake EDI$^s$ mg day$^{-1}$ | RDI or AI mg day$^{-1}$ M & F | | EAR mg day$^{-1}$ M & F | | TUL mg day$^{-1}$ M & F | PTDI mg day$^{-1}$ | % Recommended standard |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Na | 1521 | 32.5 | 6.8 | 10.3 | 1500 | 1500 | | | 2300 | | EDI$^S$/AI=0.7% |
| K | 9386 | 32.5 | 6.8 | 63.8 | 4700 | 4700 | | | | | EDI$^S$/AI=1.4% |
| Ca | 1604 | 32.5 | 6.8 | 10.9 | 1000 | 1000 | | | 2500 | | EDI$^S$/RDA=1.1% |
| Mg | 1612 | 32.5 | 6.8 | 11.0 | 420 | 320 | 350 | 265 | 350 | | EDI$^S$/RDA=2.6-3.4% |
| Cu | 2.25 | 32.5 | 6.8 | 0.02 | 0.9 | 0.9 | 0.7 | 0.7 | 10 | | EDI$^S$/RDA = 2.9% |
| Zn | 33.71 | 32.5 | 6.8 | 0.23 | 11 | 8 | 9.4 | 6.8 | 40 | | EDI$^S$/RDA = 2.1-2.9% |
| Fe | 36.91 | 32.5 | 6.8 | 0.25 | 8 | 18 | 6 | 8.1 | 45 | | EDI$^S$/RDA = 1.0-1.4% |
| Mn | 2.48 | 32.5 | 6.8 | 0.02 | 2.3 | 1.8 | | | 11 | | EDI$^S$/AI=0.9-1.1% |
| Cr | 0.421 | 32.5 | 6.8 | 0.003 | 0.035 | 0.025 | | | | | EDI$^S$/AI=8.6-12.0% |
| Ni | 0.759 | 32.5 | 6.8 | 0.005 | | | | | 1.0 | | EDI$^S$/TUL=0.5% |
| Co | 0.058 | 32.5 | 6.8 | <0.001 | | | | | | | |
| Cd | 0.044 | 32.5 | 6.8 | <0.001 | | | | | | 0.050 | EDI$^S$/PTDI<2.0% |
| Pb | 0.894 | 32.5 | 6.8 | 0.006 | | | | | | 0.215 | EDI$^S$/PTDI=2.8% |
| Hg | 2.957 | 32.5 | 6.8 | 0.020 | | | | | | 0.014 | EDI$^S$/PTDI=142% |

M: Metal; RDA: Recommended dietary allowance; EAR: Estimated Average Requirement; AI: mean intake; (TUL): Tolerable upper level; PTDI: Provisional tolerable daily intake.

With respect to Hg, the average quantity of fish consumed by ordinary Iraqis contributes 20 µg daily. Both JECFA and the Agency for Toxic Substances and Disease Registry (ASTDR) address mercury specific to its more toxic organic form of methyl mercury. The JECFA provisional maximum tolerable daily intake (PTDI) for a 60-kg adult is 13.7 µg $day^{-1}$, while the ASTDR minimal risk level (MRL) for chronic oral consumption is 18 µg $day^{-1}$for the same adult (WHO, 2004; ASTDR, 2018). If these two references are applied to fish, the daily intake of Hg by Iraqi consumers from fish alone is high (146% of PTDI and 111% of MRL) which may cause adverse health concerns to consumers. If Hg intake is recalculated assuming Iraqis consume primarily carp (*C. carpio*) which, has lower average Hg concentration of 1.681 mg $kg^{-1}$ d.w., then the daily intake of Hg from fish alone will be 82% of PTDI and 62% of MRL which are safe and recommended.

In summary, concentrations of macro and microelements in this study were close to the values reported in Tables 6 and 7, by 50-90% of the surveyed published articles for uncontaminated freshwater fish species. These directives may be suggested as guidelines for uncontaminated fish species and any extreme value (like Hg in this work) may be taken as a good indicator for contamination. Further studies are necessary to support the reliability of this hypothesis.

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