

# Determination of heavy metals and selenium contents in fish meat sold at Erbil City, Kurdistan Region, Iraq

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## Abstract

Food contamination with heavy metals may pose a serious threat to human health. Fishes are the most common seafood globally. This study aimed to evaluate the levels of heavy metals in different fishes sold in Erbil city markets. The targeted heavy metal elements were cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), mercury (Hg), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn). Seventeen dorsal meat samples were digested and subjected to metal analysis by Inductively Coupled Plasma Optical Emission Spectrometry. Co, Mn, Cu and Se were detected in all samples, while cadmium was below the detectable level in all samples. The average concentrations of targeted trace elements were  $0.03 \pm 0.016$ ,  $0.02 \pm 0.03$ ,  $0.07 \pm 0.08$ ,  $0.10 \pm 0.08$ ,  $0.03 \pm 0.03$ , and  $2.90 \pm 3.33$  mg/kg for cobalt, chromium, copper, manganese, nickel, and zinc, respectively. Lead was only found in one sample (5.88%). On the contrary, mercury was detected in all samples but in low concentration ( $0.14 \pm 0.07$ ). All detected heavy metals with specified permissible limits by FAO/WHO were significantly lower than the permissible limits. Based on detected levels of targeted heavy metals, consumption of such fish has no potential risks to human.

## Introduction

Industrial wastes, agricultural activities, and geochemical processes are the most potential sources for heavy metals pollution of environment including aquatic ecosystems (Rai, 2010). Deposition of large quantities of metals in marine bodies is a serious threat to aquatic livings and to other animals on the higher levels of various food chains. Deposition of such elements in

humans also occur especially through consumption of contaminated seafood (Baki *et al.*, 2018). Recently, the global consumption of fish has grown in parallel to the appreciation of their nutritional value. Indeed, fish meat contains essential minerals, vitamins, healthy unsaturated fatty acids, and high-quality proteins (Tilami and Sampels, 2018).

Heavy metal is a general term for a group of elements with density  $>5\text{g/cm}^3$  (Ali and Khan, 2018). Heavy metals can be divided into two categories. The essential trace elements including chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni), manganese (Mn), selenium (Se), and zinc (Zn). The second category is toxic metals and metalloids comprising cadmium (Cd), lead (Pb), and mercury (Hg). Essential trace elements, at low levels, are important for normal cellular processes, especially enzymatic reactions, in all living cells. Higher concentrations of these elements may result in toxicity and disruption of body homeostasis (Kennelly, 2018). On the contrary, no cellular functions for cadmium, lead, or mercury have been found. In fact, even very low concentrations of these elements can be toxic to cells.

Essential and nonessential elements have deleterious effects due to their bio-accumulative and non-biodegradable properties. There are two main routes by which heavy metals reach and accumulate in tissues of fish; ingestion of contaminated water or food, and via gills during respiration (Rajeshkumar and Li, 2018). Levels of heavy metals in fish reflects the levels of these elements in the water and the period of exposure (Annabi *et al.*, 2013; Nhiwatiwa *et al.*, 2011). Field and laboratory research have found the extent of accumulation in fish tissues is dependent on many factors including; the tissue, fish species, seafloor sediments, metal type, and water chemistry (Driscoll *et al.*, 1994; Korkmaz Görür *et al.*, 2012; Petrović *et al.*, 2013). Additionally, age, sex, swimming pattern, reproductive cycle, and geographical location have all been found to contribute to the bioaccumulation extent (Rajeshkumar and Li, 2018).

Numerous studies have shown that high bioaccumulation culminated in sublethal effect and death in fish populations (Almeida *et al.*, 2002; Jones *et al.*, 2001; McGeer *et al.*, 2000; Sakan *et al.*, 2007; Zeitoun & Mehana, 2014). Similarly, high concentrations of heavy metals in human cells may trigger different deleterious effects such as disruption of plasma membrane, deterioration of proteins and DNA, disruption of signal transduction systems, activating or inhibiting transcriptional

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factors, and generation of reactive oxygen species (Kim *et al.*, 2019; Wu *et al.*, 2016). These cellular injuries lead to systemic pathologies in the central nervous system, hematopoiesis, kidney function, immune system, respiratory and cardiovascular system (Kim *et al.*, 2019).

Continuous emergence of reports addressing the health hazards of heavy metals compelled many global and regional legislative bodies to specify the accepted range for such metals in different foodstuff. World Health Organization and European Food Safety Authority have set maximum permissible limits for many heavy metals. The objective of this study was to investigate the content of some toxic heavy metals and selenium in different fish species outlined in Table 1 collected from markets in Erbil city.

## Materials and Methods

### Reagents and chemicals

All the used chemicals were of analytical grade. Nitric acid (65% v/v), hydrogen peroxide ( $\text{H}_2\text{O}_2$  50% v/v) (Scharlau, Extra Pure) and distilled water were used during digestion process and sample preparation.

### Sample collection

Seventeen different fish samples were purchased and collected for this investigation from different local markets in Erbil city, Kurdistan Region (Iraq) during Jun-July 2019. Dorsal muscle was selected as a major target tissue for metal analysis because muscle tissue is known as a main storage for metals and is the most appropriate part as a food. For each fish sample, muscle tissues were separated using stainless steel knife followed by cleansing, homogenization, labelling, and storage in polypropylene tubes in the refrigerator on the same day of collection.

### Sample preparation and digestion

In this study, all samples were prepared and digested as previously published (Atobatele & Olutona, 2015; Baharom and Ishak, 2015; Ismail and Saleh, 2012; Mendil *et al.*, 2010). Briefly, dorsal muscle tissue was dried in an oven at 100 °C for 12 hours till constant weights were obtained. Each dried sample was separately crushed and homogenized by using mortar. Then, one gram of each dried sample was transferred to a digestion conical flask. Each sample was treated with 5 mL of concentrated HNO<sub>3</sub> (65 %) using a classic digestion-heater at 150°C for 20 minutes. After cooling, 3 mL of H<sub>2</sub>O<sub>2</sub> (50%) were added to each digestion conical flask. After completing digestion process, the mixture was cooled at room temperature and filtered. The filtrate was transferred to a conical flask and completed to 25 ml with 0.1N HNO<sub>3</sub> solution. Finally, the digested solution was kept in appropriate plastic bottles till analysis. Above steps were

separately repeated three times for each sample and a blank. In order to prevent contamination, all equipment were cleaned and washed well with ultra-pure nitric acid before digestion process.

### Metal analysis

Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) instrument was used to determine the level of essential elements, cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) content in digested samples. Non-essential elements; cadmium (Cd), mercury (Hg), and lead (Pb) were also investigated. The estimated levels of these metals in each sample was measured in ng·mL<sup>-1</sup> (parts per billion) and then converted to mg/kg wet weight for fish tissues. Permissible limits set by WHO were employed in comparisons with detected levels in fish samples of the present study. The maximum permissible limits were

extracted from (Baharom & Ishak, 2015; Baki *et al.*, 2018; CAC, 2019).

Limits of detection was calculated according to the following equation:

$$LOD = 3 RSDb \frac{C}{SBR}$$

Where RSDb is the relative standard deviation of 10 replicates of the blank, c is the concentration of the standard, and SBR is the signal to background ratio. Limits of detection and wavelength for each element are in Table 2. All relevant ICP operating parameters were software controlled, allowing easy selection of the optimum operating conditions according to manufacture instructions (<https://extranet.spectro.com/-/media/31793ADA-B987-4D37-B597-04AFB66C7C22.pdf>). The accuracy and precision of the method

**Table 1. Limits of detection (LOD) for the selected wavelengths (lines) for some elements.**

| Element | Wavelength (nm) | LOD 3 (µg/L) |
|---------|-----------------|--------------|
| Co      | 228.615         | 0.654        |
| Cr      | 267.716         | 0.67         |
| Cu      | 324.754         | 1.1          |
| Mn      | 257.610         | 0.08         |
| Ni      | 231.604         | 0.95         |
| Zn      | 213.856         | 0.2          |
| Cd      | 214.438         | 0.333        |
| Pb      | 220.351         | 3.44         |
| Hg      | 184.950         | 1.1          |
| Se      | 196.090         | 6.8          |

**Table 2. Characteristics of collected fish samples from Erbil city markets.**

| N  | Samples     | Species                        | ≈ weight (kg) | Origin          | Status |
|----|-------------|--------------------------------|---------------|-----------------|--------|
| 1  | Daquq fish  | <i>Cyprinus carpio</i>         | 2.5           | Kirkuk, Iraq    | Alive  |
| 2  | Daquq fish  | <i>Cyprinus carpio</i>         | 2             | Kirkuk, Iraq    | Alive  |
| 3  | Daquq fish  | <i>Cyprinus carpio</i>         | 2             | Kirkuk, Iraq    | Alive  |
| 4  | Taqtaq fish | <i>Cyprinus carpio</i>         | 2.5           | Erbil, Iraq     | Alive  |
| 5  | Taqtaq fish | <i>Cyprinus carpio</i>         | 2             | Erbil, Iraq     | Alive  |
| 6  | Taqtaq fish | <i>Cyprinus carpio</i>         | 2.5           | Erbil, Iraq     | Alive  |
| 7  | Dukan fish  | <i>Coregonus lavaretus</i>     | 1             | Sulaimani, Iraq | Alive  |
| 8  | Dukan fish  | <i>Coregonus lavaretus</i>     | 0.5           | Sulaimani, Iraq | Alive  |
| 9  | Dukan fish  | <i>Coregonus lavaretus</i>     | 2             | Sulaimani, Iraq | Alive  |
| 10 | Frozen fish | <i>Pangasius bocourti</i>      | 2             | UAE             | Frozen |
| 11 | Frozen fish | <i>Lethrinus nebulosus</i>     | 1             | UAE             | Frozen |
| 12 | Frozen fish | <i>Rastrelliger kangurata</i>  | 1             | Turkey          | Frozen |
| 13 | Frozen fish | <i>Stolephorus commersonii</i> | 0.025         | Turkey          | Frozen |
| 14 | Frozen fish | <i>Pangasius bocourti</i>      | 2             | Thailand        | Frozen |
| 15 | Frozen fish | <i>Lutjanus campechanus</i>    | 2             | Thailand        | Frozen |
| 16 | Frozen fish | <i>Labeo rohita</i>            | 2             | Myanmar         | Frozen |
| 17 | Frozen fish | <i>Clupea harengus</i>         | 1             | France          | Frozen |

UAE; United Arab Emirates.

was investigated by analyzing the standard reference material SRM 1640 (Trace Elements in Natural Water). The measured value and the certified values were in excellent agreement for all elements.

### Statistical analysis

Results of the study were subjected to statistical significance using GraphPad Prism 6 program software. One-sample t test was employed to compare the levels of heavy metals to the maximum permissible limits. Pearson correlation coefficient was used to assess the relationship between fish weight and heavy metal load. Significance level was set to 0.05.

## Results and Discussion

A total of seventeen fish samples were collected from market in Erbil city between January and July 2019. Nine samples (52.94%) were alive locally-bred carp fish, while the remaining samples were frozen products imported from foreign countries. Details of collected samples are summarized in Table 1. The weight of samples ranged from 2.5 kg to 0.025 kg with an average of  $1.65 \pm 0.74$  kg.

### Total load of heavy metals in samples

The total load of heavy metals in samples is summarized in Figure 1. The average content of heavy metals in fish muscles is  $4.14 \pm 3.35$  mg/kg with a wide range; 0.91 to 12.78 mg/kg. There is a significant difference between fish samples in terms of total content of targeted heavy metals ( $p < 0.001$ ). Poor negative correlation has been observed between fish weight and total load of targeted heavy metals ( $r^2 = -$

0.25). The highest metal load was found in samples of Dukan type. The average load of heavy metals in Dukan fish (9.15 mg/kg) is approximately three-fold higher than observed load in Daquq fish (3.6 mg/kg) and two-fold higher than Taqtaq fish (5.21 mg/kg).

### Heavy metals levels

Essential and non-essential elements that have been specified by FAO/WHO didn't exceed those limits (Table 3). Indeed, all of the detected metals were significantly lower than permissible limits ( $p < 0.001$ ). Levels of trace metal in all samples are depicted in Figure 2 while levels of detected toxic metals and selenium are plotted in Figure 3. Nickel was detected only in 11.76% of sample (2/17). Levels of Cu and Zn were  $0.07 \pm 0.08$  mg/kg and  $2.90 \pm 3.34$  mg/kg. These levels are, indeed, significantly lower than permissible limits of FAO/WHO, 3 mg/kg and 40 mg/kg, respectively ( $p < 0.001$ ).

On the other hand, cadmium was not detected in any sample, while lead (Pb) was found only in one sample (5.88%). However, mercury (Hg) was found in all samples and ranged from 0.05 to 0.30 mg/kg with an average of  $0.14 \pm 0.07$  mg/kg.

Zinc showed the highest level among targeted metals in all samples. This observation is in good agreement with a recent Nigerian study (Adebayo, 2017). In contrast, cadmium was below the detectable level in all samples, which is consistent with reports from Malaysia (Baharom & Ishak, 2015; Ismail & Saleh, 2012) and Ghana (Gbogbo *et al.*, 2018). Yet, previous studies from Iraq have detected low levels of cadmium in different locales (Al-Jubouri and Salman, 2019; Mensoor and Said, 2018). Moreover, a significantly high level was reported from Sulaimani city (Rashed, 2012). The concentrations of Cu, Mn, and Ni observed in the current study are significantly lower than found in the

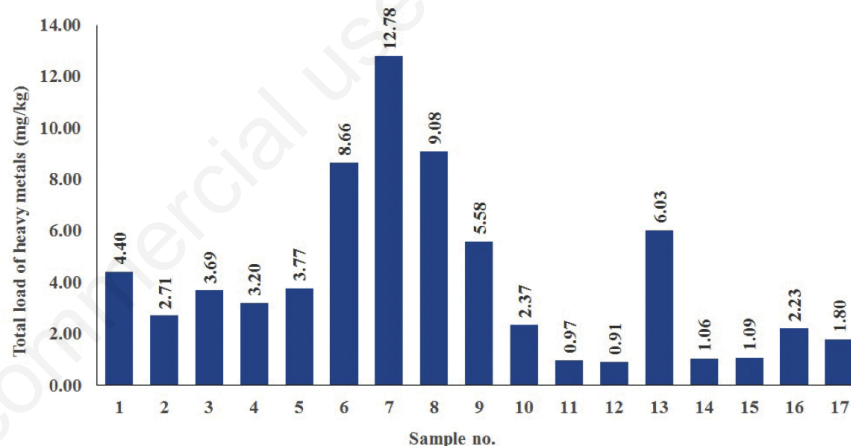


Figure 1. The total heavy metal load in fish samples.

Table 3. Comparison of mean concentration (mg/Kg) of heavy metals in fishes' sample in Erbil and other locations along with FAO/WHO standards.

|   | Trace metal elements |       |       |       |       |       | Toxic metals and selenium |        |       |       |
|---|----------------------|-------|-------|-------|-------|-------|---------------------------|--------|-------|-------|
|   | Co                   | Cr    | Cu    | Mn    | Ni    | Zn    | Se                        | Cd     | Hg    | Pb    |
| This study                                | 0.033                | 0.023 | 0.073 | 0.102 | 0.030 | 2.902 | 1.222                     | ND     | 0.137 | 0.020 |
| FAO/WHO limits <sup>a</sup>               | NS                   | 0.15  | 3     | 5.5   | 0.6   | 40    | NS                        | 0.5    | 0.5   | 0.5   |
| Maqtoof <i>et al.</i> , 2011 <sup>b</sup> | NT                   | NT    | 0.07  | NT    | NT    | 6.4   | NT                        | NT     | NT    | 0.06  |
| Rasheed, 2012 <sup>c</sup>                | NT                   | NT    | 10.08 | 4.19  | 46.71 | NT    | NT                        | 8.49   | NT    | NT    |
| Mensoor & Said, 2018 <sup>d</sup>         | NT                   | 1.07  | 0.70  | NT    | NT    | 0.75  | NT                        | 0.94   | NT    | 1.07  |
| Al-Jubouri & Salman, 2019 <sup>e</sup>    | NT                   | NT    | 0.77  | NT    | NT    | 7.32  | NT                        | 0.91   | NT    | 1.56  |
| Yabanli <i>et al.</i> , 2014 <sup>f</sup> | <0.001               | 0.018 | 0.085 | 0.259 | 0.009 | 0.371 | NT                        | <0.001 | NT    | 0.011 |
| Türkmen and Ciminli, 2007 <sup>g</sup>    | NT                   | 0.25  | NT    | NT    | NT    | NT    | 0.61                      | 0.03   | 0.00  | 0.078 |
| Adebayo, 2017 <sup>h</sup>                | NT                   | 0.18  | 0.06  | 0.15  | NT    | 11.59 | NT                        | <0.01  | NT    | 0.10  |

ND: not detected. NS: not specified. NT: not tested. <sup>a</sup>permissible limits extracted from various references (Tiimub & Afua, 2013; Hashim *et al.*, 2014; Baharom & Ishak, 2015; Rajeshkumar & Li, 2018) <sup>b</sup>samples drawn from Euphrates river in Al-Nassiriya city, Iraq. <sup>c</sup>Sulaimani city, Iraq. <sup>d</sup>Tigris river in Baghdad city, Iraq. <sup>e</sup>Tikrit city, Iraq. <sup>f</sup>Average of concentrations in two fish types from İzmir lake in southeast of Turkey. <sup>g</sup>Gölbasi lake, Turkey. <sup>h</sup>Ekiti city, Nigeria.

mentioned study conducted in Sulaimani city. Such difference is subjected to factors such as difference fish species, contamination level in fish habitat, among others (Rajeshkumar and Li, 2018). The lead content found in this study is consistent with the results reported from some fish species in Nigeria (Atobatele and Olutona, 2015).

### Comparisons of heavy metals with other studies

Variations in heavy metals levels between studies seemed to be attributed to the difference between fish species, and geographical location. Results reported from local, regional and other parts of the world are summarized in Table 3. It should be

noted that scarce of studies evaluating the same heavy metals and fish species addressed in our study is an obvious limitation. The comparison made in Table 3 is somehow limited due to lack of data. The current data were incorporated for a rough comparison and hopefully to elicit further scientific investigations of the addressed species.

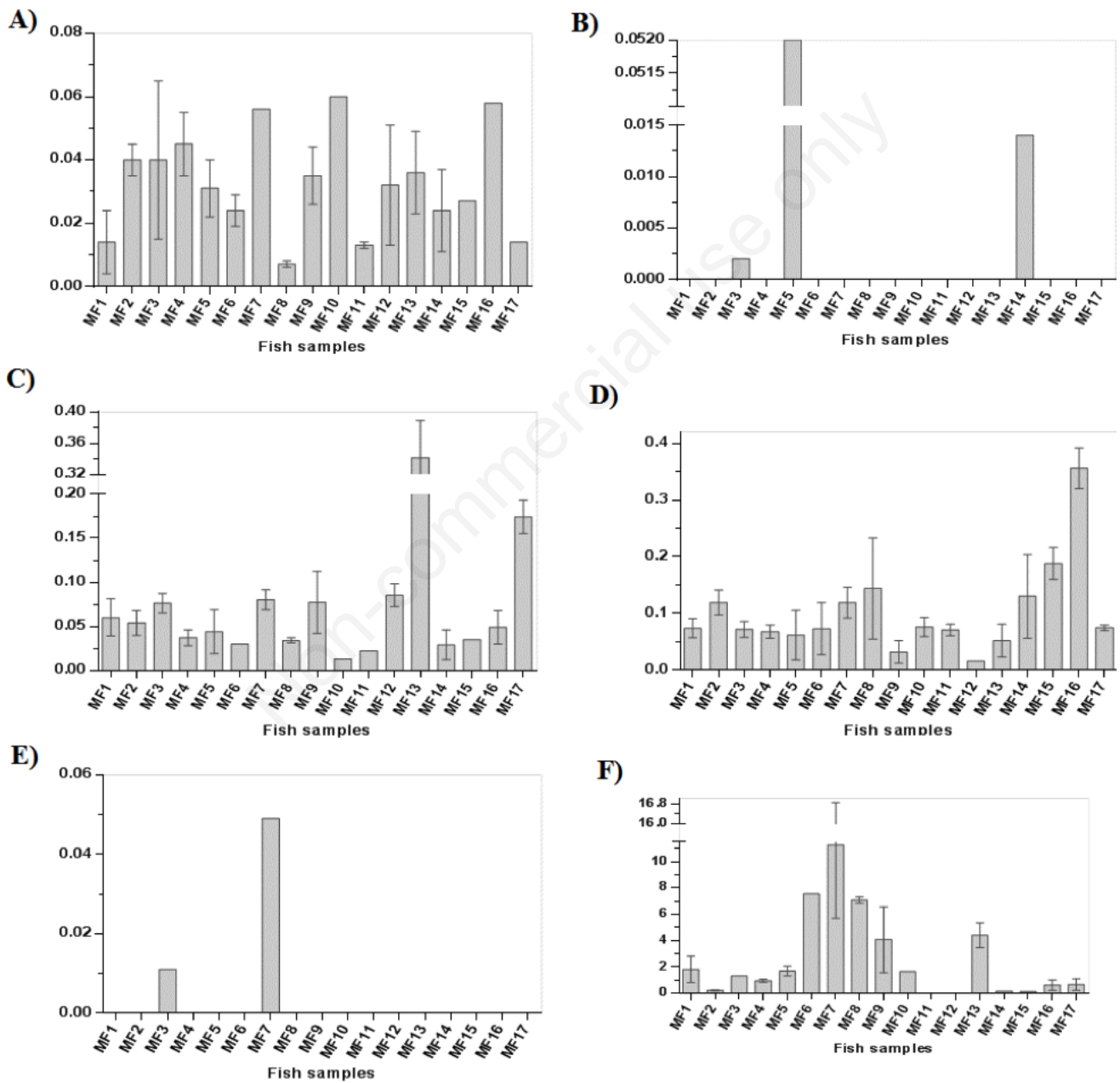


Figure 2. levels of trace elements in fish samples expressed in mg/kg. cobalt (A), chromium (B), copper (C), manganese (D), nickel (E), and zinc (F).

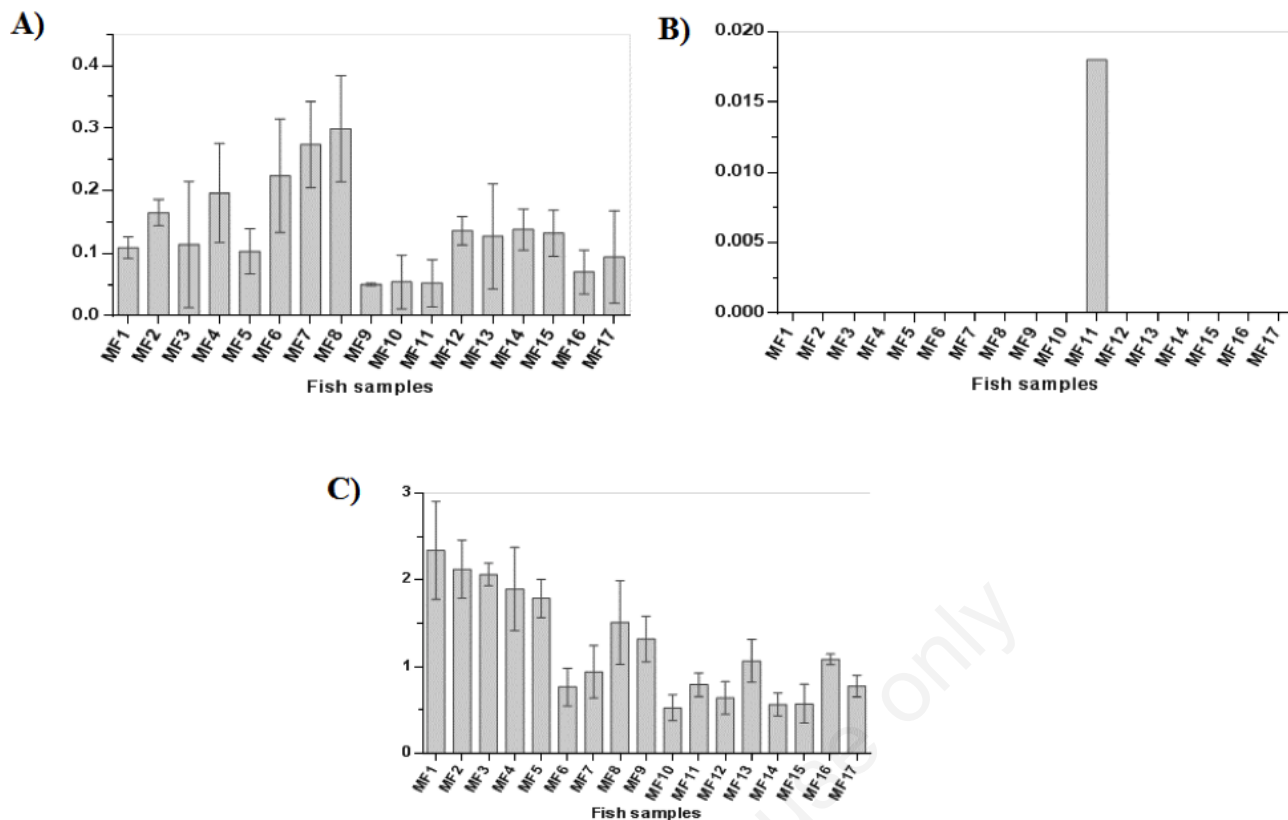


Figure 3. levels of detected toxic heavy metals and selenium in fish samples expressed in mg/kg. Mercury (A), lead (B), and selenium (C).

## Conclusions

Toxic heavy metal contents detected in fish sold in Erbil city are significantly lower than the permissible limits of FAO/WHO. Since detected heavy metals didn't exceed the permissible limits, consumers have no potential risk of heavy metal toxicity.

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