**Poll Res. 40 (3) : 1021-1029 (2021)**

ISSN 0257–8050

# CARCINOGENIC AND NON-CARCINOGENIC HUMAN HEALTH RISK ASSESSMENT OF SOME IMPORTANT TRACE ELEMENTS IN THE COMMONLY CONSUMED FROZEN FISH FILLETS *(PANGASIUS HYPOPHTHALMUS)* IN AMMAN, JORDAN

**ALA' ALI AHMAD AL-SUBEIHI**

*Department of Pharmacy, Faculty of Pharmacy, Amman Arab University, P.O. Box 2234, Amman 11953, Jordan*

(Received 20 May, 2021; Accepted 25 June, 2021)

## ABSTRACT

**Too much exposure to trace elements causesa lot of adverse health effects. This study have the objective of observing the levels of some trace elements in some frozen fish fillets samples collected from different supermarkets located in Amman, the capital of Jordan. Fourteen frozen fish fillets samples (*Pangasius hypophthalmus*) were collected and analyzed for barium (Ba), cobalt (Co), iron (Fe), lead (Pb), nickel (Ni), and chromium (Cr) using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The measured levels of the investigated elements were used to assess the potential health risk in Jordanian adults. The levels of trace elements were such that Fe>Cr >Ba>Ni > Pb > Co, with all trace elements were lower than the maximum allowable levels. The target hazard quotient (THQ) values for all quantified trace elements were less than 1, making non-carcinogenic effects insignificant to the adult population. The cancer risk (CR) factors for Ni and Cr were 17.1 and 25.2 folds above the tolerable life cancer risk ($10^{-5}$), respectively. This study indicates that intake of frozen fish fillets was probably unsafe for Jordanian adult users.**

**KEY WORDS :** Trace elements, Fish, Estimated daily intake, Health risk assessment, Carcinogenic risk, Non-carcinogenic risk, ICP-OES.

## INTRODUCTION

The continuous increase of trace element levels in environment due to agricultural and industrial activities makes these elements to get in the food products (Al-Subeihi, 2021). The persistence and non-biodegradability of trace elements raise the concern and awareness about the their health impacts (Miri *et al*., 2017). Consumption of food contaminated with toxic trace elements are generally risky to individual's health (Shariatifar *et al*., 2017). Nonessential trace elements like chromium (Cr), cadmium (Cd), nickel (Ni), mercury (Hg), and lead (Pb) are considered potential toxic elements (Makedonski *et al*., 2017; Taghavi *et al*., 2015). Administration of foodstuffs contaminated with trace elements may lead to a considerable non-occupational exposure to these elements in general population (Filippini *et al*., 2019). Nowadays, the focus on health and food safety has been growing globally and the movement of trace elements from air, soil, and water to the food products including fish has acquired the intention of doing extensive research. The existence of trace elements in fish may constitute a probable health adverse effects to individuals (Pérez Cid *et al*., 2001; Castro-González *et al*., 2008). Thus, it is crucial to identifythe content of trace elements in fish to make sure that they do not establish a health riskto general population, by maintaining their concentrations within the acceptable levels (Palaniappan *et al*., 2009). Several international organizations such as WHO, FAO, and the European Union (EU), have established maximum allowable concentrations of trace elements in food products (Xue *et al*., 2012; Sridhara *et al*., 2008). For example, the maximum acceptable limit of Pb in fish meat is 0.3 mg/kg, while for Cd is about 0.05-0.3 mg/kg as stated by the EU (EU, 2006). In addition, the international organizations currently apply a maximum allowable Hg limit of approximately 0.5 mg/kg in fish (Kimáková *et al*., 2018).

Long term intake of non-essential trace elements in considerable amounts through food may induce kidney problems, neurological disorders, heart diseases, different forms of cancer, growth retardation, increase mortality and genetic effects in exposed individuals (Shariatifar *et al*., 2020). For instance, Hg has the ability to damage the central nervous system and the kidneys (Kimáková *et al.,* 2018). Moreover, aluminum (Al) can cause neurotoxicity in humans by involving in the etiology of sporadic Alzheimer's disease (AD) and other neurodegenerative complaints (Yokel, 2012). Furthermore, arsenic (As), Ni, and Cd has been reported to cause cancer in humans (Mulware, 2013). For example, exposure to inorganic Ashas been found to induce skin and lung cancers (Pershagen, 1981), while exposure to Cd has been reported to cause lung, prostatic and renal cancers (Rahimzadeh *et al*., 2017). The target hazard quotient (THQ) and cancer risk measure (CR) were created by the United States Environmental Protection Agency (USEPA) to evaluate the health risks associated with chronic exposure to chemical pollutants (USEPA, 2011). THQ is the ratio of the quantified concentration to the oral reference dose where adverse health effects are not anticipated (Kortei *et al.,* 2020). On the other hand, CR gives insight about cancer risk after lifetime exposure to contaminants, including trace elements, which might cause cancer (Bello *et al*., 2017). Imported foods are routinely tested in Jordan under the monitoring of Jordan food and drug administration (JFDA) to make surethat the occurrence of undesirable elements doesn't surpass the maximum permissible limits. Unfortunately, in Jordan, imported fish are not usually tested for the occurrence of trace elements but sensory assessment is performed. The first objective of this research is to determine trace elements levels in imported frozen fish fillets (*Pangasius hypophthalmus)* obtained from different supermarket in Jordan by ICP-OES technique. The second objective is to make a comparison between the results obtained in the current research with those obtained from previous studies and with the international maximum permissible limits. Lastly, the potential health risk that might develop from their consumption will be evaluated and the recommendations for both consumers and the JFDA administrators will be indicated.

## EXPERIMENTAL

### Sample collection

Fourteen frozen samples of fish fillets (*Pangasius hypophthalmus*) were collected randomly from several supermarkets in Jordan. Samples were stored at -20 $^{o}$C waiting for the time of analysis. Determination of trace elements was performed based on dry weight (mg/kg).

### Materials

The Millipore water purification system (Millipore) was used as a source of the deionized water. $H_2O_2$ (30%) was purchased from JHD chemical (China) while 70 % $HNO_3$ was brought from Bio-Solve, the Netherlands. Multi-element standard solution of 1000 mg/l was bought from Accustandard, USA. All used laboratory glass ware were soaked and cleaned with 10 % $HNO_3$ overnight, then washed with deionized water, and finally dried with 99.7 % ethanol.

### Sample preparation and analysis

After allowing frozen fish samples to thaw overnight, they were dried in oven at 105 $^{o}$C awaiting for a constant weight. Dry samples were disintegrated into powder by mortar and pestle (Ezemonye *et al.,* 2019) and digested using a microwave radiation system (FSSAI, 2016). In the current research, around 0.2 g of dry sample was placed into a pressure-resistant Teflon (PTFE-TFM) flask particular for the Ethos One Milestone microwave oven. One ml $H_2O_2$ and 7 ml $HNO_3$ were transferred into each of the microwave flask containing the weighted fish sample. After that, the flasks were locked tightly and digested in accordance with the following digestion conditions: 1800 W at 180 ˝C (ramp time of 15 minutes, hold time of 15 minutes, and cool time of 15 minutes). After cooling the digested samples, they were filtered (Whitman No. 42) and brought with distilled water to a volume of 100 ml.

A thermo Fisher Scientific spectrometer (iCAP 7400 ICP-OES Due) with a glass cyclonic chamber and a burgenermira mist nebulizer was used for the analysis of trace elements in the processed fish samples. The ideal ICP-OES working parameters of nebulizer gas flow, coolant gas flow, auxiliary gas flow, and radio frequency (RF) power were adjusted to 0.5 l/min, 14.5 l/min, 0.5 l/min, and 1150 W, respectively. Each sample was tested in replicates.

### Validation of analytical method

The validation method followed in the current study was in accordance to the ICH guidelines (ICH, 1996). The validation parameters of the analytical procedure consisted of precision, linearity, % recovery, detection limit (LOD) and quantification limit (LOQ). The results of the method validation are presented in Table 1. Moreover, the analytical procedure of this research was validated using a certified reference material (CRM) of animal origin. Investigation of CRM was performed in the same way as authentic fish fillets samples.

### Risk assessment

Fish fillets samples were subjected to risk assessment after being analyzed to estimate the daily intake (EDI), the cancer risk (CR), and hazard quotient (THQ) for the non-carcinogenic risk.

### Estimation the daily intake (EDI) and Cancer risk (CR)

Ingestion is one of the major routes of exposure to trace elements in population. The *EDI* of trace elements taken by adult population through consumption of fish filletswas estimated by the following equation (Alipour and Banagar, 2018):

$$EDI = \frac{ED \times Cm \times EF \times FIR}{(TA \times BW)}$$

Where $E_D$ is the duration of exposure (60 years); $C_m$ is metal concentration in fish sample (mg/kg dry weight); $E_F$ is exposure frequency (365 days/year); $F_{IR}$ is fish's rate of ingestion (0.0252 kg/(person x day) for adults); the mean body weight (BW) of adult is assumed to be 65 kg [2]; $T_A$ is the averaging time for non-carcinogens (365 days/ year × $E_D$) (Saha *et al.*, 2016). The health risk calculations were made based on the assumption that the ingested amount of each element equals to the absorbed element's amount (USEPA, 1989) and the digestion procedure does not affect the concentration of the element (Cooper *et al.*, 1991). The exposure parameters used for risk estimations are displayed in Table 2 (USEPA, 2012).

**Table 2.** Exposure parameters used for the health risk estimations via consumption of fish (US EPA, 2012)

| Parameter | Unit | Adult |
|---|---|---|
| Body weight | kg | 75 |
| Exposure Frequency (EF) | Days/years | 365 |
| Exposure Duration (ED) | Years | 30 |
| Average Time (AT) | Days/years | |
| For carcinogenic | | 365 x 70 |
| For non-carcinogenic | | 365 x ED |

### Target hazard quotient (THQ)

Estimation of the non-carcinogenic risk for the populations which might come from the consumption of the contaminated fish was performed using the *target hazard quotient* (THQ). *THQ* was assessed by dividing the estimated daily intake (EDI) of trace elements in contaminated fish fillet relative over the reference oral dose (RfD) for each element (Mortazavi and Fard, 2017). If THQ is less than 1 then the likelihood of a trace element to cause a potential health risks decreases; on the other hand, if THQ's value exceeds 1, the chances for this trace element to act as a health hazard will be great (USEPA, 2012). THQ is calculated making use of the following equation:

$$THQ = \frac{EDI}{RfD}$$

CR is defined as the chance of incidence of any

**Table 1.** Validation parameters of the ICP OES instrumental method for the determination of Ba, Co, Fe, Pb, Ni, and Cr in frozen fish fillet samples

| Trace element | Certified value (mg/kg) | Measured value (mg/kg) | LOD (mg/kg) | LOQ (mg/kg) | Recovery (%) | Precision (mg/kg) | Linear range (mg/kg) |
|---|---|---|---|---|---|---|---|
| Ba | 3.0 | 2.85 | $1.07 \times 10^{-4}$ | $3.57 \times 10^{-4}$ | 94.93 | 0.81 | $4.5 \times 10^{-3}$ – 5.0 |
| Co | 3.0 | 2.75 | $9.10 \times 10^{-5}$ | $3.03 \times 10^{-4}$ | 91.81 | 0.40 | $1.0 \times 10^{-3}$ – 5.0 |
| Fe | 3.0 | 2.75 | $1.12 \times 10^{-3}$ | $1.82 \times 10^{-3}$ | 91.77 | 0.43 | $2.5 \times 10^{-3}$ – 5.0 |
| Pb | 3.0 | 2.73 | $3.26 \times 10^{-4}$ | $1.09 \times 10^{-3}$ | 90.99 | 0.39 | $2.5 \times 10^{-4}$ - 5.0 |
| Ni | 3.0 | 2.79 | $1.27 \times 10^{-4}$ | $2.69 \times 10^{-4}$ | 93.01 | 0.35 | $5.0 \times 10^{-4}$ - 5.0 |
| Cr | 3.0 | 2.86 | $1.98 \times 10^{-4}$ | $6.59 \times 10^{-4}$ | 95.35 | 0.45 | $1.0 \times 10^{-3}$ – 5.0 |

type of cancer after exposure to cancer-causing components during lifetime. In the current study, the CR over was predicted using the cancer slope factor according to the following formula (Peng *et al.*, 2016; Shaheen *et al.*, 2016):

$$CR = \frac{EF \ x \ ED \ x \ FIR \ x \ Cm \ x \ CSF}{(BW \ x \ TA)}$$

where CSF stands for the cancer slope factor (mg/kg/day) and other parameters had been described before. A tolerable lifetime cancer risk of $10^{-5}$was established by US EPA (Saha *et al.*, 2016).

## Statistical analysis

Microsoft Excel computation software was used to perform all statistical analysis of the current research. The following descriptive statistics were determined: range (min-max), arithmetic mean (AM), standard deviation (SD), and percentile (P). Additionally, Pearson’s correlation coefficient analysis (PCA) was performed to identify the source of trace elements in the analyzed fish samples. PCA computes the degree of the relationship between any two variables on a scale of -1 (perfect inverse relation) through 0 (no relation) to +1 (perfect sympathetic relation) (Adebiyi *et al.*, 2020). If variables have strong association between them this indicates that they might originated from the same parent source.

# RESULTS AND DISCUSSION

## Trace elements concentrations in fish samples

In the current research, Ba, Co, Fe, Pb, Ni and Cr levels were determined in fish fillets samples (*Pangasius hypophthalmus*) by ICP-OES. Some important statistics of trace elements levels in fish fillet samples were reported in Table 3. As shown in Table 3, the average levels of Ba, Co, Fe, Pb, Ni and Crwere 0.68, 0.029, 16.37, 0.084, 0.26, and 1.30 mg/kg, respectively. The concentrations of the trace elements in the examined fish fillets samples were decreasingly as Fe>Cr>Ba>Ni> Pb > Co. The concentrations of Ba, Co, Fe, Pb, Ni and Crin fish fillet samples ranged from < LOD to 2.46 mg/kg, from 0.004 to 0.072 mg/kg, from 9.03 to 32.63mg/kg, from < LOD to 0.91 mg/kg, from < LOD to 0.63 mg/kg, from 1.11 to 1.43 mg/kg respectively. All investigated trace elements in this study were found in levels above the detection limit of the ICP-OES machine, except in 7%, 28 %, and 78% of the examined samples for Ni, Ba, and Pb respectively.

Ba has been shown to cause nephropathy in

**Table 3.** Trace element concentrations with different confidence intervals. [a]

| Trace element | AM (mg/kg) | SD (mg/kg) | Min | P25 | P50 | P75 | Max |
|---|---|---|---|---|---|---|---|
| Ba | 0.68 | 0.80 | <LOD | 0.0083 | 0.452 | 1.18 | 2.46 |
| Co | 0.029 | 0.023 | 0.004 | 0.0083 | 0.026 | 0.043 | 0.072 |
| Fe | 16.37 | 6.41 | 9.03 | 11.17 | 15.57 | 19.86 | 32.63 |
| Pb | 0.084 | 0.024 | <LOD | <LOD | <LOD | <LOD | 0.91 |
| Ni | 0.26 | 0.17 | <LOD | 0.019 | 0.22 | 0.39 | 0.63 |
| Cr | 1.30 | 0.10 | 1.11 | 1.24 | 1.33 | 1.35 | 1.43 |

[a]The standard statistical analysis (Min, Max, AM, SD, and P) has been used to present the statistical variations of the data.

**Table 4.** International Guidelines levels.

| Trace element | Variation of data (mg/kg) | Mean concentration (mg/kg) | MHPRC (2013) permissible limit (mg/kg) | WHO (1989) permissible limit (mg/kg) | FAO (2000) permissible limit (mg/kg) | WHO (2000) permissible limit (mg/kg) |
|---|---|---|---|---|---|---|
| Ba | <LOD – 2.46 | 0.68 | - | - | - | - |
| Co | 0.004 – 0.072 | 0.029 | - | - | 0.5[c] | 0.5[c] |
| Fe | 9.03 – 32.63 | 16.37 | - | 100[b] | 180[c] | 109[c] |
| Pb | <LOD – 0.91 | 0.084 | 0.5[a] | 2[b] | 2[c] | 0.5[c] |
| Ni | <LOD – 0.63 | 0.26 | - | - | 55[c] | 30[c] |
| Cr | 1.11 – 1.43 | 1.3 | 2.0[a] | - | - | - |

[a](MHPRC, 2013); [b](El-moselhy *et al.*, 2014); [c](Hosseini *et al.*, 2015)

laboratory animals and it has the potential to cause hypertension in human (WHO, 2017).In the present study, the EDI of Ba was 2.64 x $10^{-4}$ (mg/kg/day),a value which was 755 fold less than the RfD of 0.2 mg/kg/day reported by US guideline (Moffet *et al.*, 2007).

Co is a vital trace elements; it acts as a cofactor for methylmalonyl-CoA mutase and methionine synthase which are important for human health. Also, Co is considered an essential part of vitamin B12 (Yamada, 2013). As indicated in Table 4, the average level of Co in this research (0.029 mg/kg) hadn't surpassed the maximum permissible Co limits in fish reported by FAO and WHO guidelines. Kwonand Lee (2001) had revealed a Co average level of 0.01 mg/kg in fish samples which was about 3 fold less than Co average level of this research (Table 5). On the other hand, Raja *et al*. (2009) had a shown concentrations of Co (0.05-0.28 mg/kg) which were higher than the mean concentration of Co in this study (Table 5).

Fe is an essential nutrient to almost all organisms, being involved in oxygen transfer, respiratory chain reactions, DNA synthesis, and immune function (Wood *et al*., 2012). Fe can be damaging when it accumulates in the tissues and can also produce toxic effects when the metal intake is excessively elevated (Jaishankar *et al*., 2014). As indicated in Table 4, the average level of Fe in this study (16.37 mg/kg) had not surpassed the maximum permissible Fe limits in fish reported by FAO and WHO guidelines. Table 5 revealed that mean concentrations of Fe of the current study were shown to be lower than the data reported by Kalay *et al.* (1999), Topcuoglu *et al*. (2002), and Yilmaz (2003). Whereas, the mean concentrations of Fe in this study were found to be comparable with finding.

Pb has been shown to cause impaired development, lower IQ, shortened attention span, hyperactivity, and mental deterioration in children. Loss of memory, nausea, insomnia, anorexia, weakness of the joints, injury to the brain, nervous system, red blood cells, and kidneys in adults (Wuanaand Okieimen, 2011). As indicated in Table 4, the average level of Pb in this research (0.084 mg/kg) was below the maximum permissible Pb limits in fish reported by FAO/WHO, FAO, WHO, and Ministry of Health of the People's Republic of China (MHPRC) guidelines. Mean Pb concentrations in the present study were found to be lower than the data of 0.67 mg/kg which was reported by Alipour *et al.*

**Table 5**. Comparison of the total elemental concentration in this study with similar studies

| Element (mg/kg) | Current study | Kalay and Canh (1999) | Kwon and Lee (2001) | Tamira *et al.* (2001) | Tuzen (2002) | Topcuoglu et al. (2002) | Bustamante et al. (2003) | Canli and Atli (2003) | Yilmaz (2003) | Raja *et al.* (2009) | Alipour *et al.* (2014) | Duran *et al.* (2014) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ba | 0.68 | - | - | - | - | - | - | - | - | - | - | - |
| Co | 0.029 | - | 0.01 | - | - | <0.05-0.40 | - | - | - | 0.05-0.28 | - | - |
| Fe | 16.37 | 59.6 -73 .4 | - | - | 9.52 -32 .4 | 30-60 | - | 19.6 - 78.4 | 29.10-93.6 | 24.1-50.3 | - | - |
| Pb | 0.084 | - | - | - | N/A | - | - | - | - | - | - | - |
| Ni | 0.26 | 4.25-6.07 | 0.02 | 0.1- 0.3 | N/V | <0.01-2.04 | 0.01 -0.1 | - | 0.32-1.72 | 0.38-1.54 | - | - |
| Cr | 1.3 | - | - | - | - | - | - | - | - | - | 0.08 | 5.97 |

(2014). Furthermore, the mean concentrations of Pb of the current study were higher than the concentrations of Pb (0.012 mg/kg) found in the study performed by Metwally and Fouad (2008).

Human body needs Ni in a very small amount which acts as a co-factor in absorption of iron from the intestine human body. On the other hand, the International Agency for Research on Cancer (IARC) has classified Ni as a moderately toxic element (Al-Mahaqeri, 2015). According to the current study, the mean concentration of Ni was 0.26 mg/kg which is lower than critical limits set by (WHO and FAO) (Table 4). Mean Ni concentrations in the present study were found to be lower than the data reported by Raja *et al.* (2009). Moreover, the mean concentration of Ni of the current study were higher than the concentrations of Ni found in the study performed by Kwon and Lee (2001) and Bustamante *et al.* (2003).

Cr has been reported to cause skin irritation and ulceration following acute exposure in human, while kidney and liver malfunctions, circulatory and nervous system damage occurs after chronic exposure (Wuana and Okieimen, 2011). Table 4 indicates that the mean concentration of Cr was below the concentration threshold set by the Ministry of Health of the People's Republic of China (MHPRC) (2.0 mg/kg) (MHPRC, 2013). The averagelevel of Cr in the current research was 1.3 mg/kg; a value above what Alipour *et al.* (2014) had stated (0.08 mg/kg). On the other hand, was below than the value reported by Duran *et al.* (2014) which was 5.97 mg/kg.

**Pearson's correlation coefficient analysis (PCA)**

The PCAanalysis results of the investigated trace elements in fish fillets samples are demonstrated in Table 6. The PCA analysis indicated a significant positive correlations between all of the tested trace elements. Significant positive relationship refers tochemical affinity, similarity in genetic origin, and/or normal levels in the investigated samples, on the other hand, negative correlation manifests that trace elements may have nonchemical similarity or initiated from other origins.

**Health risk assessment**

**Estimation of daily intake (EDI) and calculation of target hazard quotient (THQ)**

The oral reference dose (RfD) of the investigated trace elements in the current study are summarized in Table 7. In adults, the estimated daily intake (EDI) for Ba, Co, Fe, Pb, Se, Ag, Ni, and Crwere 2.64 x $10^{-4}$, 1.12 x $10^{-5}$, 6.35 x $10^{-3}$, 3.26 x $10^{-5}$, 1.00 x $10^{-4}$, and 5.04 x $10^{-4}$, respectively. These results showed that the EDI values for the tested elements were under the corresponding RfD values. As revealed in Table

**Table 6.** Values of correlation coefficient between measured trace elements.

| Pearson's r | Ba | Co | Fe | Pb | Ni | Cr |
|---|---|---|---|---|---|---|
| Ba | 1 | | | | | |
| Co | 0.975 | 1 | | | | |
| Fe | 0.970 | 0.946 | 1 | | | |
| Pb | 0.753 | 0.668 | 0.805 | 1 | | |
| Ni | 0.967 | 0.966 | 0.975 | 0.709 | 1 | |
| Cr | 0.804 | 0.863 | 0.852 | 0.459 | 0.893 | 1 |

**Table 7.** Calculated Target Hazard Quotients (THQ), and Cancer Risks (CA) of the trace elements in frozen fish fillet samples

| Trace element | Mean (mg/kg) | RfD (mg/kg bw/day) | EDI (mg/kg bw/day) | THQ | CR |
|---|---|---|---|---|---|
| Ba | 0.68 | 0.2[a] | 2.64 x $10^{-4}$ | 1.32 x $10^{-3}$ | - |
| Co | 0.029 | 0.02[b] | 1.12 x $10^{-5}$ | 5.62 x $10^{-4}$ | - |
| Fe | 16.37 | 0.7[b] | 6.35 x $10^{-3}$ | 9.07 x $10^{-3}$ | - |
| Pb | 0.084 | 0.0035[b] | 3.26 x $10^{-5}$ | 9.31 x $10^{-3}$ | 2.77 x $10^{-7}$ |
| Ni | 0.26 | 0.02[a] | 1.00 x $10^{-4}$ | 5.04 x $10^{-3}$ | 1.71 x $10^{-4}$ |
| Cr | 1.30 | 0.003[b] | 5.04 x $10^{-4}$ | 0.17 | 2.52 x $10^{-4}$ |

[a](USEPA, 2005)
[b](USEPA, 2012)

7, the calculated THQ of the noncarcinogenic trace elements (Ba, Co, Fe, and Ag) was less than 1, indicating an absence of potential health risks for the Jordanian population following consumption the frozen fish fillets containing these elements.

**Estimation of the cancer risk (CR)**

Table 7 indicated that CR factors for Pb, Ni, and Crduring a lifetime consumption of contaminated fish were 2.77 x $10^{-7}$, 1.71 x $10^{-4}$, and 2.52 x $10^{-4}$, respectively. USEPA set an acceptable limit of life cancer risk which is $10^{-5}$ (Saha, 2016). In this research, the CR of Ni and Cr was above the USEPA acceptable value, indicating that people who eatfrozen fish fillets are susceptible to cancer risk from Ni and Cr.

## CONCLUSION

In this research, the level of the sixth elements (Fe > Cr > Ba > Ni > Pb > Co) in frozen samplesof fish fillets, gathered from several shop stores in Jordan, was defined using ICP-OES instrument. The results indicated that maximum average level of the trace elementswas found for Fe, but Co was the minimum. The assessment of health risk for the sixth trace elements were investigated for adult individuals in Jordan. The analysis results revealed that the order of the noncarcinogenic toxic elements depending on THQ index was Cr>Pb>Fe>Ni>Ba >Co keeping in consideration that total THQ of all tested trace elements was less than 1. Additionally, the results displayed that the CR of Crand Ni wereand 2.52 x $10^{-4}$ and 1.71 x $10^{-4}$, respectively. It can be concluded that exposure to Cr and Ni might bring about a health risk in exposed human. Because ofpossible accumulation of these investigated elements to harmful levels, JFDA is highly advised to monitor trace elements levels in fish fillets that the population in Jordan commonly consume.

**Conflict of interest**

Ala' Ali Al-Subeihi declares that he has no conflict of interest.

**Funding**

The author would like to thank Amman Arab University for funding this current research.

## REFERENCES

Adebiyi, F.M., Ore, O.T. and Ogunjimi, I.O. 2020. Heliyon Evaluation of Human Health Risk Assessment of Potential Toxic Metals in Commonly Consumed Cray Fish (*Palaemon hastatus*) in Nigeria. *Heliyon.* 6 (October 2019): e03092. https://doi.org/10.1016/j.heliyon. 2019.e03092.

Alipour, H. and Banagar Gh R. 2018. Health Risk Assessment of Selected Heavy Metals in Some Edible Fishes from Gorgan Bay, Iran. *Iranian Journal of Fisheries Sciences.* 17 (1) : 21-34. https://doi.org/10.22092/IJFS.2018.115582.

Alipour, H., Pourkhabbaz, A. and Hassanpour, M. 2014. Estimation of potential health risks for some metallic elements by consumption of fish. *Water Quality, Exposure and Health*. 7 (2) : 179-185.

Al-Mahaqeri, A. 2015. Human Health Risk Assessment of Heavy Metals in Fish Species Collected from Catchments of Former Tin Mining. *International Journal of Research Studies in Science, Engineering and Technology.* 2 (4) : 9-21.

Al-Subeihi, A.A.A. 2021. Risk assessment of trace elements in selected imported frozen fish fillet in Jordanian market. *International Journal of Environmental Analytical Chemistry*. DOI: 10.1080/03067319.2021.1897797

Bello, S., Muhammad, B.G. and Bature, B. 2017. Total Excess Lifetime Cancer Risk Estimation from Enhanced Heavy Metals Concentrations Resulting from Tailings in Katsina Steel Rolling. *Journal of Material Sciences & Engineering.* 6 (3): 338-342. https://doi.org/10.4172/2169-0022.1000338.

Bustamante, P., Bocher, P., Cherel,Y., Miramand, P. and Caurant, F. 2003. Distribution of trace elements in the tissues of benthic and pelagic fish from the Kerguelen Islands. *The Science of the Total Environment.* 313 : 25-39.

Castro-González, M. I. and Méndez-Armenta, M. 2008. Heavy Metals: Implications Associated to Fish Consumption. *Environmental Toxicology and Pharmacology.* 26 (3): 263-271. https://doi.org/10.1016/j.etap.2008.06.001.

Duran, A., Tuzen, M. and Soylak, M. 2014. Assessment of trace metal concentrations in muscle tissue of certain commercially available fish species from Kayseri, Turkey. *Environmental Monitoring and Assessment.* 186 (7): 4619-4628.

Cooper, C.B., Doyle, M.E. and Kipp, K. 1991. Risks of Consumption of Contaminated Seafood: The Quincy Bay Case Study. *Environmental Health Perspectives.* 90: 133-140. https://doi.org/10.2307/3430857.

Eghbaljoo-Gharehgheshlaghi, H., Nabi Shariatifar, Azimeh Arab, Mahmood Alizadeh-Sani, Iraj Karimisani, Amirafshar Asdagh, Mohammadreza Rostami, Mahsa Alikord, and Majid Arabameri, 2020. The Concentration and Probabilistic Health Risk Assessment of Trace Metals in Three Type of Sesame Seeds Using ICP- OES in Iran.

*International Journal of Environmental Analytical Chemistry*. https://doi.org/10.1080/03067319.2020.1804896.

El-Moselhy, Kh. M., Othman A.I., H. El-AzemAbd and M.E.A. El-Metwally. 2014. Bioaccumulation of Heavy Metals in Some Tissues of Fish in the Red Sea , Egypt. *Egyptian Journal of Basic and Applied Sciences.* December: 1-9. https://doi.org/10.1016/j.ejbas.2014.06.001.

European Union (EU). 2006. Setting Maximum Levels for Certain Contaminants in Foodstuffs, Commission Regulation (EC) No 1881/2006; OJ L 364, 20.12.2006. 5.

Ezemonye, L.I., Adebayo, P.O., Enuneku, A.A., Tongo, I. and Ogbomida, E. 2019. Potential Health Risk Consequences of Heavy Metal Concentrations in Surface Water, Shrimp (*Macrobrachium macrobrachion*) and Fish (*Brycinus longipinnis*) from Benin River, Nigeria. *Toxicology Reports.* 6 (1): 1-9. https://doi.org/10.1016/j.toxrep.2018.11.010.

Filippini, T., Tancredi, S., Malagoli, C., Malavolti, M., Bargellini, A., Vescovi, L., Nicolini, F., and Vinceti, M. 2019. Dietary Estimated Intake of Trace Elements: Risk Assessment in an Italian Population. *Exposure and Health*, no. 0123456789. https://doi.org/10.1007/s12403-019-00324-w.

Food safety and standards authority of India (FSSAI). 2016. Ministry of health and family welfare, FDA Bhawan, Kotla Road New Delhi, 2016. www.fssai.gov.in

ICH. 1996. *Guidance for Industry Q2B Validation of Analytical Procedures: Methodology*. Vol. 20857. http://www.fda.gov/cder/guidance/index.htm%5Cnhttp://www. fda.gov/cber/guidelines.htm.

Jaishankar, M., Tenzin Tseten, NareshAnbalagan, Blessy B. Mathew, and Krishnamurthy N. Beeregowda. 2014. Toxicity, Mechanism and Health Effects of Some Heavy Metals.*Interdisciplinary Toxicology* 7 (2): 60-72. https://doi.org/10.2478/intox-2014-0009.

Kimáková, T., Kuzmová, L., Nevolná, Z., and Bencko, V. 2018. Fish and Fish Products as Risk Factors of Mercurexposure. *Annals of Agricultural and Environmental Medicine.* 25 (3): 488-93. https://doi.org/10.26444/aaem/84934.

Kortei, N.K., Heymann, M.E., Essuman, E.K., Kpodo, F.M., Akonor, P.T., Lokpo, S.Y., Boadi, N.O., Ayim-Akonor, M. and Tettey, C. 2020. Health Risk Assessment and Levels of Toxic Metals in Fishes (OreochromisNoliticus and ClariasAnguillaris) from Ankobrah and Pra Basins: Impact of Illegal Mining Activities on Food Safety. *Toxicology Reports.* 7 : 360-369. https://doi.org/10.1016/j.toxrep.2020.02.011.

Kwon, T.T. and Lee, C.W. 2001. Ecological risk assessment of sediment in wastewater discharging area by means of metal speciation. Microchemical Journal 70: 255-264

Makedonski, L., Peycheva, K. and Stancheva, M. 2017. Determination of Heavy Metals in Selected Black Sea Fish Species. *Food Control.* 72 : 313-318. https://doi.org/10.1016/j. foodcont. 2015.08.024.

Mehdi H., Seyed Mohammad BagherNabavi, Seyedeh Narges Nabavi. 2015. Heavy Metals (Cd, Co, Cu, Ni, Pb, Fe, and Hg) Content in Four Fish Commonly Consumed in Iran: Risk Assessment for the Consumers. *Environmental Monitoring and Assessment*. 187 (September): 237-242. https://doi.org/10.1007/s10661-015-4464-z.

Ministry of Health of the People's Republic of China (MHPRC). 2013. National Food Safety Standard. Maximum Levels of Contaminants in Foods (GB2762-2012). http://www. seafish.org/media/publications/China_Max_levels_of_contaminants_in_food.pdf. Accessed 14 October 2016

Metwally, M.A.A. and Fouad, I.M. 2008. Biochemical changes induced by heavy metal pollution in marine fishes at Khomse Coast, Libya. Global Veterinaria. 2 (6): 308-311.

Miri, M., Akbari, E., Amrane, A., Jafari, S.J., Eslami, H. and Taghavi, M. 2017. Health Risk Assessment of Heavy Metal Intake Due to Fish Consumption in the Sistan Region , Iran.*Environ Monit Assess.* 189 : 583-592.

Moffet, D., Smith, C., Stevens, Y., Ingerman, L., Swarts, S. and Chappell, L. 2007. Toxicological Profile for Barium and Barium Compounds. *Agency for Toxic Substances and Disease Registry*. https://www.atsdr.cdc.gov/toxprofiles/tp24.pdf%0Ahttp://stacks.cdc.gov/view/cdc /6955/.

Mortazavi, S., and Fard, P.N. 2017. Risk Assessment of Non-Carcinogenic Effects of Heavy Metals from Dez River Fish. *Iranian Journal of Health Sciences* 5 (4): 10-25.

Mulware, S.J. 2013. Trace Elements and Carcinogenicity: A Subject in Review. *3 Biotech* 3 (2): 85-96. https://doi.org/10.1007/s13205-012-0072-6.

Palaniappan, P. R. and Karthikeyan, S. 2009. Bioaccumulation and Depuration of Chromium in the Selected Organs and Whole Body Tissues of Freshwater Fish *Cirrhinus mrigala* Individually and in Binary Solutions with Nickel. *Journal of Environmental Sciences* 21 (2): 229-36. https://doi.org/10.1016/S1001-0742(08)62256-1.

Peng, Q., Nunes, L.M., Greenfield, B.K., Dang, F. and Zhong, H. 2016. Are Chinese Consumers at Risk Due to Exposure to Metals in Crayfish? A Bioaccessibility-Adjusted Probabilistic Risk Assessment. *Environment International.* 88: 261-268. https://doi.org/ 10.1016/j.envint. 2015.12.035.

Pérez Cid, B., C. Boia, L. Pombo, and E. Rebelo. 2001. Determination of Trace Metals in Fish Species of the Ria de Aveiro (Portugal) by Electrothermal Atomic Absorption Spectrometry. *Food Chemistry.* 75 (1): 93-100. https://doi.org/10.1016/S0308-8146(01)00184-4.

Pershagen, G. 1981. The Carcinogenicity of Arsenic. *Environmental Health Perspectives*. 40: 93-100.

Rahimzadeh, M.R., Rahimzadeh, M.R., Kazemi, S., and Moghadamnia, A.A. 2017. Cadmium Toxicity and Treatment: An Update. *Caspian Journal of Internal Medicine.* 8 (3): 135-45. https://doi.org/10.22088/cjim.8.3.135.

Raja, P., Veerasingam, S., Suresh, G., Marichamy, G., Venkatachalapathy, R. 2009. Heavy Metals Concentration in Four Commercially Valuable Marine Edible Fish Species from Parangipettai Coast, South East Coast of India. *International Journal of Animal and Veterinary Advances*. 1 (1): 10-14.

Saha, N., Mollah, M.Z.I., Alam, M.F. and Safiur Rahman, M. 2016. Seasonal Investigation of Heavy Metals in Marine Fishes Captured from the Bay of Bengal and the Implications for Human Health Risk Assessment. *Food Control* 70: 110-18. https://doi.org/10.1016/j.foodcont. 2016.05.040.

Shaheen, N., Nafis MdIrfan, Ishrat Nourin Khan, Saiful Islam, Md Saiful Islam, and Md Kawser Ahmed. 2016. Presence of Heavy Metals in Fruits and Vegetables: Health Risk Implications in Bangladesh. *Chemosphere* 152: 431-38. https://doi.org/10.1016/j. chemosphere.2016.02.060.

Shariatifar, N., MozaffariNejad, A.S. (Amir Khan), and Fathabad, A.E. 2017. Assessment of Heavy Metal Content in Refined and Unrefined Salts Obtained from Urmia, Iran. *Toxin Reviews* 36 (2): 89-93. https://doi.org/10.1080/15569543.2016.1262875.

Shariatifar, N., Seilani, F., Jannat, B., Nazmara, S. and Arabameri, M. 2020. The Concentration and Health Risk Assessment of Trace Elements in Commercial Soft Drinks from Iran Marketed. *International Journal of Environmental Analytical Chemistry*, 1-15. https://doi.org/10.1080/03067319.2020. 1784412.

Sridhara C., N., Kamala, C.T. and Raj, D.S.S. 2008. Assessing Risk of Heavy Metals from Consuming Food Grown on Sewage Irrigated Soils and Food Chain Transfer. *Ecotoxicology and Environmental Safety* 69 (3): 513-24. https://doi.org/10.1016/j.ecoenv. 2007.04.013.

Taghavi, M., Mohammad Ali Zazouli, M.A., Yousefi, Z. and Akbari-adergani, B. 2015. Kinetic and Isotherm Modeling of Cd (II) Adsorption by l-Cysteine Functionalized Multi-Walled Carbon Nanotubes as Adsorbent. *Environmental Monitoring and Assessment.* 187 (11). https://doi.org/10.1007/s10661-015-4911-x.

Topcuoglu, S., Kirbasoglu, C. and Gungor, N. 2002. Heavy metals in organisms and sediments from Turkish Coast of the Black Sea. *Environmental International*. 1069: 1-8

United States Environmental Protection Agency (USEPA). 1989. Risk Assessment Guidance for Superfund, Human Health Evaluation Manual Part A.

United States Environmental Protection Agency (USEPA). 2005. Toxicological Review of Barium and Compounds. US EPA, EPA/635/R-05/001.

United States Environmental Protection Agency (USEPA). 2011. USEPA Regional Screening Level (RSL) Summary Table: November 2011. http://www.epa.gov/regshwmd/risk/ human/Index.htm.

United States Environmental Protection Agency (USEPA). 2012. 2012 Edition of the drinking water standards and health advisories. EPA 822-S-12-001, Office of Water, US Environmental Protection Agency, Washington, DC.

World Health Organization, WHO (2017). Guidelines for Drinking-Water Quality: 4th Edition, Incorporating the 1st Addendum. Geneva: World Health Organization.

Wood, C.M., Farrell, A.P. and Brauner, C.J. 2012. *Homeostasis and Toxicology of Essential Metals*. Academic Press is an imprint of Elsevier. Page 520.

Wuana, R.A. and Okieimen F.E. 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *ISRN Ecology*. 2011, 1-20.

Xue, Z. J., Liu, S.Q., Liu, Y.L. and Yan, Y.L. 2012. Health Risk Assessment of Heavy Metals for Edible Parts of Vegetables Grown in Sewage-Irrigated Soils in Suburbs of Baoding City, China. *Environmental Monitoring and Assessment*. 184 (6): 3503-13. https://doi.org/10.1007/s10661-011-2204-6.

Yamada, K. 2013. Cobalt: Its Role in Health and Disease. *Metal Ions in Life Sciences*. 13 : 295-320. https://doi.org/10.1007/978-94-007-7500-8_9.

Yilmaz, A.B. 2003. Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of *Mugil cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. *Environmental Research*. 92 : 277-281

Yokel, R.A. 2012. The Pharmacokinetics and Toxicology of Aluminum in the Brain. *Current Inorganic Chemistry*. 2 : 54-63.