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HEAVY METALS AND TRACE ELEMENTS LEVELS AND ITS RISK ASSESSMENT IN TWO EDIBLE FISHES FROM WADI HANIFAH, RIYADH, SAUDI ARABIA

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ABSTRACT

African catfish (*Clarias gariepinus*) and Sabaki tilapia (*Orechromis spilurus*) were collected from Wadi Hanifah (Riyadh, Saudi Arabia) and samples of muscle, skin, and gills were analyzed for Be, Cr, Mn, Fe, Co, Ni, Pb, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, and Tl using inductively coupled plasma mass spectrometry (ICP-MS) to highlight the importance of species and tissue selection in monitoring research, contaminant studies, and human health risk assessment. All the heavy and trace elements were found to be below the international permissible standards. The Arsenic (As) levels in the muscles of both the fishes were generally higher, but none of the elements exceeded the permissible limit. The estimated daily intake (EDI) was below the reference dose (RfD) established by the US-EPA and the hazard quotient values indicated that there was no carcinogenic risk for humans.

Key word: Heavy metal, trace element, risk assessment, edible fish, and Wadi Hanifah.

INTRODUCTION

The contamination of fresh water, with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat it poses to public water supplies, but also because of the hazard to human consumption of fish (Uchida *et al*. 1961; FAO/Who, 1972; Hutton, 1987; Terra, *et al*. 2008; Copat, *et al*., 2012; Subotic, *et al*. 2013). The discharges from domestic, industrial and other man-made activity have resulted in high levels of heavy metals concentrations in freshwater (Vinodhini and Narayanan, 2008). Among the environmental pollutants, heavy metals may have major ecological consequences (Mendil, *et al*. 2006). Fishes are good indicators of trace metals pollution and potential risk of human consumption (Papagiannis, *et al*. 2004), and cannot escape from the detrimental effects of these pollutants (Olaifa, *et al*. 2004). Fishes absorb heavy and trace elements from the surrounding environment (Ginsberg and Toal, 2009), depending on a variety of factors such as the characteristics of the species under consideration, exposure period, concentration of the elements, as well as abiotic factors such as temperature, pH, salinity, and hardness (Copat *et al*. 2012). Studies on bioaccumulation of pollutants in fishes are important in determining the tolerance limits of fish species, effects of specific pollutants on fishes, and biomagnifications through food chains (Asuquo, *et al*. 2004). Fish are often considered as an important bioindicator of aquatic ecosystem, because they obtain a high trophic level and are an important source of balanced protein in the human diet (Rahman *et al*. 2012). Because of their importance in human diet, fish must be carefully screened to ensure that dangerous levels of heavy metals are not being shifted to the human population by the consuming the fish (Rahman *et al*. 2012).

To assess the contamination status of 16 heavy and trace elements in the muscle, skin, and gills, two common fish species were captured and human health risks due to consumption of particular fishes has been conducted. For that reason, we estimated the daily intake and compared it with the Provisional Tolerable Daily Intake (PTDI) recommended by the U.S. Environmental Protection Agency (US-EPA) and the Target hazard quotation (THQs) provided in the Region III risk-based concentration table (US-EPA, 2000), in order to evaluate possible alert regarding human health hazards.

MATERIALS AND METHODS

Sampling site: Wadi Hanifah is a “Wadi” or valley in the Nejd region in central Saudi Arabia. The valley runs for a length of 120 km from north to south, cutting through the city of Riyadh, the capital of Saudi Arabia. In the past, it was used as a source of water and now as a convenient means for disposing of the city’s waste water (Abdel-Baki, *et al*., 2011). In addition to the industrial wastes, the valley also receives the municipal wastes and domestic sewage from the Riyadh City. The sampling sites are located between $24^{0}25$ N – $46^{0}48$ E and $24^{0}34$ N – $46^{0}40$ E (Fig. 1).

Sample collection and preparation: Fishes were collected at two sampling sites of Wadi Hanifah using portable lift hand nets and traps as well directly from the fisherman on the spot. The fishes were killed with percussive stunning (Van de Vis *et al*., 2003). Then fishes were transferred in a cooler packed with ice block in order to maintain the freshness and later brought to the laboratory. Ten catfishes were caught from each sampling area, with average length 25.47±2.23 cm (length range 22-28 cm) and average weight of 170.94±41.54 g (weight range 115-240 g). Seven tilapia specimens were collected from each sampling area, with average length 12.24±2.414 cm (length range 8-16 cm) and average weight of 33.36±21.45 g (weight range 18-75 g). Each sample carefully dissected for its muscle, skin and gill. To prevent metal contamination, special care was taken and tissues were dissected with a special ceramic knife, scissors and plastic forceps (Miyako, California, USA). The samples (muscle, skin and gill) were then washed with distill water and cut into small pieces (2–3 cm). Then the tissues were oven dried at 65^{0}C overnight and allowed to cool at room temperature. The dried samples were powdered using a glass mortar, sieved through 1 mm mesh and stored in airtight plastic vials inside desiccators. Water sampling for the analysis of different elements concentration was done at the same two sampling sites and in the same time.

Digestion: The dried fish samples were digested according to the method of Hanson (1973) as described by Rahman, *et al*., (2012). 0.5g of dried powdered fish tissues (three replications) was taken in a digestion apparatus and 2.5 ml conc. H2SO4 and 4ml conc. HNO3 were taken. The mixture was slowly heated using a hot plate for 20 min. at 130^{o}C and allow to cool at room temperature (Rahman, 2004). The content was deionized distill water (DDW) and filtered quantitatively into a 50 ml volumetric flask.

Analytical methods: After acid digestion, concentrations were determined according to APHA (1998) through Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Perkin Elmer, NexION 300D). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono element certified reference solution ICP Standard (Merck). All laboratory plastic and glassware was cleaned by soaking overnight in a 10% nitric acid solution and then rinsed with deionized water.

Risk Assessment: The risk to human health as a result of eating these species was evaluated by calculating Estimated Daily Intake (EDI) was estimated using the following equation (Onsanit, *et al*., 2010)

$EDI = C_{fish} \times D_{fish} / BW$

Where C_{fish}= average trace element concentration in fish muscle (µg/g wet weight), D_{fish} =the average daily freshwater fish consumption (g/day) estimated suggested by FAOSTAT (2013) is only 7 g/day for Saudi, and BW = the average body weight (kg). US-EPA risk analysis, considering an adult average body weight of 70 kg (US-EPA, 2000). The hazard quotient (HQ) was calculated by dividing the estimated daily intake (EDI) by the established RfD to assess the health risk from fish consumption. There would be no obvious risk if the HQ were less than 1(Onsanit *et al*., 2010).

Statistical analysis: One-way analysis of variance tests with significant levels of 5% were conducted on each metal to test for significant differences between tissues. Differences between level means per factor were treated using Excel student's t-test. All values are expressed in mean ± standard deviation.

RESULTS

The mean trace element concentrations in three tissues (muscle, skin, and gills) of the two fish species (*C. gariepinus* and *O. Spilurus*) are presented in Table 1. The accumulation of different elements varied depending on the fish species and tissues. In both the fish species, the gills contained the highest concentration of most of the heavy metals, followed by skin, while the muscle tissue appeared to be the least preferred site for the bioaccumulation of heavy and trace metals. The levels of Be were below the detection limit in all the analyzed tissue samples and were found in very low concentration in the water samples.

The *C. gariepinus* gill tissues presented the highest mean values for Cr, Mn, Fe, Co, Pb, Cu, As, Se, Cd and Tl whereas the skin exhibited the highest mean values for Ni, Zn, Mo and Sb. Furthermore, the *C. gariepinus* muscle showed only the highest mean value for Sr. Similarly, the *O. spilurus* gills presented the highest mean values for Cr, Fe, Co, Pb, Cu, As, Se, Sr, Cd, Sb and Tl; while skin demonstrated the highest mean values for Mn, Ni, Zn, and Mo (Table 1).

Comparatively, higher concentration of Cr (8 µg/g dw) was found in the gills of *O. spilurus*, while higher and lower concentration of Mn was noted in gill (35.75 µg/g) and muscles (0.9 µg/g dw) of *C. gariepinus* respectively. Among all the heavy and trace elements examined, Fe concentration was higher in the gills then in the other tissues of both the fish species; however, there were significant differences in the Fe concentration in different tissues in both the species. The highest Co concentration was found in the gills of both the fish species. Ni showed higher concentrations in the skin of both the species. Pb concentration was found to be higher in the gills, followed by skin and muscle of both the fish species, and Cu concentration was noted to be low in all the tissues, but with significant differences. Furthermore, the relatively higher amount of Zn concentration was found in the skin of both fishes, whereas lowest Zn concentration was noted in the muscles of *C. gariepinus*. The highest As concentrations were found in the gill of *O. spilurus*, whereas there was no significant difference in the As concentrations in the muscle, skin, and gills of *C. gariepinus*.

Higher and lower concentrations of Se were found in the gills and muscle of both the fish species, respectively. In *C. gariepinus*, the muscle contained higher concentrations (3.175 µg/g dw) of Sr than the gills and skin, whereas in *O. spilurus* contained higher values of Sr than *C. gariepinus*. The Sr contained higher concentrations in gill of *O. spilurus* followed by muscle and skin. Mo and Cd were found in higher quantity in the skin of *O. spilurus.* Furthermore, higher concentration of Sb was found in the skin of both the fish species, while Tl concentration was higher in the gills and lower in other tissues of both the species.

These results showed that there were significant differences in the levels of Cr, Mn, Fe, Co, Ni, Pb, Cu, Zn, Se, Sr, Mo, Cd, Sb, and Tl among the tissues examined (muscle, skin, and gills) in *C. gariepinus* ($p<0.05$). Similarly, in *O. spilurus*, significant differences in the levels of Cr, Mn, Fe, Co, Ni, Pb, Cu, Zn, As, Se, Sr, Mo, Sb, and Tl ($p<0.05$) were observed among the tissues examined. The concentration levels of different elements found in the water samples from the highest to lowest were as follows: Fe> Zn>Ni >Sr> As > Mo>Se>Mn> Cu> Cr>Co> Sb>Pb> Tl> Cd > Be.

The average trace element concentrations in muscle were used to evaluate the human health risk assessment from fish consumption. The estimated daily intake (EDI) of trace elements is shown in Table 2. A conversion factor of 4.8 was used to transform wet weight to dry weight (Rahman *et al*., 2012). The results showed that the EDI of trace elements from freshwater fish consumption was several times lower than the reference dose (RfD) values for all trace elements studied and the values of hazard quotient were lower than 0.01, except for As (Table 2).

Table 1. Concentration (mg/kg d. w) of trace elements in different tissues. Data are mean±SD (n =7). The values with non-common letter superscript are significantly different (p<0.05) within the same row.

Element	*C. gariepinus* (µg/g)			*O. spilurus* (µg/g)			Water (µg/l)
	Muscle	Skin	Gill	Muscle	Skin	Gill	
Be	0.001±0.001^{a}	0.002±0.001^{a}	0.002±0.000 ^{a}	0.001±0.00^{a}	0.001±0.00^{a}	0.002±0.001^{a}	0.002±0.001
Cr	1.15±0.32^{a}	0.705±0.28^{b}	1.425±0.41^{a}	1.375±0.52a	7.5±1.88^{b}	8±2.41^{b}	1.692±2.072
Mn	0.9±0.59a	3.5±0.98b	35.75 ±14.08c	1.15±0.23a	8.225±2.37^{b}	11.075±3.56c	2.212+1.1850
Fe	83.75±28.36^{a}	56±23.98^{b}	104.25±41.26^{c}	43.8±15.32 ^{a}	103.15±31.58 ^{b}	171.6±43.33c	49.88±8.657
Co	1.3±0.56a	12.925±2.65b	16.225±4.89b	0.575±0.088^{a}	1.675±0.51 ^{b}	3±0.67	1.157±0.340
Ni	0.425±1.29^{a}	4.25±1.46^{b}	0.1±0.001^{c}	0.4±0.23 ^{a}	4.5±1.32 ^{b}	0.775±0.78c	9.591±2.395
Pb	7.05±3.36^{a}	41.125±11.54^{b}	75.6±11.58^{c}	3.175±0.54 ^{a}	21.22±5 ^{b}	34.525±6.48c	0.644+0.689
Cu	0.775±0.99^{a}	1.625±0.43^{b}	13±2.39^{c}	1.025±0.65 ^{a}	5.2±1.84 ^{b}	13.5±5.21c	1.895+1.765
Zn	32.25±4.97a	287.2±58.95b	38.25±5.46c	50.5±10.54 ^{a}	196.375±48.65^{b}	135.35±35.48c	11.800+4.184
As	0.7±0.18^{a}	0.775±0.31^{a}	0.825±0.24^{a}	0.875±0.36 ^{a}	1.15±0.26 ^{b}	1.375±0.44 ^{b}	4.570+0.976
Se	1.275±0.42^{a}	6.15±1.58^{b}	13.675±3.57^{c}	1.675±0.61 ^{a}	17.2±4.87 ^{b}	21.15±5.21^{b}	2.824+0.565
Sr	3.8±0.85^{a}	2.45±0.64^{b}	3.175±1.21^{c}	15.1±6.52 ^{a}	11.825±3.65 ^{b}	71.15±18.66^{c}	8.013+2.66
Mo	0.1±0.002^{a}	0.2±0.001b	0.125±0.23 ^{c}	0.05±0.002 ^{a}	0.30±0.08 ^{b}	0.225±0.001^{c}	4.189+2.432
Cd	0.15±0.02^{a}	0.5±0.05 ^{b}	0.25±0.12 ^{c}	0.175±0.047^{a}	0.75±0.25 ^{b}	0.50±0.13^{c}	0.020+0.020
Sb	0.0125±0.001^{a}	0.075±0.004 ^{b}	0.02±0.002 ^{c}	0.01±0.001 ^{a}	0.05±0.003 ^{b}	0.025±0.003^{c}	1.064+0.894
Tl	0.0025±0.002^{a}	0.0175±0.001 ^{b}	0.03±0.002 ^{c}	0.01±0.001 ^{a}	0.0125±0.002 ^{a}	0.02±0.001^{b}	0.036+0.012

^{a,b,c} The values with different letters in the same row are significantly different (Excell t-test, p<0.05).

Table 2. Daily intakes of trace elements through freshwater fish consumption by people in Riyadh, Saudi Arabia. EDI, estimated daily intake; RfD, reference doses of trace elements as established by the United States Environmental Protection Agency (2005); Hazard quotient = EDI/RfD. If the ratio is <1, there is no obvious risk. ^{a}Average concentration of inorganic As was estimated as 10% of total As (United States Food and Drug Administration, 1993).

Elements	Average concentration (µg/g ww)		EDI (µg/kg bw/day)		RfD (µg/kg bw/day)	Hazard quotient (EDI/RfD)	
	C. gariepinus	*O. spilurus*	*C. gariepinus*	*O. spilurus*		*C. gariepinus*	*O. spilurus*
Be	0.0002	0.0002	0.000	0.000	-	0.000	0.000
Cr	0.239	0.286	0.111	0.133	3	0.037	0.044
Mn	0.187	0.239	0.087	0.111	140	0.001	0.001

Fe	17.447	9.125	8.142	4.258	700	0.012	0.006
Co	0.271	0.120	0.126	0.056	0.3	0.421	0.187
Ni	0.088	0.083	0.041	0.039	1.6-5	0.025-0.008	0.024-0.007
Pb	1.469	0.661	0.685	0.308	1*	0.685	0.308
Cu	0.161	0.213	0.075	0.099	40	0.002	0.002
Zn	6.719	10.520	3.135	4.909	300	0.010	0.016
As	0.146	0.182	0.068	0.085	0.3	0.227	0.283
Se	0.266	0.349	0.124	0.163	5	0.025	0.032
Sr	0.792	3.146	0.37	1.468	600	0.001	0.002
Mo	0.021	0.010	0.01	0.005	5	0.002	0.001
Cd	0.031	0.036	0.014	0.017	1	0.014	0.017
Sb	0.003	0.002	0.001	0.001	-	-	-
Tl	0.001	0.002	0.000	0.001	0.07	0.000	0.013

*EFSA (2010) *NC = Not calculated

DISCUSSION

Heavy metals and trace elements are very important in environmental research owing to their impact on human health. Indeed, many common heavy metals (Cr, Mn, Fe, Co, Ni, Zn, As, Cd, Pb, and Cu) are known to form materials that are potentially toxic to the environment (Turkmen, *et al*., 2007; Chi, *et al*., 2007; Fallah, *et al*., 2011), and the anthropic effects of heavy metals such as Be, Se, Sr, Mo, Sb and Tl on fish and other aquatic life are less studied (Guerin, *et al*., 2011). In the present study, the values of trace elements were within the range in the muscle, skin, and gills of the freshwater fishes examined. However, the results reported in the literature are wide and depend on many external variables, which make it difficult for comparisons. Nevertheless, the distribution patterns of different elements in the tissues analyzed in this study are comparable. Most of the elements were found to be preferentially accumulated in the gills. The high metal concentrations in the gills could be owing to the formation of complex ion with the mucus, which virtually cannot be completely removed from the gill lamellae before preparing for the analysis (Khail and Faragallah, 2008). Furthermore, the adsorption of metals onto the gill surface as the first target for pollutants in water could also have a significant influence on the total metal levels in the gills. The gills are metabolically active parts that can accumulate higher levels of heavy metals, as reported in various fish species such as *Cyprinus carpio* and *Tinca tinca* from Lake Beysehir, Turkey (Khail and Faragallah, 2008) as well as *Oreochromis mossambicus* and *C. gariepinus* from Olifant River, South Africa (Marzouk, 1994). Deb and Fukushima (1999) confirmed that heavy metals may be in high concentrations in the gill, lung, and digestive gland of fishes because of their relatively high potential for metal accumulation.

Dural *et al*. (2007) and Ploetz *et al*. (2007) reported that the highest levels of Cd, Pb, Cu, and Fe were found in the gills of fish species such as *Sabanejewia aurata*, *Dicentrarchus labrax*, *Mugil cephalus,* and *Scomberomorus cavalla*. Yilmaz *et al*. (2007) reported highest levels of Cd, Co, and Cu accumulations in the gills of *Leuciscus cephalus* and *Lepomis gibbosus*, while these metal accumulations were at lower levels in the fish muscle. Similarly, high rates of accumulation of heavy metals in the gill tissue have been reported by many researchers (Celechovska, *et al*., 2007; Alhas, *et al*., 2009). The concentration of metals accumulated in the gill tissue of fishes is a fairly good indicator of the concentration of those metals in the water where the fishes live (Heath, 1987). Thus, in the present study, the gill results can be considered as very noteworthy owing to the findings of high concentrations of Fe and Zn in water. High levels of metal accumulation in the gills of both the fish species examined can be considered to be representative of the high levels of metal concentrations in the water. As gills are in constant contact with water, they are also exposed to the contaminant metals. Thus, as a result of the metals found in the water of Wadi Hanifah, which are filtered through the fish gills, high levels of metal accumulation was observed in the gill tissue.

Furthermore, in the present study, high levels of Ni, Zn, Mo, and Sb were particularly found in the skin of both the fishes, when compared with those in other tissues. This may be owing to a detoxification pathway accumulating toxic elements in the skin (Hogstrand and Haux, 1991). Similarly, Schenone *et al*. (2014) observed the accumulation of Cr, Fe, Co, Ni, Cu, Zn, Sr, Mo, Cd, Sb, Pb, and As in the skin of *Parapimelodus valenciennis* and *Prochilodus lineatus*, rather than that in the muscle. Ling *et al*. (2013) reported that the ventral and dorsal muscles with skin contained higher concentrations of metals than those without skin in two freshwater fish species. In the present study, muscles contained the least concentrations of heavy metals in both the fish species examined. The results obtained in this study are quite consistent with those reported in the literature (Khail and Faragallah, 2008; Fidan, *et al.*, 2008; Eneji, *et al*., 2011; Can, *et al*., 2012; Subotic, *et al*., 2013). According to Allen-Gill and Martynov (1995), the reason for low levels of heavy metals in fish muscles could be lower levels of binding protein in the muscles.

In the present study, the variation in the trace and heavy metal levels depended on the fish types, similar to that reported by many authors (Eneji, *et al*., 2011; Subotic, *et al*., 2013, Schenone, *et al*., 2014). In terms of comparison of the metal levels in the fish muscles, Fe, Co, Ni and Mo in *C. gariepinus* and Cr, Mn, Cu, Zn, As, Se, Sr, Cd, Sb and Tl in *O. spilurus* were found to be in higher concentrations. This result indicated that higher levels of heavy metals were accumulated in *O. spilurus* muscles, which can be concluded to have a greater capacity for metal bioaccumulation that *C. gariepinus*. Similar observation was also reported by Eneji *et al*. (2011) for *Tilapia zilli* and *C. gariepinus*. These differences in the accumulation of heavy metals may be owing to the different diet of the two species of fishes examined (omnivorous or carnivorous) as well as the difference in the growth rates, size, and length of the fishes (Linde, *et al*., 1998; Papagiannis, *et al*., 2004; Yılmaz, *et al*., 2007).

Recently, the consumption of freshwater fishes have become popular among the Saudi people, and the intake of trace elements, especially toxic elements, through freshwater fish consumption is of high concern for human health risk. To evaluate the health risk to the Saudi people through consumption of freshwater fishes, the daily intake of trace elements was estimated on the basis of the concentrations (wet wt. basis) of trace elements in the muscle of fish and daily fish consumption. In the present study, all the estimated daily intakes of Cr, Mn, Fe, Co, Ni, Pb, Cu, Zn, As, Se, Sr, Mo, Cd, Sb, and Tl were below the guideline values. Thus, the presence of these elements in the muscle of freshwater fishes may not cause any serious health risk to Saudi people. Nevertheless, the skin and gills are occasionally consumed by humans and recycled by the fishermen to feed the fish (Onsanit *et al*., 2010). As few studies have been conducted on the exposure to trace elements through freshwater fish consumption in Saudi Arabia, the data obtained in the present study could be valuable and may provide useful information for assessing the potential health risks for the Saudi people consuming freshwater fishes. As there is no guideline value or provisional limit of heavy or trace element intake (g/day/body wt), the results obtained in this study could be used to derive a guideline value for heavy and trace elements in freshwater fishes and introduce a new provisional limit for the trace element intake (daily/weekly); however, this needs to be further examined in future studies.

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