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Assessment of Accumulation of Trace Elements in Muscles, Gills, Liver and Intestine of *Clarias gariepinus* (Burchell, 1822) from Wadi Hanefah, Saudi Arabia

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ABSTRACT

The concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn was determined in the muscle, gills, liver, and an intestine of the *Clarias gariepinus* from the Wadi Hanefah (WH), Saudi Arabia. The accumulation of metals differed significantly (P<0.05) in the muscle and the skin. Zn were most abundant (55.84±4.85µg/g dry weight) and Pb was in a minimum quantity (0.22±0.02 µg/g dry weight) in the muscle. The similar pattern of elemental deposition was observed in the skin of *C. gariepinus*. The order of bioaccumulation of studied in muscle and skin of was Zn >Fe > Hg > Cu > Se > Mn > Co > Ni >Cr > As > Pb. Fe was most abundant in proximal (682.21±12.52 and 695.60±8.35 µg/g dry weight) and distal (552.46±16.75 and 574.33±10.12 µg/g dry weight) sections of liver in fish collected from upstream and downstream of WH, respectively. As and Cd was recorded in minimum quantity as 0.72±0.28 and 0.61-±0.28 µg/g dry weight recorded in the proximal and the distal section of liver, respectively. Fe was most abundant in proximal (682.21±12.52 and 695.60±8.35 µg/g dry weight) and distal (552.46±16.75 and 574.33±10.12 µg/g dry weight) sections of liver in fish collected from upstream and downstream of WH, respectively.

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SM conceived the study. ZA and SM performed the experiment. KAG analyzed the data. SM wrote the manuscript. HFAAB and FAM helped in manuscript preparation.

Key words:
Metal accumulation, *Clarias gariepinus* trace elements metal uptake, heavy metals.

INTRODUCTION

Environmental pollutants such as metals change genetic, physiological, biochemical and behavioral parameters of aquatic organisms and fish (Scott and Sloman, 2004). Though trace metals are very important for normal physiological processes, their abnormally high accumulation can be toxic to human and aquatic organisms (Uysal *et al.*, 2009; Obasohan *et al.*, 2008). Heavy metals cannot be destroyed through biological degradation and have adverse effects to the aquatic system and eventually to humans, who depend on aquatic products as a source of food (Ashraf, 2005). Fish are among the most susceptible aquatic organisms in water and sediment pollution (Mahboob *et al.*, 2014). The level of the accumulation of these metals depends upon their position in the food chain (Yilmaz *et al.*, 2007). The information about accumulation of metals in different fish tissues is, therefore, important for the supply of safe and healthy food to human beings (Al-Ghanim *et al.*, 2015). "In aquatic system, freshwater fish (main source of protein) constitute a main part of most aquatic organisms and act as bio-indicator of pollution by heavy metals. However, distribution of metals between different tissues depends on the way of exposure (environmental or dietary) and can serve as a sign of contamination (Alam *et al.*, 2007). From water, fish accumulate large amount of metals, which may be toxic for human consumption (Malakootiani *et al.*, 2011). Whereas, high level of heavy metal intake in aquatic system causes an extra stress on fish which in turn concentrate metals in metabolically active organs and tissues (Yousafzai *et al.*, 2010)". Metal pollution in fish has become a one of the major concern and many studies and environmental monitoring programs on trace metal accumulation in fish have been reported (Mahboob *et al.*, 2014; Jabeen and Chaudhry, 2010; Begum *et al.*, 2013; Zhaung *et al.*, 2013). Most of the studies on the metal pollution in fish have been mainly focused on the muscle, as the chief fish part consumed by humans, as well as on the gills, liver, kidneys, and intestine, which represent other major accumulation sites in fish (Strroelli *et al.*, 2006).

Wadi Hanefah (WH) (Fig. 1) "is one of the major natural landmarks in the middle part of the Najd plateau. It is one of the representative natural drains passes through the city of Riyadh and approximately 70% of the city is situated within its catchment area. It extends from the north of Al-Uyaynah to the south of the Al-Hair city. The watershed area of WH was estimated to be about 4400 km^2)" (Al-Ghanim, 2012)".

The fish inhabiting the drain accumulates large amounts of metals, which may be toxic for human consumption (Malakootiani *et al.*, 2011). The level of heavy metal intake in aquatic system causes an extra stress on fish that in turn concentrates metals in

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metabolically active organs and tissues (Yousafzai *et al.*, 2010).

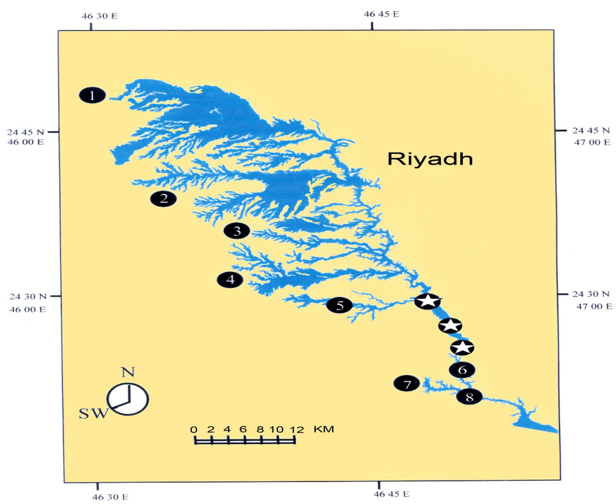

Fig. 1. Map of Wadi Hanifah channel showing (***) sampling station. 1, Alelab Dam; 2, Wadi Safar; 3, Wadi Ubayr; 4, Wadi Laban; 5, Wadi Namar; 6, Al-Hair Dam; 7, Wadi Liha; 8, Al-Hair.

Clarias gariepinus is generally considered to be one of the most important tropical freshwater fish species for aquaculture whose aquaculture potential have been documented (Dada and Wonah, 2003). It also accepts a wide range of natural and artificial food and adapts to a variety of feeding modes in expanded niches. *C. gariepinus* has high consumer preference in ranking; that's why this fish specie was selected for study from this natural reservoir.

In this study, we determine the level of concentrations of different metals in different segments of muscle, liver, and an intestine of the *Clarias gariepinus* from the Wadi Hanefah, in order to assess possible differences between them.

MATERIAL AND METHODS

Sample collection

Seven *Clarias gariepinus* of the same weight about (1600 ± 30.45 g) were collected with a help of hand net during October 2014 from the Wadi Hanefah (WH) from upstream and downstream to see the effect of industrial effluents. Fish specimens were sacrificed with a quick blow to the head, and their total body length (cm) and total body weight (g) were noted. Samples of the muscle (right dorsal muscle), skin, gill filaments, gill arch, liver, and intestine were obtained. Each liver sample was separated into two sections, proximal and distal. The intestine of catfish species is clearly differentiated into three principal regions-proximal, median and distal (Bosi *et al.*, 2006), samples from each region were sectioned. All samples were washed with distilled water and stored at −20°C for further analysis.

Sample preparation and analysis

The samples were freeze-dried using a rotary vacuum concentrator and were prepared by following the procedure reported by (Mahboob *et al.*, 2014). All reagents used in the analysis are analytical were purchased from Merck (Germany).

Chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), cadmium (Cd), and lead (Pb) were determined by using Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The blanks and calibration standard solution were also analyzed in the same way as for the samples. The instrument calibration standards were made by diluting standard (1000 ppm) supplied by Merck, Germany" (Mahboob *et al.*, 2014). The concentrations of each measured metals were corrected for response factors of both higher and lower mass internal standards using the interpolation method.

Statistical analysis

Analysis of variance (ANOVA) was performed to study the effect of seasons for the accumulation of selected metal in different organs of the fish from the different sampling sites. Statistical software (Minitab, 16.0 for Windows, Pennsylvania State University, USA) was used to perform the statistical analysis. The Turkeys multiple comparison test was used to compare means in sampling stations during wet and dry seasons in fish tissues.

RESULTS AND DISCUSSION

The average body weight of the experimental *Clarias gariepinus* specimens was 1400.60±42.64 g. The majority of specimens (75%) were male. The accumulation of Zn, Se, Ni, Mn, Hg, Fe, Cu, Cr, Co, Cd and As in the skin, muscle, liver (proximal and distal section) and intestine (anterior, middle and posterior section) showed different trends (Table I-IV).

Skin

The higher concentration of Zn, Se, Pb, Ni Mn, Hg, Fe, Cu, Cr, Co, Cd and As was recorded in the skin of *C. gariepinus* as 79.48±3.90, 2.77±0.51, 0.44±0.02, 0.78±0.06, 2.11±0.43, 0.97±0.25, 46.71±3.71, 3.66±0.68, 0.57±0.28, 0.59±0.08 and 0.57±0.09 from upstream, respectively (Table I). The higher elemental deposition was observed in the skin of *C. gariepinus* from upstream compared to downstream (Table I*)*. The differences were non-significant (p<0.05) for Mn, Co, and As concentrations in skin of *C. gariepinus* procured from upstream and downstream.

Table I.- Elemental concentrations (µg/g dry weight) ± S.E in skin of the *Clarias gariepinus* from Wadi Hanefah stations

Metal	Upstream	Downstream
Zn	92.84±5.72[a]	82.24±6.90[b]
Se	7.22±1.60[a]	5.80±1.76[b]
Pb	0.62±0.12[a]	0.49±0.10[b]
Ni	1.40±0.33[a]	0.98±0.26[b]
Mn	5.70±0.62[a]	5.01±0.77[a]
Hg	2.35±0.60[a]	1.92±0.70[b]
Fe	146.52±6.90[a]	125.40±5.80[b]
Cu	15.32±2.282[a]	12.60±2.50[b]
Cr	0.68±0.12[a]	0.51±0.15[a]
Co	0.74±0.20[a]	0.58±0.26[b]
Cd	0.48±0.14[a]	0.38±0.11[b]
As	0.55±0.18[a]	0.47±0.12[a]

The values with different superscripts in the same row are significantly different (P ≤ 0.05).

Muscle

The concentration of Zn, Se, Pb, Ni Mn, Hg, Fe, Cu, Cr, Co, Cd and As was recorded in the muscle of *C. gariepinus* collected from upstream as 55.84±4.85, 1.83±0.46, 0.22±0.02, 0.51±0.06, 1.22±0.38, 2.02±0.46, 40.30±3.41, 1.96±0.34, 0.41±0.22, 0.49±0.22 and 0.36±0.04 µg/g dry weight, respectively from upstream. The concentration of Zn, Se, Pb, Ni Mn, Hg, Fe, Cu, Cr, Co, Cd and As was recorded in the muscle of *C. gariepinus* as 45.85±2.70, 1.67±0.48, 0.18±0.01, 0.38±0.05, 1.17±0.25, 1.90±0.38, 37.71±2.90, 1.49±0.28, 0.39±0.15, 0.37±0.06, 0.23±0.04 and 0.27±0.02 µg/g dry weight, respectively from downstream (Table II). The level of As, Co, Cu, Fe, Mn, and Zn were assessed higher in the skin compared to muscle. Zn were most abundant (55.84±4.85µg/g dry weight) and Pb was in a minimum quantity (0.22±0.02 µg/g dry weight) element recorded in the muscle of *C. gariepinus* collected from upstream and downstream, respectively, of WH. The overall comparisons showed that deposition of these studied metals was higher in the skin of *C. gariepinus* from upstream compared to muscle from downstream. The order of bioaccumulation of study metals in muscle and skin of *C. gariepinus* in the skin and muscle was Zn >Fe > Hg > Cu > Se > Mn > Co > Ni >Cr > As > Pb. The accumulation of these metals was higher in skin and muscle samples in a fish collected from upstream.

Table II.- Elemental concentrations (µg/g dry weight) ± S.E in muscle of the *Clarias gariepinus* from Wadi Hanefah stations.

Metal	Upstream	Downstream
Zn	55.84±4.85[a]	45.85±2.705[b]
Se	1.83±0.46[a]	1.67±0.48[b]
Pb	0.22±0.02[a]	0.18±0.01[a]
Ni	0.51±0.06[a]	0.38±0.05[b]
Mn	1.22±0.38[a]	1.17±0.25[a]
Hg	2.02±0.46[a]	1.90±0.38[a]
Fe	40.30±3.41[a]	37.71±2.90[b]
Cu	1.96±0.34[a]	1.49±0.28[a]
Cr	0.41±0.22[a]	0.39±0.15[a]
Co	0.49±0.05[a]	0.37±0.06[b]
Cd	0.28±0.03[a]	0.23±0.04[a]
As	0.36±0.04[a]	0.27±0.02[b]

The values with different superscripts in the same row are significantly different (P ≤ 0.05).

The higher accumulation of As, Co, Cu, Fe, Mn, and Zn in the skin than in the muscle was also recorded by Al-Waher (2008) and Mahboob *et al.* (2014). Al-Waher (2008) reported that higher accumulation of in the skin might be due to the metal complexion with the mucus in this fish. Metal ions from water are able to bind to the mucus layer present on the body surface, which can lead to a higher uptake and absorption in the skin in fishes without scales (Tao *et al.*, 2000). Our results were in line with the above-mentioned workers. The muscles comparatively accumulate the less amount of metals compared to skin (Lenhardt *et al.,* 2012). The different species had maximum concentrations in either muscle or skin (Uysal *et al.,* 2009). In the present study, higher concentrations of Hg recorded in the muscle (Table II). Fu *et al.* (2010) reported skin is not an active tissue for Hg bioaccumulation. The inclusion of skin in the muscle sample reportedly reduced the concentrations of Hg detected in the muscle sample (EPA, 2000), and resultantly did not present the actual information about the acceptable metal levels in the fish meat. Several other scientists also reported differences between the skin and muscles for Cd, Cr, Ni, Pb, and Se accumulation (Storelli *et al.,* 2006; Al-Waher, 2008; Uysal *et al.,* 2009), and in the present study. Fish sampling protocols (EPA, 2000; UNEP, 1984) commonly recommend removal of skin from the muscle sample for metal analyses.

Liver

In the proximal section of liver the accumulation of various metals was recorded as: Zn 119.60±6.23 and

90.48±7.23, Se 8.75±1.71, 5.22±1.10, Pb 0.98±0.23, 0.64± 0.31, Ni 1.83±0.46, 1.17±0.41, Mn 7.05±1.70, 5.60±1.59, Hg 2.51±0.74, 1.89±0.65, Fe 682.21±12.52, 552.46±16.75, Cu 24.60±4.26, 19.61±3.36 Cr 1.02±0.32, 0.79±0.20, Co 1.59±0.48, 1.22±0.32, Cd 0.74±0.17 0.51±0.12 and As 0.72±0.28, 0.51±0.22 µg/g dry weight, from upstream and downstream, respectively. In the distal section of liver the accumulation of various metals was recorded as: Zn 125.72±7.12 and 96.59±6.90, Se 12.33±1.41, 8.32±1.52, Pb 1.14±0.18, 0.78± 0.29, Ni 2.01±0.37, 1.47±0.26, Mn 7.62±1.26, 6.10±1.67, Hg 2.74 ±0.56, 2.01±0.48, Fe 695.60± 8.35, 574.33±10.12, Cu 26.44±3.22, 22.10±3.66 Cr 1.34±0.41, 0.88±0.28, Co 1.72±0.43, 1.25±0.49, Cd 0.85±0.32, 0.61±0.28 and As 0.87±0.22, 0.62±0.20 µg/g dry weight, from upstream and downstream, respectively of WH (Table III). The overall comparison shows higher accumulation of metals in the distal section of liver of *C. gariepinus* collected from upstream of WH. The order of bioaccumulation of studied metals in proximal and distal section of liver of *C. gariepinus* was Fe >Zn > Cu > Se > Mn > Hg > Ni > Co > Pb > Cd > As (Table III). Fe was most abundant in proximal (682.21±12.52 and 695.60±8.35 µg/g dry weight) and distal (552.46±16.75 and 574.33±10.12 µg/g dry weight) sections of liver in fish collected from upstream and downstream of WH, respectively. As and Cd was recorded in minimum quantity as 0.72±0.28 and 0.61-±0.28 µg/g dry weight recorded in the proximal and the distal section of liver of *C. gariepinus* collected from upstream and downstream, respectively. Sivaperma *et al.* (2007) reported that Cd enters into the human body through food and causes toxic symptoms after ingestions of about 10 to 326 mg/kg. Our findings exhibited that there were non-significant difference in the accumulation these metals between two studied liver sections (Table III).

Intestine

The concentration of Zn, Se, Pb, Ni Mn, Hg, Fe, Cu, Cr, Co, Cd and As in the anterior section of intestine of *C. gariepinus* was determined as 92.84±5.72, 7.22±1.60, 0.62±0.12, 1.40±0.33, 5.70±0.62, 2.35±0.60, 146.52± 6.90, 15.32±2.28, 0.68±0.12, 0.74±0.20, 0.48±0.14±0.18 and 0.55 µg/g dry weight from upstream, respectively. The concentration of Zn, Se, Pb, Ni Mn, Hg, Fe, Cu, Cr, Co, Cd and As in the middle and posterior section of intestine of *C. gariepinus* was recorded as: middle section (90.22±5.11, 6.70±1.70, 0.53±0.09, 1.18±0.27, 5.26±0.75, 2.08±0.78, 142.55±6.16, 14.64±2.92, 0.60±0.26, 0.68±0.19, 0.44±0.08, 0.51±0.12) and posterior section (96.12±5.44, 7.78±2.30, 0.75±0.13, 1.32±0.41, 5.88±0.52, 2.66±0.74, 146.76±6.92, 16.28±3.56, 0.72±0.25, 0.79±0.18, 0.51±0.09 and 0.59±0.11) µg/g dry weight, respectively in fishes collected from upstream of WH (Table IV). The differences were non-significant (p>0.05) for elemental concentrations of various metals in the two sections of liver proximal and the median intestine segments in this study (Table IV). The overall comparison shows higher accumulation of metals in the posterior section of intestine of *C. gariepinus* collected from upstream of WH. The order of bioaccumulation of studied metals in anterior, middle and posterior sections of intestine of *C. gariepinus* was Fe >Zn > Cu > Se > Mn > Hg > Ni > Co >Pb > As > Cd (Table IV). Mn concentrations were comparatively lower in the middle segment of intestine (Table IV).

Table III.- Elemental concentrations (µg/g dry weight) ± S.E in liver of the *Clarias gariepinus* from Wadi Hanefah stations.

	Proximal Section		Distal Section	
	Upstream	**Downstream**	**Upstream**	**Downstream**
Zn	119.60± 6.23^b	90.48± 7.23^d	125.72± 7.12^a	96.59± 6.90^c
Se	8.75± 1.71^b	5.22± 1.10^d	12.33± 1.41^a	8.32± 1.52^c
Pb	0.98± 0.23^b	0.64± 0.31^d	1.14± 0.18^a	0.78± 0.29^c
Ni	1.83± 0.46^b	1.17± 0.41^d	2.01± 0.37^a	1.47± 0.26^c
Mn	7.05± 1.70^a	5.60± 1.59^c	7.62± 1.26^a	6.10± 1.67^b
Hg	2.51± 0.74^b	1.89± 0.65^d	2.74± 0.56^a	2.01± 0.48^c
Fe	682.21± 12.52^b	552.46± 16.75^d	695.60± 8.35^a	574.33± 10.12^c
Cu	24.60± 4.26^b	19.61± 3.36^d	26.44± 3.22^a	22.10± 3.66^c
Cr	1.02± 0.32^b	0.79± 0.20^d	1.34± 0.41^a	0.88± 0.28^c
Co	1.59± 0.48^b	1.22± 0.32^c	1.72± 0.43^a	1.25± 0.49^c
Cd	0.74± 0.17^b	0.51± 0.12^d	0.85± 0.32^a	0.61± 0.28^c
As	0.72± 0.28^b	0.51± 0.22^e	0.87± 0.22^a	0.62± 0.20^c

The values with different superscripts in the same row are significantly different ($P \leq 0.05$).

Cd was recorded in minimum quantity as 0.51±0.09 in the middle section of intestine of *C. gariepinus* collected from downstream. A more accumulation of metals detected in the liver and the intestine of *C. gariepinus* from the upstream of WH. The muscle and the skin exhibit significantly different patterns for elemental accumulation as compared to liver and intestine. Comparatively higher concentration of Cd, Co, Cr, Cu, Fe, Hg, and Se in the intestine and liver are probably due

Table IV.- Elemental concentrations (µg/g dry weight) ± S.E in intestine of the *Clarias gariepinus* from Wadi Hanefah stations.

Metal	Anterior section of intestine		Middle section of intestine		Posterior section of intestine	
	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
Zn	92.84±5.72^{b}	82.24±6.90^{c}	90.22±5.11^{b}	80.12±6.10^{a}	96.12±5.40^{a}	84.28±6.52^{c}
Se	7.22±1.60^{b}	5.80±1.76^{e}	6.70±1.70^{d}	5.40±1.51^{e}	7.78±2.30^{a}	6.08±1.88^{c}
Pb	0.62±0.12^{b}	0.49±0.10^{d}	0.53.±0.09^{c}	0.46±0.11^{d}	0.75±0.13^{a}	0.54±0.18^{c}
Ni	1.40±0.33^{a}	0.98±0.26^{c}	1.18±0.27^{b}	0.89±0.21^{d}	1.32±0.41^{a}	1.13±0.31^{b}
Mn	5.70±0.62^{b}	5.01±0.77^{d}	5.26±0.75^{c}	4.80±0.55^{e}	5.88±0.52^{a}	5.06±0.70^{d}
Hg	2.35±0.60^{b}	1.92±0.70^{d}	2.08±0.78^{c}	1.85±0.69^{e}	2.66±0.74^{a}	2.06±0.58^{c}
Fe	146.52±6.90^{b}	125.40±5.80^{d}	142.55±6.16^{c}	122.22±5.80^{e}	146.76±6.92^{a}	126.70±5.89^{d}
Cu	15.32±2.28^{b}	12.60±2.50^{e}	14.64±2.92^{c}	11.12±3.25^{e}	16.28±3.56^{a}	13.82±2.75^{d}
Cr	0.68±0.12^{b}	0.51±0.15^{e}	0.60±0.26^{c}	0.55±0.18^{d}	0.72±0.25^{a}	0.54±0.23^{d}
Co	0.74±0.20^{b}	0.58±0.26^{d}	0.68±0.19^{c}	0.55±0.18^{d}	0.79±0.18^{a}	0.63±0.20^{c}
Cd	0.48±0.14^{b}	0.38±0.11^{e}	0.44±0.08^{d}	0.33±0.11^{e}	0.51±0.09^{a}	0.42±0.07^{c}
AS	0.55±0.18^{b}	0.47±0.12^{d}	0.51±0.08^{c}	0.43±0.09^{e}	0.59±0.11^{a}	0.52±0.08^{c}

The value with a different letters in the same column is different ($p \leq 0.05$)

to the fact these are main depository region in this fish (Storelli *et al.*, 2006). Olowoyo *et al*. (2010) reported that the level of the wide-ranging metals from one specie to another and from one location to another. Biological responses and pollutant concentrations in fish are related to cyclic physiological changes that are linked not only to food availability. In spite of repeated efforts we could not find any support from the literature, this issue was not determined in any of the previous studies. Assessment of metal accumulation in the intestine indicated that Co, Mn, and Zn concentrations in the anterior section differed from those in the two upper intestine sections, while there were no significant differences recorded between the middle and posterior sections. These differences in deposition pattern among the anterior, middle and posterior intestine sections were may be due to the differences in their activity in fish. The findings of the present study may be an initial report on this issue. We are of the view that there is a dire need to conduct further study to find an appropriate sampling method for various fish tissues, whether the skin is to be included with the muscle sample and exact part of the intestine may be sampled.

Statistical analysis exhibited significant differences ($p<0.05$) among skin, muscle, liver and intestine for the accumulation of these metals. The order of bioaccumulation of these metals in skin, muscle, liver and intestine of *C. gariepinus* was Fe >Zn > Cu > Se > Mn> Hg> Ni > Co >Pb > As > Cd.

No comprehensive information on the standardized approach about the sampling of fish tissue is available in the literature. We conducted a small literature survey to know more about this issue. According to Crafford and Avenant-Oldewage (2010), authors either included or removed the skin from the muscle sample, but did not report which sampling method is practically more useful. No reports are available about which part of the liver was included in the sample. Alam *et al*. (2007) reported the distribution of metals in different tissues depends on the way of exposure (environmental or dietary).

CONCLUSIONS

The elemental accumulation levels of some metal ions may have toxic effects on humans, some of them are necessary for sustaining metabolism of the human body, even though high concentrations of all metal ions can pose a threat to human health.

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