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DETERMINATION OF SOME HEAVY METALS IN THREE FISH SPECIES FROM DUHOK CITY MARKETS IN KURDISTAN OF IRAQ

Shahyan S. Ahmed [a] *, Mahmood A. Hasan [a]

[a] Dept. of Chemistry, College of Science, University of Duhok, Kurdistan Region, Iraq (shahyan.sahil; mahmood.ali@uod.ac)

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ABSTRACT:

In this study, the concentrations of some heavy metals including Al, Cu, Fe, Mn, Zn, Cd, Co, Cr, Ni and Pb were determined in the muscles of three commercial fish species available in Duhok city markets in two seasons, using inductively coupled plasma-optical emission spectrometry (ICP-OES), after the wet digestion process. Lowest and highest mean values of metals in µg/g (dry weight) were as follows: Al: Under Detection Limit (UDL)-3.71, Cu: UDL-4.00, Fe: 5.40-21.44, Mn: UDL-3.45, Zn: 13.38-140.11, while Cd, Co, Cr, Ni, and Pb were not detected. The highest levels of Al, Fe and Zn were recorded in Shukhat fish species, and the highest levels of Cu and Mn were recorded in Shabout in the winter season. The highest level of all metals was recorded for Zn (172.8 µg/g) with the highest mean value of (140.11 µg/g) in Shukhat species during winter. Most heavy metal concentrations were below the international permissible limits for fish, however, some of the maximum and mean values of metals were above the international standards. Fe concentrations were above the maximum allowable limits set by WHO (1999). Zn concentrations were above the maximum permissible limits set by joint FAO/WHO (1989) and FAO (2012).

KEYWORDS: Heavy Metal; Fish; ICP-OES; Wet Digestion.

1. INTRODUCTION

Pollution involves the destruction of the natural condition of the environment by human activities (Wafi, 2015). In recent years considerable attention has been paid to the issues of environmental pollution by a wide diversity of chemical pollutants, including the heavy metals (Rajeshkumar,*et al.*, 2017). Heavy metals are attributed to the set of metals and metalloids that have an atomic density of more than 4 g/cm^3, they are also known as trace elements since they present in biological systems in minute concentrations (Muzyed, 2011). It is familiar that some of the trace metals are fundamental for biological processes and are needed for biota to develop and accomplish their life cycle; however, they can be toxic as their concentrations override those necessary for a correct nutritional response (Ahmed, *et al*., 2015). Because heavy metals are not metabolized thus, they are persistent and bioaccumulated (Mohammadnabizadeh, *et al*., 2014). The aquatic environmental pollution by heavy metals including rivers has become a global matter of great and scientific concern since they are non-degradable and most of them have toxic effects on organisms which cause danger to human consumption of fishery resources (Muiruri, 2013; Subotić, *et* al., 2013).The pursuing programs and studies on heavy metals in aquatic systems are of high significance (Asante,2013). Rivers can be polluted by both natural activities such as soil erosion, natural weathering of the earth's crust, atmospheric deposition, run-off from adjacent agricultural lands and anthropogenic activities such as industrial effluent and sewage discharges, accidental chemical waste spills, insect or disease control agents applied to crops, mining and smelting activities. (Jaishankar, et al.,2014; Jayaprakash, et al., 2015; Arulkumar, et al., 2017; Jia, et *al*., 2017).

Relying on the heavy metals concentrations they may have valuable or noxious effects on plant, animal and human life. Metals as (Cu, Fe, Mn and Zn) are fundamental metals (essential) due to their remarkable role in biological systems, whereas (Hg, Pb, and Cd) are toxic, even in trace concentrations (Subotić, *et al.*, 2013; Tüzen, 2003;Alturiqi and Albedair, 2012; Perugini, *et al*., 2014; Makedonski, *et al*.,2015). Under certain circumstances, heavy metals can be bioconcentrated in fish tissues and/or biomagnified to serious levels through dietary exposure and as a result transferred to humans via their diet (Low, *et al*., 2015). Because fish is a substantial part of the diet for human, it is not surprising that the quality and the integrity aspects of fish are of special concern to human health (Rajeshkumar,*et al*., 2017).In the human being, heavy metals accumulation has serious effects on the brain, liver, kidneys, lungs, and muscles (Arantes,*et al*., 2016). In recent years the consumption of fish has increased significantly due to that fish is a significant food source of macronutrients like carbohydrates, animal protein, fatty acids, vitamins (as vitamin D and vitamin B_{12}) (Julshamn, *et al*.,2011), and polyunsaturated fatty acids and micronutrients (copper, zinc, iron, and selenium) for human health (Arulkumar, *et al*., 2017). In addition, they contain the unsaturated omega-3 fatty acids that are known to contribute to good health (Perugini, *et al*., 2014), and fish could be a great source of iodine and fluorine (Pirestani, *et al*.,2014).

This study aimed to estimate the concentration of heavy metals (Al, Cu, Fe, Mn, Zn, Cd, Co, Cr, Pb, Ni and) in the fish species available in Duhok city markets which consumed largely by consumers and to investigate if there are any seasonal variations in their concentrations.

* Corresponding author

2. MATERIALS AND METHODS

2.1 Instrumentation and Chemicals:

Concentrations of Al, Cu, Fe, Mn, Zn, Cd, Co, Cr, Cu, Ni and Pb were determined by ICP-OES (Perkin Elmer Optima 2100 DV). Instrumental conditions were summarized in Table I. Standard solutions of metal ions were prepared from their stock solutions of 1000 µg mL^{-1} (High Purity Standards-Charleston; SC. Standards). Lower concentrations were prepared by appropriate dilutions from stock solutions with distilled water. Deionized water was used in experiments (Atlas Filter, Italy). Analytical balance (Sartorius, Germany), hot plate (Stuart SB500, UK), oven (Qumai, China) were available in the laboratory. HNO_3, $HClO_4$ and H_2O_2 were supplied from Sigma-Aldrich (Darmstadt, Germany). To check the accuracy of the ICP-OES method, NIST 1643e (trace elements in water) supplied from National Institute of Science and technology, USA used as standard reference material and NWTM-15 (fortified water sample) supplied from High Purity Standards, USA used as certified reference material.

Table 1: Instrumental conditions for ICP-OES

Parameter	Conditions	
RF Power	1450 W	
Plasma gas flow rate, L/min	15	
Auxiliary gas flow rate, L/min	0.2	
Nebulizer gas flow rate, L/min	0.8	
Sample flow rate, mL/min	1.5	
View mode	Axial	
Read	Peak area	
Source equilibration time, sec.	15	
Read delay, sec.	50	
Replicates	3	
Background correction	2-point (manual point correction)	
Spray chamber	Scott type spray chamber	
Nebulizer	Cross-flow gem tip nebulizer (HF resistant)	
Detector	CCD	
Purge gas	Nitrogen	
Shear gas	Air	
Plasma Gas	Argon	
Wavelength, nm	Cr (267.716) Pb (220.353) Ni (231.604) Cd (228.802) Mn (257.610)	Cu (327.393) Fe(238.204) Zn (206.200) Co (228.616) Al (396.153)

2.2 Sampling and sample preparation:

A total of 30 of three fish species from Tigris River near Faidah Township were purchased during the winter (March 2018) and 30 fish of the same species were purchased during the summer (July 2018). From each type, 10 fishes were purchased as in Table 2. The total length (cm) and the body-wet weight (g) of all specimens were measured.

Each fish was dissected by the fishmonger and six muscle pieces were cut from every individual fish, two near the head, two from the trunk and two near the tail using ceramic and stainless-steel knives. The samples were kept in falcon plastic bags and put into an icebox, brought to the laboratory and stored in a deep freezer at -20 ^{o}C until being ready for analysis.

Table 2: Details of fish samples

No.	Scientific name	Common Name	TL (cm) mean	BW (g) mean	Date
1	*Cyprinuscarpio*	Common Carp S1	47.2	1845	12/3/2018
2	*Arabibarbusgrypus*	Shabout S1	56.9	1510	15/3/2018
3	*Carassiuscarassius*	ShukhatS1	26.4	300	
4	Cyprinuscarpio	Common Carp S2	43.6	1330	10/7/2018
5	Arabibarbusgrypus	Shabout S2	38.2	400	
6	Carassiuscarassius	ShukhatS2	25.2	215	

TL = Total Length, BW= BodyWeight, S1= Winter season, S2 = Summer season

2.3 Sample Treatment

All muscle samples were thawed, washed with de-ionized water, 10 g of each sample were dried at 110oC in the oven to a constant weight (Hashim, *et al*.,2014; Küpeli, *et al*.,2014),ground by mortar and pestles to be homogenized, kept in plastic cup tubes and stored in the refrigerator until the time of digestion. Moisture content for all the analyzed tissue samples was calculated using the difference between the wet and dried weights (Anandkumar, *et al*., 2018).

From each sample, one gram of homogenized dried muscle was taken and a mixture of 6 ml of (1:1) 69.5% HNO_3 and 70% $HClO_4$ acids was added. The solutions were heated on hotplate at 72 ^{o}C for 30 minutes, a clear solution was obtained (Al-Najare, *et al*., 2015), the solutions removed from the hotplate and cooled, for lipid digestion 2 ml of 50% H_2O_2 was added to each sample and heated for 15 min at 72^{o}C (Hashim, *et al*.,2014; Negi and Maurya,2015).

The samples were filtered through (Macherey-Nagel) (4-12µm) filter papers and diluted to 50 ml by deionized water in volumetric flasks (50ml), (Al-Najare, *et al*., 2015). The samples were kept in glass reagent bottles in the refrigerator until the time of metal analysis by ICP-OES. The final concentration of each metal in (µg/g) dry weight was calculated using the formula:

Metal concentrations in sample (µg/g) = ICP reading (mg/L) * Dilution Factor

Where: Dilution factor = dilution volume / weight of dried sample digested

Dilution volume = 50 ml

Weight of dried sample digested = 1 gm.

The data were analysed by the Statistical Analysis System (SAS) software and the means comparisons were done by Duncan's Multiple Ranges Test under 5%, which was claimed by (SAS, 2009).

3. RESULTS

The total moisture content (H_2O %) for all the analyzed muscle tissue samples was calculated and the results are shown in Table 3.

Table 3: Total moisture content of fish muscles

Season	Fish species	Moisture content % (n=10) H_2O%
Winter	Common Carp, *Cyprinuscarpio*	76.84%
	Shabout, *Arabibarbusgrypus*	80.57%
	Shukhat, *Carassiuscarassius*	79.86%
Summer	Common Carp, *Cyprinuscarpio*	77.66%
	Shabout, *Arabibarbusgrypus*	79.12%
	Shukhat, *Carassiuscarassius*	80.32%

n = number of replicates

3.1 Analytical Characteristic:

The Analytical characteristics (Linear range, mg/L, Slope, Intercept, r^2, limit of detection (LOD) (ppm or mg/L) and limit of quantitation (LOQ) (ppm or mg/L) of the measured heavy metals are given Table 4.

Table 4: Analytical characteristics of the measured elements

Element	Method	Linear range, mg/L	Slope	Intercept	r^2	LOD (ppm or mg/L)	LOQ (ppm or mg/L)
Al	Linear Calculated Calibration Interpolation	**0.05-80**	**169700**	**-3661.7**	**0.9999**	**0.0012**	**0.0040**
Cd		**0.05-10**	**9291**	**-322.3**	**0.9999**	**0.0038**	**0.0125**
Co		**0.05-10**	**3706**	**326.8**	**0.9884**	**0.0009**	**0.0030**
Cr		**0.05-10**	**6328**	**-142.1**	**0.9999**	**0.0016**	**0.0053**
Cu		**0.05-10**	**16020**	**3001**	**0.9993**	**0.0009**	**0.0030**
Fe		**0.05-10**	**9023**	**451.0**	**0.9948**	**0.0029**	**0.0096**
Mn		**0.05-10**	**59040**	**-278.1**	**0.9999**	**0.0019**	**0.0063**
Ni		**0.05-10**	**1239**	**7.9**	**0.9903**	**0.0019**	**0.0063**
Pb		**0.05-10**	**358.7**	**-22.1**	**0.9985**	**0.0041**	**0.0135**
Zn		**0.05-5.0**	**9817**	**634.3**	**0.9635**	**0.0011**	**0.0036**

Accuracy of the method was checked by applying two certified reference samples. Mean recoveries of the analysed metals were between 96.73 and 101.45%, indicating a good agreement between certified and observed values; the results are shown in Table 5.

Table 5: Accuracy of the method

Element	NIST 1643e			NWTM-15		
	Certified value (µg/L)	Observed value (µg/L)	Recovery (%)	Certified value (µg/L)	Observed value (µg/L)	Recovery (%)
Al	141.8±8.6	138.296±8.215	97.53%	21.7	21.209±1.098	97.74%
Cu	22.76±0.31	22.089±0.232	97.05%	18.3	17.856±1.296	97.57%
Fe	98.1±1.4	97.652±1.102	99.50%	25.0	25.362±1.658	101.45%
Mn	38.97±0.45	38.198±0.632	98.00%	18.4	18.102±1.182	98.38%
Zn	78.5±2.2	77.963±2.145	99.32%	-	-	-
Cd	6.568±0.073	6.365±0.064	96.91%	13.2	13.106±0.896	99.30%
Co	27.06±0.32	26.185±0.489	96.77%	15.1	14.856±1.085	98.38%
Cr	20.40±0.24	20.236±0.199	99.20%	17.2	17.365±1.265	100.96%
Ni	62.41±0.69	61.416±0.700	98.41%	18.1	18.123±1.085	100.13%
Pb	19.63±0.21	19.065±0.303	97.12%	11.8	11.414±0.942	96.73%

3.2 The concentration of metals in fish muscles of different species

Results of the ICP-OES analysis of heavy metals concentrations and statistical analysis in various fish species during this two-season study are given in Table 6 (Cd, Co, Cr, Ni and Pb were under detection limits during the two seasons).

Table 6: Al, Cu, Fe, Mn and Zn concentrations (µg/g dry weight) in fish samples

Season	Fish Species	Al		Cu		Fe		Mn		Zn	
		Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
Winter	Common Carp S1 (Tigris)	1.45-2.70	1.84±0.136	1.80-2.45	2.07±0.062	11.80-22.60	16.20±1.118	-	-	5.10-20.60	13.38 ±1.958
	Shabout S1	2.45-3.25	2.77±0.090	3.25-4.95	4.00±0.178	4.95-10.10	6.24±0.587	2.45-4.30	3.45±0.166	19.95-39.00	27.58 ±1.951
	ShukhatS1	1.80-4.90	3.71±0.273	3.25-4.10	3.84±0.100	15.30-30.00	21.44±1.307	2.45-2.95	2.65±0.052	100.3-172.8	140.11±6.902
Summer	Common Carp S2 (Tigris)	-	-	-	-	5.10-11.30	7.99 ±0.735	1.85-2.75	2.22±0.099	72.60-118.25	98.43 ±4.091
	Shabout S2	1.15-2.10	1.71±0.106	1.10-2.10	1.63±0.097	2.25-9.25	5.40 ±0.559	1.95-3.25	2.51±0.132	13.25-60.60	34.79±4.728
	ShukhatS2	-	-	1.95-3.25	2.53±0.139	9.95-17.60	12.04±0.786	-	-	49.95-82.60	62.96±2.736

(-): Under Detection Limit (UDL), S1: Season one (winter) and S2: Season two (summer)

Aluminum: The highest level of Al (4.90 µg/g) and the highest mean value (3.71 µg/g) were detected in Shukhat in winter. Significant differences ($p \leq 0.05$) were observed for aluminum mean values in fishes collected in winter.

Seasonally, the highest concentration was found in Shukhat in winter and lowest in summer Shabout. There was a significant difference ($p \leq 0.05$) in Al mean concentrations between the two seasons for Shabout species.

Copper: The copper concentrations ranged from not detected (ND) in Common Carp S2 to the highest concentration of 4.95 µg/g in Shabout S1 with a highest average value of 4.00 µg/g in Shabout S1. A significant difference ($p \leq 0.05$) was found in Cu levels between the two seasons for Shabout and Shukhat fish species. During winter, copper levels in fish species showed no significant difference ($p>0.05$) among Common Carp and shukhat, whereas, the average concentrations of Cu in summer for Shabout and Shukhat were significantly different ($p \leq 0.05$).

Iron: The concentrations of iron showed the minimum value of 2.25µg/g in Shabout S2 and the maximum value of 30.00µg/g in ShukhatS1 with the highest mean value of 21.44µg/g. The mean concentrations of Common Carp and Shukhat varied significantly ($p \leq 0.05$) between the two seasons, whereas Shabout during the two seasons showed no significant difference ($p>0.05$). In summer, statistical results revealed that Fe concentrations in Common Carp and Shabout were not significantly different ($p>0.05$), but both were significantly different ($p \leq 0.05$) from Shukhat species.

Manganese: Manganese concentrations in Common CarpS1 and ShukhatS2 were under the limit of detection, while the highest level (4.30 µg/g) with a highestaverage value of (3.45 µg/g) recorded in Shabout S1. Statistical analysis of data showed a significant difference ($p \leq 0.05$) in manganese mean concentration in Shabout between winter and summer. In summer, Mn mean concentrations in both Common Carp and Shabout were not significantly different ($p>0.05$).

Zinc: The concentration of zinc varied among all fish species studied and in both seasons. The lowest concentration of Zn was (5.10 µg/g) in Common Carp S1 and the highest value (172.80 µg/g) observed in ShukhatS1, which also had the highest average value (140.11 µg/g). The levels of zinc in Common Carp and Shukhat statistically were significantly different ($p \leq 0.05$) between winter and summer, however, Zn concentrations insignificantly different ($p>0.05$) in Shabout between the two seasons. Comparing Zn mean levels in both seasons among Common Carp, Shabout and shukhat, statistical results indicated significant variation ($p \leq 0.05$).

4. DISCUSSION

The results of this study indicated that fish species collected in winter had higher heavy metals concentrations than those collected in summer, except for Mn in Common Carp, in addition to Zn in Common Carp and Shabout that had higher concentrations in summer, this may be due to that fishes collected in summer were of smaller size (weight and length) compared to those collected in winter. Asante showed that there was a positive correlation between fish sizes and metal concentrations (Asante, 2013). Yi and Zhang demonstrated that in most cases there was a positive correlation between fish size and metal levels (Yi and Zhang, 2012). Caglar et al. found that there was a positive correlation between heavy metal accumulation in muscle and fish size (Caglar,*et al*., 2019). Another explanation for this variation is that, during late spring and early summer increased fluvial discharges from rainfall and melting snow contribute to raising the water level of the river, resulting in dilution and decrease in metal concentrations in the river water (Varol and Sünbül, 2018).

Differences in metal concentrations at a particular location may be related to the seasonal changes of the organism's tissues weight rather than to any change in the metal content of the organism (Bahnasawy,*et al.,*2009).Furthermore, the late winter and early spring (in the middle of March) in which fishes were purchased is the spawning season in the region and at this time fishes have increased fat and proteins in their bodies, which are concentrated with high levels of trace metals, giving rise to higher metal concentrations in fish species (Hantoush,*et al.,*2012).

In the current study, the concentrations of the heavy metals Cd, Co, Cr, Pb and Ni were not detected since their concentrations in fish muscles were very low and under the limit of detection, this may be due to that, these elements are of minute amounts and could not be detected by the ICP-OES instrument. It could also be related to the fact that Tigris River in this region is not polluted to a degree that can cause bioaccumulation of these metals in fish muscles, because manufactories, industrial plants or mining processes are not present on the river yet near the fishing site. Another reason which can contribute is that the fish muscles accumulate the least amount of heavy metals if compared to active metabolite organs as gills, liver and kidney (Balasim,2013), (Abarshi,*et al*., 2017), (El-Moselhy,*et al*.,2014), (Nwani, *et al*., 2009),the muscle tissue usually has the lowest metal levels in fish (Mohammadnabizadeh,*et al*., 2014).

The maximum permissible limits of heavy metals (Cu, Fe, Mn, Zn) in fish muscles (ppm) according to international standards; FAO 2003 retrieved 2012, WHO 1996, FAO/WHO 1989, FAO 2009, WHO 1999 and WHO 1993 are given table 7.

Table 7: Maximum permissible limits of heavy metals in fish muscles (ppm) according to international standards.

Metal	Standard Limits ppm	Organization	Reference
Cu	30	FAO 2003 retrieved 2012	Baharom, and Ishak, 2015
		WHO 1996	Jasim, and Shkhaier, 2016
		FAO/WHO 1989	Abarshi et al., 2017
Fe	50	FAO 2009	Al-Najare, et al., 2015
	4.5	WHO 1999	Nasir and Al-Najar, 2015
	186	FAO/WHO 1989	Abarshi et al., 2017
Mn	8	FAO 2009	Al-Najare, et al., 2015
	12.97	FAO/WHO 1989	Abarshi et al., 2017
Zn	40	FAO 2003 retrieved 2012	Baharom, and Ishak, 2015
	150	WHO 1993	Balasim, 2013
	92.7	FAO/WHO 1989	Abarshi et al., 2017

5. CONCLUSION

This investigation showed that the concentrations of the metals aluminum, copper, manganese, iron and zinc were different in the muscles of studied fish species during winter and summer seasons. The concentrations of the heavy metals cadmium, chromium, lead, cobalt and nickel were not detected since their concentrations in fish muscles were very low and under the limit of detection. The observations of this study demonstrated that fish species collected in winter had higher concentrations of heavy metals than those collected in summer, except for magnesium in Common Carp, in addition to zinc concentration in Common Carp and Shabout that had higher concentrations in summer. The highest metal mean concentrations were either in Shabout or Shukhat species during winter season. Shabout had the highest concentration of copper and manganese, whereas Shukhat contained the highest levels of aluminum, iron and zinc.

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