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Bioaccumulation of Heavy Metals in Freshwater Fish Species Retailed in Kayseri Region: Potential Public Health Hazard of Toxic Metals

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Abstract: In this study, it was aimed to investigate the heavy metal (Al, Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Se, V, Zn) accumulations in the muscles of freshwater fish retailed in the Kayseri region by inductively coupled plasma mass spectrometry (ICP-MS). For this purpose, a total of 91 edible freshwater fish, including 37 *Cyprinus carpio*, 27 *Sander lucioperca*, 6 *Tinca tinca*, 5 *Esox lucius*, 4 *Squalius cephalus*, 5 *Copeata tinca*, 3 *Silurus glanis*, and 4 *Oncorhynchus mykiss* species, were used. Statistical analyses such as principal component, cluster, and correlation analysis were done to evaluate the obtained data. The order of mean concentrations (mg kg^{-1} wet wt.) of metals in all fish meat samples were Al (204) > Zn (10.2) > Fe (6.43) > Cu (0.666) > Hg (0.589) > Ni (0.556) > Pb (0.497) > Cr (0.396) > Ag (0.291) >Se (0.144) > As (0.074) > Co (0.043) > Cd (0.037) >V (0.027). Hg, Cd, and Pb concentrations in some samples exceeded the permissible legal limits, whereas As concentrations was lower than maximum allowable limits. Ninety-one fish samples were categorized into five categories by principal component analysis (PCA) with Eigenvalues higher than 1.00. Cluster analysis results showed that the pollution with metal pairs analyzed might be from similar sources. In conclusion, in this study in some fish samples, the levels of some toxic elements (Hg, Cd, and Pb) were higher than permitted legal limits. Therefore, it could be suggested that the consumption of these fish might pose a hazard for public health.

Keywords: Freshwater fish, Heavy metals, HCA, ICP-MS, PCA

Kayseri Bölgesinde Perakende Satışı Yapılan Tatlı Su Balık Türlerinde Ağır Metallerin Birikimi: Toksik Metallerin Potansiyel Halk Sağlığı Tehlikesi

Özet: Bu çalışmada, Kayseri bölgesinde perakende satışı yapılan tatlı su balıklarının kaslarında ağır metal (Al, Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Se, V, Zn) birikimlerinin indüktif eşleşmiş plazma kütle spektrometresi (ICP-MS) ile araştırılması amaçlandı. Bu amaçla 37 adet *Cyprinus carpio*, 27 adet *Sander lucioperca*, 6 adet *Tinca tinca*, 5 adet *Esox lucius*, 4 adet *Squalius cephalus*, 5 adet *Copeata tinca*, 3 adet *Silurus glanis* ve 4 adet *Oncorhynchus mykiss* türü olmak üzere toplam 91 yenilebilir tatlı su balığı kullanıldı. Elde edilen verileri değerlendirmek için temel bileşen, küme ve korelasyon analizi gibi istatistiksel analizler yapıldı. Tüm metallerin numunelerdeki derişimleri (mg kg^{-1} yaş ağırlık) sırasıyla; Al (204) > Zn (10.2) > Fe (6.43) > Cu (0.666) > Hg (0.589) > Ni (0.556) > Pb (0.497) > Cr (0.396) > Ag (0.291) > Se (0.144) > As (0.074) > Co (0.043) > Cd (0.037) > V (0.027) idi. Bazı numunelerdeki Hg, Cd ve Pb derişimleri yasal limitleri aşarken, As derişimleri izin verilen maksimum limitlerin altındaydı. Temel bileşen analizine göre (PCA), 91 balık örneği özdeğerleri 1.00'den yüksek olan beş kategoriye ayrıldı. Küme analizi sonuçları, analiz edilen metal çiftleri ile kirliliğin benzer kaynaklardan olabileceğini gösterdi. Sonuç olarak, bu çalışmada bazı balık örneklerinde bazı toksik elementlerin (Hg, Cd ve Pb) seviyeleri yasal izin verilen limitlerin üzerinde bulundu. Bu nedenle, bu balıkların tüketilmesinin halk sağlığı açısından tehlike oluşturabileceği sonucuna varıldı.

Anahtar Kelimeler: Tatlı su balığı, Ağır metaller, HCA, ICP-MS, PCA

1.Introduction

Many kinds of industrial wastes, traffic, and agricultural activities lead to freshwater pollution, of which the most important and common are heavy metals. However, fish are known to be bio-indicators for heavy metals in aquatic ecosystems. In other words, fish tissue tends to amass heavy metals (1,2). Contamination of water resources by heavy metals constitutes significant threats for ecosystem safety and aquatic organisms and human health. When heavy metals are accumulated in water, they can be transferred to the food chain, and it might pose a public health risk due to their ability to bioaccumulate, toxicity, and the fact that they cannot be eliminated from the body (3-6).

The toxic metal level in fish depends on fish age, size (length and weight), sex, dietary habit, location, amount of time exposed to the elements, and environmental conditions

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(water chemistry, salinity, hardness, temperature, and contaminants) (3, 5, 7). Metals can be classified as necessary for different physiological and biochemical functions (copper, zinc, selenium, iron, cobalt, magnesium, manganese, molybdenum, nickel, chromium, vanadium) and potentially toxic (arsenic, cadmium, lead, mercury, and silver) (8-10). Fish are important foodstuff for their ability to collect different elements in their muscles (4). However, because they tend to lose salts and gain water, freshwater fish are exposed to heavy metal accumulation more than marine fish (11). Several studies about metal contamination in fish muscles have been reported using different analytical techniques, such as atomic absorption spectrometry (AAS) (4,5,10), chemical vapor generation-atomic fluorescence spectrometry (CVG-AFS) (12), inductively coupled plasma-optical emission spectrometry (ICP-OES) (13, 14) and recently there is limited data about heavy metal pollution in fish muscles by inductively coupled plasma-mass spectrometry (ICP-MS) (10, 15, 16). ICP-MS, known as a reliable method for multi-element analysis in food samples and used to identify the sample groups of the measured variables, are hierarchical clustering analysis (HCA) and PCA (17).

This study was designed i) to detect the Al, Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Se, V, and Zn levels of eight edible freshwater fish species in Kayseri city of Türkiye by ICP-MS, ii) to realize PCA, HCA, and correlation analyses by datum obtained from fish meat samples, and iii) to investigate the relationships among heavy metal concentrations in fish meats.

2. Materials and Methods

2.1. Instrumentation

An ICP-MS instrument (ICP-MS Agilent 7500a, Agilent Technologies, Tokyo, Japan) equipped with an autosampler was used in this study. The instrumental conditions were radio frequency power 1300 W, sample depth 7.6 mm; torch-H-0.5 mm, torch-V 0.7 mm, carrier gas 1.13 L min^{-1}, auxiliary gas flow rate 0.9 L min^{-1}, plasma gas flow rate 15 L min^{-1}, nebulizer pump 0.12 rps, spray chamber temperature 2°C, and interface cones of nickel. Before each experiment, the instrument was tuned for daily performance using the Agilent ICP-MS tuning solution of 10 µg L^{-1} (Ce, Co, Li, Tl, and Y). ICP-MS was applied for ^{27}Al, ^{51}V, ^{53}Cr, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{107}Ag, ^{111}Cd, ^{202}Hg, ^{208}Pb determinations in standards and the digests of the analyzed samples. A Berghofmws-4 microwave system (Berghof Speedwave MWS four digestion system, Germany) with closed Teflon vessels was used for microwave digestion of the samples.

2.2. Reagents

The reagents used in the experiment were analytical grade. Ultra-high purity (UHP) water (18.2 MΩ cm) was used to prepare all solutions. A multi-element calibration standard solution of 10 µg mL^{-1} (Agilent Technologies, USA) was applied to prepare multi-element standard solutions. A mixed standard solution of Rh, Sc, and Bi of 200 µg L^{-1} was applied as an internal standard to ensure the instrument's stability and check instrumental drift and non-spectral interferences. Concentrated HNO_3 (70%) and H_2O_2 (30–32%) were supplied by Merck (Germany). Dogfish Liver as reference material (DOLT-4) was acquired from the National Research Council, Ottawa, Ontario, Canada.

2.3. Sample collection

In this study, a total of 91 edible freshwater fish samples of 8 species (37 *Cyprinus carpio*, 27 *Sander lucioperca*, 6 *Tinca tinca*, 5 *Esox lucius*, 4 *Squalius cephalus*, 5 *Copeata tinca*, 3 *Silurus glanis*, and 4 *Oncorhynchus mykiss*) were purchased from a local fisherman in Kayseri, Turkey, between February and May 2018. Their length and weight ranged from 19 to 64 cm and 72 to 1836 g, respectively (Table 1). Upon collection, the fish were immediately transferred into the icebox and conveyed into the laboratory, and then protocol numbers were given. Before dissection, they were allowed to thaw, and anthropometric measurements were taken. The edible muscles from these organisms were cut and stored at -20 °C. Before heavy metal analysis, the frozen fish samples were thawed in the refrigerator at +4 ºC for 12 hours. After the epaxial muscle tissues of the fish were separated and washed with UHP water to prevent cross-contamination and placed in different petri dishes. Petri dishes were kept in an oven at 55ºC for 12 ± 1 hour until the samples reached a constant weighing weight. The samples were ground into a homogenized powder with a porcelain mortar and pestle then transferred into falcon tubes, covered with paraffin, and stored in a desiccator.

2.4. Microwave digestion procedure

Briefly, 0.20 g of homogenized and powdered fish meat was digested with 2 mL of H_2O_2 (30%) and 8 mL of HNO_3 (65%) in a microwave digestion system (Berghof microwave system, Germany). The digestion program consisted of one step with potency: T(ºC): 200, P(bar): 40, power (%): 90, Ta (min):15 and time (min): 40. After cooling for about 20 min, the digest was diluted to 25 mL by adding UHP water. The levels of Al, V, Cr, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Cd, Hg, and Pb in the clear solutions were assessed using an Agilent 7500a ICP-MS with interior standards. The CRM (Dogfish Liver, DOLT-4) was dissolved with the same digestion method.

Table 1. Fish species and their length and weight range

Fish species	Number of samples (n)	Length range (cm)	Weight range (g)
Tinca tinca	6	22-34	251-513
Cyprinus carpio	37	21.5-50	178-1836
Sander lucioperca	27	25-37	134-492
Esox lucius	5	37-47	378-725
Squalius cephalus	4	32-36	456-691
Copeata tinca	5	19-39	72-708
Silurus glanis	3	48-64	692-1640
Oncorhynchus mykiss	4	23.5-26.5	174-282

2.5. Quality control and assurance

The microwave digestion/ICP-MS method precision was validated by examination of the Dogfish Liver (DOLT-4). The levels of elements agreed with the DOLT-4, and the recoveries of elements ranged from 90% to 112% (Table 2). The limits of detection (LOD) were calculated as the ratio of the three standard deviations (SD) of the blank signals to the slope (b) of the calibration curve (3SD/b, n=10). The LODs were 4.46, 0.04, 1.50, 2.08, 0.96, 1.50, 0.12, 0.71, 0.15, 0.03, 0.07, 0.31, 0.64, and 0.72 µg L^{-1} for Fe, Al, Zn, Cu, Ni, Cr, V, Pb, As, Cd, Co, Se, Hg and Ag in fish samples, respectively. The determination coefficients for calibration curves of the metals were found to be ≥ 0.99.

Table 2. Determination of elements in Dogfish Liver certified reference material (DOLT-4)

	Concentration (µg g^{-1})		
	Certified[a]	**Found[b]**	**R(%)**
Al[c]	200	215 ±10	108
V[c]	0.6	0.56 ± 0.01	93
Cr[c]	1.4	1.52 ± 0.02	109
Co[c]	0.25	0.28 ± 0.02	112
As	9.66 ± 0.62	9.55 ± 0.16	99
Cd	24.3 ± 0.8	24.2 ±0.3	100
Cu	31.2 ± 1.1	31.7±0.4	102
Fe	1833 ± 75	1914±65	104
Pb	0.16 ± 0.04	0.16±0.02	100
Hg	2.58 ± 0.22	2.31±0.02	90
Ni	0.97 ± 0.11	0.94±0.03	97
Se	8.3 ± 1.3	8.3±0.2	100
Ag	0.93 ± 0.07	0.86±0.01	92
Zn	116 ± 6	113±1	97

a: At 95 % confidence level, **b:** n=3, **c:** Information value

2.6. Statistical analysis

The SPSS 22 package for windows was used for all analyses. The mean and standard deviation of metal concentrations in meats of fish species were calculated. The Pearson correlation analysis reflected the degree of the linear relationship between metals (18). Multivariate analyses, including PCA and CA, were used to assess the relationships among the fish samples and metal accumulations. The varimax method was used with PCA. Eigenvalue criterion over 1.00 was used to decide the number of principal components. HCA was conducted with Ward's method, and a squared Euclidean distance was used as a distance measure. A Z score transformation was performed before cluster analysis.

3. Results and Discussion

3.1. Heavy metal levels in fish meats

This study observed the decreasing trend between Hg and Pb concentration and increased fish length and weight (Figs. 1a and 1b). In addition, the relationships between concentrations of Hg in *C. carpio* and *S. lucioperca* muscles are given in Fig. 1c and Fig.1d, respectively. It was revealed that the order of mean concentrations (mg/kg wet wt.) of metals in all fish meat samples were Al (204) > Zn (10.2) >

Fe (6.43) > Cu (0.666) > Hg (0.589) > Ni (0.556) > Pb (0.497) > Cr (0.396) > Ag (0.291) >Se (0.144) > As (0.074) > Co (0.043) > Cd (0.037) >V (0.027). Al showed the highest concentrations for all studied samples with a high standard deviation. The elevated Al levels may be because of the natural conditions of the soil (1). The accumulation of elements in fish meat samples is affected by many factors such as season, biological diversity, nutrition resource, water chemistry, salinity, temperature, contaminants, and food processing methods (19).

As shown in Table 3, the highest metal concentrations (mg kg^{-1}) in all fish muscles were as follows: V: 0.102, Se: 0.265 and Co: 0.083 in *Tinca tinca*; Al: 555, Cu: 1.746 and As: 0.114 in *S. lucioperca*; Zn: 34.4 and Hg:1.383 in *E. lucius*; Cr: 0.847, Ni:1.229, Ag: 2.557 and Cd :0.101 in *S. cephalus*; and Fe: 7.68 and Pb :1.483 in *S. glanis*. The European Food Safety Authority (EFSA) reports a tolerable daily intake of 0.9 mg/day for Cu in adults. The average intake level for Zn in Europe is reported to be 13 mg/day and 9 mg/day for men and women, respectively (20). The mean concentrations of Cu and Zn were lower than their permissible limit levels in fish samples. The Zn concentration was higher than its permissible limit level in one *E. lucius* and the four *S. lucioperca.*

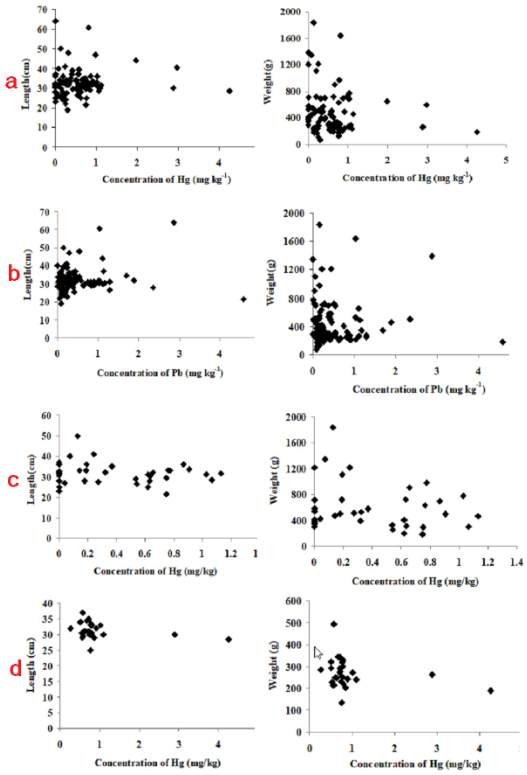

Figure. 1 Accumulation of (a) Hg and (b) Pb elements in fish muscles according to the fish sizes (n=91) and accumulation of Hg in (c) *C. carpio* (n=37) and (d) *S. lucioperca* (n=27) muscles according to the fish sizes.

3.2. Toxic elements

3.2.1. Mercury

In the present study, the concentration of Hg in all the fish species was in the range of 0.183 to 1.383 mg kg^{-1}, with an average of 0.589 mg kg^{-1}. The highest Hg concentration was determined as 4.26 mg kg^{-1} *in S. lucioperca*, followed by 2.97 mg kg^{-1} in *E. lucius*, 2.89 mg kg^{-1} in *S. lucioperca*, and 1.98 mg kg^{-1} in *E. Lucius* samples. The mean Hg concentrations for fish species were decreased in the following order: *E. lucius* > *S. lucioperca*> *S. cephalus*> *C. carpio*> *S. glanis*> *C. tinca*> *Tinca tinca*> *O. mykiss* (Table 3). Finally, Hg concentrations determined in fish muscle samples were found to be higher than 1.0 mg kg^{-1} for ten samples and higher than 0.5 mg kg^{-1} for forty samples set by the TFC (0.5-1 mg kg^{-1}) (21), and the EFSA (4 µg/kg per week) (20) and the European Union's Regulation (EU) (0.5 and 1.0 mg kg^{-1}) (22) in this study. Also, the maximum Hg level in food authorized for human consumption is 0.5 µg/kg according to the World Health Organization (WHO) guideline (23). The Hg concentration found in our study was relatively higher than those of Biland□iζ et al. (24) and Velusamy et al. (7); however, they were lower than those of Keshavarzi et al. (2) and Carvalho et al. (19). Mercury is a very toxic element that causes severe contamination. Fish get Hg through feeding, which can be determined by the diet quality, fish size, environmental factors, and water quality parameters (7).

Table 3. Heavy metal concentrations (μg g^{-1}) in eight freshwater fish species collected from Kayseri, Turkey

Species	n		Al	V	Cr	Fe	Co	Ni	Cu	Zn	As	Se	Ag	Cd	Hg	Pb
Tinca tinca	6	Mean	4.48	0.102	0.203	0.854	0.083	0.285	0.095	4.33	0.106	0.265	0.140	0.092	0.262	0.096
		SD	2.11	0.016	0.040	0.961	0.029	0.390	0.232	8.69	0.033	0.072	0.113	0.046	0.121	0.043
C. carpio	37	Mean	55.9	0.024	0.289	7.54	0.022	0.50	0.190	1.949	0.059	0.125	0.097	0.044	0.398	0.492
		SD	189	0.030	0.330	2.80	0.030	0.486	0.370	4.049	0.050	0.086	0.164	0.108	0.361	0.833
S. lucioperca	27	Mean	555	0.016	0.611	7.49	0.080	0.665	1.746	22.7	0.114	0.206	0.380	0.021	0.916	0.602
		SD	2443	0.035	0.431	6.00	0.162	0.756	7.414	30.0	0.062	0.081	0.840	0.064	0.808	0.407
E. lucius	5	Mean	70.0	0.032	0.222	3.132	0.033	0.636	0.430	34.4	0.068	0.070	0.275	0.031	1.383	0.363
		SD	91.1	0.047	0.159	2.492	0.038	0.533	0.462	54.4	0.052	0.086	0.558	0.048	1.080	0.424
S. cephalus	4	Mean	250	0.053	0.847	7.253	0.053	1.229	0.736	9.84	0.068	0.118	2.557	0.101	0.514	0.609
		SD	457	0.038	0.338	5.108	0.086	0.586	0.970	17.9	0.060	0.067	5.001	0.120	0.387	0.866
C. tinca	5	Mean	6.34	0.019	0.117	3.60	ND	0.194	0.143	1.018	0.006	0.018	0.029	0.016	0.350	0.139
		SD	2.40	0.041	0.032	1.34	ND	0.210	0.276	0.526	0.007	0.041	0.056	0.035	0.204	0.059
S. glanis	3	Mean	27.2	0.007	0.657	7.68	0.013	0.628	ND	0.976	0.011	0.125	0.021	0.002	0.373	1.483
		SD	18.8	0.007	0.264	2.32	0.012	0.331	ND	0.748	0.011	0.109	0.022	0.002	0.407	1.228
O. mykiss	4	Mean	8.05	ND	0.146	3.29	0.012	0.410	ND	0.394	0.055	0.010	0.004	ND	0.183	0.195
		SD	4.27	ND	0.014	0.68	0.017	0.516	ND	0.382	0.043	0.015	0.009	ND	0.046	0.147

n: sample number **ND**: not detected

3.2.2. Arsenic

Arsenic is assigned as a Group 1 human carcinogen by the WHO (25). The contaminated water and seafood could result in arsenic exposure to humans and adverse health effects such as skin rash, toxic cardiomyopathy, abdominal pain, vomiting, and diarrhea (26). The mean concentration of As in this study was found to be 0.074 mg kg^{-1}. The highest values of As were observed in *S. lucioperca* (0.114 mg kg^{-1}) while the lowest values were in *C. tinca* (0.006 mg kg^{-1}). It was found that the mean concentration of As in ten fish samples was below the detection limit (Table 3). The concentrations of As in fish muscles in this study were much lower than the limit values set by WHO (5 mg kg^{-1}) and the TFC (0.020 mg kg^{-1}). In this study, the mean of As concentrations in fish samples was significantly lower than previously reported by Shakeri et al. (27) and Okati et al. (28).

3.2.3. Cadmium

In the present study, the mean Cd concentration (0.037 mg kg^{-1}) was not over the permissible level of 0.050 mg kg^{-1}, 0.5 μg g^{-1} and 0.05-0.1 mg kg^{-1} set by the TFC (21), WHO (23), and EU (22), respectively. The Cd concentrations in 2 *S. lucioperca*, 2 *S. cephalus*, 5 *C. carpio*, 4 *Tinca*, and 1 *E. Lucius* muscles were found to be higher than the limits set by the TFC (21). In this study, the highest mean concentration of Cd was in *S. cephalus* muscle tissue (0.101 mg kg^{-1}), while the lowest mean concentration of Cd was detected in the *S. glani*s muscle tissue (0.002 mg kg^{-1}). In addition, the Cd concentrations in *O. mykiss* muscle tissue were below the detection limit (Table 2). The maximum Cd accumulation was detected in two C. carpio samples to be 0.488 mg g^{-1} and 0.408 mg g^{-1}.

3.2.4. Lead

Our results show that Pb concentrations are highest in *S. glanis* muscle (1.483 mg kg^{-1}), whereas it is lowest in *Tinca*

muscle (0.096 mg kg^{-1}). The Pb content in the muscles of the fish analyzed in the study can be given in the following order with an average of 0.589 mg kg^{-1}: *S. glanis*> *S. cephalus*> *S. lucioper*ca> *C. carpio*> *E. lucius* > *O. mykiss*> *C. tinca*> *Tinca* (Table 3). In 14 fish muscles, Pb levels higher than 1.0 mg kg^{-1} were found. However, the permissible limit for Pb concentration in the TFC (21), WHO (23), and EFSA (29) for fish meat is 0.3 mg kg^{-1}. The accumulation range of Pb in our study was like earlier studies conducted by Keshavarzi et al. (2) and Li et al. (10). Whereas significantly higher results were reported by Rahman et al. (4) who detected Pb in fish samples with concentrations ranging from 1.76 to 10.27 mg kg^{-1}.

3.3. Correlation analysis

The Pearson correlation analysis was performed to identify the origin of the element's contents in the eight different species of 91 fish meat. A correlation matrix was calculated for the metals analyzed in fish species to identify the common origin of metals. As shown in Table 4, a high positive correlation (r=0.760) was determined between the length and weight of the fish samples. The contents of Se in fish muscle samples were negatively correlated with a total weight (r= -0.235, $p<0.05$). There were no significant correlations ($p<0.05$) between the content of Cu, Cr, Ag, Co, and Hg and fish sizes (length and weight). Similarly, no significant relationships between fish size and Cu were found by Yi and Zhang (30). Zuliani et al. (16) recorded a negative correlation between Cr and fish size, while Arulkumar et al. (5) reported a remarkable negative correlation (r= – 0.328 for Cd, r= – 0.192 for Pb, and r= – 0.064 for Cu) between the fish size and heavy metal (Cd, Pb, and Cu) concentrations.

In this study, most of the metal pairs significantly positively correlated with each other, from which Co-Cu (0.687), Cr-As (0.564), Cr-Co (0.557), As-Se (0.479), and Al-Cr (0.443) pairs demonstrated remarkable positive correlation, whereas Fe-V (-0.392) and Fe-Co (-0.359) pairs indicated considerable negative correlations at the 99% confidence level (Table 4).

Table 4. Correlation analysis of heavy metals among meats of the eight fish species (n=91)

	Al	**V**	**Cr**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**As**	**Se**	**Ag**	**Cd**	**Hg**	**Pb**	**Length**	**Weight**
Al	1															
V	0.131	1														
Cr	**0.443****	0.211*	1													
Fe	-0.180	**-0.392****	0.076	1												
Co	**0.293****	**0.502****	**0.557****	**-0.359****	1											
Ni	0.185	**0.217***	**0.411****	0.027	**0.365****	1										
Cu	0.055	0.203	**0.381****	-.0165	**0.687****	**0.299****	1									
Zn	0.027	-0.123	0.098	0.058	0.015	-0.093	0.050	1								
As	**0.438****	**0.264***	**0.564****	-0.003	**0.390****	0.205	**0.282****	**0.349****	1							
Se	0.232*	0.115	**0.289****	0.047	**0.314****	**0.233***	**0.299****	0.103	**0.479****	1						
Ag	**0.426****	**0.212***	**0.327****	-0.179	**0.291****	0.076	0.150	0.195	**0.347****	0.095	1					
Cd	0.060	**0.422****	**0.211***	-.0121	**0.209***	0.101	-0.026	-0.094	0.119	0.027	-0.032	1				
Hg	0.060	-0.185	0.004	-0.058	0.050	0.197	0.095	**0.313****	0.088	0.156	0.121	-0.048	1			
Pb	0.028	-0.148	0.166	**0.217***	0.036	-0.004	-0.063	0.236*	-0.007	-0.116	-0.008	0.204	0.052	1		
Length	-0.054	-0.069	0.122	0.098	-0.089	0.022	-0.035	0.109	-0.105	-0.086	0.028	-0.144	0.049	0.121	1	
Weight	-0.086	0.034	0.044	0.044	-0.148	-0.044	-0.076	-0.098	-0.153	**-0.235***	-0.001	-0.039	-0.148	-0.004	**0.760****	1

Bold numbers show significant correlations, Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level.

3.4. Multivariate analysis

The Kaiser–Meyer–Olkin (KMO) measure was implemented to investigate the suitability of the data for principal component analysis. KMO test shows the proportion of variance and should be above 0.5 for sampling adequacy (17,30). In this study, KMO was found to be 0.651. The data considered for this statistical treatment was obtained by measuring the concentration of fourteen elements, in triplicate, in 91 samples of fish muscles from different places.

Five PCs with eigenvalues greater than 1.0 (Kaiser Criterion) with a total contribution of 66.8% were taken out to identify the probable resources of metals in the fish muscles (Table 4). The metals in the same PC reflect a similar behavior for their origin and/or sources. As listed in Table 5, the first principal component (PC1 17.6 %) had strong positive loadings for Cr (0.56), Co (0.69), Ni (0.69), Cu (0.76), and Se (0.56). The second principal component (PC2 33.4%) expressed high positive loadings for Al (0.79), Cr (0.56), As (0.71) and Ag (0.69). High positive loading on PC3 (46.1 %) was found for V (0.65) and Co (0.47), whereas Zn (0.76) and Hg (0.75) had high positive loading on PC4 (56.8%). Moreover, PC5 (66.8%) was associated with Cd (0.73) and Pb (0.75). The relations among the heavy metals based on the first three PCs are presented in Fig. 2a. These results might suggest pollution arises from common origins or similar geochemical behavior of these metals (31).

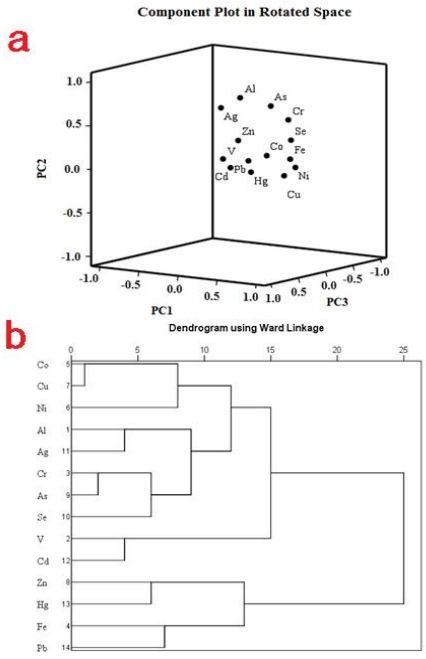

Figure 2. (a) A loading plot of three PCs of heavy metals and (b) dendrogram obtained from hierarchical cluster analysis of the 14 heavy metals in eight fish species.

Table 5. Varimax rotated loadings and communalities for the fish meats (n=91)

Component						
						Communalities
	1	**2**	**3**	**4**	**5**	**(h^2)**
Al	0.071	**0.793**	0.077	-0.064	-0.001	0.644
V	0.256	0.192	**0.654**	-0.321	0.255	0.698
Cr	**0.566**	**0.564**	-0.098	-0.057	0.310	0.747
Fe	0.044	-0.053	**-0.877**	-0.072	0.112	0.792
Co	**0.693**	0.249	**0.479**	0.040	0.170	0.802
Ni	**0.694**	0.037	-0.044	-0.053	0.093	0.496
Cu	**0.762**	-0.007	0.260	0.169	-0.091	0.685
Zn	-0.073	0.263	-0.093	**0.763**	0.130	0.682
As	0.386	**0.713**	-0.033	0.131	0.063	0.680
Se	**0.567**	0.323	-0.145	0.053	-0.210	0.494
Ag	-0.032	**0.696**	0.280	0.204	-0.058	0.609
Cd	0.090	0.021	0.281	-0.218	**0.734**	0.674
Hg	0.181	-0.057	0.036	**0.755**	-0.083	0.614
Pb	-0.069	0.003	-0.274	0.299	**0.752**	0.735
Eigenvalue	3.68	1.84	1.37	1.31	1.15	
% of variance	17.6	15.9	12.7	10.7	10.0	
Cumulative %	17.6	33.4	46.1	56.8	66.8	

Bold numbers represent significant loading values (≥0.479)

In general, anthropogenic inputs from agricultural and industrial practices, such as pesticides and chemical fertilizers, wastewater irrigation, and residues from metalliferous mining, increase the contamination levels of toxic metals such as Hg Zn, Cd, and Pb in surface waters. Fish meats collect Hg due to feeding and could be affected by the size and diet of fish, ecological factors, and water quality parameters (5, 7, 15, 16, 32, 33).

3.5. Cluster analysis

In this study, heavy metals detected in fish muscle tissue samples were categorized into two main clusters and four subclusters, as shown in a dendrogram (Fig. 2b). Cluster 1 revealed the high association of Co-Cu, Al-Ag, Cr-As, and V-Cd pairs, while Cluster 2 demonstrated the relation of Zn-Hg and Fe-Pb. These results indicated that the pollution with metal pairs analyzed might be from similar sources. These clusters show similarity with PCA results on the origins of metals for fish muscles.

4. Conclusions

The current study's results demonstrate that the order of mean concentrations (mg kg^{-1} wet wt.) of the metals in fish muscle samples were Al>Zn>Fe>Cu>Hg> Ni>Pb>Cr>Ag> Se>As>Co>Cd>V. Based on the results, in some fish samples, the levels of some toxic elements (Hg, Cd, and Pb) were higher than permitted legal limits. It can be stated that element content in the examined fish species varies from each other. This might be related to environmental and agronomic circumstances and versatility exposure to

pollution. The correlation, PCA, and cluster analysis results indicated that the metal bioaccumulation varied between fish sizes and species, and heavy metal pollution in fish might be caused by the same environment or similar geochemical behavior of metals. These fish might be a significant concern to human consumption due to their toxicological effects. Therefore, establishing standardized monitoring systems for determining heavy metal content in fish and fishery products are needed to protect consumer health.

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Consent to Participate: Informed consent was obtained from all individual participants included in the study.

Consent for Publication: The participants have consented to the submission of the interview results to the journal.

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