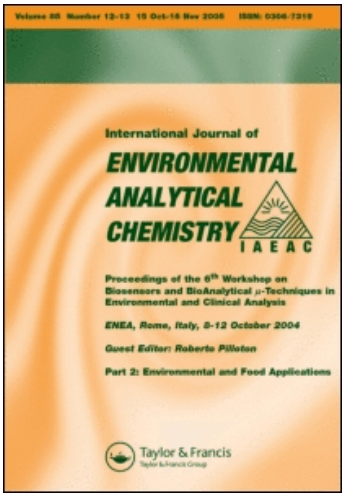

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Trace Elements Determination in some Species of Fish Commonly Consumed in Pakistan

J. H. Zaidi[a]; I. H. Qureshi[a]; M. Arif[a]; I. Fatima[a]
[a] Nuclear Chemistry Division, Pakistan Institute of Nuclear Science and Technology, Islamabad, Pakistan

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TRACE ELEMENTS DETERMINATION IN SOME SPECIES OF FISH COMMONLY CONSUMED IN PAKISTAN

J. H. ZAIDI, I. H. QURESHI, M. ARIF and I. FATIMA

Nuclear Chemistry Division, Pakistan Institute of Nuclear Science and Technology, P.O. Nilore, Islamabad, Pakistan

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Extensive use of some of the fresh water fish species as one of the main food items in Pakistan entails the evaluation of trace element contents in them. Instrumental neutron activation analysis (INAA) and atomic absorption spectrometry (AAS) were utilized to determine the concentration of 30 trace elements (essential, toxic and nonessential) in five different species commonly consumed. This data will serve as baseline values and will be helpful to monitor the degree of future contamination. Species belonging to Cyprinidae family were found to contain relatively high amounts of essential and toxic elements. The estimated dietary intake through fish species show that these may be considered as an appreciable source of trace element intake due to their extensive consumption.

KEY WORDS: Fish, trace elements, INAA, AAS, toxic elements, essential elements, dietary intake.

INTRODUCTION

Metal metabolism and metal toxicology are gaining ever increasing interest.[1] Trace element levels have been measured extensively in individual food articles and integrated human diets.[2-4] The list of essential elements has been steadily increasing (K, Na, Mg, Mn, Ca, Cr, Fe, Ni, Co, Cu and Zn). Though very little is known about their specific biological functions, still their nutritional significance has been established.[5] Along with the essential trace elements, food also contains some toxic elements (Hg, Se, As, Pb, Cd, Cl, Br etc.), which if present in relatively high amounts may adversely affect the biochemical system.[6,7] Some of the effects of toxic elements and the protective effects of essential elements might be attributed to competition for binding sites on ligands that have important roles in homeostasis. Interaction among the elements can occur at different levels: during the consumption of food, during the process of absorption, within the cells as part of enzyme systems, during the catabolic phases of metabolism. The excess of some element can lead to substitution of some other element at important molecular sites, or, more generally, to halter levels of other elements.Therefore, from the nutritional view point the focus of interest is the adequacy of the daily intake of all essential elements by the population at large. The toxicologists are interested in the intake of amounts of toxic elements. Food[8] being the main source of intake of these elements, it is significant to monitor the concentrations of toxic and essential elements in various food items of daily consumption.

Various food items have been analysed, for establishing base-line levels of trace elements, in our laboratory.[4,9-12] In the present work a set of five species of fish,

commonly consumed in Pakistan, have been analysed. The list of these species is given in Table 1, along with their zoological names and respective families.[13] Barbus is a genus of freshwater fishes, containing numerous species often called barb or barbel. The African and Asian species, such as mahseer, are esteemed food and sport fishes. Labeo is a genus containing numerous species of African and Asian river fishes of the carp family. They are bottom feeders and eat algae and small animals. Rohu (*L. Rohita*) of subcontinent is esteemed for food and sport and is cultured in ponds. Kalbano (*L. Calbasu*) is about 12 cm long and is black with red tail. This fish is called "Shark" after its appearance rather than disposition. Mastacembelus is a genus of freshwater spiny eels. They are carnivorous, and have a long, movable snout and a row of spines preceeding the soft portion of the dorsal fin. Bam (*M. Armatus*) may be up to about 90 cm long but most are much smaller. It is commonly consumed in Pakistan. Hilsa is a genus of saltwater fishes that swim up rivers. Palla (*H. Ilisha*) is rather deep bodied and have a notch in the upper jaw that receives the tip of the lower. The flesh of these fishes is considered very good though bony.

Fish species were analysed for 30 toxic, essential and non-essential, elements employing instrumental neutron activation analysis (INAA) and atomic absorption spectrometry (AAS) techniques. INAA technique has been extensively used for this purpose[14,15] due to its high precision, accuracy and sensitivity. However, under the normal mode is not suited for the determination of Pb and Cd. Therefore, AAS was employed for the investigation of these elements.

EXPERIMENTAL

Sampling and sample preparation

All the five species (see Table 1) were collected at random from local markets of Rawalpindi/Islamabad area in sufficient quantities and sun-dried after removal of their internal organs, head, fins, gills and tail. The dried samples were then chopped into pieces with the aid of a stainless steel knife (steam cleaned). Only the edible muscle tissue samples were used for analysis. The sample pieces were further dried using a Christ Beta A freeze dryer until a constant weight was obtained (dry weight). The dried samples were ground separately using a suitable grinder with PTFE-coated blades to avoid contamination. The powdered samples were thoroughly mixed and homogenized in a vibrating mill with three-dimensional motion. Representative samples for analysis were stored in pre-cleaned polyethylene capped bottles. Homogeneity of the samples was tested by analyzing Mn and K contents; the variation was found to be less than 3% for both the elements for a minimum sample weight of 250 mg.

Table 1 Zoological classification and nomenclature of the fish species analyzed.

Family	*Zoological name*[13]	*Common name*
Cyprinidae	Barbus Putitora	Mahaseer
	Labeo Rohita	Rohu
	Labeo Calbasu	Kalbano
Mastacembelidae	Mastacembelus Armatus	Bam
Clupeidae	Hilsa Ilisha	Palla

Preparation of standard for AAS determinations

For AAS determinations, stock solutions of Pb and Cd (1000 mg/l) were prepared by dissolving the appropriate amount of spec-pure metal oxides in purified nitric acid and diluting to the required volume with deionized distilled water. Fresh working standards for calibration purposes were always prepared by serial dilution of the stock solutions before use.

Neutron irradiations

Samples, each weighing about 250 mg, were taken in triplicate and heat sealed in pre-cleaned polyethylene and silica vials for short and long irradiations, respectively. The silica vials were placed in NRX type irradiation capsules and cold welded. A 27 kW Tank-in-Pool type research reactor (PARR-II) and a 10 MW Swimming Pool type research reactor (PARR-I) were used for short and long neutron irradiations, respectively, of the samples and standards. The thermal neutron flux densities at the irradiation sites of PARR-I and PARR-II were of the order of 5×10^{13} n cm^{-2} sec^{-1} and 10^{12} n cm^{-2} sec^{-1}, respectively. Thermal neutron flux monitors were inserted between the samples and the standards to monitor the fluctuations in the thermal neutron flux gradient; which were found to be insignificant. The samples along with appropriate amounts of IAEA standards Fish Flesh Homogenate [MA-A-2 (TM)] and Mixed Human Diet (H-9) were irradiated for 2 min to 24 h according to the requirement utilizing both the reactors. The irradiated samples and standards were transferred to pre-weighed polyethylene vials and re-weighed to determine the exact weight.

Gamma-ray measurement and analysis

The gamma-ray spectra of the samples and standards were measured, after appropriate cooling (5 min to 4 weeks, see Table 2), for varying times ranging from 2 min to 16 h employing a 4 k series 85 Canberra multichannel analyzer (model 8503) coupled with ORTEC coaxial 30 cm^3 Ge(Li) detector. The system has a resolution of 2.1 keV for 1332.5 keV peak of ^{60}Co and peak/compton ratio of 40:1. The data, transferred from MCA to central computer facility, was processed employing indigenously developed programmes. The peaks of all the elements investigated, with the exception of Zn and Hg, were well resolved and interference-free. The full energy peak areas of 1115 keV from ^{65}Zn and 279.2 keV from ^{203}Hg were determined, after subtracting contributions from ^{46}Sc and ^{75}Se respectively, as described elsewhere.[11]

AAS determination of Pb and Cd

Samples weighing 500 mg were taken in a 100 ml flask fitted with a 30 cm long air condenser. 5 ml of ultrapure nitric acid was added to the sample and the mixture was then heated at 80°C for 30 min. After cooling, 3.5 ml of 70% $HClO_4$ was added and then the mixture was heated at 250°C with occasional shaking till white fumes evolved. The clear solution obtained was cooled and transferred into a 25 ml measuring flask and the volume was made up with water. A blank was prepared under similar conditions. The reliability of this wet ashing procedure has already been established.[16]

Table 2 Optimum experimental conditions and nuclear data[17] employed for the analysis.

Isotope	*Half life*	*γ-peak used (keV)*	*Irradiation time*	*Cooling time*
^{28}Al	2.24 m	1778.98	2 min	–
^{27}Mg	9.46 m	843.7, 1014.4	2 min	5 min
^{38}Cl	37.20 m	1642.4, 2167.5	2 min	30 min
^{56}Mn	2.58 h	846.6	2 min	2 hours
^{42}K	12.40 h	1524.7	2 min	2 hours
^{24}Na	15.00 h	1368.5	2 min	2 hours
^{82}Br	35.40 h	776.5	24 hours	2 days
^{76}As	26.30 h	559.1	24 hours	2 days
^{140}La	1.68 d	1596.5, 328.7	24 hours	2 days
^{99}Mo	2.75 d	739.5, 181.1	24 hours	2 days
^{122}Sb	2.70 d	564.1	24 hours	2 days
^{47}Ca	4.54 d	1297.1	24 hours	2 days
^{131}Ba	11.80 d	496.2	24 hours	4 days
^{117m}Sn	13.61 d	158.5	24 hours	1 week
^{86}Rb	18.60 d	1078.8	24 hours	2 weeks
^{51}Cr	27.80 d	320.1	24 hours	2 weeks
^{169}Yb	32.02 d	177.2, 197.9	24 hours	2 weeks
^{181}Hf	42.50 d	482.0	24 hours	2 weeks
^{59}Fe	44.60 d	1099.3, 1291.6	24 hours	2 weeks
^{203}Hg	46.60 d	279.2	24 hours	2 weeks
^{95}Zr	64.02 d	756.7, 724.2	24 hours	2 weeks
^{46}Sc	83.90 d	889.3, 1120.5	24 hours	2 weeks
^{75}Se	120.00 d	264.5, 135.9	24 hours	4 weeks
^{65}Zn	243.80 d	1115.5	24 hours	4 weeks
^{110m}Ag	249.76 d	657.7, 884.6	24 hours	4 weeks
^{134}Cs	2.04 y	795.8	24 hours	4 weeks
^{60}Co	5.26 y	1173.2, 1332.5	24 hours	4 weeks
^{152}Eu	13.20 y	1408.0	24 hours	4 weeks

AAS was carried out using a Hitachi Model Z-8000 spectrometer with a Zeeman-effect background correction mode and equipped with a graphite furnace and auto-sampler. Argon was used as an inert purging gas; the flow was interrupted during the atomisation step. Signal evaluation was based on integrated absorbance values. The conditions for the instrumental determinations were optimised and listed in Table 3. The measurements of Pb and Cd with a electrothermal atomisation technique.

RESULTS AND DISCUSSION

The optimized conditions for INAA analyses of fish species were the same as described earlier[12] and are listed in Table 2 along with the nuclear data[17] for ready reference. Accordingly, the radionuclides of short half-lives, i.e. ^{28}Al, ^{27}Mg, ^{38}Cl, ^{56}Mn, ^{42}K and ^{24}Na were measured employing short irradiations and the radionuclides of relatively long half-lives, i.e. ^{82}Br, ^{76}As, ^{122}Sb, ^{117m}Sn, ^{51}Cr, ^{59}Fe, ^{46}Sc, ^{65}Zn, ^{60}Co, ^{47}Ca, ^{131}Ba, ^{110m}Ag, ^{134}Cs, ^{99}Mo, ^{86}Rb, ^{95}Zr, ^{169}Yb, ^{140}La, ^{181}Hf, ^{152}Eu, ^{203}Hg and ^{75}Se, were measured employing long irradiations along with appropriate cooling times. The precision and accuracy of the method was rechecked[12] by analyzing the IAEA SRMs Fish Flesh [MA-A-2(TM)] and Mixed Human Diet (H–9). Our values are in fairly good agreement with certified values as shown in Table 4.

Table 3 Experimental AAS conditions.

Analytical conditions		*Pb*	*Cd*
Lamp current (mA)		7.5	7.5
Wavelength (nm)		283.3	228.8
Slit width (nm)		1.3	1.3
Carrier gas flow (ml/min)		100	100
Sample volume (µl)		10	10
Heating programme:			
Drying:	temp. (°C)	80–120	80–120
	time (sec.)	30	30
Ashing:	temp. (°C)	400	300
	time (sec.)	30	30
Atomiz:	temp. (°C)	2100	1700
	time (sec.)	7	7
Cleaning:	temp. (°C)	3000	2600
	time (sec.)	3	3

Table 4 Analysis of IAEA reference materials (concentrations in µg/g)[a].

Element	*MA–A–2(TM)*		*Mixed human diet (H–9)*	
	Our values	*IAEA values*	*Our values*	*IAEA values*
Fe	56 ± 2	54 ± 1	32.9 ± 2.0	33.5 ± 2.2
Mn	0.87 ± 0.05	0.81 ± 0.04	12.1 ± 0.9	11.8 ± 0.8
Co[b]	82 ± 7	80 ± 10	50 ± 6	43 ± 5
Zn	32 ± 1	33 ± 1	27.3 ± 1.5	27.5 ± 1.8
Cr	1.4 ± 0.1	1.3 ± 0.1	0.16 ± 0.02	0.15 ± 0.04
K	14394 ± 398	–	8295 ± 620	8300 ± 664
Hg[b]	475 ± 25	470 ± 20	5.0 ± 1.0	5.0 ± 1.0
Se	1.67 ± 0.21	1.70 ± 0.3	0.12 ± 0.01	0.11 ± 0.01
As[b]	2590 ± 130	2600 ± 100	90 ± 30	88 ± 32
Sb[b]	6 ± 1	5 ± 1	11 ± 0.6	(12)
Cl	1132 ± 70	–	12590 ± 1570	12500 ± 1500
Cs[b]	40 ± 0.2	–	30 ± 0.2	(25)
Rb	11 ± 2	–	8.2 ± 0.5	8.0 ± 0.6

[a] Values in parentheses are uncertified.
[b] Concentrations in ng/g.

The concentrations were determined on dry weight basis and the results, as averages of at least six determinations with standard deviations around mean values, are given in Table 5. It can be seen that the species of Cyprinidae family are not only rich in essential elements but also have relatively higher amounts of toxic elements. Mahaseer contains, relatively, higher amounts of Ca, Cl and Br as compared to the rest of species analyzed. Rohu has higher amounts of Ba and Cd. Kalbano contains higher amounts of Cr, Fe, Na, Hg and Pb. Whereas Bam contains higher amounts of Co, Zn, Mg and Sb and Palla contains higher amounts of Mn, K and As. These variations are likely to be due to the migratory nature and feeding habits of the different species of fish or various species have tendencies to concentrate certain elements in their tissues more than the surrounding medium. The comparison of our average values with the values of some

Table 5 Trace element concentration in some species of fish (in μg/g on dry weight basis).

Element	*Mahaseer*	*Rohu*	*Kalbano*	*Bam*	*Palla*
Cr[a]	160 ± 6	78 ± 4	213 ± 10	201 ± 9	61 ± 3
Mn	0.61 ± 0.03	0.76 ± 0.04	0.39 ± 0.01	0.99 ± 0.05	1.11 ± 0.05
Fe	32 ± 2	24 ± 1	84 ± 4	25 ± 1	16 ± 0.7
Co[a]	55 ± 2	24 ± 0.8	71 ± 3	84 ± 4	63 ± 3
Zn	16.3 ± 0.7	13 ± 0.5	24.5 ± 1.1	26.5 ± 1.2	12.2 ± 0.4
Na	1960 ± 98	1760 ± 80	2850 ± 140	2660 ± 136	2250 ± 110
K	13340 ± 1000	13790 ± 1100	14890 ± 1200	16600 ± 1300	16650 ± 1350
Ba	1.85 ± 0.07	1.90 ± 0.07	1.66 ± 0.05	1.25 ± 0.04	1.56 ± 0.06
Ca	3520 ± 110	589 ± 24	240 ± 9	1461 ± 45	260 ± 10
Ag	2.4 ± 0.08	1.9 ± 0.06	2.1 ± 0.07	2.2 ± 0.06	1.6 ± 0.04
Mg	265 ± 10	123 ± 5	165 ± 6	715 ± 25	112 ± 4
Al	82.5 ± 4	27.2 ± 2	11.0 ± 0.5	25.1 ± 1.5	28.2 ± 1.8
Sn[a]	18 ± 1	13 ± 0.6	8 ± 0.3	6 ± 0.1	11 ± 0.4
As	0.31 ± 0.01	0.34 ± 0.01	0.29 ± 0.006	0.24 ± 0.007	0.67 ± 0.02
Se	0.20 ± 0.02	0.13 ± 0.01	0.22 ± 0.02	0.18 ± 0.02	0.31 ± 0.02
Hg[a]	11 ± 1	6 ± 1	42 ± 2	19 ± 2	6 ± 1
Sb[a]	20 ± 2	110 ± 10	33 ± 2	130 ± 10	39 ± 3
Pb	5.2 ± 0.2	5.5 ± 0.25	7.2 ± 0.3	1.6 ± 0.04	2.4 ± 0.1
Cd[a]	203 ± 8	350 ± 15	250 ± 9	210 ± 8	110 ± 4
Cl	4341 ± 278	1362 ± 32	2647 ± 115	1080 ± 60	579 ± 23
Br	8.4 ± 0.5	2.4 ± 0.2	3.0 ± 0.2	1.2 ± 0.2	2.1 ± 0.2
Hf[a]	37 ± 2	30 ± 1.5	19 ± 1	43 ± 2	7 ± 0.5
Rb	5.1 ± 0.2	2.7 ± 0.1	0.65 ± 0.02	10.1 ± 1.0	2.7 ± 0.6
Yb[a]	25 ± 2	18 ± 1	40 ± 2	26 ± 2	21 ± 1
La[a]	15 ± 1	38 ± 2	13 ± 1	9 ± 0.2	8 ±0.2
Mo[a]	12 ± 0.7	22 ± 1	13 ± 0.8	8 ± 0.4	5 ± 0.2
Zr	1.8 ± 0.07	2.7 ± 0.1	3.8 ± 0.13	2.1 ± 0.08	3.6 ± 0.14
Cs[a]	50 ± 2	63 ± 4	40 ± 2	198 ± 8	24 ± 2
Sc[a]	33 ± 2	42 ± 2	22 ± 2	260 ± 30	42 ± 4
Eu[a]	27 ± 2	38 ± 2	19 ± 2	39 ± 1	44 ± 3

[a] Concentration in ng/g.

Table 6 Comparison of data on trace elements in some species of fish (concentration in μg/g on dry weight basis).

Element	*Our. av. values*	*Sharif et al.*[18]	*Tallandini et al.*[19]
As	0.37	3.234	–
Se	0.21	4.385	1.6
Hg	0.02	0.341	–
Fe	36	–	66
Mn	0.77	–	2.1
Zn	18.5	59.59	42.3
Cr	0.14	1.007	3.3

elements reported in literaure are given in Table 6. Our values are much less than reported by Sharif *et al.*[18] and Tallandini *et al.*[19] The difference may be attributed to the facts that the species compared are not the same and species analyzed by Sharif *et al.* and Tallandini *et al.* are marine fish while species analyzed in present work are fresh water fish.

The weekly dietary intake values for essential and toxic elements through different species of fish have been estimated assuming that a person ingests 20 g (dry weight) of each of the varieties per week and are given in Table 7 along with the suggested weekly requirement/tolerance limits.[20] The estimated intake of Fe, Cr and Na through Kalbano is maximum in comparison with the rest of the species, whereas intake of Mn is minimum. The intake of Mn, Co and K through Bam and Palla are comparable. Intake of As and Cd is maximum through Palla and Rohu respectively, whereas the intake of Hg and Pb through Kalbano is maximum. The total estimated intake of Cr, Mn, Fe, Co and Zn corresponds to 1.02%, 0.22%, 2.6%, 0.05% and 1.76%, respectively, of the upper limits of the suggested weekly requirements. The estimated intake of toxic elements is well below the tolerance limits.

CONCLUSION

This paper provides the base-line values of certain essential and toxic elements in some of the commonly consumed species of fish in Rawalpindi/Islamabad area. The Cyprinidae family species are not only rich in essential elements but also contain relatively higher amounts of some of the toxic elements. Estimation of weekly dietary intake shows that fish species are appreciable source of trace element intake. The intake of toxic trace elements is with in the safe limits recommended by joint WHO/FAO expert committees.[3] Comparing all the species investigated it can be seen that Kalbano, Palla and Bam are rich in Cr/Fe, Mn and Co/Zn, respectively. The results reported in the present work may prove useful in the fields of nutrition and food technology. The nutritive importance of trace elements has been much emphasised but work in the direction of correlation between difficiency of trace elements and different diseases is not only desirable but needed too.

Table 7 Dietary intake values of trace elements through species of fish (expressed in μg/week, person).

Element	*Mahaseer*	*Rohu*	*Kalbano*	*Bam*	*Palla*	*Total intake*	*Weekly requirement[a] /tolerance[20]*
Cr	3.2	1.56	4.26	4.02	1.22	14.26	0.07–1.4
Mn	12.2	15.2	7.8	19.8	22.2	77.2	3.5–35
Fe	640	480	1680	500	320	3620	70–140
Co	1.1	0.48	1.42	1.68	1.26	5.94	1–12
Zn	326	260	490	530	244	1850	56–105
Na[a]	39.2	35.2	57.0	53.2	45.0	229.6	805–21000
K[a]	267	276	298	332	333	1506	2100–35000
As	6.2	6.8	5.8	4.8	13.4	37	2.8
Se	4	2.6	4.4	3.6	6.2	20.8	1.4
Hg	0.22	0.12	0.84	0.38	0.12	1.68	0.28
Cd	4.0	7.0	5.0	4.2	2.2	22.4	0.35–1.05
Pb	104	110	144	32	48	438	0.7–2.1
Sb	0.4	2.2	0.66	2.6	0.78	6.64	–
Cl	87	27	53	22	12	201	1400–3500
Br	168	48	60	24	42	342	7

[a] Values expressed in mg/week, person.
All values expressed on dry weight basis.
Weekly ingestion of each of the species taken as 20 g.

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