

Measurement of Toxic Elements in Fish: An Indicator of Aquatic Environment

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Summary: Fish is a good source of protein and extensively used as a food item in Pakistan, this entails the evaluation of toxic elements (Hg, Se, As, Pb, Cd and Br) in five different species commonly consumed. Instrumental Neutron Activation Analysis (INAA) and Atomic Absorption Spectrometry (AAS) were utilized for the analysis. Our results were compared with results reported from other countries. The weekly dietary intake of toxic elements through these species has been estimated, which is well below the tolerance limit. The reliability of the techniques has been established by the use of standard reference materials. This data will serve as the baseline values and will be helpful to monitor the degree of future contamination.

Introduction

In developing countries due to the rapid industrialization and less attention paid for the protection of the aquatic environment, anthropogenic contamination is increasing. To protect the marine biota regular monitoring of toxic elements in the aquatic environment is necessary as this affects the human health. The nutritional significance of trace elements has been well established and is necessary for various metabolic processes and toxic elements (As, Hg, Se, Pb, Cd, Br etc), if present in relatively higher amounts adversely affect these processes [1, 2]. The excess of some elements can lead to substitution of some other elements at important molecular sites; therefore, the toxicologists are interested in monitoring the intake of toxic elements through food and related products [3]. Food being the main source of intake of these elements, it is significant to monitor the concentrations of toxic elements in various food items of daily consumption. Trace element levels in individual food articles [4, 5] and integrated human diet [6] have been measured for nutritional adequacy and safety evaluation. Extensive use of fish as one of the main food items in Pakistan entails the evaluation of toxic trace element contents in them and in turn gives us idea about the aquatic media of our country. In the present investigation, a set of five fresh water species of fish (Table-1) most commonly used in Pakistan have been analyzed using instrumental neutron activation (INAA) and atomic absorption spectrometry (AAS) techniques. The results of this study will help in

generating data needed for the assessment of toxic metal intake from this source.

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

Family	Zoological Name	Common Name
Cyprinidae	Barbus macrolepis	Mahaseer
	Labeo Rohita	Rohu
	Labeo Calbasu	Kalbano
Mastacembelidae	Mastacembelus Armatus	Bam
Clupeidae	Hilsa Hisha	Palla

Results and Discussion

The four toxic elements As, Hg, Br and Sb in five species of fresh water fish were measured using INAA, while Pb and Cd were measured by AAS. The optimized conditions for INAA analysis of fish species are listed in Table-2 along with the nuclear data for ready reference [7]. Analyzing the IAEA SRMs Fish Flesh [MA-A-2(TM)] and Mixed Human Diet (H-9) rechecked the precision and accuracy of the method. Our values are in fairly good agreement with certified values. Dry weight toxic element concentrations from different species are recorded in Table-3. The analytical results are averages of six determinations with standard deviations around mean values. Toxic elements concentration varied markedly in different species, these variations may be due to the migratory nature and feeding habits of the different species of fish or various species have tendencies to concentrate certain

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Table-2: Toxic element concentration in some species of fish (ng/g on dry weight basis).

Element	Mahaseer	Rohu	Kalbano	Bam	Palla
As	330 ± 15.4	366 ± 14.9	298 ± 13.2	253 ± 17	693 ± 33
Hg	12.2 ± 2.2	8.2 ± 1.5	38.1 ± 4.3	18.6 ± 2.1	9.8 ± 1.6
Pb	662 ± 75	596 ± 64	932 ± 85	268 ± 37	343 ± 33
Cd	214 ± 10.2	356 ± 15.1	260 ± 13.6	198 ± 11.2	132 ± 9.9
Br ⁻	8.5 ± 0.5	3.1 ± 0.2	4.5 ± 0.2	2.2 ± 0.2	3.4 ± 0.2
Sb	25.6 ± 2.2	118 ± 10	29.5 ± 3.1	136 ± 9.2	39 ± 3.2

*Concentration in µg/g

Table-3: Comparison of toxic elements in fish with international data (concentration in µg/g on dry weight basis)

Element	Our av. values	Sharif <i>et al.</i> [8]	Voegborlo <i>et al.</i> [9]
As	0.37	3.234	0.18
Hg	0.02	0.341	0.29
Pb	0.56	-	0.28
Cd	0.232	-	-

elements in their tissues more than others. Hilsa Ilisha (Palla) is a genus of salt water fish that swim up river, the results show that this specie has high concentration 693 ± 33 ng/g of arsenic almost two folds of other species but five times less than in a reports from Sharif *et al.* [8] and greater than Voegborlo *et al.* [9] (Table-3). Arsenic concentration in fish muscles has shown increase with age [10]. It is difficult to assess the toxicity of arsenic in fish as in marine organism it is generally present in organic form which is less toxic than inorganic arsenic [11]. Fish of Cyprinidae family, mahaseer, rohu and kalbano have relatively higher amount of Pb, Cd and Br. The concentration of Hg varies from 8.2 ± 1.5 to 38.1 ± 4.3 ng/g, highest is in kalbano in comparison to other four varieties. This fish is present in slow moving waters of rivers and feeds on plants filamentous algae and diatoms [12]. Concentration of Sb in Bam fish is highest among the species analyzed 0.136 ± 10 µg/g.

The weekly dietary intake values of toxic elements through these varieties of fish have also been calculated assuming that a person ingests 20 g (dry weight) of each of the varieties per week and are given in Table-4. Intake of Cd and Sb through Rohu is maximum, whereas the intake of Hg and Pb through Kalbano is greater than others. The joint Food and Agricultural Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives has suggested a provisional tolerable intake of Hg is 0.3 mg, 400-500 µg of Cd and 3 mg of Pb per week in human food [13]. The estimated intake of toxic elements through the reported fish species is well below the tolerance limits.

Table-4: Dietary intake values of toxic elements through species of fish (expressed in µg/ week, person).

Element	Mahaseer	Rahu	Kalbano	Bam	Palla	Total intake	Weekly requirement ^{*/} Tolerance ¹⁴
As	6.6	7.32	5.96	5.06	13.86	38.8	2.8
Hg	0.24	0.16	0.76	0.37	0.19	1.72	0.28
Pb	13.2	11.9	18.6	5.36	6.86	55.9	0.7-2.1
Cd	4.28	7.12	5.2	3.96	2.64	23.2	0.35-1.05
Br	170	62	90	44	68	434	7
Sb	0.51	2.36	0.59	2.72	0.78	6.96	-

*Values expressed in mg/week/person

All values expressed on dry weight basis.

Weekly ingestion of each species taken as 20 g.

Experimental

Sampling and Sample Preparation

Fresh water fish samples of five different species (Table-1) were collected randomly from local markets of Rawalpindi/Islamabad area in sufficient quantities. Samples were washed with distilled water and the edible muscle tissues were used for analysis. The samples were freeze dried until a constant weight was attained (dry weight). The dried samples were ground separately. The powdered samples were thoroughly mixed and homogenized and stored in pre-cleaned polyethylene bottles. Five randomly selected aliquots were analysed for their Mn contents in order to check the homogeneity of the sample. The results showed about 4 % variation around mean value certifying the homogeneity of the prepared fish samples.

Preparation of Standard for AAS Determinations

For AAS determination, 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 Irradiation

Fish samples each weighing about 250 mg, were taken in triplicate and heat-sealed in pre-cleaned silica vials for irradiations. The silica vials were placed in NRX type irradiation capsules and cold-welded. A 10 MW swimming pool type research reactor (PARR-I) was used for neutron irradiations of the samples and standard. The thermal flux density at

the irradiation sites of PARR-I was $7 \times 10^{13} \text{ n cm}^{-2} \text{ sec}^{-1}$. 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 5 h. The irradiated samples and standards were transferred to pre-weighed polyethylene vials and re-weighed to determine the exact weight.

γ -Ray Spectrometric Analysis

The γ -ray spectra of the fish samples and standards were measured after appropriate cooling, for varying time from 24 hrs to 2 weeks (Table-5) employing a 4k series 85 Canberra multichannel analyzer (model 8503) coupled with Eurisys Mesures coaxial 245 cm³ high purity germanium detector. The detector was housed inside a thick lead shield to reduce the background of the system. The system has a resolution of 1.9 keV for the 1332.5 keV γ -ray peaks of ⁶⁰Co and a peak to Compton ratio of 70:1.

Table-5: Optimum experimental conditions and nuclear data employed for the analysis.

Isotope	Half life	γ -peak used (keV)	Cooling time
⁷⁶ As	26.30 h	559.1	24 hrs.
⁸² Br	35.40 h	776.5	24 hrs.
¹²² Sb	2.70 d	564.1	2 days
²⁰³ Hg	46.60 d	279.2	2 weeks

AAS Determination of Pb and Cd

Samples weighing 500 mg were taken for digestion in a 100 ml flask fitted with a 30 cm long air condenser. 5 ml of ultra pure 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 % HClO₄ 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 water was added. Appropriate amounts of IAEA and NIST CRMs and a blank were also prepared under similar conditions. The reliability of this wet ashing procedure has already been established [14].

AAS was carried out using a Hitachi Model Z-8000 spectrometer with Zeeman effect background correction mode and equipped with a graphite furnace and auto-sampler. Argon was used as an inert purging gas. The optimized conditions for the

instrumental determinations are same as described elsewhere [15].

Conclusion

Five varieties of fish commonly consumed in Pakistan were analyzed for toxic trace elements using INAA and AAS techniques to evaluate the contamination in the aquatic environment. The results show that the intake of toxic elements is within the safe limits recommended by joint WHO/FAO expert committee [13] and RDA, USA [14]. This data can be utilized by the national food and nutrition organization for planning and establishing legislations and for the baseline values as well as future contamination monitoring studies. The study is being extended to investigate trace elements in other species of fish in different aquatic medium.

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