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## Analytical Methods

# Accumulation of arsenic in different fresh water fish species – potential contribution to high arsenic intakes

Abdul Qadir Shah, Tasneem Gul Kazi<sup>\*</sup>, Muhammad Balal Arain, Muhammad Khan Jamali, Hassan Imran Afridi, Nusrat Jalbani, Jamil Ahmed Baig, Ghulam Abbas Kandhro

National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan

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#### ABSTRACT

The aim of the study was to evaluate total arsenic (As) in five tissues (gills, mouthpiece, intestine, liver and muscles) of 10 fish species caught from As contaminated Manchar Lake ( $26^{\circ}3'N$ :  $67^{\circ}6'E$ ) Sindh Pakistan during 2006–2007. The total As concentration was determined by hydride generation atomic absorption spectrometry (HG-AAS), prior to microwave assisted acid digestion. The certified reference material DORM-2 (dogfish muscle) was used to check the quality control of the technique. The good agreement with the certified value at 95% confidence limit confirmed the validity of As determination method. The limit of detection (LOD) and limit of quantitation (LOQ) of As were 0.034 and 0.11  $\mu$ g/g, respectively.

The As concentration ranges in different tissues were obtained as: gills (1.01–10.4), mouth pieces (1.01–18.6), intestine (1.01–11.2), liver (3.51–10.9) and in muscles (2.12–15.2)  $\mu$ g/g on dried basis. The bioac-cumulation factor (BAF) for As in fish muscles were found in two ranges (4.88–7.2) and (17.6–35.3). The contribution of the daily intake of As, based on the consumption of 250 g fresh fish muscles per day was found in the range of 0.1–0.76  $\mu$ g, higher than WHO tolerable limit.

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## 1. Introduction

Contamination of aquatic ecosystems (e.g. lakes, rivers and underground water) with As has been receiving worldwide attention and has become a challenge for world scientists (Bhattacharya, Jacks, Ahmed, Routh, & Khan, 2002). The effect of As exposure on human health was observed in population of south and southeastern Asia, particularly in Bangladesh, India and Taiwan (Al Rmalli, Haris, Harrington, & Ayub, 2005; Das et al., 2004; Hall et al., 2006; Lin & Liao, 2008).

In different areas of Pakistan, people are facing the As related severe public health disaster like India and other neighboring countries. In Pakistan As concentration was high in ground and surface water used as a drinking water (Shrestha, 2002). In Sindh province, 16–36% of population has been exposed to As contaminated water with over 10–50 ppb (Ahmad, Kahlown, Tahir, & Rashid, 2004). Manchar Lake (Sindh, Pakistan) is a biggest Asian Lake and main source of water for domestic, irrigation and fishing purposes. The Main Nara Valley Drain is a most important source of As

enrichment in this lake, as reported in our previous work (Arain et al., 2008).

Arsenic exposure has been related to the appearance of some types of cancer. Arsenic is a known carcinogen in humans, causing lung, liver, skin and bladder cancer (Kapaj, Peterson, Liber, & Bhat-tacharya, 2006).

The most serious sources of As pollution include emissions and wastewater of the ore mining and processing industry, dye manufacture facilities, tanneries, thermal power plants, and application of certain insecticides, herbicides and pesticides (Sarkar & Datta, 2004).

Water pollution leads to fish contaminated with toxic metals, from many sources, e.g. industrial and domestic waste water, natural runoff and contributory rivers (Marcursen, Holm, Ha, & Dalgard, 2007; Rashed, 2001). Arsenic has a considerable tendency to accumulate in bottom sediments (Smedley & Kinniburgh, 2002). Essentially, fish assimilate metals (also As) by ingestion of particulate material suspended in water, ingestion of food, ion-exchange of dissolved metals across lipophilic membranes, e.g., the gills, and adsorption on tissue and membrane surfaces. Metal distribution between the different tissues depends on the mode of exposure, i.e., dietary and/or aqueous exposure, and can serve as a pollution indicator (Alam et al., 2002). The bioaccumulation of metals is therefore an index of the pollution status of the relevant water body and is a useful tool studying the biological role of the





<sup>\*</sup> Corresponding author. Tel.: +92 022 2771379; fax: +92 022 2771560.

*E-mail addresses*: aqshah07@yahoo.com (A.Q. Shah), tgkazi@yahoo.com (T.G. Kazi), bilal\_ku2004@yahoo.com (M.B. Arain), mkhanjamali@yahoo.com (M.K. Jamali), hassanimranafridi@yahoo.com (H.I. Afridi), nusratjalbani\_21@yahoo.com (N. Jalbani), jab\_mughal@yahoo.com (J.A. Baig), gakandhro@yahoo.com (G.A. Kandhro).

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metals present at elevated levels in aquatic organisms, especially fish (Tariq, Jaffor, & Ashraf, 1991).

In order to determine the total As concentration in different fish tissues, it is imperative to assure complete mineralization of the samples, unless using non-destructive technique (Jalbani et al., 2007).

In recent years, microwave digestion processes have been used, owing to the advantages of this technique, which include speed of digestion and less possibility of contamination during the process (Soylak, Tuzen, Narin, & Sari, 2004). Different techniques have been used for As determination at trace levels, such as electrothermal atomic absorption spectrometry (ETAAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) (Gong, Xiufen, Mingsheng, Corinna, & Le Chris, 2002). The generation of hydride is one of the most important procedure for the determination of As (Moretto & Cadore, 2004).

The aim of the present study is to determine the total As concentration in gill, mouth piece, intestine, liver and muscle tissues of 10 edible fish species, collected from As contaminated Manchar Lake, Pakistan. All fish species are edible and local people (75% fishermen) used daily, while these fishes are frequently used in their diet in other parts of Pakistan especially in Sindh. The fish tissues (dry basis) were acid digested by microwave oven. Quality control was assured by the analysis of samples, reagent blank, procedural blanks and standard reference material. The estimated daily intake (EDI) of As by adults consuming different understudied fish species were also evaluated for possible human health risks.

## 2. Experimental

## 2.1. Sample collection

The 10 different fish species were sampled in December 2006– May 2007 by professional fishermen from different stations, located at both ends of Manchar Lake, scientific and common names are reported in Table 1. These fishes were differ according to their eating habits (*Mirgala, Thaila, Seenghara, Bam* and *Gonia* were *omnivorous*, whereas *Calbasu, Reba, Gullio, Dayo, Rahu* belongs to the herbivorous group Has-Schon, Bogut, & Strelec, 2006).

All fish species were collected at sizes 20–45 cm with weight range 750–1500 g and assigned individual identification numbers. At the end of each sampling effort, all the samples were wrapped in plastic bags, placed in polyethylene bags, held in an ice box, and refrigerated at -4 °C until further treatment. In first step, the fishes were dissected and separated gills, mouthpiece, intestine, liver and muscles. The livers were separated from gallbladders while intestines were cleaned by squeezing out the contents, making a longitudinal incision and scraping and blotting to remove As-containing food particles and feces. All tissues were washed with physiological saline and deionized water repeatedly and were stored in rewashed polyethylene bottles at -20 °C till further analysis and preparation.

## 2.2. Analytical

Ultra pure water, obtained from an ELGA lab water system (Bucks, UK) was used throughout the experiment. Concentrated HCl, HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were analytical reagent grade from Merck (Darmstadt, Germany) and were checked for possible trace contamination. Calibrations were prepared for each analytical session using certified stock standard solution of As 1000 ppm, Fluka Kamica (Bushs, Switzerland). Appropriate dilutions were made from this solution with 0.1 M HCl whenever necessary. The methods were validated by certified reference material, NRCC DORM-2 (dogfish Muscle) from the National Research Council of Canada (Ottawa, Ontario Canada).

All glassware and polyethylene bottles were thoroughly washed and then soaked overnight in 5 M HNO<sub>3</sub>, thoroughly rinsed with distilled and deionized water before use. Solution of sodium tetrahydroborate was prepared by dissolving NaBH<sub>4</sub> powder Acros Organics (New Jersey, USA) in 0.05 M KOH.

Total As determined by Perkin–Elmer A Analyst 700 atomic absorption spectrometer equipped with a deuterium background corrector and a MHS-15 hydride generation system, Perkin–Elmer Corp., Perkin–Elmer (Shelton, CT, USA). The operating parameters for working of As hollow cathode lamp were set as recommended by the manufacturer. Milestone Microwave System (Bergamo, Italy) was used for samples digestion.

#### 2.3. Digestion methods

## 2.3.1. Conventional wet acid digestion method (CAD)

Replicate six samples of (0.2 g) of CRM (DORM-2) and triplicate 1.2 g sub samples of different tissues of each fish species (n = 20) were directly weighed into Pyrex flasks separately. Added 5 ml of a freshly prepared mixture of concentrated HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> (2:1, v/v) and kept for 10 min at room temperature, then the content of flasks were heated on an electric hot plate at 60–70 °C for 2–3 h until clear, transparent digests were obtained. Final solutions were made up to 10 ml with 0.1 M HCl. The final solutions were collected in polyethylene flasks and kept at 4 °C until further analysis. As was determined by hydride generation AAS. Blank digestions were also carried out.

#### 2.3.2. Microwave acid digestion method (MAD)

A microwave assisted digestion procedure was carried out in order to achieve a shorter digestion time. About 0.2 g replicates six samples of CRM, while 0.2 g triplicate (dry weight) of different tis-

Table 1

Estimation of arsenic in different parts of fish species by microwave assisted and conventional acid digestion methods (µg/g) on dried basis (n = 100)

Scientific name with common name	Gills	Mouth piece	Intestine	Liver	Muscles
Labeo calbasu (Calbasu)	$9.7 \pm 0.5^{a} (10.1 \pm 0.5)$	18.2 ± 0.5 (18.6 ± 0.3)	8.9 ± 0.74 (9.6 ± 0.71)	$5.6 \pm 0.04 \ (6.1 \pm 0.31)$	9.1 ± 0.9 (9.3 ± 0.1)
Cirrhinus mrigala (Mrigala)	1.1 ± 0.13 (1.4 ± 0.15)	1.0 ± 0.13 (1.3 ± 0.13)	6.9 ± 0.22 (7.3 ± 0.31)	8.3 ± 0.11 (8.8 ± 0.05)	2.0 ± 0.11 (2.2 ± 0.21)
Cirrhinus reba (Reba)	0.79 ± 0.06 (1.1 ± 0.13)	$1.0 \pm 0.07 (1.2 \pm 0.13)$	6.5 ± 0.05 (7.3 ± 0.31)	9.3 ± 0.05 (9.7 ± 0.03)	2.6 ± 0.09 (2.7 ± 0.11)
Mystus gullio (Gullio)	7.6 ± 0.5 (8.0 ± 1.3)	10.1 ± 1.3 (11.0 ± 1.5)	9.9 ± 1.3 (10.3 ± 1.1)	4.8 ± 0.12 (5.3 ± 0.08)	8.2 ± 1.4 (8.6 ± 1.2)
Catla catla (Thaila)	6.5 ± 0.5 (7.7 ± 1.1)	14.6 ± 0.90 (15.1 ± 0.81)	$10.4 \pm 1.1 \ (11.0 \pm 0.9)$	3.5 ± 0.21 (3.9 ± 0.18)	14.8 ± 0.80 (15.2 ± 0.25)
Mystus seenghara (Senghara)	$10.6 \pm 0.14 (11.0 \pm 0.21)$	12.0 ± 0.9 (12.5 ± 0.2)	9.9 ± 0.81 (10.6 ± 0.78)	$4.4 \pm 0.07 (5.0 \pm 0.13)$	12.0 ± 0.37 (12.1 ± 0.37)
Mastacembelus armatus (Bam)	$0.86 \pm 0.04 \ (1.2 \pm 0.05)$	$1.5 \pm 0.14 (1.9 \pm 0.15)$	0.58 ± 0.11 (1.0 ± 0.21)	8.8 ± 0.13 (9.4 ± 0.05)	$3.0 \pm 0.06 (3.1 \pm 0.09)$
Tilapia mossambicus (Dayo)	$1.4 \pm 0.14 (2.1 \pm 0.15)$	$1.7 \pm 0.15 (2.1 \pm 0.21)$	1.8 ± 0.11 (2.5 ± 0.12)	$9.0 \pm 0.03 \ (9.6 \pm 0.14)$	2.3 ± 0.21 (2.4 ± 0.30)
Labeo rohita (Rahu)	9.8 ± 0.41 (10.4 ± 0.51)	11.0 ± 0.51 (11.4 ± 0.68)	10.9 ± 0.41 (11.2 ± 0.37)	$8.5 \pm 0.04 \ (9.0 \pm 0.11)$	7.3 ± 0.30 (7.6 ± 0.31)
Labeo gonius (Gonia)	$4.0 \pm 0.11 (4.4 \pm 0.51)$	$2.1 \pm 0.15 \ (2.5 \pm 0.20)$	3.7 ± 0.11 (4.1 ± 0.12)	$10.1 \pm 0.05 \ (10.9 \pm 0.12)$	$2.0 \pm 0.26 (2.1 \pm 0.31)$

Key = <sup>a</sup>conventional digestion method.

Values in parenthesis obtained by microwave digestion method.

sues of each studied fish samples were directly weighed into Teflon PTFE flasks. Added to each flask 2 ml of a freshly prepared mixture of concentrated HNO<sub>3</sub> and  $H_2O_2$  (2:1, v/v) and kept for 10 min at room temperature, then placed the flasks in covered PTFE container. It was then heated following a one-stage digestion programmed at 80% of total power (900 W), 1–2 min time required for all fish tissues. The digestion flasks were cooled; the resulting solution was evaporated to semidried mass to remove excess acid, and then diluted to 10.0 ml 0.1 M HCl as stock sample solution.

#### 2.4. Analysis

For As determination a second dilution was made by taking different aliquots of the master stock sample solutions and diluting with 1.5% HCl. Arsine gas was generated from this solution on the addition of 2% sodium borohydride (reagent grade) and 10% potassium iodide (Welz & Sucmanova, 1993). Solutions were swept by argon gas stream into preheated quartz tube atomizer installed over a burner with lean, blue air-acetylene flame. Analyses were performed in peak height mode to determine absorbance. A blank extraction (without sample) was carried out throughout the complete procedure. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal, from an appropriate reagent blank. All experiments were conducted at room temperature (30 °C) following the well-established laboratory protocols. Deionized water or a dilute acid solution can be aspirated between samples (but experimentation is required to ascertain what produces the best reproducibility). To stabilize the HGAAS system the acidic blank is often flowed through the sample inlet tube for 5 or 10 min although the longer this goes on, the more acidic waste is produced.

Statistical analyses were performed using computer program Microsoft Excel 2000 (Microsoft Corp., Redmond, WA) and Minitab 13.2 (Minitab Inc., State College, PA).

#### Table 2

Validation of microwave assisted (MAD) and conventional acid digestion methods (CAD) for determination of As ( $\mu$ g/g) against CRM DORM-2

Element	Certified $\bar{x} \pm s^a$	MAD $\bar{x} \pm s$	% recovery	CAD $\bar{x} \pm s$	% recovery
As	18.0 ± 1.1	18.1 ± 0.85	100.6	17.8 ± 0.72	98.8

Key = <sup>a</sup>Mean ± standard deviation.

#### 2.5. Quality assurance-quality control

The method was assured by the analysis of triplicate samples, reagent blank, procedural blanks and standard reference material. The detection and quantitation limits were calculated by  $LOD = 3 \times \frac{s}{m}$  and  $LOD = 10 \times \frac{s}{m}$ , respectively, where s is the standard deviation of 10 measurements of a reagent blank and m is the slope of the calibration graph corresponding to As was  $0.452 \pm 0.014 \,\mu\text{g/ml}$  and good precision could be seen for the calibration. The calculated LOD was 0.034  $\mu$ g/g and LOQ was 0.11  $\mu$ g/g. The precision of the methods, expressed as the relative standard deviation (RSD) of 8 independent analyses of the same sample, provided values were less than 10% for the determination of total As. The accuracy of analytical method was performed with standard reference material DORM-2 (dogfish muscles) with chemical composition and analyte concentration, match those of the samples under investigation (Table 2). Application of paired *t*-test also showed that there is no difference between two methods. The paired *t*-test was calculated for n - 1 = 5 degrees of freedom,  $t_{exp}$ (0.146) was less than the  $t_{crit}$  (2.57) at a confidence interval of 95% (P = 0.05).

The method was also validated by spiking the muscle tissues of fish Gonia in triplicate at four concentration levels 0.5, 1.0, 1.5, 2.0  $\mu$ g/ml and were analysed by HG AAS. Average recovery of As spikes from fish muscles matrix were in the range of 98.0–99.5%.

Mean values and standard deviations were calculated for five tissues of each fish species, containing each 30 experimental data. Due of the numerous experimental data, the approach to the discussion of our results are rather complex. At first glance of intact data (Table 1 and Fig. 1), it is evident that there are great differences in As concentration according to the fish species, as well as tissue analyzed.

## 3. Results and discussion

#### 3.1. As levels in Manchar Lake

The lake water and sediment samples have high level of total As, ranged  $(60.4-101.8 \ \mu g/l)$  and  $(13.2-17.7 \ \mu g/g)$ , respectively (Arain et al., 2008). The concentration in lake water is 6.0–10 times greater than permissible limit of As, recommended by WHO  $(10 \ \mu g/l)$  for drinking water. The pollution level of Manchar Lake seems to become worse, information on the background levels of

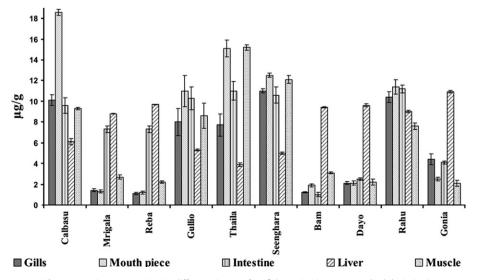


Fig. 1. Arsenic concentration in different tissues of 10 fish species (mean ± standard deviation).

As in the fish species will continue to be needed, contributing to the effective monitoring of both environmental quality and the health of organisms inhabiting the lake ecosystem.

#### 3.2. Arsenic in fish tissues

The 10 fish species were collected from Manchar Lake, which are commonly consumed by local and people residing in other areas of Pakistan. These fish species were used as a bioassay indicator for the lake pollution with high concentration of As. The mean values and standard deviations of As concentration in analyzed tissues of 10 fish species are given in Table 1. The broad distributions of As concentration in different tissues of 10 fish species were found in the range of, gills (1.01–10.4), mouth piece (1.01–18.6), intestine (1.01–11.2), liver (3.51–10.9), muscle (2.12–15.2)  $\mu$ g/g. According to these data, great variation in accumulation of As among same tissues of understudied fishes were observed. The highest level of As was observed in gills of *Mysteus seenghara* (11.2  $\mu$ g/g); mouth piece of *Labeo calbasu* (18.6  $\mu$ g/g); intestine of *Labeo rohita* (11.2  $\mu$ g/g); liver of *Labeo gonius* (10.9  $\mu$ g/g) while in muscles of fish *Catla Catla* (15.2  $\mu$ g/g).

The calculated mean values on As contents in the different tissues of fish species were compared with the literature data. Has-Schon et al. (2006), analyzed As in five fish species from River Neretva, Croatia (muscles (0.038-0.309), gills (0.005-0.255) and liver 0.005–0.227) µg/g. Delgado-Andrade, Navarro, Lopez, and Lopez (2003) reported total As in fish (n = 25) from south-east Spain and found total As in muscles (0.396–12.58) µg/g. Rosemond, Xie, and Liber (2008) analysed five freshwater fish species from Back Bay near Yellowknife, NT, Canada, and reported data of total arsenic in muscles (0.57-1.15), intestine (1.48-8.92), and liver (0.42-2.25) µg/g. Jankong et al. (2007), analysed arsenic content of freshwater fish from arsenic-contaminated and non-contaminated sites in Thailand, and reports high arsenic concentrations in the edible muscle tissue (0.051-0.806) µg/g. Al Rmalli et al. (2005), analysed arsenic in foodstuffs on sale in the United Kingdom and imported from Bangladesh found total arsenic in fish muscles (0.097–1.32) ug/g: Moretto and Cadore (2004) developed a method to analyse total arsenic in food samples, reported As in fish muscles, 0.27-10.3  $\mu$ g/g. The found values of As concentration in different tissues of understudied fish species were high, as compare to the literature reported values of As concentration in different tissues of fishes except the result reported by Delgado-Andrade et al. (2003) and Moretto and Cadore (2004).

The estimated daily intake of total As in different understudied fish species for individuals of study area, on the consumption of 250 g fish muscles, was found in the range of  $1.75-12.7 \mu g/kg$  body weight/day (Table 3). Although the total As intake was high, while taking into account that the inorganic As in muscles of fishes represents a minimal portion of the total As content (Devesa et al.,

 Table 3

 Bioaccumulation factor of arsenic concentrations in muscle tissue and intake of arsenic by consumption of 250 g of fish muscles/kg body weight/day

Common name of fishes	$\bar{x} \pm s \; (\mu g/g)$ Wet weight	BAF = total As in muscles/As in water (86 μg/l)	µg of As/kg body weight/day
Calbasu	$1.86 \pm 0.02$	21.6	7.75 ± ±0.06
Mrigala	$0.54 \pm 0.04$	6.27	$2.25 \pm 0.05$
Reba	$0.44 \pm 0.02$	5.11	$1.83 \pm 0.02$
Gullio	1.72 ± 0.24	20.0	7.17 ± 0.57
Thaila	$3.04 \pm 0.05$	35.3	$12.7 \pm 0.07$
Seenghara	$2.42 \pm 0.07$	28.1	10.08 ± 0.3
Bam	$0.62 \pm 0.02$	7.20	$2.58 \pm 0.07$
Dayo	$0.44 \pm 0.06$	5.11	1.83 ± 0.22
Rahu	$1.52 \pm 0.06$	17.6	6.33 ± 0.09
Gonia	$0.42 \pm 0.06$	4.88	$1.75 \pm 0.07$

2001). It is known that the As and its species bioavailability in foods is variable. Consequently, future research will be required to obtain a better knowledge of how specific factors, traditional cooking processes, ingredients of foods as well as consumption of surface and ground water contains high level of As (average value  $80.6 \mu g/l$ ), especially in understudy area of Pakistan, can create health problems.

### 3.3. Bioaccumulation of As in different fish species

Arsenic accumulation in different muscle tissue of 10 fish species showed significant differences in As concentrations (Fig. 1). As concentration in the intestine and liver are higher in most of the cases than As concentration in muscle tissues, these results are consistent with other study (Suhendrayatna, Ohki, Nakajima, & Maeda, 2001). The *Mystus gullio, C. catla* and *M. seenghara* were an exception because in their gills, liver and intestine As concentration were lower than their muscle tissues. This finding is in agreement with literature data (Chan & Huff, 1997), indicating that methylation capacity changes with exposure, the periods of high metabolism and As excretion alternate with periods when As is accumulating in tissues.

In present study we calculated the bioaccumulation factor of fish muscles, mainly due to consumed as edible part of fishes. Variation in accumulation of total As in muscles tissues (due to consumable part of fishes) of under studied fish species are shown in Table 3. The lower level of As were found to be 2.1, 2.2, 2.4, 2.7 and 3.1 µg/g in the muscles tissues of L. gonius, Cirrhinus mrigala, Tilapia mossambicus, Cirrhinus reba and Mastacembelus aramatus, respectively. The bioaccumulation factor was found in the range of (4.88–7.2) also shows the similar accumulation of As. The high level of As was observed as 7.6, 8.6, 9.3, 12.1 and 15.2 µg/g in L. rohita, Mystus gullio, L. calbasu, M. seenghara and C. catla, respectively. Among all fish spices, two fish species M. seenghara and C. catla, accumulates high concentration of As as compared to others. The highest BAF was observed in C. catla (35.3). The variation in bioaccumulation of As in different species collected from same locality is consistent with other study (Williams, Schoof, Yager, & Goodrich, 2006).

## 4. Conclusion

The microwave acid digestion has been demonstrated to be an efficient methodology for determination of total As by hydride generation atomic absorption spectrometry. The accuracy of methods were checked with certified sample and by spiky recovery test. The good analytical features of the method allow for its application for routine analysis of large number of samples and a variety of foodstuff since there are no risks for interferences or matrix effect.

The results of this experiment may be used to guide environmental monitoring programs that assess the bioavailability of As in freshwater fishes. Analyses of As in muscle, liver, intestine are recommended for evaluating the bioavailability of As to freshwater fishes. In general, total As concentrations included in the present study are important for the provision of nutritional information on the background As levels in lake water. The As in soluble or suspension form which are finely tend to settle down to the bottom which may be taken by the aquatic organism and their contribution via different fish species to the daily intake in the Pakistani diet. Among understudied 10 fishes, evidently some fish species have high accumulation factor of As in muscles, e.g. L. calbasu, M. seenghara. and C. catla. Therefore, they may be potentially harmful as the constituent of human food. It is recommended that meals prepared in the analyzed fishes do not contain inner organs (intestine), as well as gills (alternatively, the whole head). Our investigation suggests that among freshwater fish species, those fishes in which less As were present especially in muscles, therefore recommended for human diets. However, people in rural areas near by the sampling lake cooked fish with As contaminated lake water, may increasing the concentration of As intake.

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