



Hazardous impact of arsenic on tissues of same fish species collected from two ecosystem

Abdul Qadir Shah^a, Tasneem Gul Kazi^{a,*}, Mohammad Balal Arain^a, Jameel Ahmed Baig^a, Hassan Imran Afridi^a, Ghulam Abbas Kandhro^a, Sumaira Khan^a, Mohammad Khan Jamali^b

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

^b Government degree college Usta Mohammad Balochistan Pakistan

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ABSTRACT

The purpose of this paper is to develop a database of fish tissues and to evaluate concentration of arsenic (As) in five tissues of fish species collected from Manchar Lake Pakistan and to compare concentration of As in fish tissues of same fish species collected from the Indus River, Pakistan. A sensitive and precise, hydride generation atomic absorption spectrometry (HG AAS) method is presented for the determination of total Arsenic (As). Microwave acid-assisted digestion (MAD) procedure based on the mixture $\text{HNO}_3/\text{H}_2\text{O}_2$ was evaluated. The method was successfully validated against CRM DORM-2 (dogfish muscle). Quantitative As recovery in CRM (DORM-2) was obtained and no statistical differences were found at 95% level by applying the *t*-test. The limit of detection (LOD) and limit of quantitation (LOQ), for As were established as 0.022 and $0.063 \mu\text{g g}^{-1}$, respectively. The results of this study indicated that As concentration in fish tissues from the Indus River are generally lower than in tissues of fishes from Manchar Lake. Arsenic concentrations in fish tissues of Indus River are although above the respective human health-based concentrations.

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1. Introduction

Arsenic is a toxic element for humans and it is commonly associated with serious health disruptions [1]. Total diet As studies carried out in various countries have shown that fish and shell fish are the most significant dietary source of As, accounting for nearly three quarters of total intake [2,3]. The concentration of As was found in environmental samples, mainly in waters, where inorganic form is predominant [4,5]. Arsenic exposure has been related to the appearance of some types of cancer [6]. A report on an assessment of the cancer risk associated with consumption of oysters caused a panic among consumers in Taiwan [7]. Some of these human health effects currently observed in population of south and southeastern Asia, particularly in countries such as Bangladesh and India [8]. Besides the direct exposure of humans to As through drinking contaminated water, the As might also be biologically available to aquatic organisms, such as fish which are used as human food

thereby providing an additional source of As. Arsenic has a considerable tendency to accumulate in bottom sediments [9]. For this reason, issues related to As content in aquatic organisms and sea fish in particular, have attracted considerable attentions. The relevance of this As intake will depend on the concentration of As accumulated by the fish [10].

During recent years, serious concern has been voiced about the rapidly deteriorating state of fresh water bodies with respect to toxic metals pollution. Fishes are often at the top of the aquatic food chain and accumulate large amounts of some metals from the water [11]. Water pollution leads to fish contamination with toxic metals from many sources, e.g., industrial and domestic wastewater, natural runoff and contributory rivers [12,13]. Fishes, living in polluted water may accumulate toxic trace metals via their food chains, they assimilate metals by ingestion of particulate material suspended in water, ion exchange of dissolved metals across lipophilic membranes, e.g., the gills, adsorption on tissue and membranes surfaces [14]. The bioaccumulation of metals is therefore, an index of the pollution status of the relevant water body [15].

The pollution level of big fresh water lake (Manchar Lake) Sindh, Pakistan, seems to become worse due to anthropogenic sources [16]. Indus River is polluted through three sources: municipal wastewater discharges, industrial wastewater discharges and return-agriculture flows through drainage structures. Most of the cities and towns of Sindh discharge their municipal wastewater into

* Corresponding author. Tel.: +92 22 2771379; fax: +92 22 2771560.

E-mail addresses: aqshah07@yahoo.com (A.Q. Shah), tgkazi@yahoo.com (T.G. Kazi), bilal_KU2004@yahoo.com (M.B. Arain), jab_mughal@yahoo.com (J.A. Baig), hassanimranafriidi@yahoo.com (H.I. Afridi), gakandhro@yahoo.com (G.A. Kandhro), skhanzai@gmail.com (S. Khan), mkhanjamali@yahoo.com (M.K. Jamali).

the Indus River. Due to water pollution in Indus River, the species diversity of fish has been reduced [17].

Many researchers have presented the elemental contents in various tissues, such as liver, kidneys, gills, gonads and muscles of fishes [18]. In present work, five different tissues of 10 species were analyzed. In order to determine the total As concentration in different tissues, it is imperative to assure complete mineralization of the samples. The acid digestion induced by microwave energy is a well-established methodology as it has been recently reviewed [19,20]. The advantages of this technique are, speed of digestion and lesser possibility of contamination during the process. Different techniques have been used for determination of As at trace levels, such as electrothermal atomic absorption spectrometry (ETAAS), atomic fluorescence spectrometry (AFS), inductively coupled plasma mass spectrometry (ICP-MS) [21,22]. The hydride generation is one of the most important technique for the determination of As. This technique coupled with atomic absorption spectrometry provides the analytical chemist with an excellent tool for detecting and quantifying hydride forming elements [23].

The aim of the present study is to evaluate the total As concentration by hydride generation atomic absorption spectrometry (HG AAS) prior to microwave assisted acid digestion in 10 fish species collected from the polluted Manchar Lake and for comparative purpose same species were also sampled from Indus River, Pakistan. The accuracy and precision of the proposed technique established with certified reference material. Subsequently, the contribution of these fish species to the total daily intake of total As was evaluated.

2. Materials and methods

2.1. Sample collection and pretreatment

Two hundred individual samples of 10 freshwater fish species, *Rita rita*, *Wallago attu*, *Notopterus notopterus*, *Heteropneustes fossilis*, *Channa striata*, *Channa marulius*, *Notopterus chitala*, *Ompok bimaculatus*, *Ompok pabda*, *Glossogobius giurus* were collected during 2007–2008 from contaminated Lake ($n = 100$) and Indus River ($n = 100$), Sindh, Pakistan and assigned individual identification numbers. At the end of each sampling effort, all the samples were wrapped in plastic bags and kept in an ice box. In laboratory, the fishes were dissected and separated gills, mouthpiece, intestine, liver and muscles. All tissues were washed several times with deionized water. The livers were separated from gallbladder, rinsed thoroughly with physiological saline and deionized water. The intestines were cleaned by squeezing out the contents, making a longitudinal incision, scraping and blotting to remove As containing food particles and feces. Each tissue of fishes was dried for 48 h in an oven at 65 °C to a constant weight. Then, they were minced in the vibrational agate ball mill for 5 min using a power of 60%. The powdered samples were sieved through a nylon sieve to obtain particle size 65 μm . The powder obtained for sample preparation was stored in closed polyethylene tubes and maintained in a refrigerator at -4°C until analysis.

All collected fish species have different eating habits, *R. rita*, *W. attu*, *C. striata*, *C. marulius*, *N. chitala*, *Ompok bimaculatus* and *G. giurus* were carnivorous and *N. notopterus*, *H. fossilis* and *O. bimaculatus* were omnivorous.

2.2. Reagents and glassware

Ultra pure water (0.05 $\mu\text{S cm}^{-1}$ resistivity) obtained from an ELGA lab water system (Bucks, UK) was used throughout the experiment. Concentrated HCl, HNO_3 and H_2O_2 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 mg L^{-1} , obtained from Fluka Kamica (Buchs, Switzerland). Appropriate dilutions were made with 0.1 mol L^{-1} HCl whenever necessary. The technique was 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 soaked over night in 5 mol L^{-1} HNO_3 , then thoroughly rinsed with distilled and de-ionized water before use. Solutions of sodium tetra hydroborate were prepared by dissolving NaBH_4 powder Acros Organics (NJ, USA) in 0.05 mol L^{-1} KOH.

2.3. Apparatus

Total As determined by PerkinElmer A Analyst 700 atomic absorption spectrometer equipped with a deuterium background corrector and a MHS-15 hydride generation system, PerkinElmer Corp., (Shelton, CT, USA). The operating parameters for working element were set as recommended by the manufacturers. A PM023 domestic programmable PEL microwave oven (PEL Japan) was operated for sample digestion.

2.4. Digestion method

2.4.1. Conventional wet acid digestion method (CAD)

Replicate six samples of CRM (DORM-2) and triplicate sub samples of different tissues of each fish species ($n = 30$) were directly weighed (0.2 g) in Pyrex flasks separately, added 5 ml of freshly prepared mixture of concentrated $\text{HNO}_3\text{-H}_2\text{O}_2$ (2:1,v/v) and kept for 10 min at room temperature, then the content of the flasks were heated on an electric hot plate at 60–70 °C for 2–3 h until clear, transparent digests were obtained. Finally, solutions were made up to 10 ml with 0.1 mol L^{-1} HCl. The solutions were collected in polyethylene flasks and kept at -4°C till further analysis. Blank digestions were also carried out.

2.4.2. Microwave assisted acid digestion method (MAD)

A microwave assisted acid digestion procedure was carried out in order to achieve a shorter digestion time. Weighed 0.2 g replicates six samples of CRM, while 0.2 g triplicate (dry weight) of different tissues of each studied fish samples directly into Teflon PTFE flasks. Added to each flask 2 ml of a freshly prepared mixture of concentrated HNO_3 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 one-stage digestion programmed at 80% of total power (900 W); 1–2 min 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 with 0.1 mol L^{-1} HCl, as stock sample solution.

For As determination a second dilution was made by taking different aliquots of the master stock sample solutions and added 10% KI for pre reduction of As(V) to As(III) then diluted with 0.15 mol L^{-1} HCl. Arsine gas was generated from this solution on the addition of 0.8 mol L^{-1} of sodium borohydride (reagent grade) [24]. Solutions swept by an argon gas stream in to pre-heated quartz tube atomizer installed over a burner with lean, blue air-acetylene flame. Analyses were performed in peak height mode to determine absorbances. A blank extraction (without sample) was carried out through 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 dilute acid solution can be aspirated between samples.

Table 1
Validation of the proposed method for determination of As against CRM (DORM-2) ($\mu\text{g/g}$).

Certified value	MAD $\bar{x} \pm \frac{ts}{\sqrt{n}}$ ^a	%Recovery ^c	CAD $\bar{x} \pm \frac{ts}{\sqrt{n}}$ ^a	%Recovery	Paired t -test: $t_{\text{experimental}}$ ^b
18.0 \pm 1.1	18.1 \pm 0.32	100.6	17.8 \pm 0.72	98.8	0.146

^a Average value \pm confidence interval ($P=0.05$).

^b Paired t -test between MAE vs CAD, degree of freedom ($n-1$) = 5, t_{critical} at 95% confidence limit = 2.57.

^c % Recovery = $\frac{[\text{certified value}]}{[\text{MAD}]}$ \times 100.

Table 2
Recovery of arsenic spiked in gills tissue of fish specie *Rita rita*.

S. no.:	Fish specie	MDM values Mean \pm S.D. ($\mu\text{g g}^{-1}$)	As spiked ($\mu\text{g L}^{-1}$)	Obtained values	Recovery of As (%)
1		10.4 \pm 0.50	0	10.4 \pm 0.50	–
2	<i>Rita rita</i>		10.0	29.5 \pm 1.23	98.3
3			20.0	39.6 \pm 1.87	99.0
4			40.0	49.3 \pm 2.45	98.6

Table 3
Estimation of arsenic in tissues of same fish species of Manchar Lake and Indus River Pakistan. ($\mu\text{g/g}$ on dried basis).

Fish species	Gills	Mouth piece	Intestine	Liver	Muscles
<i>Rita rita</i>	9.25 \pm 0.40 ^a (10.4 \pm 0.50)	5.49 \pm 0.41 (6.10 \pm 0.21)	10.1 \pm 0.50 (11.4 \pm 0.68)	10.0 \pm 0.41 (11.2 \pm 0.37)	6.58 \pm 0.30 (7.48 \pm 0.31)
<i>Wallago attu</i>	4.00 \pm 0.11 (4.40 \pm 0.50)	2.27 \pm 0.15 (2.50 \pm 0.20)	3.69 \pm 0.11 (4.10 \pm 0.12)	6.64 \pm 0.22 (7.3 \pm 0.31)	1.92 \pm 0.26 (2.11 \pm 0.31)
<i>Notopterus notopterus</i>	1.01 \pm 0.06 (1.14 \pm 0.13)	1.10 \pm 0.07 (1.20 \pm 0.13)	6.49 \pm 0.05 (7.30 \pm 0.31)	9.82 \pm 0.05 (9.70 \pm 0.03)	2.43 \pm 0.09 (2.70 \pm 0.11)
<i>Heteropneustes fossilis</i>	7.25 \pm 0.52 (8.24 \pm 0.33)	4.71 \pm 0.12 (5.34 \pm 0.08)	9.06 \pm 0.43 (10.3 \pm 0.31)	9.91 \pm 0.38 (11.2 \pm 0.25)	7.68 \pm 1.4 (8.63 \pm 1.2)
<i>Channa striata</i>	6.96 \pm 0.50 (7.74 \pm 1.1)	3.26 \pm 0.21 (3.63 \pm 0.18)	9.57 \pm 1.1 (11.0 \pm 0.9)	10.9 \pm 0.90 (12.3 \pm 0.81)	9.65 \pm 0.80 (11.1 \pm 0.25)
<i>Channa marulius</i>	9.79 \pm 0.14 (11.3 \pm 0.21)	4.46 \pm 0.07 (5.02 \pm 0.13)	9.32 \pm 0.81 (10.6 \pm 0.78)	11.1 \pm 0.9 (12.5 \pm 0.2)	12.2 \pm 0.37 (14.1 \pm 0.37)
<i>Notopterus chitala</i>	1.12 \pm 0.04 (1.24 \pm 0.05)	0.96 \pm 0.11 (1.05 \pm 0.21)	1.74 \pm 0.14 (1.92 \pm 0.15)	8.55 \pm 0.13 (9.42 \pm 0.05)	2.82 \pm 0.06 (3.12 \pm 0.09)
<i>Ompok bimaculatus</i>	1.95 \pm 0.14 (2.15 \pm 0.15)	1.92 \pm 0.15 (2.12 \pm 0.21)	1.31 \pm 0.11 (2.53 \pm 0.12)	8.74 \pm 0.03 (9.61 \pm 0.14)	3.96 \pm 0.21 (4.42 \pm 0.30)
<i>Ompok pabda</i>	9.04 \pm 0.41 (10.4 \pm 0.51)	6.72 \pm 0.30 (7.64 \pm 0.31)	9.96 \pm 0.41 (11.2 \pm 0.37)	10.2 \pm 0.51 (11.4 \pm 0.68)	8.85 \pm 0.04 (10.3 \pm 0.11)
<i>Glossogobius giuris</i>	1.03 \pm 0.11 (1.19 \pm 0.12)	2.29 \pm 0.15 (2.55 \pm 0.20)	3.92 \pm 0.11 (4.44 \pm 0.51)	9.91 \pm 0.05 (10.9 \pm 0.12)	2.73 \pm 0.26 (3.01 \pm 0.31)

() Values in parenthesis obtained from fishes collected from Manchar Lake.

^a Fishes collected from Indus river Pakistan.

2.5. Statistical analysis

The accuracy of the technique was verified by determining total As in CRM (DORM-2) and the results showed good agreement with the certified values (Table 1). Two-tailed t -test was used to compare the means of related (paired) samples in order to ascertain that both methods showed insignificant results at 95% confidence level. The calculated t -value was compared with the theoretical value at $\alpha=0.05$, which were smaller than the theoretical value, it means that, at the chosen significance level, no difference between the two methods was found.

3. Result and discussion

The accuracy of the HG AAS technique and the reliability of the data were confirmed by analyzing certified reference material. The results were found within the certified values for the As with good precision at 95% confidence limit (Table 1). The accuracy of methodology was also ensured by standard addition method. The triplicate samples of gill tissues of a fish species *R. rita* were spiked prior to the microwave and conventional acid digestion at three concentration levels 10.0, 20.0 and 40 $\mu\text{g L}^{-1}$, respectively. In this experiment, the volume made up to 25 ml in volumetric flasks and were analysed by HG AAS. Percentage recovery of As spikes with gill tissues matrixes was observed in the range of 98.3–99.0% (Table 2). Mean values and standard deviations were calculated for five tissues of each fish species, containing 30 experimental data. The concentrations of As in five tissues of edible fresh water fish species of both sampling sites were reported in Table 3.

The concentrations of As in different tissues of 10 fish species collected from Manchar Lake were found in the range as, gills (1.10–11.0), mouth piece (1.05–7.64), intestine (1.90–11.4), liver (7.30–12.5) and muscle (2.11–14.1) $\mu\text{g g}^{-1}$ where as concentrations

of As in tissues of fishes from Indus River was observed in range as gills (1.01–9.79), mouthpiece (0.966–6.72), intestine (1.74–10.0), liver (6.64–11.1) and muscle (1.92–12.2) $\mu\text{g g}^{-1}$. It was observed that fishes collected from Manchar Lake accumulated high concentration of As, as compared to fishes from Indus River (Fig. 1). The pollution level of Manchar Lake seems to become worse, the high level of As in water was 102 $\mu\text{g L}^{-1}$ and in sediments samples was 17.7 mg kg^{-1} [25]. Where as highest concentration of As in water and sediments at various location of Indus River was found 18.5 $\mu\text{g L}^{-1}$ and 7.45 mg kg^{-1} , respectively. It was reported that concentration of toxic metals in sediments and water reflected a possible correlation between metal content of fish and sediments [26]. According to these data, great variations in accumulation of As in same tissues of fishes were observed. The highest accumulation of As, tissues wise was found as, 11.0 $\mu\text{g g}^{-1}$ in gills of *C. marulius*, 7.61 $\mu\text{g g}^{-1}$ in mouth piece of *O. pabda*, 11.4 $\mu\text{g g}^{-1}$ in intestine of

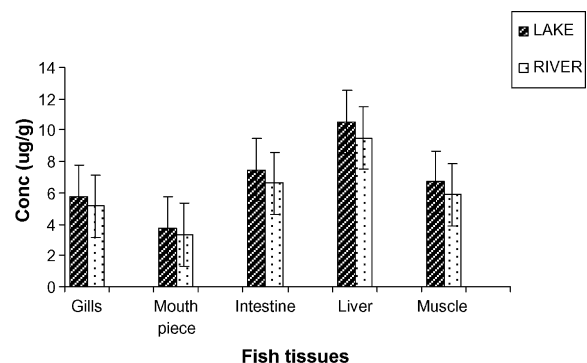


Fig. 1. Comparison of arsenic in fish tissues of Manchar Lake and River Indus, Pakistan.

Table 4
Daily intake of Arsenic by consumption of 250 g of fish muscles/kg body weight/day.

Fish species	μg of As/kg body weight/day	
	Manchar Lake	Indus river
<i>Rita rita</i>	6.23 \pm 0.13	5.48 \pm 0.09
<i>Wallago attu</i>	1.75 \pm 0.24	1.61 \pm 0.49
<i>Notopterus notopterus</i>	2.25 \pm 0.09	2.02 \pm 0.31
<i>Heteropneustes fossilis</i>	7.16 \pm 0.41	6.43 \pm 0.42
<i>Channa striata</i>	9.25 \pm 0.39	8.04 \pm 0.67
<i>Channa marulius</i>	11.7 \pm 0.50	10.1 \pm 0.37
<i>Notopterus chitala</i>	2.58 \pm 0.26	2.35 \pm 0.28
<i>Ompok bimaculatus</i>	3.67 \pm 0.19	3.32 \pm 0.29
<i>Ompok pabda</i>	10.3 \pm 0.31	7.37 \pm 0.43
<i>Glossogobius giuris</i>	2.52 \pm 0.19	2.25 \pm 0.19

R. rita, 12.5 $\mu\text{g g}^{-1}$ in liver of *C. marulius* and 14.1 $\mu\text{g g}^{-1}$ in muscles of *C. marulius*. These variations in high levels in different tissues of fish species were related to their accumulative capacity. The concentration of As in the intestine and liver tissues were higher in most cases than As concentration in muscles tissues, consistency with the study of Suhendrayatna et al. [27], while *N. notopterus*, *N. chitala* were an exceptions because As concentration were lower in their gills and intestine than their muscle tissue, consisted with other study [28]. The high concentration of As were detected in muscles of *R. rita*, *H. fossilis*, *C. marulius* and *O. pabda*. In present study it was observed that some fish species showed great affinity for As. Therefore, it may be potentially harmful to consume those fishes, which contains high As concentration in edible part (muscles).

We calculated that a person (weighing 60 kg) consuming an average of 250 g per day of muscles of fishes either obtained from Manchar Lake or from Indus River getting (1.75–11.5) and (1.61–10.1) μg total arsenic kg body weight $^{-1}$ day $^{-1}$ (Table 4), these values are above (except two species), the Joint Food and Agriculture Organization of the United Nations/World Health Organization tolerable daily intake of 2.14 $\mu\text{g kg body weight}^{-1}$ day $^{-1}$ [29]. On the basis of previous reports on inorganic As in food composites [30,31], we also assumed that at least 50% of the total As in our studied samples is inorganic As. The inhabitants that tend to eat more (including children, people aged 55 and older) may face doses up to much higher, constituting a sizable proportion of their tolerable daily intake.

It was recommended that when meals prepared from the fishes do not contain inner organs (intestine) as well as gills (alternatively the whole head). Our investigation suggested that among fresh water fish species, those fishes in which less As were present especially in muscles, therefore, can be recommended for human diets. However, this presumption should be further tested on some other types of fishes. However, people lives in rural areas near by the sampling lake, cooked fishmeal using water of As contaminated lake, caused high As intake [25].

4. Conclusion

Arsenic was determined by using two digestion methods, microwave acid and conventional wet digestion method in 10 fish species collected from river and polluted lake. The accuracy of methods were checked with certified reference material and by spike recovery test for As. The HG AAS technique is highly suitable for the determination of total As in fishes. The good analytical features of the technique allow for its application to routine analysis for large number of samples and variety of foodstuff since there are no risks for interferences or matrix effect.

Information on the levels of As in the fish species should be properly maintained, which help for the effective monitoring of

both environmental quality and the health of organisms inhabiting in lake and river ecosystem. The collected data indicate that fresh water fishes are responsible for a significant source of As to human population. The arsenic concentrations found in this study lead us to conclude that the public's exposure to arsenic from fishes demands reconsideration by regulatory agencies.

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