APPLICATION OF PIXE, NAA AND OTHER TECHNIQUES FOR THE DETERMINATION OF TOXIC ELEMENTS IN FOODSTUFFS AND DRINKING WATER IN BANGLADESH

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Abstract

An investigation was conducted on the status of trace and minor elements in some foodstuffs, meats and also drinking water in Bangladesh, using proton induced X-ray emission (PIXE), radioisotope-induced X-ray fluorescence (XRF), atomic absorption spectrophotometry (AAS) and also ion-selective electrode techniques. The elemental concentrations of K, Ca, Mn, Fe, Ni, Cu, Zn, As, Br, Rb and Sr, were determined in the samples by comparison with X-ray yield curves constructed from IAEA and NBS standard reference materials. Cadmium and zinc in the drinking water of Bangladesh was determined by AAS followed by solvent extraction technique. The total iodine content in different types of vegetables was determined by alkali ashing followed by the ion-selective electrode technique. The results indicate that none of the food regimes and also drinking water investigated here is burdened with heavy metals beyond permissible limits. The significance of the results is discussed in relation to human health and diseases. Three IAEA (2-1, 2-2 and 2-3) secondary reference standard samples were also analyzed for intercomparison studied using PIXE and ASV techniques.

1. INTRODUCTION

The investigation of trace elements in biological, agricultural, industrial and environmental matrices has gained increasing demand because of their role in health and diseases. Trace elements, either toxic or essential, reach the human body through foods, drinks, ambient air and other occupational and accidental exposures. The usefulness of essential trace elements lies in the fact that they act as co-factors in enzyme metabolism. From the view point of safety assessment, it is therefore necessary to have information on the levels of both toxic and essential elements in foodstuffs and to compare the results with maximum permissible concentrations specified in relevant national and international regulations. It is, therefore, customary to monitor food items regularly for heavy metal contamination. In this context, the modern analytical techniques such as PIXE, Neutron Activation Analysis (NAA), Atomic Absorption Spectrophotometry (AAS), Anodic Stripping Voltametry (ASV), *etc.* have made this monitoring programme more fruitful for regulatory purposes.

The basic food items of the daily consumption of all the population of Bangladesh are rice, wheat, pulses, vegetables, meat, fish, milk, poultry and diary products. During the last two years of the research programme, all the common food items except meat, such as cereals, vegetables, fish, milk and egg, were studied and documented but in this

report, meat was also included. This report deals with the results obtained during the last and final contract period.

2. EXPERIMENTAL MEASUREMENTS

2.1. Sample preparation

2.1.1. Meat

Meat (beef and mutton) and liver (beef) samples were collected from the local market of Dhaka city. The collected samples (250 g) were cut into small pieces with a tantalum knife and washed under tap water to remove blood and other fluid materials. After several washes with distilled water followed by distilled deionized water, the samples were wiped with white tissue paper to remove the excess water and then air dried. Finally, the fresh weight of the materials was taken in a thoroughly cleaned porcelain dish. An aliquot of the homogenate sample was freeze dried, ground to fine powder and then preserved for subsequent analysis.

2.1.2. Fish

The sweet water fish samples, (500 g) after cleaning and cutting into small pieces, were thoroughly cleaned with distilled water and finally with distilled deionized water. The excess water was removed and the samples were then air dried to obtain the fresh weight. Finally, they were oven dried at $105 \pm 5\,^{\circ}C$ to enable easy powdering them and preserved in a desiccator.

Finally, pellets were made from all the powdered samples with a hand press pellet maker for PIXE and XRF analysis.

2.1.3. Vegetables

Vegetable samples collected from the local market were cleaned, dried, powdered and then preserved for the analysis of iodine by ion-selective method.

2.1.4. Drinking water

Water samples were collected from Sylhet and Mymensingh, the two major cities of Bangladesh, in clean and dry polyethylene bottles. After collection, each of the samples was acidified with 1% supra pure HNO_3 to prevent hydrolysis and surface adsorption.

2.2 Methods of Analysis

2.2.1. PIXE measurements

In this analysis, the external beam technique was applied to determine minor and trace elements in the samples. The samples were irradiated with 2.0 MeV proton beams (energy on the target) obtained from the 3 MeV Van de Graaff Accelerator at the Atomic Energy Centre, Dhaka (AECD). The irradiations were performed with 30 nA beam intensity for 40 μc charge at 45°C with respect to the beam direction. The characteristic X-rays

were detected with a 30 mm sq Si(Li) Ortec detector having resolutions of 170 eV at 5.9 KeV and analyzed with a 1024 multichannel analyzer (Canberra) and other NIM electronics. All the peak areas were integrated manually, assuming linear background under peak of interest.

2.2.2. XRF measurements

A radioisotope-induced X-ray fluorescence system was used for XRF analysis where a 10 mCi cadmium-109 annular source was used for excitation. The samples were excited for 5000 seconds in the form of pellets, 100 mg weight with 10 mm diameter. All X-ray spectral data acquisition and processing were similar to those mentioned under PIXE measurements.

2.2.3. AAS measurements

Supply and tube-well water in some major cities/towns were collected for the determination of cadmium and zinc contents in these samples. Zinc was measured directly in all the water samples using air-acetylene flame with combination hollow cathode lamp and Perkin-Elmer-560 AAS spectrophotometer. But cadmium was determined after chelation with sodium diethyldithiocarbamate followed by chloroform extraction. Finally, the extractant was evaporated and then treated with 10 ml of 6M HCl and 1 ml of 70% $HClO_4$ for the estimation of cadmium by atomic absorption spectrophotometry.

2.2.4. Ion-selective electrode (ISE) measurements

The iodine content in vegetables was determined by an Orion Ion Analyzer (Model 470A) using iodide electrode. A known quantity (0.5 g) of the powdered vegetable sample along with 0.5 ml of 4M KOH solution was taken in a platinum crucible. The mixture was dried at 105°C for 20 hours in an oven and then heated at 150°C for 30 minutes in a muffle furnace. Finally, the temperature was raised to 500°C and kept constant for one hour to complete the reaction. The residue was digested with 10 ml of distilled deionized water on a water bath, filtered and then diluted to a volume of suitable concentration. All the measurements were taken by an ion Analyzer and the concentration of total iodine was calculated by comparison with the calibration curve constructed from sodium iodide standard solution.

2.2.5. Concentration calibration

The number of X-rays per ppm of the elements of interest per micro coulomb of charge vs atomic number of the elements is defined as the concentration calibration. The IAEA and NBS standard reference materials were used without any further treatment for concentration calibration in all the analyses. It was observed that a single multielement biological standard can be used for all other biological specimens as this was verified previously from this laboratory [1,2].

3. RESULTS AND DISCUSSIONS

3.1. Fish

Out of the five sweet water fish species, (two kachki and Gura chingri) were prepared with the gut while the other three varieties (Puti, Mola and Prawn) prepared without gut for their analyses. The results are shown in Table I. Under the present experimental conditions of X-ray excitation, eight elements (K, Ca, Mn Fe, Ni, Cu, Zn and As) were possible to be determined in these samples. Of the known toxic elements, only arsenic in prawn was found above the detection limit and the content is 5.04 ± 09 mg/Kg (fresh weight basis). The maximum permissible limit of arsenic in fish is 3.0 mg/Kg (fresh weight basis). Prawns caught from U.K. coastal areas were found to contain as much as 170 mg/kg (fresh weight basis). The essential trace elements (Fe, Ni, Cu and Zn) were found in all the species except copper in Mala. The highest concentration of iron (54.0 mg/Kg) was found in Mola while the range of copper was 0.50 - 17.0 mg/Kg in Gura chingri. The range of zinc in sweet water fish is found to be 15.2 - 62.1 mg/Kg. The environmental factors, as well as the difference in species could be the reasons for these variations. In any case, the sweet water fish species studied here do not appear to have excess heavy metal load.

3.2. Meat

In the present study, 10 varieties of beefs, 5 varieties of mutton and 1 liver (beef) samples were investigated, and in all the cases, the samples were collected from the local market of Dhaka city. The concentration of about 8 minor (Ca, Mn, Fe, Ni, Cu, Zn, As and Sr) and 9 (Ca, Mn, Fe, Ni, Cu, Zn, As, Br and Rb) trace elements was determined in meat and liver samples using the PIXE method. But in the case of XRF method, the concentration of 5 (Fe, Cu, Zn, As and Rb) elements can be determined in the meat samples. In all the cases, the concentration was determined using a concentration calibration curve constructed from the NBS Bovine Liver (SRM-1577), NBS Orchard Leave (SRM-1571) and IAEA standard MA-A-2. The results are listed in Table II and Table III. The XRF method was applied to determine arsenic both in meat and liver samples and the content ranged from 0.41-0.88 mg/Kg (fresh weight basis) with the average value of 0.68 mg/Kg (fresh weight basis). The average value of arsenic in English foods specially in meat (uncooked pork, beef and lamb), is reported to be 0.1 to 0.05 mg/Kg (fresh weight basis). Most human foods contain less than 0.5 mg/Kg arsenic and rarely exceed 1.0 mg/Kg on the fresh weight basis. Barring the possibilities of contamination, the present values of arsenic appear to be high.

The levels of iron, cooper and zinc are found to be comparable with the values reported in Thai meat. In other words, in terms of essential trace elements, animal protein especially meat, is the best source of such type of food which are mainly consumed by the middle and high income group of the Bangladeshi population. The poor consumptions of animal protein in food habit by the vast population in Bangladesh has created malnutrition in the country amongst which child mortality within 1-5 years of age is quite high. The deficiency of nutritive foods causes poor growth, poor health and many diseases and ultimately, face a slow and miserable death.

3.3. Drinking water

During the second contract period of our programme, cadmium and zinc levels in the source and supply waters of some major cities of Bangladesh, namely, Dhaka, Khulna, Chittagong and Rajshahi were studied and presented at the second coordination meeting. This year (1987-1988), the study has been extended to two other major cities, namely, Mymensingh and Sylhet to assess and complete the quality control survey of overall water supplies in the major cities of Bangladesh. All the water samples were collected in a cleaned polyethylene bottles and then analyzed by flame atomic absorption spectrophotometry using air-acetylene flame. The results are shown in Table IV. Spectroscopically, pure standard salts (Johnson Matthey) were used for concentration calibration. Analysis of zinc and cadmium was carried out because of their interaction with the living system.

Cadmium could not be detected in any of the supply and source waters of the major cities of Bangladesh except Dhaka under the present experimental conditions. The average value of zinc in Mymensingh supply waters was 159 μg/L for six samples while 44 μg/L for 5 samples in Sylhet.

3.4. Iodine in vegetables

The total iodine content in different types of vegetables was determined by alkali ashing procedure followed by the ion-selective electrode technique and the results are given in Table V. This technique offers some advantages over the traditional alkali dry ashing and acid digestion in low iodine losses, simplicity and rapidity. The alkali dry ashing also does require large sample volumes for analysis.

About 12 varieties of vegetables (Table V) were investigated in this study for the determination of total iodine content. The highest value of iodine was observed in Lalsakh (20.4 mg/Kg) with a range of 0.21-20.4 mg/Kg. The iodine concentrations in human foods in general are exceedingly variable, mainly due to differences in the content and availability of iodine in the soil, water and to the amount and nature of the fertilizers applied. Barakat [4] in a study of iodine content in the edible portions of 15 vegetables, found large variations between samples of the same vegetables, presumably reflecting differences in the availability of iodine in the soil.

3.5. Analysis of IAEA secondary reference materials

The analysis of IAEA reference sample 2-1, 2-2 and 2-3 was taken up in order to develop the compatibility in PIXE, XRF and other related techniques and to establish their validity by comparing the results obtained from other participants. The samples were analyzed as such without further treatment. They were analyzed using external beam PIXE method and irradiated in the form of pellets (50 mg, 7 mm diameter and 1 mm thick) with 2 MeV protons for 40 μc charge.

A radioisotope-induced X-ray fluorescence (RIXFA) system was also used for XRF analysis where a 10 mCi Cd-109 annular source was used for excitation. The samples were excited for 5000 seconds in the form of pellets, 100 mg weight with 10 mm diameter.

For concentration calibration, IAEA standard Reference Material MA-A-2, NBS Orchard Leave (SRM-1571) and Bovine Liver (SRM-1577) were used. Under the present experimental conditions, 8 elements (Ca, Mn, Fe, Ni, Cu, Zn, As and Br), including Rb in IAEA (2-3) were estimated and the results obtained are given in Table VI. The PIXE procedure was validated by analyzing Mn, Fe and Zn by atomic absorption spectrophotometry after dry ashing at 500°C.

Anodic Stripping Voltametry (ASV) has been undertaken to estimate the level of Cd and Pb in the reference materials. This technique is sensitive even at a very low concentration for the elements (Cd and Pb). As an extra precautions for ultra trace level measurements, all the chemicals used in this experiment were supra pure grade. The Table VII illustrates the results of analysis of reference materials and secondary reference materials using this technique. The concentration of Cd and Pb in reference materials is found to be in good agreement with the certified values.

4. CONCLUSIONS

The different groups of food items such as fish, meat, cereals, vegetables, milk and egg commonly consumed in Bangladesh have been investigated by PIXE, XRF, AAS and ASV to obtained base line data on the level of trace elements in these foodstuffs. Drinking water supplies of major cities of Bangladesh is also included in this Co-ordinated Research Programme (CRP) for its importance as a source of trace elements. The important toxic and essential trace elements of interest in foodstuffs are Cd, Hg, Cu, As, Cr, Fe, Pb, I and Zn. From the base line data obtained, there is practically no indication of potential health hazards from these foodstuffs and drinking water supplies. Moreover, evaluation of base line data provides an idea about the nutritional quality of these food items in terms of trace elements. The present programme also helps substantially to develop analytical capabilities which can offer analytical quality control services and to provide validation support for National monitoring programme.

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TABLE I. TRACE ELEMENT COMPOSITION OF SOME SWEET WATER FISH AND PRAWN (mg/kg, fresh weight basis)*

Element	Puntius stigma (Puti)	Amnly pharyngodon (Mola)	Bicamugil cascasia (Kachki)	Macrobracsium lammarrei (Gura chingri)	Plaemon carcinus (Prawn)	Range
K	2382 ± 166	1502 ± 102	---	1426 ± 90	3389 ± 140	1436 - 3389
Ca	6066 ± 42	4003 ± 30	6181 ± 16	7537 ± 41	770 ± 10	770 - 7537
Mn	5.29 ± 0.26	4.43 ± 0.12	7.09 ± 0.15	2.99 ± 0.21	1.36 ± 0.17	1.36 - 7.09
Fe	29.2 ± 0.3	54.0 ± 0.1	16.4 ± 0.1	18.7 ± 0.2	39.1 ± 0.3	16.4 - 54.0
Ni	1.43 ± 0.12	4.24 ± 0.08	3.95 ± 0.10	0.65 ± 0.21	1.92 ± 0.16	0.65 - 1.43
Cu	0.66 ± 0.12	---	0.50 ± 0.08	17.0 ± 0.2	5.33 ± 0.08	0.50 - 17.0
Zn	42.6 ± 0.4	44.1 ± 0.1	62.1 ± 0.4	34.0 ± 0.3	15.2 ± 0.1	15.2 - 62.1
As	---	---	---	---	5.04 ± 0.09	---
Br	2.47 ± 0.23	1.03 ± 0.11	3.90 ± 0.12	11.3 ± 0.1	17.9 ± 0.1	1.03 - 17.9
Rb	2.84 ± 0.32	1.34 ± 0.30	1.68 ± 0.17	6.64 ± 0.33	6.27 ± 0.14	1.34 - 6.64
Sr	12.8 ± 0.4	13.6 ± 0.3	15.1 ±	46.3 ± 0.2	6.55 ± 0.17	6.55 - 46.3

* Uncertainties are due to counting statistics.

The dry weight factor varied from 4.4 - 5.0 at 105 ± 5°C.

TABLE II. MINOR AND TRACE ELEMENT CONCENTRATIONS IN MEAT AND LIVER (BEEF) USING PIXE* ($\mu g/g$ fresh weight basis)

	Ca	Mn	Fe	Ni	Cu	Zn	As	Br	Rb	Sr
BM - 1	37.25 ± 1.46	0.35 ± 0.09	19.97 ± 0.24	1.78 ± 0.05	0.95 ± 0.09	48.70 ± 0.49	0.69 ± 0.04	---	---	1.55 ± 0.25
BM - 2	36.28 ± 1.46	0.65 ± 0.07	23.37 ± 0.24	4.17 ± 0.13	1.2 ± .2	51.86 ± 0.49	0.71 ± 0.05	---	---	1.37 ± 0.2
BM - 3	21.18 ± 0.97	---	9.74 ± 0.24	0.98 ± 0.08	1.1 ± 0.2	53.79 ± 0.24	0.61 ± 0.05	---	---	1.12 ± 0.2
BM - 4	40.42 ± 1.46	---	25.08 ± 0.24	3.30 ± 0.08	0.93 ± 0.14	38.22 ± 0.24	0.66 ± 0.05	---	---	0.96 ± 0.14
BM - 5	22.16 ± 1.22	0.43 ± 0.05	12.42 ± 0.24	4.01 ± 0.14	1.4 ± 0.2	46.51 ± 0.49	0.72 ± 0.06	---	---	1.02 ± 0.2
BM - 6	28.24 ± 1.70	---	14.12 ± 0.24	0.70 ± 0.11	0.53 ± 0.11	32.87 ± 0.45	0.68 ± 0.04	---	---	0.25 ± 0.12
BL - 7	30.92 ± 1.22	2.03 ± 0.12	58.92 ± 0.49	0.46 ± 0.08	2.71 ± 0.11	34.09 ± 0.24	0.81 ± 0.09	1.05 ± 0.13	3.94 ± C.30	---

* Uncertainties are due to counting statistics.

The dry weight factor varied from 23.5 - 24.0 (%).

TABLE III. TRACE ELEMENT CONTENTS IN MEAT (MUTTON AND BEEF) USING XRF* (μg/g fresh weight basis)

	Fe	Cu	Zn	As	Rb
MM - 1	25.25 ± 1.75	3.58 ± 0.41	35.50 ± 0.5	0.69 ± 0.04	1.35 ± 0.08
MM - 2	32.50 ± 1.75	2.90 ± 0.37	37.50 ± 0.5	0.88 ± 0.08	1.42 ± 0.08
MM - 3	25.75 ± 1.50	2.29 ± 0.26	22.50 ± 0.5	0.86 ± 0.07	1.10 ± 0.04
MM - 4	33.00 ± 1.75	2.06 ± 0.25	25.50 ± 0.3	0.75 ± 0.08	1.17 ± 0.04
MM - 5	25.50 ± 1.5	1.61 ± 0.23	19.00 ± 0.25	0.61 ± 0.03	1.02 ± 0.04
BM - 1	20.95 ± 1.60	2.50 ± 0.37	14.94 ± 0.44	0.64 ± 0.06	1.35 ± 0.08
BM - 2	30.50 ± 1.75	2.94 ± 0.38	29.50 ± 0.50	0.41 ± 0.06	4.94 ± 0.11
BM - 3	30.50 ± 1.75	2.71 ± 0.27	13.24 ± 0.31	0.67 ± 0.07	1.70 ± 0.05
BM - 4	34.25 ± 1.75	3.11 ± 0.30	12.10 ± 0.31	0.56 ± 0.06	2.48 ± 0.06
Range	20.95 - 34.25	1.61 - 3.58	12.10 - 37.50	0.41 - 0.88	1.02 - 4.94

* Uncertainties are due to counting statistics.

TABLE IV. TRACE ELEMENT CONTENTS IN DRINKING WATERS OF SIX BIG CITIES IN BANGLADESH. CONCENTRATIONS ARE EXPRESSED IN μg/L

Type of sample	No.	Location	Parameter	Cd	Zn
Source Water	17	Dhaka	Range	BDL	13-301
			Mean (n)		57.94 (16)
Supply Water	27	Dhaka	Range	<1.27 - 1.58	18 - 3800
			Mean (n)	1.29 (7)	181 (24)
Tubewell Water	5	Dhaka	Range	1.71 - 2.24	42 - 370
			Mean (n)		203 (5)
Supply Water	5	Chittagong	Range	BDL	44 - 170
			Mean (n)		85 (5)
Supply Water	6	Rajshahi	Range	BDL	38 - 580
			Mean (n)		272 (6)
Supply Water	5	Khulna	Range	BDL	16 - 250
			Mean (n)		75 (5)
Supply Water	6	Mymensingh	Range	BDL	20 - 653
			Mean (n)		159 (6)
Tubewell Water	5	Sylhet	Range	BDL	14 - 125
			Mean (n)		44 (5)
MDL				1.27	

BDL Below detection limit
MDL Minimum detection limit
No. Number of samples analyzed
n Element found in the number of samples

TABLE V. IODINE LEVEL OF SOME VARIETIES OF VEGETABLES IN BANGLADESH (edible portion, dry weight basis)

Name of vegetables	Concentration (mg/kg)
Pulwal (Trichosanthes dioeca)	1.17
Lady's finger (Hibscus esculentus)	3.6
Bitter gourd (Lagenaria vulagaris)	0.37
Spinach (chenopodiaceae)	9.3
Radish (Raphanus sativus)	8.27
Brinjal (Solanum melongena)	11.23
Field bean (Dolichos lablab)	5.0
Cauliflower (Brassica oleracea var. botrytis	0.83
Sweet gourd (Cucurbitapepo)	0.37
Balsam apple (Momordica charantia)	0.30
Lal sak (Amaranthus gangeticus)	20.4
Potato (Solanum tuberosum)	0.57

TABLE VI. CONCENTRATION OF MINOR AND TRACE ELEMENTS IN IAEA SECONDARY REFERENCE SAMPLES (μg/g) USING PIXE*

Sample	Ca	Mn	Fe	Ni	Cu	Zn	As	Br	Rb
IAEA (2-1)	48.8 ± 3.5	12.52 ± 0.46	20.94 ± 0.52	2.10 ± 0.21	1.87 ± 0.21	14.64 ± 0.47	2.94 ± 0.35	7.38 ± 0.68	---
		13.65 ± 0.15	20.73 ± 0.73			14.49 ± 0.23			
IAEA (2-2)	148.0 ± 6.0	9.91 ± 0.52	33.67 ± 0.74	3.99 ± 0.34	5.34 ± 0.41	12.86 ± 0.51	2.99 ± 0.40	7.16 ± 0.71	---
		9.20 ± 0.22	34.25 ± 0.56			14.33 ± 0.57			
IAEA (2-3)	555.0 ± 10.0	14.08 ± 0.57	41.29 ± 0.77	4.24 ± 0.37	1.67 ± 0.29	34.05 ± 0.78	2.95 ± 0.38	10.07 ± 0.81	10.27 ± 1.12
		12.65 ± 0.35	39.0 ± 0.61			32.13 ± 0.33			

* Uncertainties are due to counting statistics and the values in parentheses are obtained by AAS.

TABLE VIIA. ANALYSIS OF STANDARD REFERENCE MATERIALS (SRM) USING ASV TECHNIQUE. CONCENTRATION IN μg/g

Element	Spinach (NBS - 1570)		Bovine Liver (NBS - 1577)	
	This analysis	Certified value	This analysis	Certified value
Cd	1.44 ± 0.23	(1.5)	0.27 ± 0.0007	0.27 ± 0.04
Pb	1.67 ± 0.16	1.2 ± 0.20	0.36 ± 0.46	0.34 ± 0.08

TABLE VIIB. ANALYSIS OF IAEA SECONDARY REFERENCE MATERIALS USING ASV TECHNIQUE. CONCENTRATION IN μg/g.

Element	IAEA (2-1)	IAEA (2-2)	IAEA (2-3)
Cd	0.017 ± 0.003	0.015 ± 0.001	0.05 ± 0.003
Pb	0.073 ± 0.004	0.050 ± 0.002	1.00 ± 0.007