Arsenic and Other Trace Elements in Bangladeshi Foods and Non-Foods and Their Relationship to Human Health

PhD Thesis

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DEDICATION

The thesis is dedicated to my loving mother, ***Mrs. Salma Matouk***, who gave her love and prayers, for everything she sacrificed in her life for me. Without her love and prayers, it would not have been easy for me to come this far.

It is also dedicated to my beloved wife ***Mrs. Hawa Takali***, who gave me continuous support during this time. Finally my thanks go to my children ***Fatima, Wafa, Osama, Aya, Dwaa*** who have been constant source of joy and pleasure. Without their love and prayers I would never have made it this far.

ABSTRACT

It is estimated that over 30 million people are exposed to arsenic from drinking contaminated groundwater in Bangladesh. Furthermore, due to the use of contaminated water for irrigation purposes, arsenic and other toxic elements are entering the food chain of Bangladeshis. In this thesis, the total levels of toxic elements (As, Cd, Pb) and essential elements (Mn, Se, Zn) in 1,120 samples of Bangladeshi foods (including rice, vegetables, fish) and non-foods (betel quid and baked clay) imported into the United Kingdom were determined. From this analysis, it is concluded that Bangladeshis are exposed to high levels of toxic elements. Inorganic arsenic levels in Bangladeshi rice can be very high, especially from regions with high arsenic in groundwater. However, there is a lack of studies in the literature regarding arsenic levels in rice from regions in Bangladesh with relatively low levels of arsenic in the groundwater. Therefore, rice from one such region (Sylhet district) was analysed. The results indicated that boro (mean 71.7 µg/kg) and aman (mean 85.7 µg/kg) rice from Sylhet contained between 2 to 4-fold lower levels of arsenic, compared to other regions of Bangladesh thus far reported in the literature. Arsenic speciation was carried out on a selection of rice (aromatic and non-aromatic) from Sylhet region and this revealed 70% (mean value) as inorganic arsenic (As^{III} and As^{V}), which is similar to rice grown in other regions of Bangladesh. Importantly, it was found that the arsenic levels of aromatic rice (mean 48.5 µg/kg) from Sylhet region was over 40% lower than that of non-aromatic rice (mean 81 µg/kg). The aromatic rice also contained higher levels of essential elements (such as Se and Zn). It was calculated that for an individual consuming 0.5 kg of rice per day, switching from consumption of non-aromatic rice to aromatic rice would increase Se and Zn intake by 46% and 23% respectively.

Arsenic speciation was also carried out on other Bangladeshi food and non-food items, including fish, betel quid and baked clay, to obtain a better insight into exposure to toxic arsenic species. High levels of arsenic (range 3.8-13.1 mg/kg) and lead (range 21-26.7 mg/kg) were detected in the baked clay samples, which are consumed by some Bangladeshi women in an ancient practice known as geophagy. The efficiency of arsenic extraction from baked clay was 33% of the total arsenic present and the main arsenic species present was inorganic As^{V} (100%); As^{III} was not detected in these samples. Millions of Bangladeshis chew betel quid and this contained predominantly As^{III} species (extraction efficiency was 100%). Arsenic and lead intake from eating baked clay could exceed the provisional maximum tolerable daily intake (PMTDI) by 2- and 5-fold respectively. For the first time, arsenic speciation in Bangladeshi fish is reported. Hilsha, which is a very popular fish in Bangladesh, contained 2.55 mg/day (mean value) of total arsenic. Extraction efficiencies (%) were 59 – 89 for fish flesh, over 69% of arsenic present in the extract was dimethyl arsenic acid (DMA) species with about 11% arsenobetaine (AsBet) and 19% arsenosugar. These studies reveal that rice, betel quids and baked clay can be a significant source of exposure to inorganic arsenic and DMA in Bangladeshis.

Exposure to cadmium is linked with kidney disease and over 20 million people in Bangladesh suffer from chronic kidney disease. Results obtained showed that the daily intake of cadmium by the Bangladeshi population from baked clay (mean 17 µg/day), rice (mean 18.6 µg/day) and certain leafy vegetables (mean 12 µg/day) was higher total daily intake compared to other countries. Surprisingly, puffed rice, which is commonly consumed by Bangladeshis, contained much higher levels of cadmium (mean 67.9 µg/kg) and lead (mean 98 µg/kg), compared to uncooked rice (cadmium, 37.2 µg/kg; lead, 18.9 µg/kg). This may be related to the illegal practice of using urea for whitening puffed rice in Bangladesh.

Exposure to manganese in the Bangladeshi population through drinking water has been previously highlighted as a possible health problem, although the intake from foods and non-food has not been reported. The daily manganese intake by Bangladeshis was calculated to be 20.3 mg/day, which is higher than any other country in the world thus far reported. Betel quid components have high levels of manganese and this was reflected by higher urinary manganese the mean urinary Mn levels in chewers (1.93 µg/L, SD 1.8) was significantly higher (3.1 fold; $P = 0.009$) compared to non-chewers (0.62 µg/L, SD 0.4). Bangladeshi women who eat baked clay and chew betel quids are likely to be exposed to high levels of arsenic, lead and other toxic elements. This is particularly of concern for pregnant women as these metals can be transferred to the unborn baby through the placenta.

For assessing the risk of exposure to toxic elements versus intake of essential elements from the same foods, a Food Toxicity Scale (FTS) was devised in order to

identify foods that are beneficial or harmful. FTS values were obtained by calculating the toxic elements : essential elements ratio (As:Se, As:Zn etc) and the value obtained subsequently multiplied by toxic element concentration and the quantity of the particular food consumed per day. The higher the FTS value, the greater the risk of exposure to harmful elements. Rice and leafy vegetables had the highest FTS values, partly because large quantities of these foods are consumed. However, lentils and animal products (such as small fish) had relatively lower FTS values compared to other foods.

Total daily intake of arsenic (306 µg/day), selenium (90.4 µg/day), cadmium (34.6 µg/day), lead (74.4 µg/day), manganese (20.3 mg/day) and zinc (11.2 mg/day) in the Bangladeshi population was calculated. The intake of arsenic and manganese exceeds the PMTDI for these elements. Water was the highest source of arsenic exposure in Bangladeshis followed by rice. For cadmium and lead, rice and leafy vegetables were the key contributors to the daily intake.

The results presented in this thesis show that Bangladeshis are exposed to high levels of toxic elements and how modifications can be made to their diet to not only reduce their exposure to toxic elements but also increase the intake of essential elements. This could be achieved by a combination of the following: (i) reducing the intake of rice (by about 50%); (ii) switching to eating aromatic rice; (iii) increasing the intake of animal products (meat, fish etc.); (iv) decreasing the intake of certain leafy vegetables; and (v) stopping or reducing the practice of eating baked clay and chewing betel quid.

DECLARATION

I declare that the work described in this thesis is original work undertaken by myself for the Doctor of Philosophy degree, at the School of Allied Health Science, Faculty of Health and Life Sciences, De Montfort University, Leicester, United Kingdom.

Shaban Wanis Al-Rmalli

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PUBLICATIONS

PUBLISHED PAPERS

1. S.W. Al-Rmalli, R.O. Jenkins and P.I. Haris. Dietary Intake of Cadmium from Bangladeshi Foods. *Journal of Food Science*, 2012, 77(1): T26-T33.
2. S.W. Al-Rmalli, R.O. Jenkins and P.I. Haris. Betel Quid Chewing Elevates Human Exposure to Arsenic, Cadmium and Lead. *Journal of Hazardous Materials*, 2011, 190: 69-74.
3. S.W. Al-Rmalli, R.O. Jenkins and P.I. Haris. Betel Quid Chewing As a Source of Manganese Exposure: Total Daily Intake of Manganese in a Bangladeshi Population. *BMC Public Health*, 2011, 11:85-94.
4. S.W. Al-Rmalli, R.O. Jenkins, M.J. Watts and P.I. Haris. Risk of Human Exposure to Arsenic and Other Toxic Elements from Geophagy: Trace Element Analysis of Baked Clay Using Inductively Coupled Plasma Mass Spectrometry. *Environmental Health*, 2010, 9:79-85.

CONFERENCE PUBLICATIONS

1. S.W. Al-Rmalli, R.O. Jenkins and P.I. Haris, Health Risk from Human Ingestion of Arsenic from Charred Soil in Bangladesh, SEGH2010, 27th June – 2nd July, Galway, Ireland.

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ABBREVIATIONS

Arsenite	As^{III}
Arsenate	As^{V}
Monomethylarsonic acid	MMA(V)
Dimethylarsinic acid	DMA(V)
Monomethylarsine oxide	MMAO(III)
Dimethylarsenic acid glutathione	DMA(III)GS
Arsenobetaine	AsBet
Arsenocholine	AsChol
Trimethylarsine oxide	TMAO
Tetramethylarsonium ion	TMAs
Arsine	AsH_3
Total Daily Intake	TDI
Provisional Maximum Tolerable Daily Intake	PMTDI
Target Hazard Quotient	THQ
Food Toxicity Scale	FTS
Inductively Coupled Plasma Mass Spectrometry	ICP-MS
Graphite Furnace Atomic Absorption Spectrometry	GF-AAS
Food Frequency Questionnaire	FFQ
Standard deviation	SD

1 INTRODUCTION

Bangladesh, a country in the south of Asia with a population of about 150 million is one of a number of countries that has high arsenic contamination in the groundwater. The statistics available on the arsenic contamination in groundwater indicate that 52 districts, around 80% of the area of Bangladesh (Karim and Begam, 1999), are contaminated and estimates show that around 30 million people are at risk (WHO, 2008). Patients with melanosis (blackening of skin), leuco-melanosis (white and black spots side by side), keratosis (hardening of palms and soles), hyperkeratosis, gangrene and skin cancers have been identified in Bangladesh (Tanabe *et al.*, 2001; Hafeman *et al.*, 2006). Arsenic may be entering the food chain due to the use of arsenic contaminated water for irrigation purposes in Bangladesh (Al-Rmalli *et al.*, 2005; Meharg *et al.*, 2009).

Beside arsenic, high levels of other trace elements have been detected in Bangladeshi groundwater. For example, recently manganese in groundwater has been identified as another element that may pose health risks in Bangladeshi populations through drinking manganese contaminated groundwater (Frisbie *et al.*, 2009). In a study of Bangladesh, selenium deficiency has been reported in Bangladeshi diet (Spallholz *et al.*, 2004). Also it has reported that children and women in Bangladeshi villages have low serum zinc concentrations (Kongsbak *et al.*, 2006; Li *et al.*, 2008). More recently, some studies have recommended zinc-biofortified rice as a solution to Zn deficiency amongst Bangladeshis (Arsenault *et al.*, 2010, Mayer, 2010; Lindstrom *et al.*, 2011). In this thesis, a comprehensive study of Bangladeshi foods and non-foods, which are on sale in the UK markets, was achieved. Arsenic and other trace elements (Cd, Mn, Pb, Se and Zn) in these foods and non-foods were determined; also arsenic speciation was carried out for some of these samples.

1.1 Physical and chemical properties of trace elements

1.1.1 Arsenic

Arsenic (As) is widely distributed in the Earth's crust (O'Neill, 1990). It is a metal-like grey material and is usually found in the environment combined with other elements such as oxygen, chloride and sulphur. Arsenic combined with these elements is called inorganic arsenic. However, when combined with carbon and hydrogen and involves an arsenic-carbon bond it is referred to as organoarsenic (Ashoworth, 1991; O'Neill, 1990). The oxidation states of arsenic are –3, 0, +3 and +5, of which As(0) and As^{III} are characteristic of reducing environments. The complex anions AsO_2^-, AsO_4^{3-}, $HAsO_4^{2-}$ and $HAsO_3^-$ are the most common mobile forms of arsenic. Table 1-1 lists of some properties of arsenic.

Arsenic compounds (inorganic and organic) are white or colourless powders that do not evaporate. Some of the most important arsenic species found in the environment include the two oxidation states arsenite (As^{III}) and arsenate (As^{V}). Organic species of arsenic include monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine oxide (TMAO), arsenobetaine (AsBet) and arsenocholine (AsChol).

Table 1-1: Some properties of As, Cd and Mn (compiled from Lenntech, 2011)

Property	**As**	**Cd**	**Mn**
Atomic Number	33	48	25
Atomic Weight	74.9216	112.4	54.9380
Electronic Configuration	[Ar] $3d^{10}4s^24p^3$	[Kr] $4d^{10}5s^2$	[Ar] $3d^54s^2$
Valency	-3, 0, +3 and +5	0 and +2	0, +2 and +7
Ionic Radius (Å)	0.58 (+3)	0.97 (+2)	0.8 (+2)
Ionization Potential (V)	- 0.3	- 0.402	- 1.05
Density	5.72	8.7	7.43
Melting Point (^oC)	814	321	1247
Boiling Point (^oC)	615	767	2061
Energy of first ionisation (kJ/mol)	947	866	716
Electronegativity	2.0	1.7	1.5

1.1.2 Cadmium

Cadmium (Cd) is mainly present in the earth's crust, usually found combined with zinc. It also occurs as a by-product of zinc, lead and copper extraction from different minerals. About 3% of the impurity CdS can be produced as a by-product of the smelting of zinc from its ore, sphelerite (ZnS). Cadmium enters the environment mainly through industrial and mining activities, and it is also found in manures and pesticides. Table 1-1 lists some properties of cadmium.

1.1.3 Manganese

Manganese (Mn) is an abundant element in the earth's crust. It is present in soil, water and food. It is a pinkish-grey element and it is chemically active. It is also a hard metal and is very brittle. It is hard to melt, but easily oxidised. It can burn in oxygen and reacts with water. Manganese is a key component of low-cost stainless steel formulations. Other manganese compounds are manganese dioxide (used as a catalyst), potassium permanganate (oxidizing agent), manganese oxide (MnO) and manganese carbonate ($MnCO_3$). Both inorganic and organic manganese compounds are present in the environment, inorganic form is the most common. Table 1-1 lists some properties of manganese.

1.1.4 Lead

Lead (Pb) is a bluish white glossy metal. It is very soft, highly malleable, spongy, and a relatively poor conductor of electricity. It is a passive metal and very resistant to corrosion but tarnishes upon exposure to air. It is very rare to find lead metal in nature in its metallic form. However, lead can be found as an ore with zinc, silver and copper, but the main mineral of lead is Galena (PbS). Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements. Table 1-2 lists some properties of lead.

1.1.5 Selenium

Selenium (Se) is a non metallic chemical element, the main selenium forms are a red amorphous powder, a red crystalline material, and a grey crystalline metallike. It has good conductivity of electricity and it is used in photo and solar cells. It is mainly used in the glass industry. 15% of selenium production is used in supplements as sodium selenite.

Some selenium compounds are added to anti-dandruff shampoos. Selenium is rarely present on the surface of this planet and it is present in the atmosphere as methyl derivatives. Both inorganic and organic selenium compounds can be found in the environment. Table 1-2 lists some properties of selenium.

Table 1-2: Some properties of Pb, Se and Zn (compiled from Lenntech, 2011)

Property	**Pb**	**Se**	**Zn**
Atomic Number	82	34	30
Atomic Weight	207.2	78.96	65.37
Electronic Configuration	[Xe] $4f^{14}5d^{10}6s^26p^2$	[Ar] $3d^{10}4s^24p^4$	[Ar] $3d^54s^2$ 0,+2
Valency	0 and +2	-2, 0, +2 and +6	and +4
Ionic Radius (Å)	1.32 (+2)	1.98 (-2)	1.38 (+2)
Ionization Potential (V)	-	-0.77V	- 0.763
Density	11.34	4.79	7.11
Melting Point (^{o}C)	327	217	420
Boiling Point (^{o}C)	1755	688	907
Energy of first ionisation (kJ/mol)	715.4	940.7	904.5
Electronegativity	1.8	2.4	1.6

1.1.6 Zinc

Zinc (Zn) is an abundant element that can be found in soil, water and food. It is a lustrous bluish-white metal, which is brittle and crystalline at ordinary temperatures. When heated between 110 °C and 150 °C it becomes ductile and malleable. It is a reactive metal that combines well with oxygen and other non-metals. Table 1-2 lists some properties of zinc.

1.2 Trace elements in the environment

1.2.1 Arsenic in the environment

Arsenic is a naturally occurring element in minerals and soils, and thus is potentially airborne, and may subsequently be deposited on land and in water through wind, dust particles, leaching and water runoff. Another potential cause of arsenic in the air and in soil is volcanic eruptions. Particularly, the substance is linked with those ores mined for metals, namely copper and lead, and therefore, during ore mining and smelting processes, the element may enter into the environment. Moreover, there is the potential release into the atmosphere as a direct result of active incinerators and coal-fired power plants owing to coal and waste products commonly comprising some degree of arsenic (Bhumbla & Keefer, 1994).

Importantly, it is recognised that, once in the environment, arsenic cannot be destroyed; rather, its state can only be changed or may otherwise adhere to particles. Moreover, its form be changed through reacting with various molecules, including air, oxygen, soil or water, or alternatively through bacteria-related actions inherent in sediment or soil. With this in mind, it is recognised that arsenic released as a result of combustion processes is commonly affixed minute particles, as highlighted by Bhumbla & Keefer (1994), whilst arsenic found in the case of wind-borne soil is commonly found in larger particles. Notably, such particles can be washed out of the air by rain or may otherwise settle to the ground (PHE, 1994). Importantly, prior to being washed away, arsenic attached to small particles may remain in the air for long periods of time and be transported great distances. Furthermore, some arsenic may become dissolved in water, and so contamination can occur in the case of groundwater, lakes, rivers, snow or otherwise through the discharge of industrial wastes. Some arsenic will become adhered to sediment or soil particles on the bottom of lakes or rivers, with some subsequently carried by the water. Essentially, therefore, the majority of arsenic ends up in sediment or soil (Bhumbla and Keefer, 1994; Naidu *et al.*, 2006).

Speciation analysis has been described as an analytical process concerned with measuring and/or establishing the volume of one or more distinctive chemical elements within a sample (Templeton, 2000), with chemical speciation of environmental arsenic considered valuable owing to its toxicity, which markedly depends on the species it creates. Importantly, arsenite (As^{III}) and arsenate (As^{V}) are created as a result of the weathering of those minerals containing arsenic, which may, under conditions such as *in vivo* human body and anaerobic environment may be easily reduced to arsenite and absorbed by algae, animals, bacteria, fungi and higher plants to organic compounds, namely dimethylarsinic acid (DMA^{V}) and monomethylarsonic acid (MMA^{V}). A number of other arsenic compounds to have been identified in the context of living organisms, such as marine biota, include, amongst others, arsenobetaine (AsBet), arsenocholine (AsChol), trimethylarsine oxide (TMAO), the tetramethylarsonium ion (TMAs) and arsenosugars (Craing, 2003). With this in mind, the table below (Table 1-3) provides a number of inorganic and organic arsenic species' names and structures.

1.2.1.1 Arsenic in soil

In soil specifically, arsenic is seen to occur predominantly as an inorganic species, although it has the potential to bind to organic substances, as highlighted by Bhumbla &Keefer (1994) and Naidu *et al.* (2006). In the context of oxidising conditions, within aerobic environments, arsenate (As^{V}) is regarded as a stable species, and is keenly sorbed onto clays, iron and manganese oxides/hydroxides and organic matter. Essentially, in iron-rich soil, arsenic advances as ferric arsenic, whilst under reducing conditions, arsenite (As^{III}) is acknowledged as the fundamental arsenic element. With this taken into account, it is recognised that inorganic arsenic compounds can be methylated through microorganisms, creating under oxidising conditions, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO). The different types of arsenic present in soils ultimately depends on the form and degree of the soil's sorbing elements, the redox potential, as well as the overall pH. Accordingly, arsenic in the soil of a number of different countries have been acknowledged as being within a range of 0.1–40 mg/kg, comprising an overall mean of 6 mg/kg (Bowen, 1979; Naidu *et al.*, 2006).

Table 1-3: Names and structures of main naturally occurring inorganic and organic arsenic species

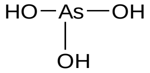
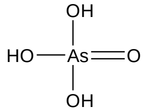
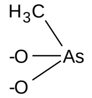
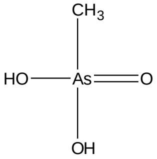
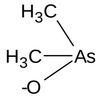
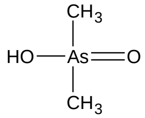
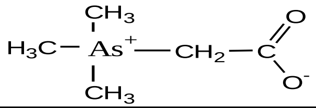
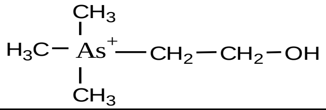
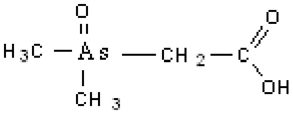
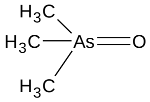
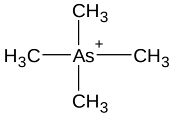
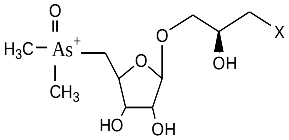
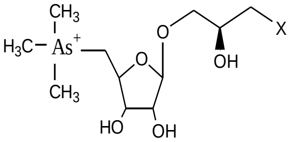
Compound name	**Formula**	**Structure**
Arsenious acid (As^{III})	H_3AsO_3	
Arsenic acid (As^{V})	H_3AsO_4	
Monomethylarsonite (MMA^{III})	$CH_3AsO_2^{2-}$	
Monomethylarsonate (MMA^{V})	$CH_3AsO(OH)_2$	
Dimethylarsinite (DMA^{III})	$(CH_3)_2AsO^-$	
Dimethylarsinate (DMA^{V})	$(CH_3)_2AsO(OH)$	
Arsenobetaine (AsBet)	$(CH_3)_3As^+CH_2COO^-$	
Arsenocholine (AsChol)	$(CH_3)_3As^+(CH_2)_2OH$	
Dimethylarsinoyl acetic acid (DMAA)	$(CH_3)_2AsOCH_2COOH$	

Table 1-3 continued.

Compound name	**Formula**	**Structure**
Trimethylarsine oxide (TMAO)	$(CH_3)_2AsO$	
Tetramethylarsonium Ion (TMAs)	$(CH_3)_4As^+$	
Arsenosugars: Dimethylarsinoylriboside derivatives		 X= SO_3H, OSO_3H, OH, $OPO_3HCH_2CH(OH)CH_2OH$
Arsenosugars: Trimethylarsonioriboside derivatives		 X = SO_3H, OSO_3H, OH, $OPO_3HCH_2CH(OH)CH_2OH$

1.2.1.2 Arsenic in water

In water, it is acknowledged that arsenic can experience a number of different complicated transformations, such as biotransformation, ligand exchange, oxidation-reduction reactions and precipitation. However, there is a lack of data concerning such reactions' rate constants, although the elements most predominantly impacting the processes in water are recognised as being the composition and distribution of the biota,iron concentrations, metal sulphide and sulphide ion concentrations, oxidation-reduction potential, pH, temperature and salinity (Wakao *et al.*, 1988; Naidu *et al.*, 2006).

In water, arsenic is known to occur in the form of, for example, arsenous acid (H_3AsO_3) and arsenic acid (H_3AsO_4), with suchtypes predominantly occurring as As^V in oxidising environments, namely surface water and as As^{III} under reducing conditions, including groundwater, which may comprise high concentrations of arsenic when water is in an anaerobic state. Nevertheless, the decrease of arsenate to arsenite is sluggish, and thus the former is commonly identified in reducing environments. Furthermore, arsenite oxidation in oxidising environments is relatively slow; thus, arsenite may be identified in oxidising environments. Notably, as highlighted by various scholars (Irgolic, 1994; Craig, 2003; Naidu *et al.*, 2006), methylated species (MMA and DMA) are not commonly found to be present in water supplies. Such organic arsenic complexes are usually established as a result of inorganic arsenic through microbial actions or otherwise presented through implementation as herbicides, as highlighted by the US National Research Council (1999). In terms of natural waters and their pH range, the main aqueous arsenate species include dihydrogen arsenate ($H_2AsO_4^-$) and hydrogen arsenate ($HAsO_4^{2-}$), with the main arsenite species known to be arsenous acid (H_3AsO_3). Arsenic's field of stability can be seen in Figure 1-1, as well as its species in regard to hydrogen ions (pH) activity and electrons (oxidation-reduction) (Eh) activity. Notably, arsenic solubility is somewhat reliant on sulphur speciation; thus, the greater the degree of total sulphur concentration, the more prevalent arsenic minerals' solubility. In this regard, it is true to state that arsenic is therefore more inclined to be more soluble in oxidising conditions. Thermodynamic information is summarised in an Eh (reduction potential) - pH diagram (Figure1-1) for a system including sulfur (adapted from Ferguson, and J. Gavis, 1972).

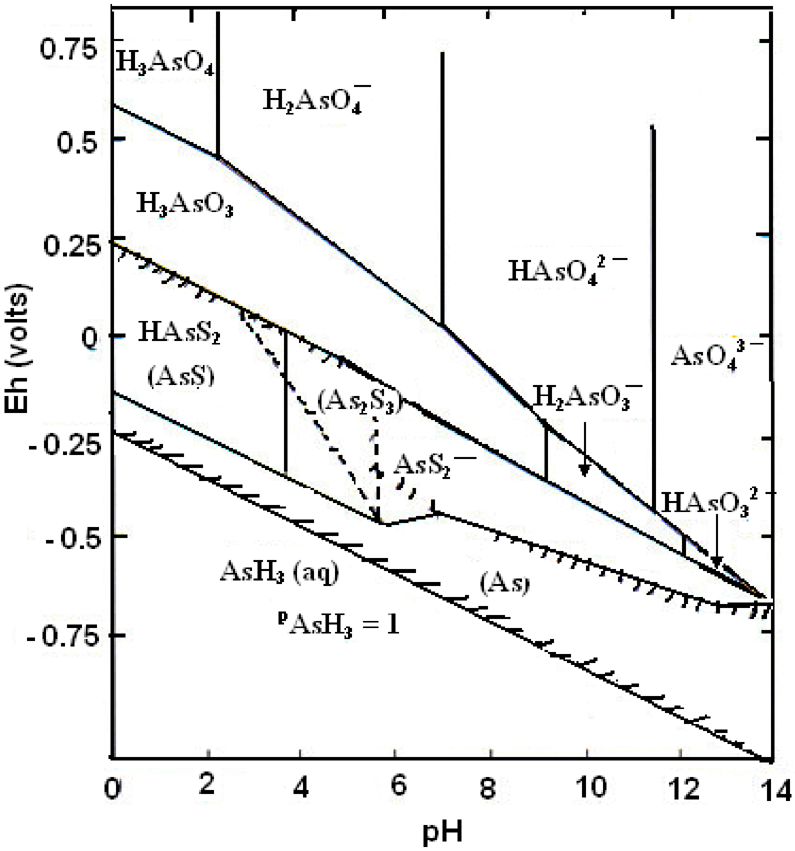


Figure 1.1: Eh-pH diagram for arsenic at 25°C, one atmosphere with total arsenic 10^{-5} mol/L and total sulphur 10^{-3} mol/L. Solid species are enclosed in parentheses in cross-hatched area, which indicates solubility less than $10^{-5.3}$ mol/L (adapted from Ferguson and Gavis 1972).

Arsenic content in unpolluted freshwater typically ranges from 1 to 10 µg/L. Levels may increase to 100 - 5000 µg/L in areas of sulfide mineralization and mining. The World Health Organization (WHO) maximum permissible limit in drinking water is 50 µg/L and the recommended value, is 10 µg/L (WHO 2001).

1.2.1.3 Arsenic in plants

In plant tissues, arsenic is seen to accumulate, with the amount of arsenic depending almost completely on the volume of arsenic to which the plant becomes exposed. In this way, plants' arsenic concentration is dependent upon the soil's levels of arsenic contamination, and thus varies from approximately 0.01 to approximately 1.5 µg/g (dry weight basis) in the case of those plants living in uncontaminated soils (Pendias and Pendias, 1984; Naidu *et al.*, 2006). With this taken into account, it is true to state that there is little opportunity for animals to be affected when consuming such vegetation simply owing to the fact that, before toxic concentrations can appear, plant injury is seen to occur (Feed Additive Compendium, 1975; Naidu *et al.*, 2006).

It is known that the level of arsenic in the soil can be established by considering the tissue of edible plants, with high levels commonly found when the plant is grown in soils contaminated by arsenic. Generally, however, individual plants differ in terms of their arsenic uptake capacity. For instance, those plants grown in soils with smaller particles, namely clays comprising high contents of iron/aluminium and clay-mineral, are generally more likely to contain lower levels of arsenic than when grown in soils with larger particles, such as sandy loams or sands when all soil types comprise the same concentrations of arsenic. Notably, the arsenic uptake of plants may circumvent the soil-root route through foliage absorption, with overall plant arsenic concentrations seen to be lower than in the soils in which they are grown (O'Neill, 1990; Naidu *et al.*, 2006). Although it is recognised that the quantities of arsenic taken up do vary in terms of the plant type, it is nevertheless recognised that, generally, terrestrial plant arsenic levels are much lower when compared with the soils in which they grow, whilst some aquatic macrophytes may accrue arsenic levels not dissimilar to those found in their sediments (O'Neill, 1990). In specific regard to arsenic levels in grass, high levels of arsenic have been established in England mine piles, with levels reaching up to approximately 3460 mg/kg of dry weight. In the context of some urban soil (20 mgAs/kg), levels have been found to amount to 3 mgAs/kg dry weight (O'Neill, 1990). Furthermore, in the case of dredged soil (8 mgAs/kg) for growing crops, a number of results have been found in regard to concentration factors (mgAs/kg of dry weight): radish (1.0) > grass (0.33) > lettuce (0.26) > carrot (0.17) > potato tuber (0.07) > spring wheat grain (0.04). Generally speaking, roots are seen to comprise greater levels of arsenic when compared with the plant's stems, leaves or produce (O'Neil, 1990). Table 1-4 lists various plant species accompanied by excessive arsenic concentrations due to excessive levels in their soils from various industrial processes (Pendias and Pendias, 1984; Naidu *et al.*, 2006).

Table 1-4: Levels of Arsenic in Plants Grown at Contaminated Sites (adapted from (Pendias and Pendias, 1984)

Country	**Site and pollution source**	**Plant species**	**Plant part**	**(mg/kg dry wt.)**
Canada	Mining or mineralized area	Douglas fir	Stems	140-8200
UK		Grass	Tops	460-6640
Canada	Metal-processing industry	Grass	Whole	0.5-62
Canada		Tree	Foliage	27-2740
Japan		Rice	Leaves	7-18
Norway		Hay	Whole	0.3-2.6
Canada	Battery manufacturer	Tree	Foliages	16-387
Japan	Sludged or irrigated field	Brown rice	Grain	1.2
Canada	Application of arsenical pesticides	Turnip	Roots	1.08
Canada		Potato	Tuber peels	1.10
Canada		Carrot	Roots	0.26

Furthermore, the level of arsenic uptake witnessed is also dependent on the soil's presence of arsenic species, with researches carried out on bean roots demonstrating a number of different uptakes, as highlighted here in descending order, first providing the species most assimilated arsenate> arsenite> monomethylarsonate> dimethylarsinate. During the conduction of tests and experiments, it was found that, in the context of two different rice cultivars, the phytoavailability of arsenic was in the order DMAA< As^{V}< MMAA< As^{III} (Marin *et al.*, 1993; Naidu *et al.*, 2006). On the other hand, in another research carried out by Carbonell-Barrachina *et al.*(1998), the order of DMAA< MMAA< As^{V}< As^{III} for two wetland plant species was identified. There is consensus amongst the researches that As^{V}, As^{III} and MMAA were generally found in the roots, although DMAA was also found in shoots. With this in mind, there is the theory that the uptake of arsenic is witnessed inertly with phosphate uptake (O'Neill, 1990). Table 1-5 lists arsenic concentrations in common food plants (for humans and animals) (Pendias and Pendias, 1984; Naidu *et al.*, 2006).

Table 1-5: Arsenic Concentrations in Common Food Plants (adapted from Pendias and Pendias, 1984)

Plant	**Tissue sample**	**Arsenic concentration (µg/kg dry wt.)**
Barley	Grains	3-18
Oats	Grains	10
Wheat	Grains	3-10
Brown rice	Grains	110-200
Sweet corn	Grains	30-400
Snap beans	Pods	7-100
Cabbage	Leaves	20-50
Spinach	Leaves	200-1500
Lettuce	Leaves	20-250
Carrot	Roots	40-80
Onion	Bulbs	50-200
Potato	Tubers	30-200
Tomato	Fruits	9-120
Apple	Fruits	50-200
Orange	Fruits	11-50
Edible mushroom	Whole	280
Clover	Tops	20-160
Grass	Tops	280-330

As a result of the availability of arsenic to the plant, pH levels can increase up to 5.5, consistent with soil acidity, wherein aluminium and iron oxides are known to become more soluble (Campbell *et al.*, 1985). In this vein, there is much support that augmented levels of arsenic uptake is seen in lettuce in the case of high-pH soils, with the concentrations of arsenic seen to be as much as 3–4 times greater in lettuce grown in soils with a pH of 8 than in soils with a pH of 6, for example (O'Neill, 1990). With this in mind, it can be stated that the phytotoxicity of arsenic is significantly dependent on the species of the plant, water availability and soil characteristics (O'Neill, 1990; Naidu *et al.*, 2006).

1.2.1.4 Arsenic in fish

On a global scale, it is known that fish is one of the most fundamental of foods consumed by human beings, with Bangladesh recognised as one of the countries consuming this food. With this in mind, it is noted that fish is able to accumulate significant levels of arsenic as a result of water contamination, with the subsequent consumption of fish having the potential to cause arsenic to run through the food chain. During the course of various studies, arsenic has been identified in various different levels, with higher concentrations found in seawater fish compared with freshwater fish (FSA, 2005; Shah *et al.*, 2009; Sirot *et al.*, 2009). For example, during the conduction of a survey within the UK, it was found that the average levels of arsenic in fish was 3.214 mg/kg; nevertheless, organic arsenic was recognised as the main arsenic species, such as arsenobetaine, with the average inorganic arsenic in the UK study 0.016 mg/kg (FSA, 2005). Such information provide a good comparison with the European information of arsenic in seafood and fish, which notably fluctuated up to 18 mg/kg to less than 0.1 mg/kg (European Commission, 2004). Markedly, in the case of both seafood and fish, the organic forms of arsenic—essentially arsenobetaine (AsBet), arsenocholine (AsChol), MMA and DMA—are predominant (Schoof *et al.*, 1999; Borak and Hosgood, 2007).

1.2.2 Cadmium in the environment

There is a common association between zinc ores, i.e. sulphate and carbonate, and cadmium, and is also recognised as a by-product of the other metals' refining processes. Nevertheless, through the production of various metals, namely copper, lead and zinc, cadmium is commonly found in the environment (WHO, 2010a), as well as in plants, soil and water. Notably, after 1945, great volumes of cadmium were released into the atmosphere either as an effluent or as dust, subsequently being deposited into fresh water. In addition, notable levels of cadmium entered soil as a direct result of agricultural and industrial activities (Streppler, 1991).

Cadmium is only found in very low levels (less than 1 µg/L) in uncontaminated waters, although soils, on the other hand, are believed to contain up to 5 mg/kg. Such levels increase as a result of human activities, resulting in significant contamination around the world, as highlighted by Thornton (1992). Moreover, the atmosphere receives the elements as vapour as a result of industrial operations (WHO, 2010a), which subsequently experiences an immediate reaction, thus producing cadmium oxide in the air, reacting with water vapour, carbon dioxide and other gases to produce various cadmium salts, including CdS and $CdCO_3$. Such salts are not able to dissolve in water, but, with the passing of time, change form and become water soluble, namely through converting into $Cd(NO_3)_2$ and $CdCl_2$. With this in mind, a number of different human industrial activities are recognised as adding to the environmental contamination of cadmium (WHO, 1992), with the presence of $CdSO_4$ in soil demonstrating that lettuce leaves have Cd levels amounting to800 mg/kg. Furthermore, cadmium contamination is also recognised as stemming from fertilisers (WHO, 1992).

1.2.3 Manganese in the environment

For animal, human and plant life, manganese is recognised as fundamental, important for development, growth, and the preservation of good health, and is known to enter the environment through a number of different methods, such as via air and water. Notably, in the aquatic environment, manganese occurs in the forms of Mn^{II} and Mn^{IV}, which notably change in relation to abiotic or microbial mediators, for example (WHO, 2010).

Soil is known to comprise high concentrations of manganese, which markedly travels to air, soil or water. In regard to soil, manganese solubility is established according to redox potential and pH (WHO, 2010c). Notably, during recent times, it has been recognised that manganese has been identified as another element potentially causing health problems amongst Bangladeshi populations through water consumption (Frisbie *et al.*, 2009). In this regard, Mn levels higher than the World Health Organisation standard (0.400 mg/L) are known to have been identified in the groundwater of Bangladesh(Hafeman *et al.*, 2007; Frisbie *et al.*,2009; Ljung *et al.*, 2009).

1.2.4 Lead in the environment

At the Earth's crust, lead is known to occur in low concentrations, predominantly as PbS. Nevertheless, the main incidence of lead within the environment is essentially the outcome of human activities, namely electronic waste, lead recycling, leaded glass manufacture, mining, refining, smelting, and use in water pipes and solder. In addition, another cause of environmental lead contamination is owing to volcanoes. Furthermore, a number of other natural activities, including geochemical weathering, may cause lead to infiltrate soil, water and sediment (WHO, 2010b). Accordingly, water can demonstrate high levels of lead contamination, particularly in those areas making use of plumbing systems utilising lead pipes.

1.2.5 Selenium in the environment

At various concentrations, selenium is found in soil as a result of human activities, such as mining, and volcano activity. Overall, low concentrations of selenium are found in soils, although such levels are taken up by plants. Furthermore, levels found in water are present as selenite and selenate, both of which are inorganic chemical forms. Importantly, the species present depends on the water pH.

Upon the movement of water through rocks and soils, the concentration of selenium is known to increase, and further accumulates in plants, which depends on environmental factors and the plant species. In this regard, plants may be a source of selenium for humans and animals through vegetables and other plant produce (Glover *et al*., 1979). With this taken into account, some organic selenium compounds include methylselenol, mehylselenite, trimethylselenonium ion, Se-Methonine, selenodiglutathione, and are supposed to be present in human fluids; however, thus far, only the trimethylselenonium ion has been found in human urine (Francesconi and Pannier, 2004).

1.2.6 Zinc in the environment

Zinc is an important component to humans in terms of enzymatic function (WHO, 2001b), and is found in great volumes at the Earth's crust, present in a number of different minerals and rocks at varying levels. ZnS is considered to be the most important ore mineral. The transportation of zinc into the atmosphere depends on the overall size of the particle. It has the capacity to either accumulate in soil or dissolve in water, although solubility depends on the pH, with acidic soils recognised as decreasing the potential of Zn to absorb in soil. Markedly, amongst plants, soils and water, zinc is present, with levels in soil, for example, ranging between 10 and 300 mg/kg. In the case of fresh water, however, levels are much lower at between 0.1 and 50 µg/L, with sea water levels believed to be between 0.002 and 0.1 µg/L.

1.3 Metabolism and toxicity of trace elements in humans

Trace elements can be divided into two groups, essential and toxic elements. Toxic elements have adverse effect on the human health, leading to death if absorbed in high amounts (Figure 1-2). However, essential elements can play very important roles in human health, but they also can become toxic when taken in excess (Figure 1-3).

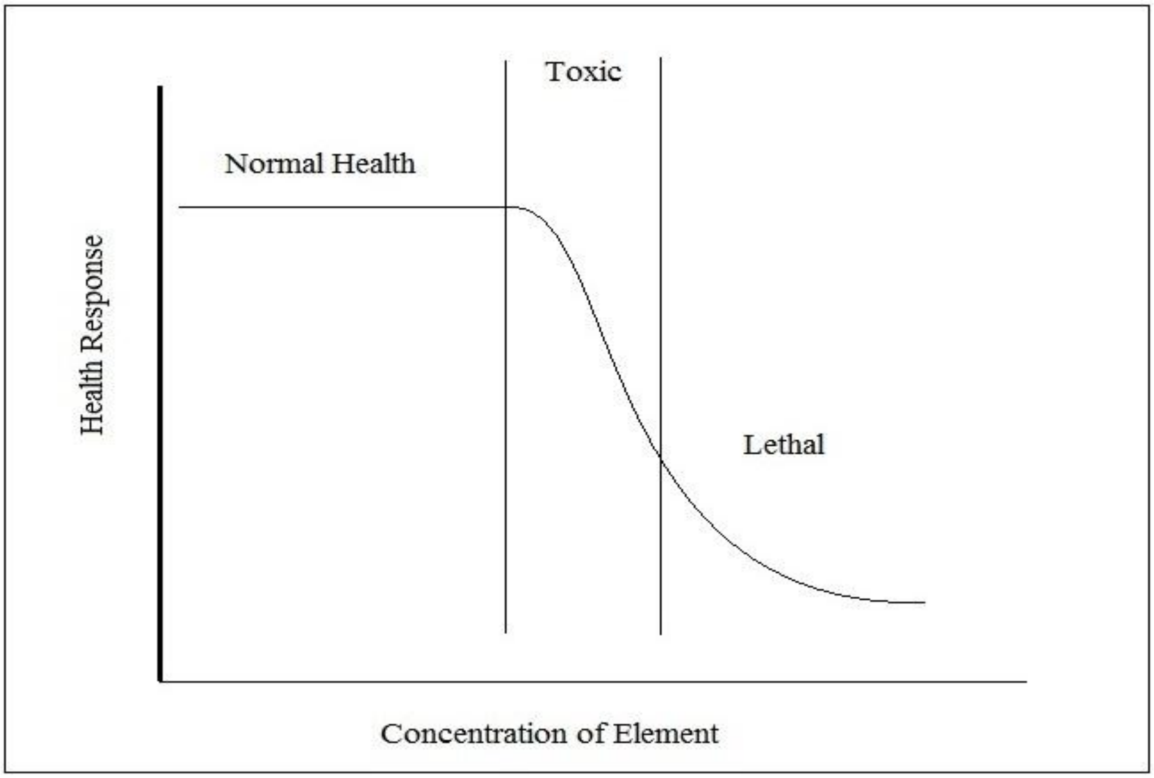


Figure 1.2: Health response for toxic element (adapted from Fergusson, 1990).

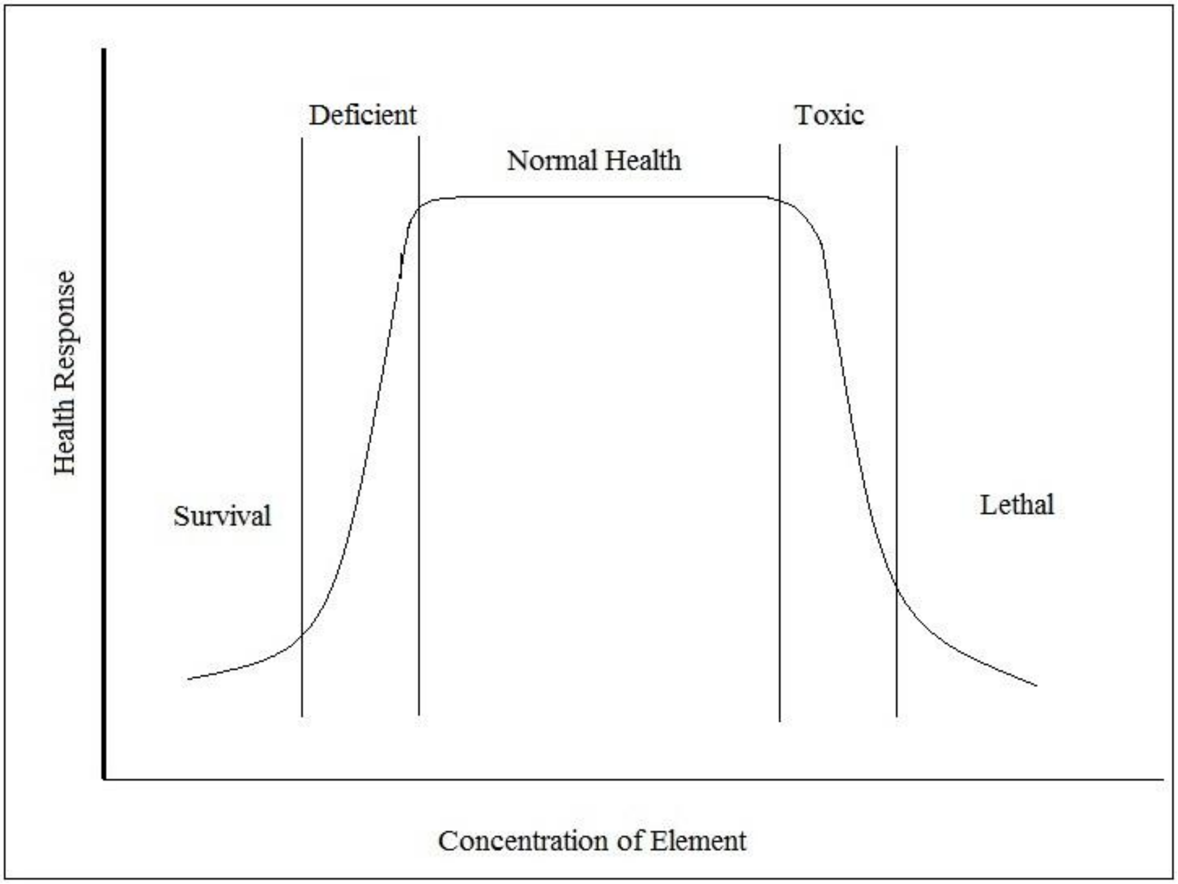


Figure 1.3: Health response for essential element (adapted from Fregusson, 1990).

1.3.1 Metabolism and toxicity of arsenic

1.3.1.1 Metabolism

In the context of *in vivo,* the metabolic routes for arsenic are dictated by two notable factors, including oxidation and reduction reactions between As^{V} and As^{III} in the plasma; and methylation reactions occurring successively in the liver. With this in mind, in humans, the inorganic arsenic is methylated [MMA and DMA] *in vivo*. There is the rapid reduction of arsenate, resulting in arsenite, which is subsequently partially methylated. Notably, methylation seems to be most apparent in the liver, wherein arsenic methyltransferase enzymes facilitatethe overall process of methylation with *S*-adenosylmethionine, with the glutathione (GSH) and methyl donor as an essential co-factor (Wang *et al.*, 2006; Buchet &Lauwerys, 1987). Researches carried out on human samples imply that methylation might be limited at a dose of approximately 0.2–1 mg each day (0.003–0.015 mgAs/kg.b.w.day) (Buchet and Lauwerys, 1987; Marcus and Rispin, 1988; Wang *et al.*, 2006). In regard to such researches, arsenic in regard to humans is considered in terms of cleaning and decontamination, with various different types of both organic and inorganic arsenic species (arsenicals) identified as being present in environmental media, foodstuffs and water. With this in mind, it is recognised that humans are commonly exposed to such forms of arsenic. With this in mind, each of the arsenic forms has different bioavailability and physico-chemical properties; thus, researches surrounding kinetics and metabolism of arsenicals in both humans and animals are not simple or straightforward topics. Markedly, inorganic arsenic compounds of As^{III} are, in the context of tissue components, known to be reactive, although methylated arsenic compounds are viewed as being less sensitive, and are therefore excreted at a rapid speed (Craig, 2003; Nagvi, 1994).

The intake of arsenic can occur through inhalation via numerous sources, including fumes and dust, or otherwise through ingestion, which may occur through food, soil, beverages and water. In this regard, only a limited number of studies have considered dermal absorption. Thus far, the information available suggests that dermal absorption rates, overall, are considered to be much lower, i.e. less than 10%; regardless, however, in specific regard to certain types of arsenic, it remains that there may be higher rates (WHO, 2001). In this vein, it is stated that inorganic arsenic forms [As^{III} and As^{V}], in the context of animal samples utilised in a laboratory environment, are known to have crossed the placenta, as highlighted by WHO (2001). Such a deduction has been further supported by the work of Concha *et al*. (1998), who states that similar levels of arsenic have been recognised in maternal blood and cord blood (approx. 9 μg/L) in regard to maternal-infant pairs open to drinking water with high levels of arsenic (approx. 200 μg/L).

In specific regard to animals, the organic arsenic form (DMA) is recognised as being the main metabolite; in the context of humans, on the other hand, urinary excretion occurs under normal conditions; in other words, without undue inorganic arsenic ingestion, of approximately 20% inorganic arsenic, 20% MMA and 60% DMA (Vahter *et al*., 1984). In this regard, it is known that inorganic arsenic is methylated to MMA and DMA *in vivo*. On the other hand, during circulation in plasma, MMA is partially absorbed, with such absorbed MMA further methylated to DMA. This is then excreted mainly in an unchanged form (Mandal and Suzuki, 2002).

Overall, a number of different arsenic exposure biomarkers have been the target of much research, such as the overall concentrations of arsenic found in blood, hair, nails and serum, and the total metabolites of arsenic—total arsenic or speciated metabolites—in urine; however, it is recognised that low levels of arsenic are excreted through other means, including via human milk, as highlighted by Concha *et al*., 1998). Importantly, it is recognised that, in urine, the concentration of arsenic may be apparent as a result of inhalation or ingestion exposure, and may therefore provide some degree of insight concerning the total dose absorbed (Yamauchi &Fowler, 1994; Naidu *et al.*, 2006; Kippler *et al.*, 2009).

In relation to a number of researches carried out in the recent past, the concentration of arsenic in total urine can be considered as a biomarker of the exposure to arsenic of late. This method, however, is not commonly utilised owing to the fact that various organoarsenicals, such as arsenobetaine, which is a virtually non-toxic compound, as found to be present in large volumes in various foodstuffs, are generally found in an unchanged state in urine (Kaise and Fukui, 1992; Le *et al.*, 1994; Lai *et al.*, 2004). Owing to the fact that human consumption of seafood, such as bivalves, crustaceans, marine fish and seaweed, for example, is believed to be correlated with an enhanced volume of arsenic in urinary excretions, as emphasised by Buchet *et al*. (1996), under such conditions, the examination of inorganic arsenic exposure may actually mean inorganic arsenic exposure is over estimated.

1.3.1.2 Toxicity

Overall arsenic toxicity ultimately depends on its state of oxidation and chemical form. Of all arsenic species, arsine (AsH_3) is known to be the most toxic, with inorganic arsenic forms [As^{III} and As^{V}] following subsequently, both of which are toxic more so than organoarsenicals. With this taken into account, a number of vital systems within the body are affected through the accumulation of the arsenic species, with chronic exposure to inorganic arsenic potentially causing various health-related problems in terms of cardiovascular system, gastrointestinal and respiratory tracts, hematopoietic system, liver, nervous system, and skin. Moreover, Vega *et al*. (2001) have noted the levels of arsenic species toxicity in human keratinocytes cultures and ranked them in the following order: As^{III}> monomethylarsine oxide MMAO(III) > complex dimethylarsinous acid with glutathione DMA(III)GS > DMA(V) > MMA(V) > As^{V}.

Importantly, the principal detoxification pathway is the methylation of arsenic compounds (Craig, 2003), although it has been implied through various information that methylated metabolites may be only partially accountablewhen considering negative outcomes in regard to arsenic exposure (Vega *et al.*, 2001). Furthermore, it has also been reported by Maeda (1994) that As^{V} is toxic to not only animals but also to humans, but can be more toxic in terms of vegetation. Moreover, in those geographic areas of West Bengal (India) known to be affected by arsenic, Chaterjee *et al.* (1995) have emphasised that the most toxic species, As^{III}, is known to exist in the groundwater at a level of approximately 50% total arsenic level. Such volumes cause a number of different skin ailments, such as blackfoot disease, hyperkeratosis, pigmentation changes, and skin cancers(see Figure 1-4), all of which have shown some form of correlation to medicines and drinking water containing arsenic and the consumption of such (WHO, 2001). Toxic effects of arsenic on human beings are summarised in Table1-6.

Figure 1.4: Long-term effect of arsenic in drinking water on feet and hands (Harvard, 2011).

Biological availability, toxicity and transport mechanisms are all considered to be significantly dependent on arsenic's physico-chemical form. This is potentially rationalised as being owing to the fact that As^{III} is reserved in the human body for a longer period of time through affixing to the sulphydryl groups (-SH). Such types are common compounds of enzymes in the human body, all of which are known to impact the overall rate at which metabolic reactions are witnessed. Essentially, alongside enhanced methylation, toxicity declines. Monomethylarsonic acid MMA^{V} is considered to be far more toxic than dimethylarsinic acid DMA^{V}, although it remains that both arsenocholine (AsChol) and arsenobetaine (AsBet) are recognised as being non-toxic species (Craig, 2003).

Table 1-6: Toxic effect of arsenic on different parts of the human body (adapted from Fergusson, 1990).

Affected area	**Toxic effect**
Skin	Hyperpigmentation, hyperkeratosis, black foot disease, gangrene, skin cancer
Lung	Lung cancer (needs confirmation)
Liver	Cirrhosis, heamongioendothioma
Kidneys	Renal readsorption problems
Blood system	Inhibits biosynthesis of porphyrin, affects white blood cells
Reproductive system	Spontaneous abortions
Peripheral nervous system	Peripheral neuropathy, paralysis, loss of hearing
Gastrointestinal tract	Damage to intestine, intense pain

1.3.2 Metabolism and toxicity of cadmium

In the human body, cadmium is known to accrue, particular in the lungs and kidneys, as recognised by Bernard (2008). This has the potential to result in kidney renal tubular damage. Importantly, humans may become exposed to cadmium from a number of sources, such as food and soil, with the occupational exposure of workers potentially arising within the mining industry. With this in mind, it is possible that cadmium may result in some degree of kidney dysfunction, consequently resulting in the re-absorption of various elements, namely amino acids, glucose and proteins (Jarup *et al.*, 1998). During recent times, researches carried out on both animal and human samples have shown that skeletal damage (osteoporosis) may be a dangerous and very serious cadmium exposure-related outcome. In this same vein, the lungs may also be affected as a direct result of exposure to cadmium (WHO, 1992). A number of other cadmium exposure-centred outcomes may be recognised, including instabilities in terms of calcium metabolism, hypercalciuria, and the occurrence of kidney stones. With this in mind, it is noted by the International Agency for Research on Cancer (IARC) that cadmium is categorised in Class 1, ‘The agent (mixture) is carcinogenic to human’s (IARC, 1993). Furthermore, in direct regard to industrial exposure, there is a relation between lung cancer and prostate cancer.

As can be seen from reviewing related texts, the epidemiological data linking cadmium and lung cancer are more apparent than for prostate cancer, whilst correlations between cadmium and liver, kidney and stomach cancer still remains indistinct (Waalkes, 2000).

1.3.3 Metabolism and toxicity of manganese

It is known that a number of fundamental metabolic functions in humans are facilitated through manganese, including energy metabolism, enzyme activation, immunological system and nervous system, and is also known to be essential in blood clotting, the control of cellular energy, and tissue growth, as noted by Erikson & Aschner (2003). Notably, there are three main metabolic functions associated with manganese: primarily, it stimulates the gluconeogenic enzymes pyruvate carboxylase and isocitrate dehydrogenase; secondly, it provides the mitochondrial membranes with protection; and finally, glycosyl transferase, which is involved in saccharide synthesis, is activated through manganese (Zlotkin *et al*., 1995). Accordingly, the consumption of manganese is important, with diet playing the main role in this. With this in mind, the deficiency of manganese has undergone much research in the context of animals, with the finding established that diet is able to impact the production of both hyaluronic acid and heparin (Zlotkin *et al.*, 1995). In addition, it has also been stated that manganese is known to be a neurotoxic element: for instance, in France, a number of employees exposed to manganese in great concentrations, such as through smelting and mining, have experienced various ailments believed to be a result, including limb tremor, muscle weakness and salivation (Santamaria, 2008). In this regard, it is recognised that manganese toxicity has the potential to cause brain damage through such issues, with disease referred to as Manganism and recognised as being not dissimilar to Parkinson’s disease (Santamaria, 2008).

1.3.4 Metabolism and toxicity of lead

It is also known that lead is able to induce various biological impacts depending on the exposure, both in terms of duration and level. As a result of such exposure, morphological changes and death are both possible owing to the inhibition of enzyme production. With this stated, it is recognised that children are more susceptible to the impacts of lead owing to behavioural, neurological and metabolic reasons. More importantly, lead is able to affect the central nervous system, which is an affect applicable to both adults and children. In this vein, epidemiological researches imply that minimal exposure of a foetus and developing child may notable result in damage to the learning capacity and the neuropsychological development, for example(Goyer, 1986). Furthermore, following long-term exposure to lead, a number of neurobehavioural changes have also been identified, with kidney function also impacted as a result.

1.3.5 Metabolism and toxicity of selenium

Although there is not a wealth of understanding concerning the metabolism of selenium in the human body, it is nevertheless considered to be a fundamental element, which occurs in body tissues affixed to proteins, i.e. selenoproteins, as highlighted by Sunde (2000). Importantly, in the body, 50% of all selenium can be found in skeletal muscles, although erythrocytes, the immune system and platelets are also known to contain high volumes. The chemical is excreted from the body through the urine (Burk and Levander, 2002). *S*-methylselenocysteine is the major selenocompound in Se-enriched plants, namelybroccoli flowers, garlic, onions, and sprouts; however, Se-Cys mainly derives from meat sources, as noted by Whanger (2002). Markedly, a number of selenocompounds are comprised within selenium, all of which arecommonly found in the urine, i.e. selenite, selenate, methylselenol, mehylselenite, trimethylselenonium ion, Se-Met, selenodiglutathione. Nevertheless,it remains that only the trimethylselenonium ion has been found in human urine, as supported by the work of Francesconi & Pannier (2004). In addition, it has been recognised that selenium plays a role in the decrease of arsenic toxicity owing to the two elements behaving as metabolic antipodes (Hsueh *et al.*, 2003).

The deficiency of selenium was initially established in China, with the disease believed to be instigated through low Se levels in soil in Keshan (China). Accordingly, there was a low intake of Se, sometimes lower than10 μgSe/day. In contrast, high levels can be toxic to people, although this ultimately depends on the species of selenium. In this regard, it is true to state that chronic Se toxicity in the context of humans results in selenosis, which is an illnesscharacterised throughabnormal functioning of the nervous system, fingernail changes and brittleness, garlic breath, gastrointestinal disturbances, hair loss and skin rash. Moreover, toxicity is also linked with thyroid hormones syntheses (Goldhaber, 2003).

1.3.6 Metabolism and toxicity of zinc

In regard to zinc, it is recognised that the importance of this mineral has undergone much study and has been well documented. It is known that, in humans, there are between 2 g and 3 g of zinc found, with in excess of 200 enzymes requiring zinc in order for their activities to continue. Furthermore, a healthy immune system requires zinc, which can help to ensure skin problems are overcome. Owing to its importance, it is believed that human adults should take 15mg of zinc each day; deficiency can result in hair loss, night blindness, loss of smell, being open to infections, skin problems and an under-performing immune system. In contrast, however, there are various problems associated with a high intake of zinc, including diarrhoea, dizziness, drowsiness, hallucinations, nausea, and weakness of the immune system (Sigel, 1983; Harper *et al.*, 1990; Prasad, 1988).

1.4 Trace elements in foodstuffs

Arsenic in groundwater is of common occurrence in various parts of the world, e.g. Argentina, Bangladesh, Canada, Chile, China, Finland, Hungary, Japan, Mexico, Southern Thailand, Taiwan, United Kingdom, USA, Vietnam and India (Mandal and Suzuki, 2002) (Figure 1-5). Widespread elevated concentrations of arsenic in groundwater usually result from natural processes, although human activities can also be responsible for high concentration.

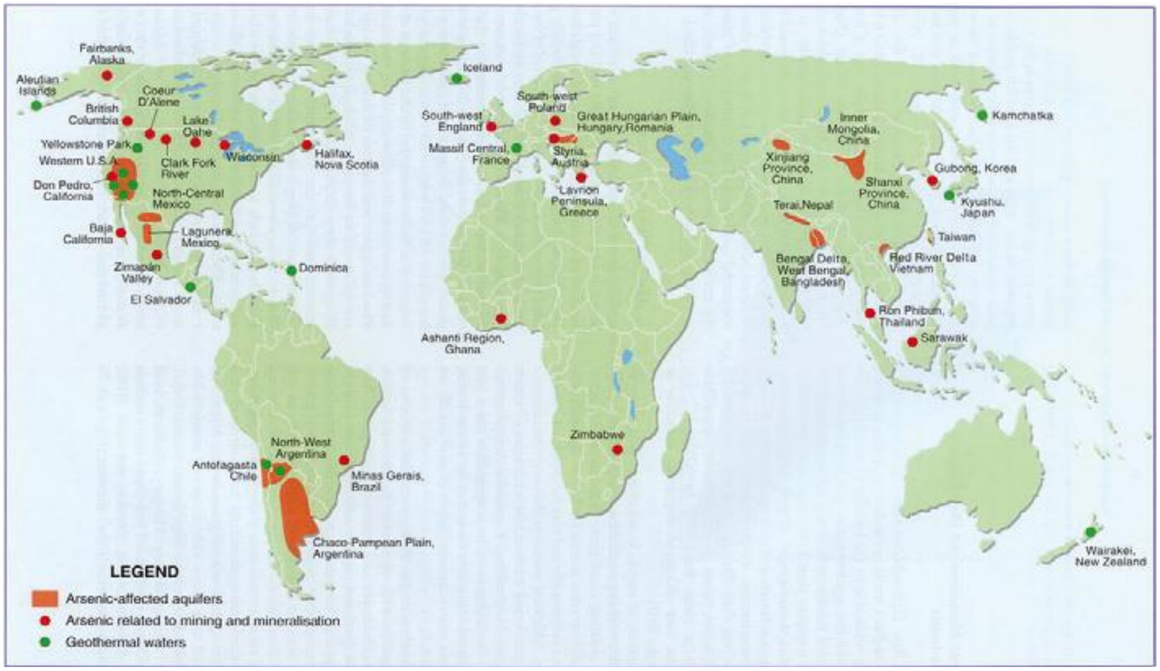


Figure 1.5: World map presents regions with documented arsenic problems in groundwater (As > 50 µg L^{-1}). Source of diagram: British Geological Survey (BGS, 2001).

1.4.1 Arsenic in foods

Notably, the majority of foodstuffs do contain some form of chemical, whether natural or synthetic, all of which could pose a number of different toxicity-related issues to consumers. In regard to food toxicology, there are natural contaminants and those intentionally introduced, the latter of which may include food additives, pesticides and veterinary drugs. Importantly, metal concentrations can be increased through the release of hazardous pollutants into the environment, subsequently meaning food supply is contaminated. In this regard, metal contamination may be seen to occur during both the production and handling of food; therefore, in addition to vegetation growth, there may be various factors resulting in the contamination of food.

In regard to arsenic, a number of foods do contain the chemical although in less toxic states. Food is widely acknowledged as being one of the most common sources of arsenic (WHO, 1982), with seafood and fish recognised as containing the greatest concentrations owing to water contamination, although arsenic, in this regard, is regarded as being less harmful owing to it being an organic form (Francesconi and Edmonds, 1994). Nevertheless, following digestion, methyated arsenic compounds are formed from arsenosugars, and are regarding as being far more toxic forms. On the other hand, humans are more likely to be negatively affected by inorganic arsenic forms. More specifically, following ingestion, approximately 80% of arsenobetaine is excreted from the human body in an unchanged form (Kaise *et al.*, 1985; Lai *et al.*, 2004).

Notably, in food, both inorganic and organic states of arsenic are found to be present, with reports emphasising that seafood and fish may amasslarge volumes of arsenic as a direct result of their environment (Ebisuda *et al.*, 2002). For instance, it is considered that Bangladeshis consume a number of different types of fish in their diet, although few researches have thus far highlighted levels ofarsenic and their speciation in Bangladeshi fish (for more details, see Chapter 4).

A number of researches—such as Tusdu *et al.*(1995), Debeka *et al.* (1993), Gundern (1995), MAFF (2000), Roychowdhury *et al.* (2003), Alam *et al.* (2003), Das *et al.* (2004) and Al-Rmalli *et al.* (2005), to name a few—have established arsenic as being present in foodstuffs, with contamination-related information based on analytical determinations of substances levels in certain foods. In this regard, the data concerning food consumption have been garnered through the conduction of dietary surveys. In this regard, three different methods may be utilised with the aim of examining and evaluating dietary exposure to a substance: firstly, Total Diet Study (TDS), sometimes referred to as the Market Basket Approach; secondly, the Duplicate-Meal Study (DMS), commonly referred to as Duplicate Portion Method; and finally, individual food items' selective analysis.

Moreover, a number of other researches postulate that valuable evaluations carried out between intake predictions, subsequently used for different research designs, are not endorsed owing to the fact that, in a number of different instances, researches are not seen to have been dependably comparable, such as in terms of methodology and sample age groups, for example. Nevertheless, predicted dietary intake established through two different approaches, namely TDS and DMS, have been previously contrasted by Tsuda *et al.* (1995), who have found no notable disparities between the two different approaches.

1.4.2 Cadmium and Lead in foods

It is known that there is a presence of cadmium within the environment, although, in the context of Bangladesh, the concentrations were found to be relatively low in drinking water (Frisbie *et al.*, 2002). Nevertheless, in those areas viewed as being heavily contaminated, river and well waters may be markedly contaminated by cadmium. Importantly, in this regard, environmental cadmium exposure is, on a global scale, mainly attributable to food, with airborne cadmium atmospheric deposition resulting from the application of fertilisers containing the chemical, mining activities, and sewage sludge on farming land resulting in soils contamination and enhanced levels of cadmium uptake by vegetables and crops grown for human consumption. Markedly, it is recognised that all foods contain cadmium in various degrees, including cereals, fish and vegetables (Kachenko and Singh 2006; Karavoltsos *et al.*, 2008). In consideration of the intake levels of cadmium, this various between countries, with the UK demonstrating 13 μg per day whilst Bangladesh, on the other hand, is approximately 9.5μg (Alam *et al.*, 2003; Ysart *et al.*, 1999).

Furthermore, it is recognised that lead is a toxic element, and can be identified in a number of different foodstuffs, including vegetables. Notably, the main source of Pb contamination is food, with leafy crops known to amass high volumes of Pb when compared with non-leafy ones, i.e. rice and other cereals. Importantly, it is recognised that exposure to high volumes of lead is responsible for a number of different health-related issues, including lung cancer (De Palma *et al.*, 2008), immnuotoxicity (Mishre, 2009) and neurotoxicity (Verstraeten *et al.*, 2008). With this in mind, as a result of enhanced industrialisation, which goes hand-in-hand with the utilisation of Pb-based chemicals and fuels, Bangladesh is experiencing greater exposure, which is quickly being recognised as a notable problem for the country, with school-aged children found to have high levels of Pb in their blood (Kaiser *et al.*, 2001). However, no study for Bangladeshi food has been done (for more details see Chapters 4 and 7).

1.4.3 Manganese in foods

Manganese has been identified in all different food groups, including cereals, tea leaves and vegetables, with a number of researches having highlighted a keen link between children's intellectual performance and Mn exposure through drinking water (Brown &Foos, 2009), as well as levels of infant mortality (Hafeman *et al.*, 2007). During more recent times, Ljung *et al*. (2009) considered the levels of Mn in drinking water and the link with a number of different biomarkers during the earlier stages of pregnancy, targeting Bangladeshi women as the sample. Although there has been much investigation into the relationship between Mn intake from water and urine and blood concentrations, thus far, on strong link has been established, which may be rationalised through the failure to consider Mn intake from foods, despite the wide acknowledged that the main sources of Mn are cereals, tea leaves and vegetables.

1.4.4 Selenium and Zinc in foods

It is known that selenium levels range from low to high levels in different foods, such as Brazil nuts, cucumber, grain, mushroom, shellfish and wheat (Ogra *et al.*, 2004). Furthermore, plants—mainly leafy vegetables—are known to comprise high levels of zinc, with levels reaching concentrations of 301 mg/kg in lettuce grown in contaminated soil, compared with 77 mg/kg in lettuce from non-contaminated soil, with levels of zinc found in other vegetables ranging between 0.4 to 35 mg/kg in non-contaminated soil compared to 4 to 400 mg/kg for contaminated soil, as reported by (WHO, 1992b).

1.4.5 Trace elements in non-foods

The deliberate eating of non-food or non-nutritive substances is known as pica (Woywodt and Kiss, 2002). Many different types of pica have been described in the literature such as ingestion of baby powder, charcoal, calcium hydroxide (lime), ash, uncooked starch and ice (Young *et al.*, 2008). Betel quid and tobacco chewing are the most common type of non-food consumption. Geophagy is also one of the most common types of pica and involves deliberate eating of earth such as soil and clay. It is an ancient practice that is still widespread in many parts of the world such as in Asia, Africa and South America, North America and parts of Europe (Ghorbani, 2008). Many diseases have been associated with the chewing betel quid including oral cancer (particularly cancer of the bucal and labial mucosa) (Nandakumar *et al.*, 1990; Sankaranarayanan *et al.*, 1998) and oral submucous fibrosis (Auluck *et al.*, 2008). Chewing *Piper* betel leaves and betel nut, either with or without tobacco, can cause cytogenetic changes in the oral epithelium; it is a strong factor for development of oral submucous fibrosis and mouth cancer (Vanwyk *et al.*, 1993).

Very little work has been done on the intake of arsenic and other toxic elements (Cd, Pb), from non-food sources such as betel quid chewing (for more details, see Chapter 5) and baked clay (for more details, see Chapter 6), in populations that are exposed to arsenic through drinking water. Betel quid and their components are widely consumed in arsenic contaminated regions of Bangladesh and India. Exposure to As, Cd, Pb and other elements from betel quids have not been previously considered despite the fact that millions of people in these regions consume it on a daily basis. Only a few studies have investigated arsenic intake from various types of foods consumed in Bangladesh (Alam *et al.*, 2003; Das *et al.*, 2004; Al-Rmalli *et al.*, 2005), however, these studies did not include contribution from non-foods such as betel quids and baked clay. *Piper betel* leaves and Areca nut are the main components in betel quid, arsenic in *Piper betel* leaves was measured by Al-Rmalli *et al.* (2005) and arsenic in areca nut was recently measured by Signes *et al.* (2008a). In contrast, no previous studies have reported arsenic content and its species in betel quid. Hence, it is very important to monitor the impact of this chewing material on different populations.

1.5 Risk assessment of Arsenic

Particularly, across the globe, instances of arsenic poisoning have been witnessed, with sources including both natural and industrial. There has been much documentation surrounding the contamination of arsenic in the well waters of the south-west coast of Taiwan (1961–1985) (Thornton, 1997), with the arsenic content of the well water examined and subsequently found to be in the range of 0.01–1.82 mg/L. Moreover, in 37 villages comprising more than 40,000 individuals, chronic arseniosis was identified, in addition to in excess of 7,000 cases of blackfoot disease, hyperpigmentation, as well as cancer in the bladder, kidney, liver, lung, skin and prostate (Tsai *et al.*, 1998).

It has been established by Smith *et al.*(2000) that, in northern Chile, there have been increased occurrences of arsenic-induced bladder and lung cancer and skin lesions, as well as mortality associated with the former. Markedly, in a number of different Chinese regions, including Xinjiang Uygur A.R., Inner Mongolia and Shanxi, endemic arsenicosis was found during the 1980s. Moreover, in the case of these areas’ groundwater, arsenic concentration was found to be in the range of 220–2000 µg/L, with levelsincreasing up to 4400 µg/L in various areas, as noted by Lianfang &Jianghong (1994). As a result, a significant portion of the population were exposed to chronic arsenic poisoning as a direct result of consuming well water comprising arsenic in high volumes. Furthermore, in China, more than 2 million people were exposed to high arsenic volumes, with more than 20,000 Chinese patients developing arsenicosis in 2000 alone (Sun *et al.*, 2001).

In the context of America, of 992 drinking water samples gathered from households in New Hampshire, with the highest concentrations subsequently recognised in New Hampshire bedrock wells, amounting to approximately 50 µg/L. Notably, the concentrations of arsenic were in the range of 0.003–180 µg/L, with water from domestic wells found to have notably greater volumes of arsenic when compared with water from municipal sources (Peters *et al.*, 1999). Furthermore, in Japan in 1994, arsenic was found to be over the allowableconcentrations for drinking water use (0.01 mg/L) in well waters, with high concentrations found to be amounting to 0.293 mg/L, which is a markedly high level when contrasted alongside other arsenic-containing well waters in the context of other Japanese regions (Kondo *et al.*, 1999).

Bangladesh, is known to have high levels of arsenic contamination in its groundwater, with contamination levels of groundwater reaching more than 50 µg/L amongst 41 of 64 districts (Samanta *et al.*, 1999). Groundwater with elevated arsenic concentrations (>50 μg/L) has also been recognised in regard to sedimentary aquifers in a number of different geographic locations across the globe (Mandal and Suzuki, 2002). With this in mind, during recent years, extensive arsenic occurrence in groundwater from Bengal Delta Plain in Bangladesh and the adjoining state of West Bengal, India, has been recognised as a fundamental area of concern. It is known that should arsenic-rich groundwater be used for irrigation, there is the likelihood of arsenic bioaccumulation in crops, which then means such contamination is witnessed across the human food chain. With this taken into consideration, the levels of arsenic recognised in rice amongst those areas contaminated are believed to be 2–3 times greater than in areas not affected by contamination, as noted by Hironaka &Ahmed (2003). Moreover, in specific consideration to Bangladesh, the main source of the population's water—for drinking and cooking purposes—is from tubewell water extracted from shallow aquifers (Ali *et al.*, 2003).

The arsenic concentration of rice produced in arsenic-contaminated area is 2 to 3-fold higher than that produced in unaffected districts (Hironaka and Ahmed, 2003). In Bangladesh tubewell water extracted from shallow aquifers is the primary source of drinking/cooking water for most of its population, particularly the rural population (Ali *et al.*, 2003) (for more details, see Chapter 3).

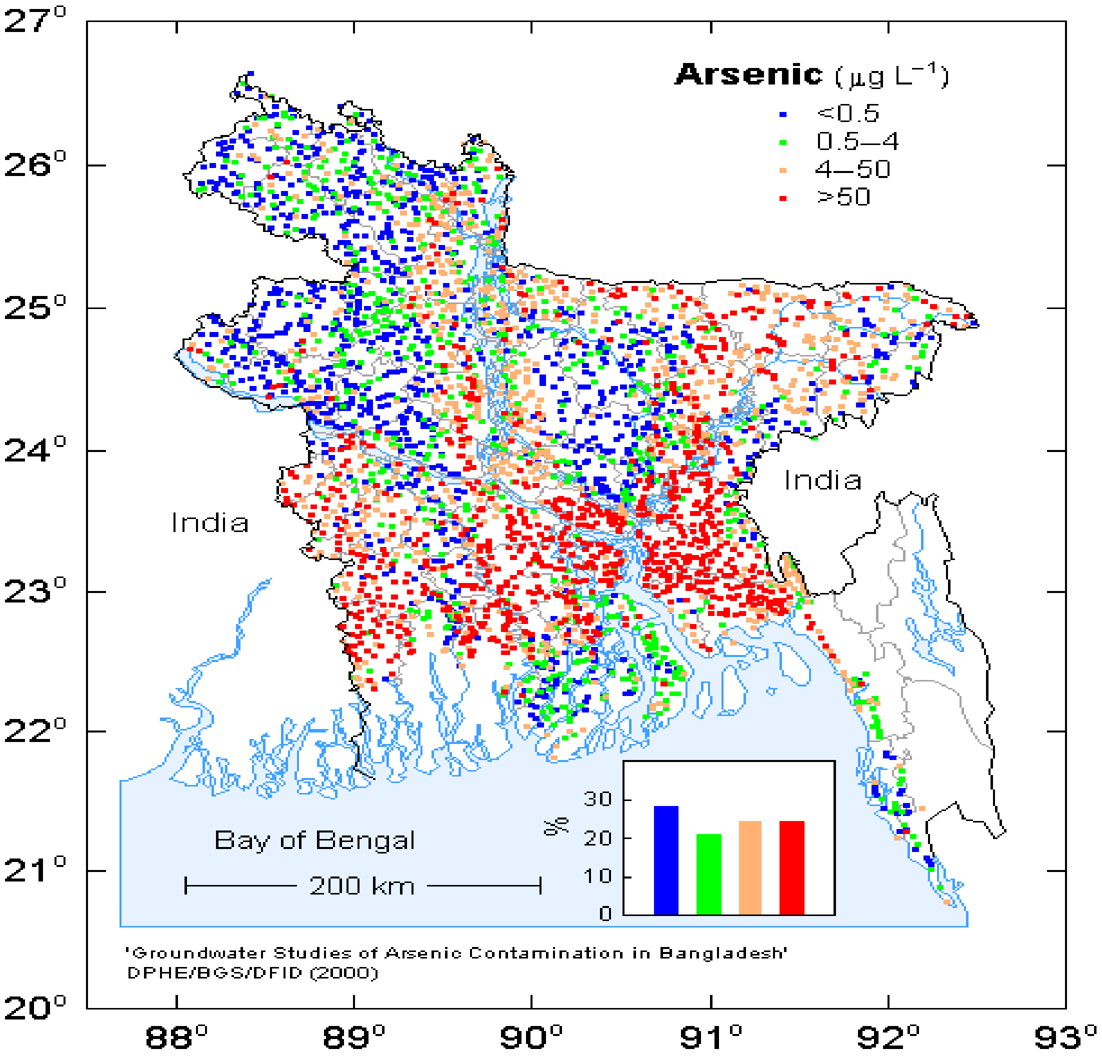


Figure 1.6: Map of Bangladesh presenting regions with documented arsenic problems in groundwater. Source of diagram: British Geological Survey (BGS, 2001)

From the literature survey, many studies have investigated trace element levels in foods around the world; some of them have detected arsenic in Bangladeshi foods and groundwater. Majority of these studies have focused on arsenic levels in groundwater and others detected arsenic in some foods, especially rice, from contaminated areas of Bangladesh. However, few studies focused on arsenic levels in rice and vegetables from non-contaminated areas of Bangladesh. Furthermore, there are no systematic studies in the literature that compared arsenic levels in aromatic and non-aromatic rice. Also very few studies have measured other trace elements (both toxic and essential elements) in Bangladeshi food including rice, vegetables.

Although fish is the major animal product in the Bangladeshi diet, no previous studies have determined arsenic and its species in Bangladeshi fish. There are virtually no studies in the literature that have considered the arsenic intake from ingestion of non-food items by Bangladeshis such for example the determination of arsenic and its species in betel quids and baked clay (non-foods). Consumption of these non-foods are very common (especially betel quid chewing) amongst Bangladeshis and they can affect the estimation of the total daily intake of arsenic for Bangladeshis. The total daily intakes of other trace elements such as lead, manganese, selenium and zinc for Bangladeshis have not been previously investigated although this important for evaluating the health of humans, especially those that are exposed to high levels of arsenic.

1.6 Aims of the study

This thesis investigates the potential of human exposure to toxic elements (such as arsenic) and evaluates the deficiency of essential elements (such as selenium and zinc) through the consumption of Bangladeshi foods and non-foods.

Objectives of this thesis are as follows:

- To measure levels of arsenic, cadmium, lead, manganese, selenium and zinc in Bangladeshi foods and non-foods including rice, vegetables, fish, betel quids and baked clay.
- To identify arsenic species, in a selection of Bangladeshi foods and non-foods including rice, fish, betel quids and baked clay.
- To determine and estimate the daily intakes of arsenic, cadmium, lead, manganese, selenium and zinc from individual foods and non-foods and also to estimate the total daily intakes of these elements for Bangladesh.
- To study arsenic, cadmium, lead, manganese, selenium and zinc in urine of Bangladeshi volunteers and compare these levels between betel quid chewer and non-chewers.
- To estimate risk assessments of the Bangladeshi diet by estimating the Provisional Maximum Tolerable Daily Intakes (PMDTIs) and Target Hazard Quotients (THQs) of arsenic, cadmium, lead, manganese, selenium and zinc for all Bangladeshi foods and non-foods.

2 ANALYTICAL METHODS

2.1 Instrumental techniques

2.1.1 Introduction

A number of different analytical approaches have been applied in order to establish the overall levels of trace elements contamination in non-foods and foods, includingapproaches such as inductively coupled plasma mass spectrometry (ICP-MS), which are regarded as being appropriate for all elements, inductively coupled plasma atomic emission spectrometry (ICP-AES), hydride generation atomic absorption spectrometry to measure arsenic, selenium and their speciation (HG-AAS) (Vinas *et al.*, 2003), hydride generation inductively coupled plasma mass spectrometry (HG-ICP-MS), (Munoz *et al.*, 1999; Roychowdhury *et al.*, 2002; Judith *et al.*, 2002; Gallagher *et al.*, 2001) and graphite furnace atomic absorption spectrometry GF-AAS (Schoof *et al.*, 1999). In the context of this research, there was the utilisation of ICP-MS in order to establish the levels of arsenic and other trace elements in Bangladeshi non-foods and foods, as well as high-performance liquid chromatography (HPLC) used in combination with ICP-MS (HPLC-ICP-MS) with the objective to distinguish between and accordingly calculate arsenic species in the context of such samples. Furthermore, HG-AAS with Zeeman background correction was adopted with the aim of establishing the concentrations of arsenic in food samples.

Markedly, ICP-MS is a commonly implemented method for identifying trace elements in environmental samples, such as biological, soil and water samples. The method ensures highprecision compared with many other techniques (Gallagher *et al.*, 2001).

2.1.2 Inductively coupled plasma mass spectrometry (ICP-MS)

Furthermore, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is recognised as an analytical approach applied for elemental determinations in different types of samples. Importantly, ICP-MS is recognised for its high precision and sensitivity, as well as its low detection limits, and are therefore considered to be at least parallel but otherwise better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS). It is recognised that ICP-MS is commonly utilised in both geochemical and chemical laboratories. Moreover, as well as having a low detection threshold, the tool also offers a number of other benefits, including the capacity to deal with both complicated and simple matrices, regardless of possible matrix interferences, owing to ICP-source hightemperatures. Moreover, it also facilitates the gathering of isotopic data. More specifically, ICP-MS blends togethera high-temperature ICP (Inductively Coupled Plasma) source alongside a mass spectrometer (MS), and is thus a toolable to be used alongside high-performance liquid chromatography (HPLC), which is known to be extremely valuable in terms of dividing and establishing element speciation, i.e. selenium and arsenic (Inoue *et al.*, 1994).

Markedly, the plasma is produced into a low-pressure interface comprising both a skimmer cone and sampler, with argon gas flowing within the ICP torch's concentric channels. There is a radio-frequency (RF) generator, which is linked with the RF load coil and, upon the supply of energy to the load coil from the generator, at the end of the plasma torch, magnetic fields and oscillating electric are established (see Figure 2-1). Accordingly, in order to ensure element interference is avoided, such as As^{75}, which may interfere with ^{40}Ar and ^{35}Cl, nitrogen gas is used. Undoubtedly, it is recognised that HPLC is a widespread analysis approach widely implemented owing to its ease of use and also owing to the fact that it is not limited in terms of the sample’s thermal stability or volatility. Moreover, HPLC is commonly utilised in order to establish the identification, purification, quantification and separation of various compounds.

Figure 2.1: Plasma torch of the ICP-MS instrument (Thermo-Fisher).

Figure 2.2 shows a photograph of the ICP-MS [A Thermo-Fisher Scientific X-SeriesII instrument equipped with CCTED (collision cell technology with energy discrimination), USA] instrument at Nottingham University. This setup was used for the determination of total arsenic in digested solution of foods (including rice, vegetables, fish etc), non foods (such as betel quid and baked clay) and human urine samples (more details are provided in section 2.2.4).

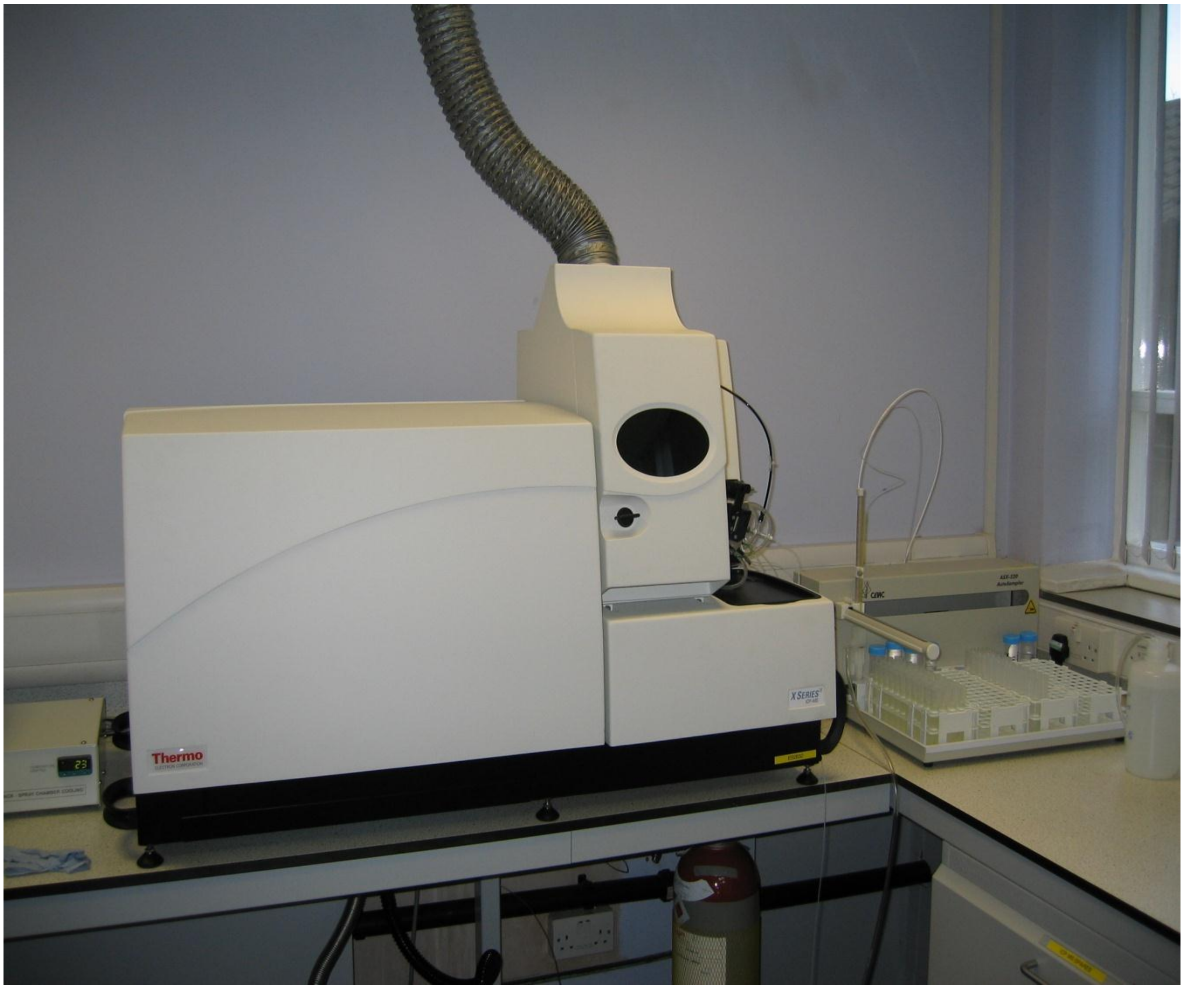


Figure 2.2: Inductively coupled plasma mass spectrometer (A Thermo-Fisher Scientific X-SeriesII instrument).

Figure 2.3 shows a photograph of the Agilent 7500 ICP-MS (Agilent Technologies, UK) instrument at British geological survey (Keyworth, UK). This setup was used for arsenic speciation analysis in rice, fish and baked clay samples (more details are provided in section 2.2.7).

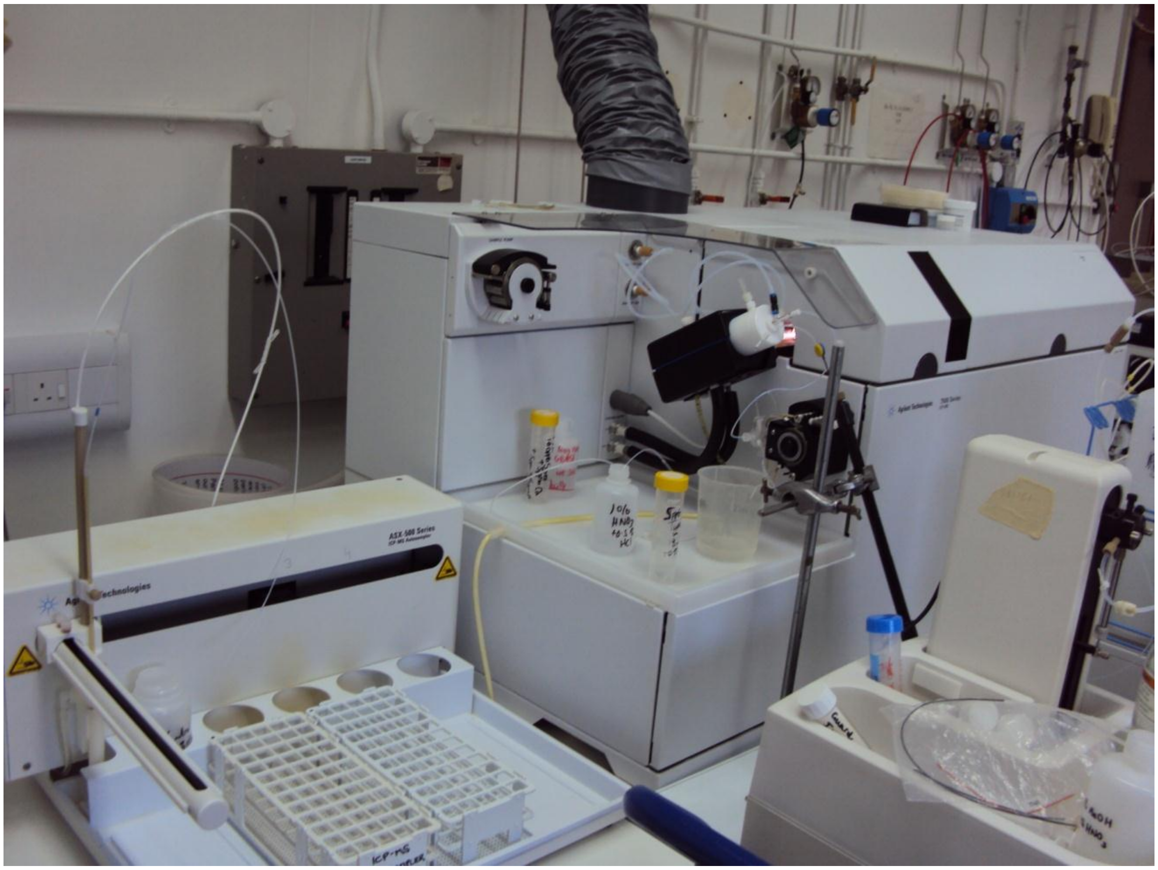


Figure 2.3: Inductively coupled plasma mass spectrometer (Agilent 7500 ICP-MS, Agilent Technologies, UK).

2.1.3 Atomic absorption spectrometry

Atomic absorption spectrometry measures the absorbance in the flame. Atomic absorption spectrometry is applicable to the determination of most elements of the periodic table (almost all metals and metalloids and some non-metals) in a wide variety of samples, including biological, clinical, environmental, food and geological. Hence it is one of the most commonly used techniques for elemental analysis. Two types of atom cells have been commonly used for AAS. The flame is widely used because of its ease of use for elemental analysis in the part per million (mg/L) levels. However, the use of a graphite furnace as the atomizer is used when a limited sample volume is available or when lower analyte concentrations (part per billion, µg/L level) are present in the sample.

The fundamentals of quantitative atomic absorption measurement for the determination of elements depend upon the absorption of radiation by free ground state atoms. A monochromatic light beam, with intensity (I^o) delivered by the source, enters the cell containing the gaseous analyte (Figure 2-4). The transmitted beam (I) then passes into a detection system that converts the light beam into an electrical signal. With an analyte present in a cell, the transmitted beam is less intense than the incident beam (Eq.1).

$$T\ (\text{ Transmittance }) = \frac{I}{I^o} \cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots (1)$$

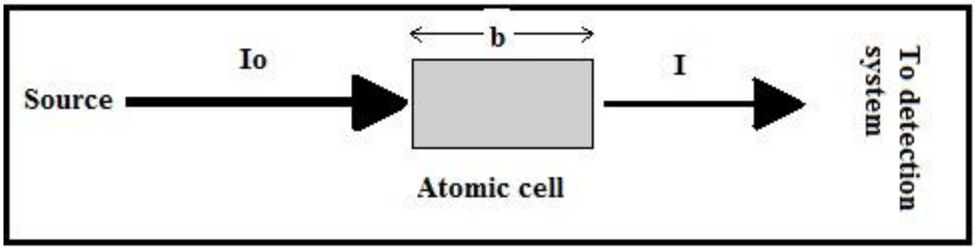


Figure 2.4: Schematic diagram of atomic absorption process, where b is the length of the atom cell, Io is the incident intensity, and I is the transmitted intensity.

The absorbance (Eq.2), derived from the logarithm of T (Eq.1), obeys the Beer-Lambert Law.

$A = \log 1/T$ or $\log (I°)/I$ ··(2)

Note that absorbance increases as transmittance decreases, indicating that as more atoms are present in the cell, the absorbance increases. The quantitative relationship between absorbance and concentration (c, g/L solution) is described by the Beer-Lambert law (Eq.3):

$A = \varepsilon b c$ ···(3)

Where A is absorbance; ε is the absorption coefficient, unique to the atoms of the analyte at a specified wavelength; b is the optical path length and c is the concentration of the analyte (Beaty *et al.*, 1993).

2.1.4 Graphite furnace atomic absorption spectrometry (GF-AAS)

Graphite furnace atomic absorption spectrometry (GF-AAS) (Figure 2-5) is also known by various other acronyms, including electrothermal atomic absorption spectrometry (ETAAS). In GF-AAS, samples (solid, liquid, or gas) are deposited in a small graphite tube, which can then be heated to vaporize and atomise the analyte (Butcher and Suedden, 1998). This instrument was used to measure As levels in some foods such as rice and vegetables.

Figure 2.5: Graphite furnace atomic absorption spectrometry (Varian model 220-Z), GF- and HG-AAS.

2.1.5 High Performance Liquid Chromatography (HPLC)

Importantly, it is recognised that, through the use of HPLC, chemical separations can be achieved through making use of the fact that various compounds comprise a number of different polarities, thus resulting in various migration rates within a particular column and mobile phase system. Essentially, the mobile phase and choice of stationary phase dictates the degree and extent to which separation is recognised.

A number of different techniques have been implemented for the purpose of distinguishing between arsenic species, the main one of which is ion exchange chromatography; this is an approach based on positively or negatively charged species' interactions with a stationary phase comprising an anionic functional group (cation exchange) ora cationic functional group (anion exchange).

Furthermore, as well as the ion exchange HPLC column, there are also reversed phase columns comprising a stationary phase, established from silica bonded chemically with an alkylsilyl compound to give a non-polar, hydrophobic surface (Lim, 1986). With this in mind, separation is owing mainly to polar interactions occurring between the stationary phase surface. Notably, micellar liquid chromatography has been utilised for the purpose of achieving the separation of arsenic species (Londesborough *et al.*, 1999). Furthermore, it is also recognised that one ideal technique for separating small charged organometallic ions (organoselenium and organoarsenic) is ion exchange chromatography, although this may also be valuable for metalloproteins fractionation (Szpunar, 2000).

Upon the selection of the ion chromatography column, the mobile phase needs to be enhanced and improved in consideration of individual species' separation. Notably, the mobile phase includes ions able to impact those interactions occurring between the stationary phase and species of interest; thus, separation optimisation can occur through adjusting the concentration and the pH. Figure 2-7 shows a schematic HPLC chromatographic system and Figure 2-8 shows HPLC instrument.

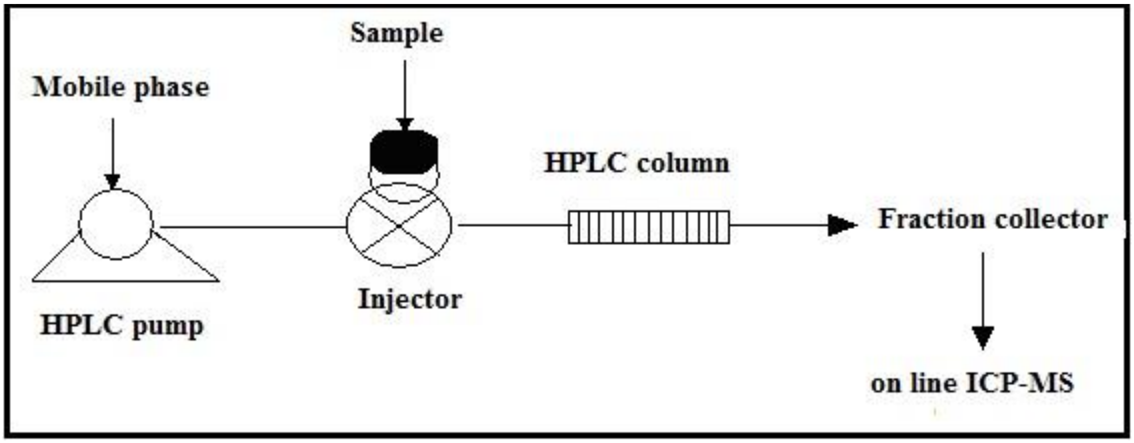


Figure 2.6: Block diagram of HPLC – ICP-MS.

Figure 2.7: High performance liquid chromatography (HPLC) (GP50-2 pump, Dionex, USA).

2.1.6 X-ray Fluorescence Spectroscopy (XRFS)

X-ray fluorescence spectroscopy utilises multi-element determination in a number of different environmental, geological and industrial materials, and is commonly implemented in laboratory settings with the aim of calculating the presence of elements in clay and soil samples. In addition to a large calibration range, extending a 1000 ppm for most trace elements, its detection limits in the sub-ppm or ppm range are possible together with large calibration range. Notably, the samples of soil are arranged into a dry, fine powder state and accordingly made into pellets with the use of an inert binder, with such samples commonly prepared with the aim of conducting a trace element analysis. Nevertheless, a number of key elements are examined through fused glass bead analysis. Samples were analysed using an energy dispersive instrument (PANalytical Axios advanced WD-XRFS) (Figure 2-9).

Figure 2.8: Energy dispersive X-Ray Fluorescence Spectrometry (XRFS) (PANalytical Axios advanced WD-XRFS).

2.1.7 Microwave digestion

In the context of organic and analytical laboratory practice, microwave heating has become widely used and is now recognised as being an extremely valuable and non-polluting approach of activation. With this in mind, it is recognised that microwaves are electromagnetic waves comprising a frequency range of 100 MHz–3 GHz; thus, such energy has the potential to act as non-ionising radiation, thus triggering ion molecular motion and dipole rotation; however, this is not known to impact overall molecular structure (Chemat *et al.*, 2004). Markedly, the use of a microwave digester (Figure 2-10) may be considered in mind of various environmental samples' digestion owing to its capacity to deliver a cleaner, faster, more reproducible, more accurate and safer approach to sample preparation alternatives.

Figure 2.9: CEM, Microwave digestion MARS Xpress, USA.

Trace metal analysis has become increasingly important to many areas of scientific work. Open and close microwave systems were used for digesting different samples with different concentration of nitric acid to determine arsenic and other trace elements. Some studies (Heitemper *et al.*, 2001; Helgesen and Larsen, 1998; Bohari *et al.*, 2002) have used microwave heating to determine arsenic in foodstuffs. In this study, microwave heating was used to digest some foodstuff sample because this method is faster and cleaner.

2.2 Experimental procedures

2.2.1 Sample collection

2.2.1.1 Foods and non-foods

Different types of foods and non-foods which are widely consumed in Bangladesh were analysed. Foods including rice and vegetables, fruits, freshwater fish, betel quid and its components [*Piper betel* leaves, areca nut, lime, tobacco and flavoured tobacoo (*zarda*)] and baked clay (called Sikor) were purchased from the UK based ethnic shops in the cities of Leicester, Birmingham, London and Luton during the months of September 2008 and June 2010. The products analysed in this study were mainly of Bangladeshi origin and are popular within Bangladeshi communities living in the UK and in Bangladesh.

2.2.1.2 Urine sample collection and study population

Ethical approval from De Montfort University, Faculty of Health & Life Sciences, Ethics committee, was obtained for a study investigating the dietary and life-style habits of different ethnic groups in the United Kingdom including members of the Bangladeshi community. This involved the use of a questionnaire and collection of urine samples. The samples were collected and kept at -20 ^{o}C until further analysis. Informed consent was obtained from the volunteers prior to sample collection and questionnaire completion. The questionnaires and samples from the Bangladeshis were divided into two groups (betel quid chewer and non-chewers). Urine samples from 37 volunteers (15 chewers and 22 non-chewers) were collected from Leicester city in the UK during September 2009. The age of volunteers was in the range 28-71 years (median; 47 years for males and 36 years for females). All these volunteers reported to be non-smokers and did not consume alcohol. The ratio of male:female was 2.5:1 for both groups combined, but was 1:1 for the chewers.

2.2.2 Pre-treatment of samples

Food samples were treated before digestion in different ways. Cereals including rice and puffed rice were ground using a coffee grinder and then kept for analysis. However, vegetables were washed three times with tap water and then with distilled water, before being dried in an oven at 80 ^{o}C overnight. The samples were then ground to a powder for analysis. The present moisture content for all samples was calculated by measuring lost weight before and after drying. Fish samples were cleaned and then dried using a freeze dryer. Frozen fish samples were used and drying of the fish samples overnight by using the freeze dryer. Then the fish samples were ground and prepared for analysis.

Non-foods were treated as the following: *Piper betel* leaves were washed with water three times, dried in an oven overnight and then ground. However, betel nut, tobacco, lime and zarda were ground as a powder for analysis. Powder was prepared from baked clay and kept for analysis.

Urine samples were collected in clean polypropylene tubes and immediately kept in a freezer at -20 °C to avoid any contamination. The samples were then melted and used for analysis.

2.2.2.1 Betel quid preparation

Some betel quid samples (ordinary and sweet) were collected from ethnic shops. However, betel quids were also prepared in the laboratory by combining different chewing components in proportions that are commonly used in commercial preparations. Additional information from betel quid chewers was also used for this preparation. There is no literature data on precise quantities of the various components of betel quids that make up a typical betel quid. In the current study, one leaf (approximately 1 g dry weight), combined with areca nut (approximately 4 g), lime (approximately 0.4 g) and tobacco (0.4 g – either tobacco leaves or *zarda*) we used. The quantities of these materials used in the quid can vary and some chewers do not include tobacco or lime in their quids. Ordinary quid contains *Piper betel* leaf, areca nut, some lime and some tobacco. Sweet quid contains similar components as the ordinary quid but with additional components such as pan masala, coconut, cumin and flavoured tobacco (*zarda)*. The betel quids were dried overnight in an oven at 80 °C and then ground with a grinder before digestion for analysis using ICP-MS. Dry weight of betel quids ranged from 5.5 to 11 g with an average of 7 g. This average weight, together with the mean trace elements concentrations of ordinary betel quids was used for calculating the Provisional Maximum Tolerable Daily Intakes (PMTDIs) and Target Hazard Quotients (THQs).

2.2.3 Sample digestion

2.2.3.1 Food sample analysis

Cereal (rice), vegetables (leafy and non-leafy), fruits and fish samples were digested. A dry ground weight (0.3 - 0.5 g) of sample was mixed with 4 ml of 70% nitric acid (HNO_3) Romil-UpA (Romil Ltd., Cambridge, UK) and 2 ml of hydrogen peroxide (H_2O_2) and then digested for 40 minutes using a microwave digester at a total pressure of 20 bars and a maximum temperature of 125 °C (Anton Paar - Multiwave 3000 Microwave Sample Preparation System, Graz, Austria). The digested solution was evaporated to dryness and then diluted to 25 ml in volumetric flasks with ultra pure water Romil-UpS (Romil Ltd., Cambridge, UK) for analysis. Some of these foods were digested using a microwave digestion unit at a maximum temperature of 170 °C (CEM, Microwave digestion MAR *X*press, Matthews, NC, USA).

2.2.3.2 Betel quid analysis

All chewing components including ordinary and sweet betel quids were digested by microwave assisted digestion in ultra pure 70% $HNO_{3.}$ A known weight (0.2 – 0.4 g) of dried, ground sample was mixed with 4 ml of 70% nitric acid and 2 ml H_2O_2 and then digested for 40 minutes using a microwave digester at a total pressure of 20 bars and a maximum temperature of 125 °C (Anton Paar - Multiwave 3000 Microwave Sample Preparation System, Austria). The solution was evaporated and then made up to 25 ml in volumetric flasks with ultra pure water for analysis.

2.2.3.3 Urine analysis

Aliquots (3 – 4 ml) of urine samples were digested with ultra pure 70% HNO_3 (2 ml). The mixture was heated overnight at 90 °C and then evaporated to near dryness. Subsequently, 2 ml of pure 70% HNO_3 and 2 ml of 30% H_2O_2 were added to the sample and heated again for four hours. Finally the sample was evaporated to near dryness and then diluted with ultra pure water for analysis.

2.2.3.4 Baked clay (Sikor) analysis

Sikor samples were digested by microwave assisted digestion in *aqua regia* (1:3 of ultra pure 70% HNO_3 and pure 37% HCl were used). A selected weight (0.1 g) of sample was mixed with 5ml of aqua regia overnight and then digested for three hours using a microwave digestion unit at a maximum temperature of 170 °C (CEM, Microwave digestion MAR *X*press, USA). The solution was made up to 100 ml in volumetric flasks with ultra pure water for analysis.

2.2.4 Elemental determination

2.2.4.1 Determination of arsenic concentrations in foods by GF-AAS

The atomic absorption spectrometer (Varian model 220-Z) was used in conjunction with a graphite–furnace atomizer (GTA-110) equipped with Zeeman background correction. Arsenic was determined at the 193.7 nm wavelength, using a 1000±3 mg/L standard solution of arsenic (CPI, International, USA) diluted to 65 µg/L with 1% nitric acid. The calibration standards in the range 0 to 26.05 µg/L were prepared on line via the automix facility on the autosampler. Palladium 1000 mg/L was used as the matrix modifier. The instrumental parameters used for the GF-AAS analysis are shown in Tables (2-3 and 2-3).

Table 2-1: Temperature Programme for GF-AAS Analysis.

Instrumental parameters	
Lamp type / current (mA)	hollow cathode/12.0
Wavelength (nm)	193.7
Slit width (nm)	0.2
Measurement mode	Peak height
Background correction	BC on (Zeeman)

Table 2-2: Instrumental Conditions Used for GF-AAS Analysis of Arsenic.

Step	**Temp (^{o}C)**	**Time (S)**	**Flow (L/min)**	**Gas type**	**Read**
1	85	5.0	3	Nitrogen	No
2	95	40.0	3	Nitrogen	No
3	120	10.0	3	Nitrogen	No
4	1400	5.0	3	Nitrogen	No
5	1400	1.0	3	Nitrogen	No
6	1400	2.0	0	Nitrogen	No
7	2600	0.6	0	Nitrogen	Yes
8	2600	2.0	0	Nitrogen	Yes
9	2600	2.0	3	Nitrogen	No

2.2.4.2 Determination of arsenic concentrations in food and non-food by ICP-MS

Concentrations of trace elements in the digested samples were determined by inductively coupled plasma mass spectrometer (ICP-MS) at Nottingham University. A Thermo-Fisher Scientific X-SeriesII instrument equipped with CCTED (collision cell technology with energy discrimination) Scandium (50 µg/L), Rhodium (10 µg/L) and Iridium (5 µg/L) in the preferred matrix of 2% HNO_3 was used. The instrument parameters used are as follows: forward power (1,404 W), hexa-pole bias (-18.0 V), pole bias (-14.0 V), reaction cell gas flow rate (4 ml/min), nebuliser (Carrier gas) flow rate (0.82 L/min), extraction lens (-129.4 V), quadru-pole dwell times (20 ms). External calibration standards for elements were prepared in the range 0.0-100 µg/L (ppb). Samples were introduced via a covered autosampler (Cetac ASX-520) through a concentric glass venture nebuliser (Thermo Fisher Scientific Inc., Waltham, MA, USA). The data processing was undertaken using a Plasmalab software (version 2.5.4; Thermo-Fisher Scientific, UK) set to employ separate calibration blocks and internal cross-calibration where required. Some raw data of food samples using ICP-MS instrument were reported in this thesis (Appendix 1).

2.2.5 Quality control and standard reference material

In this study, all the sample masses were measured to an accuracy of ± 0.1 mg. Elemental concentrations obtained by ICP-MS technique were evaluated by the use of certified reference materials and were found to be in good agreement with the certified values of the references material. The analytical procedure and the reliability of the digestion process of samples were validated by analysing of different types of standard reference materials (see Table 2-4 and 2-5). The average recoveries of reference material ranged between 87 to

105% for all measurement runs excluding baked clay. Average recoveries of baked clay ranged between 75 to 101%. For urine analysis, standard references urine [Human urine CRM (NIES No.18) and Seronorm trace elements urine] were used and the recoveries were 91 to 105% of certified values.

Table 2-3: Certified reference materials used in this study.

Certified Reference Material	**Foods and non-foods**
Rice flour (NIES No.10-b)	Cereal and rice analysis
Typical Japanese food (NIES No.27)	Fish and all foods
Tomato leaves (NIST 1573a)	Vegetables, fruits, betel quid
Sargasso seaweed (NIES No.9)	For all foods and betel quid
Tuna fish tissue (BCR-627)	Arsenic species in fish
Montana I soil (2710a)	Total elements in baked clay
Soil reference material (BCSS-1)	Arsenic species in baked clay
Human urine CRM (NIES No.18)	Urine analysis
Seronorm trace elements urine	Urine analysis

Table 2-4: As, Cd, Mn, Pb, Se, and Zn (µg/kg) content in standard references material.

Reference material	Element	Certified value	Found value
Rice flour (NIES No. 10-b)	*As*	0.110	0.103
	Cd	0.320 ± 0.02	0.33 ± 0.04
	Mn	31.5 ± 1.6	29.8 ± 2.8
	Se	0.02	0.021
	Zn	22.3 ± 0.9	24.4 ± 1.3
Seaweed (NIES No. 9)	*As*	115 ± 9	105 ± 12
	Cd	0.15 ± 0.02	0.16 ± 0.05
	Mn	21.2 ± 1.0	23.4 ± 1.7
	Pb	1.35 ± 0.05	1.45 ± 0.07
	Se	0.05	0.06
	Zn	15.6 ± 1.2	13.9 ± 1.6
Typical Japanese diet (NIES No.27)	*As*	0.60 ± 0.04	0.56 ± 0.05
	Cd	0.069 ± 0.009	0.07 ± 0.01
	Mn	8.9 ± 0.2	8.7 ± 0.6
	Se	0.25 ± 0.02	0.28 ± 0.07
	Zn	20.9 ± 0.9	18.7 ± 1.2
Tomato levels (NIST 1573a)	*As*	0.112 ± 0.004	0.115 ± 0.007
	Cd	1.52 ± 0.04	1.47 ± 0.08
	Mn	246 ± 8	232 ± 12
	Se	0.054 ± 0.003	0.051 ± 0.005
	Zn	30.9 ± 0.7	28.6 ± 0.6
Montana I soil (2710a)	*As*	1540 ± 100	1430 ± 90
	Cd	12.3± 0.3	11.3± 0.7
	Mn	2140± 60	1610 ± 70
	Pb	5520 ± 30	5580 ± 240
	Se	1.0	1.5
	Zn	4180 ± 150	3760 ± 80

For quality control of arsenic speciation in different types of foods and non-foods the following were used; (i) for rice speciation, rice flour (NIES No.10-b) was used and extraction recovery was 100%. (ii) For fish speciation, Tuna tissue (BCR-627) was analysed and the extraction recovery was 99%.

Also for baked clay speciation, soil reference material (BCSS-1), which has known total arsenic and arsenic species content, was used. Both As^{III} and As^{V} were spiked to the soil reference material (BCSS-1) before extraction and then extracted using the same method that was used for Sikor samples extraction. The spike recoveries were up to 100% for both arsenic species with linearity $r^2 = 1$ (see Figures 2-16 and 2-17). The mean recovery of total arsenic extraction from the soil reference material (BCSS-1) was 75 ± 5%, whereas, the mean recovery of arsenic species was 76 ± 6%. Very low detection limits of < 0.1 mg/kg were obtained for arsenic species.

2.2.6 Limit of detection

Detection limits for trace elements were evaluated by the analysis of ten replicate determinations of the reagent blank; blank samples were measured with the food samples for calibrating the instruments. The results of blanks for each digestion group were used to calculate the limit of detection (LOD) and the limit of quantification (LOQ) using the following equations:

$$\text{LOD} = 3 \times \text{SD} \quad \text{and} \quad \text{LOQ} = 10 \times \text{SD}$$

where SD is the standard deviation.

Table 2-6 shows the LOD and LOQ calculations of ten measurements of the blank (1% v/v HNO_3, which was used for foods, betel quid and urine) (Thomsen *et al.*, 2003). However, table 2-7 shows LOD and LOQ of *aqua regia* blank used for baked clay and lime.

Table 2-5: Determination of LOD and LOQ for As, Cd, Mn, Pb, Se and Zn in 1% HNO3 blank solution using ICP-MS.

Blank sample	**As**	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
Mean (µg/L)	0.03	0.00	0.011	0.001	0.01	0.04
SD	0.01	0.00	0.06	0.002	0.015	0.03
LOD = 3 x SD	0.03	0.00	0.18	0.006	0.045	0.09
LOQ = 10 x SD	0.10	0.00	0.60	0.020	0.15	0.30

Table 2-6: Determination of LOD and LOQ for As, Cd, Mn, Pb, Se and Zn in *aqua regia* blank solution using ICP-MS.

Blank sample	**As**	**Cd**	**Fe**	**Mn**	**Pb**	**Zn**
Mean (mg/L)	0.86	0.003	32.6	0.88	2.70	7.40
SD	0.033	0.0006	1.3	0.002	0.015	0.03
LOD = 3 x SD	0.10	0.002	3.90	0.10	0.31	1.30
LOQ = 10 x SD	0.33	0.006	13.00	0.33	1.03	4.33

2.2.7 Arsenic speciation section

2.2.7.1 Arsenic speciation of rice, fish and betel quid

A previously published method was used for the extraction of arsenic from rice and other food (Narukawa *et al.*, 2008). Brifely, accurate weight (0.2 – 0.5 g) of the relevant sample was transferred to a Teflon tube. Subsequently 10 ml of pure water was added and the extraction process was carried out using a microwave digester. Samples were digested for 30 minutes at 80 ^{o}C, and then 5 ml of pure water was added and filtered through a 0.45 µm filter. The arsenic in the extract was immediately determined using HPLC-ICP-MS and the method used is described below in section (2.2.7.3). Calibration curve of arsenic species including AsBet, As^{III}, DMA, MMA and As^{V} (as shown in Figures 2-11 to 2-15). Also the standard addition method was used in this study by spiking arsenic standards to the samples (see Figures 2-16 to 2-17). Figure 2-18 shows the chromatogram of arsenic speciation in mixed standard solutions which were used for rice, betel quid, Sikor and fish speciation.

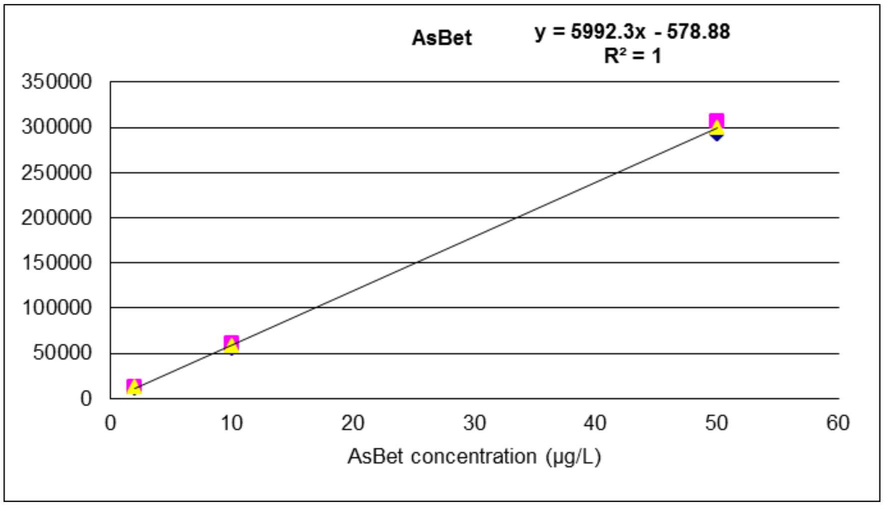


Figure 2.10: Calibration curve of AsBet species, the mean value was used.

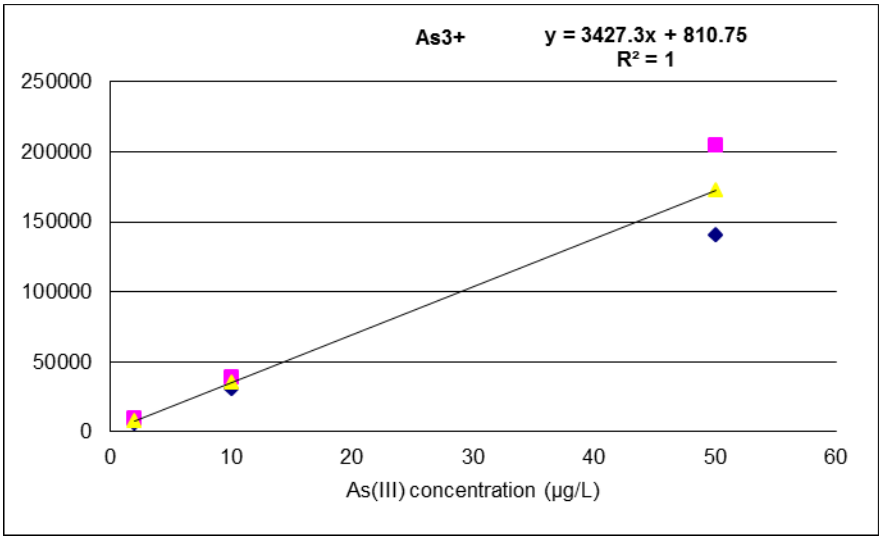


Figure 2.11: Calibration curve of As^{III} species, the mean value was used.

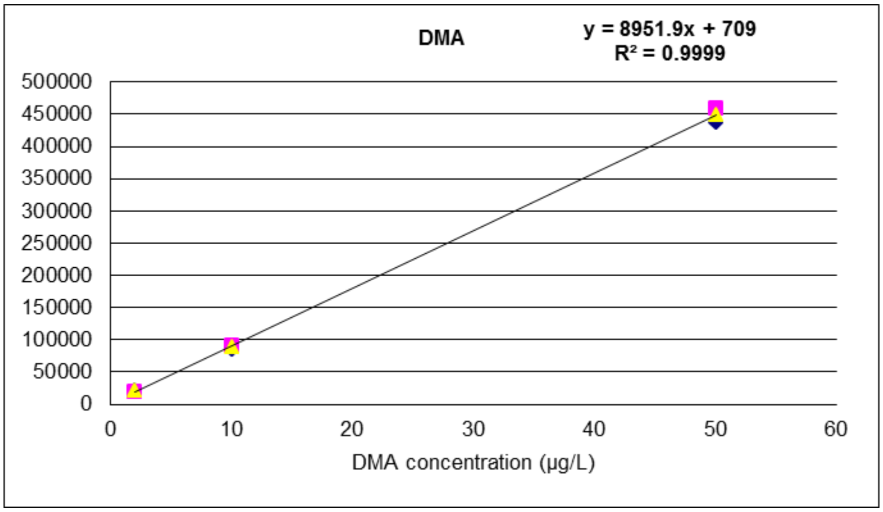


Figure 2.12: Calibration curve of DMA species, the mean value was used.

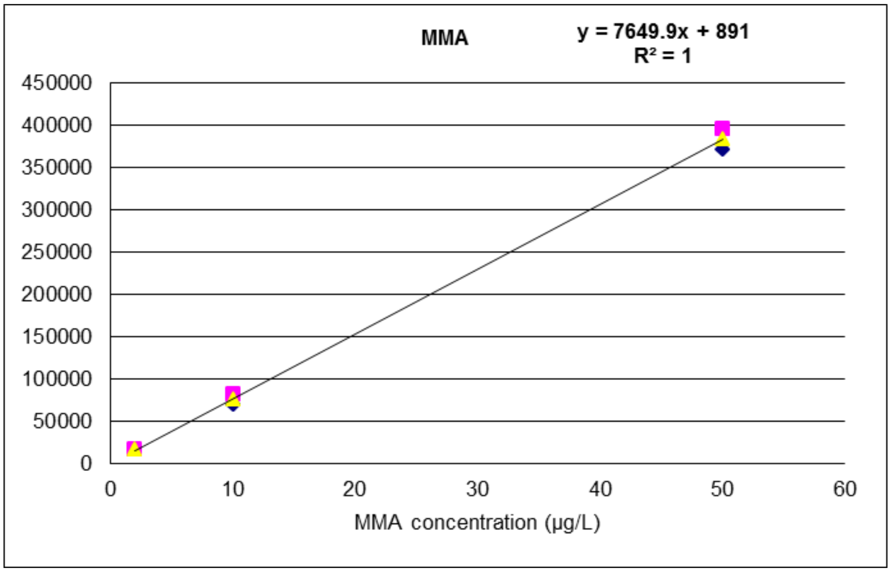


Figure 2.13: Calibration curve of MMA species, the mean value was used.

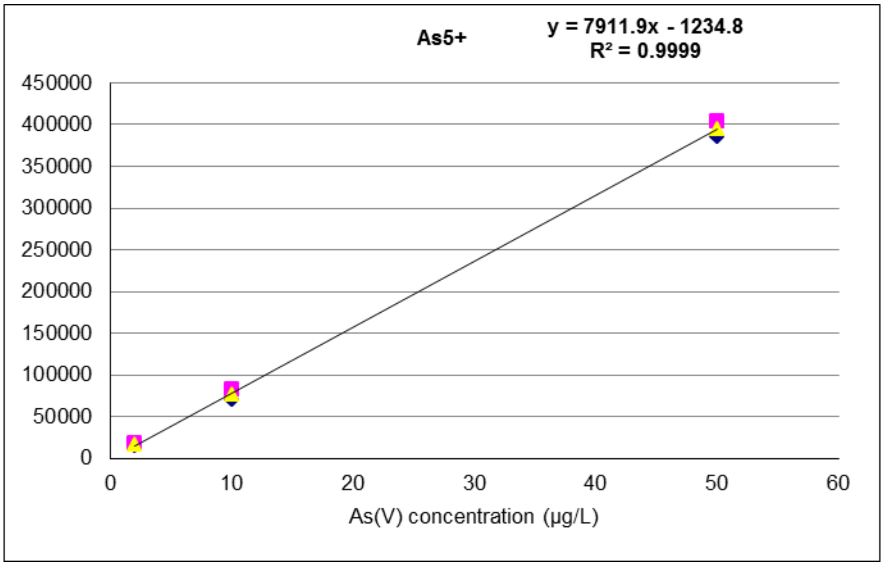


Figure 2.14: Calibration curve of As^{V} species, the mean value was used.

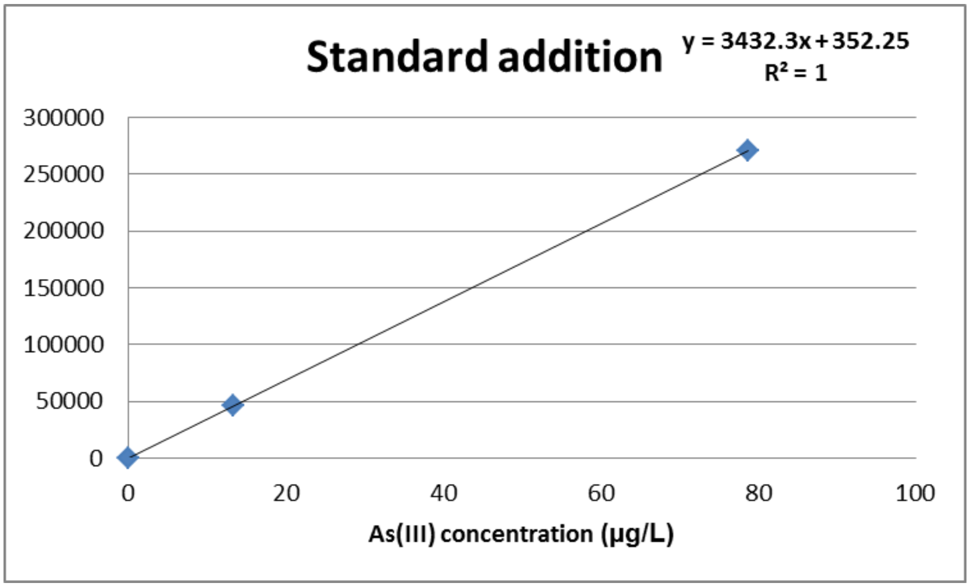


Figure 2.15: Calibration curve of standard addition of As^{III} species, the mean value was used.

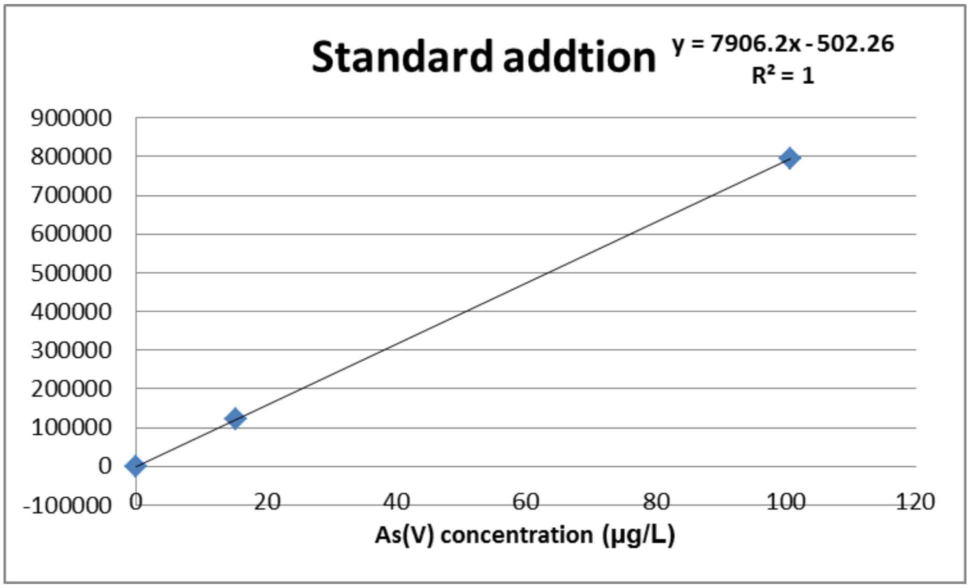


Figure 2.16: Calibration curve of standard addition of As^V species, the mean value was used.

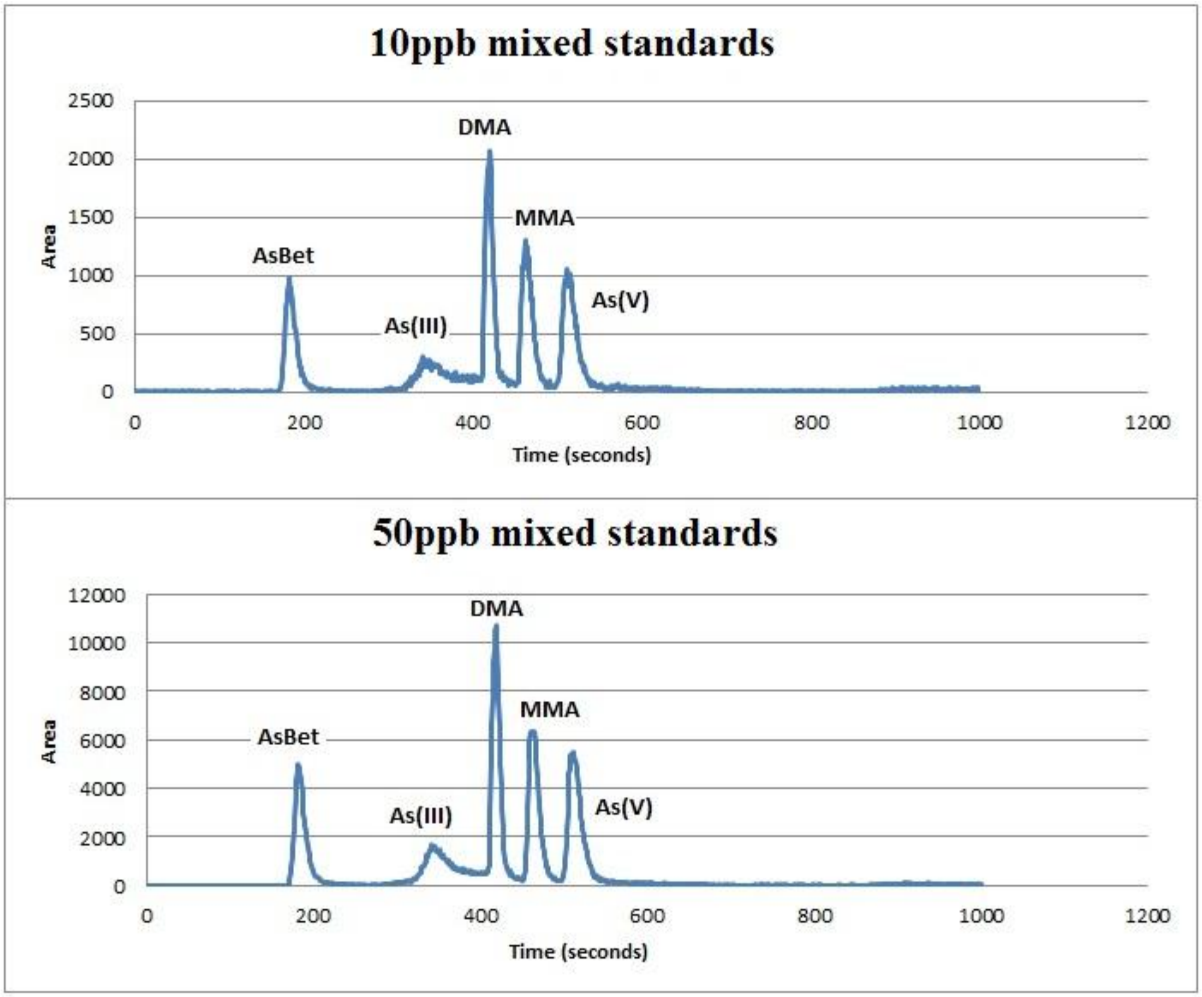


Figure 2.17: Chromatograms of arsenic speciation in mixed standard solutions using HPLC-ICP-MS; ppb: µg/L.

2.2.7.2 Arsenic speciation of baked clay

A previously published method was used for the extraction of arsenic from baked clay (Sikor) (Button and watts, 2008). Briefly, 0.1 - 0.2 g of Sikor sample was weighed into a 30 ml bottle. To this was added 10 ml of H_3PO_4 (1M)/ ascorbic acid (0.5M) mixture. This was then mixed for four hours using an orbital shaker at 200 rpm in order to extract the arsenic. Then 20 ml of Millipore water was added to dilute the mixture, dilution was carried out rapidly to avoid conversion from As^{III} to As^{V} (Gallardo *et al.* 2002). The solution extract was subsequently centrifuged for 15 minutes at 4000 rpm and the supernatant collected. As levels (total and As species) in all the extracted samples were analysed immediately using HPLC-ICP-MS instrument. Calibration curve of arsenic species including AsBet, As^{III}, DMA, MMA and As^{V} were obtained. Furthermore, standard addition method was used in this study by spiking arsenic standards to the samples (see Figures 2-11 to 2-17).

2.2.7.3 Determination of arsenic species in food and non-food samples

Detailed elemental speciation analysis was only conducted for As. For this, the total content of As in a solution, extracted from rice, fish, betel quid and Sikor samples, were determined using an Agilent 7500 ICP-MS (Agilent Technologies, (UK) (see Figure 2-3). This instrument was fitted with micro flow concentric nebulizer and quartz Scott-type chamber. Helium (4 L/min was used for collision cell and tellurium (50 µg/L) was used as internal standard. Subsequently, the solution was subjected to analysis using HPLC (GP50-2 pump, Dionex, USA) coupled to an ICP-MS instrument (Agilent Technologies, UK) in order to determine the As species (Figure 2-19). Details of HPLC conditions used are similar to that previously reported (Watts *et al.* 2008). Anion exchange column (Hamilton PRP-X100, 250 x 4.1 mm, 10 µm) was used to separate the As species. Ammonium nitrate was used as anion exchange mobile phase at pH 8.65.

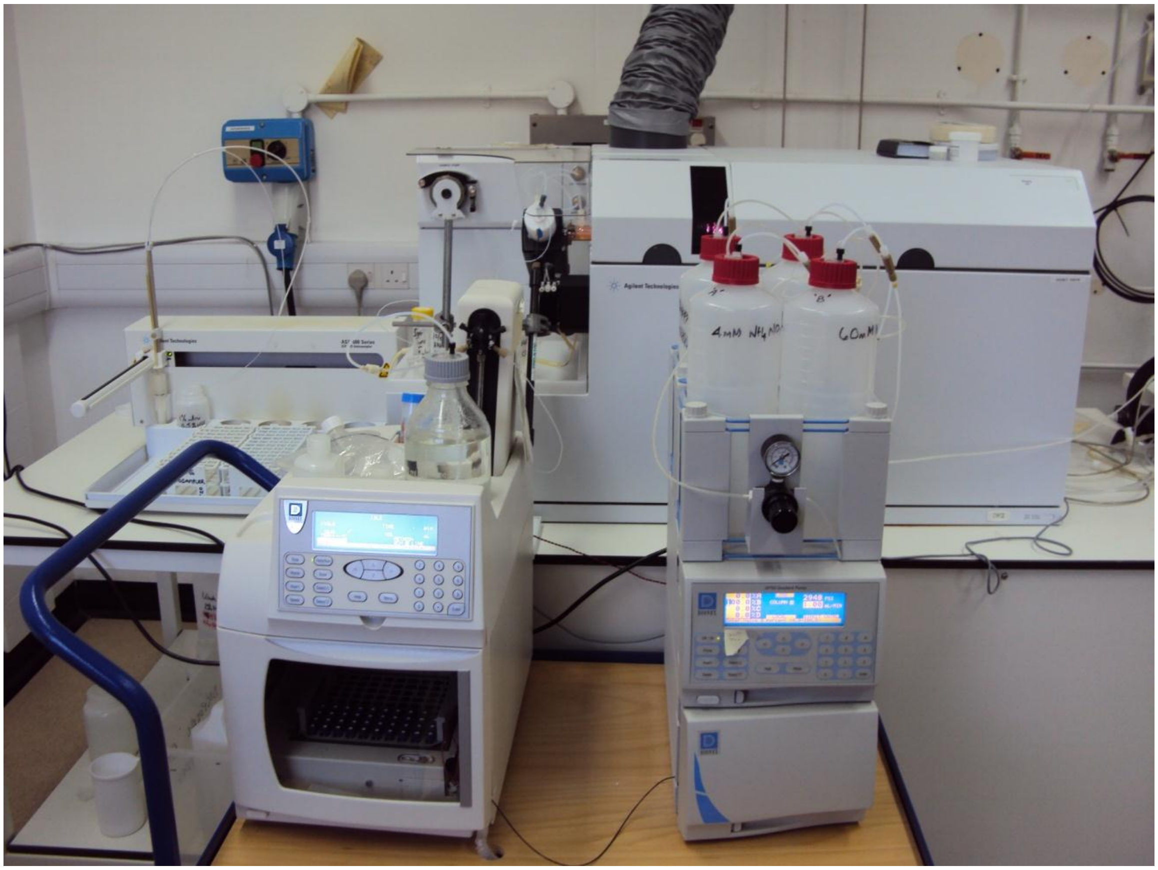


Figure 2.18: HPLC (GP50-2 pump, Dionex, USA) coupled to ICP-MS (Agilent 7500 ICP-MS, Agilent Technologies, UK).

2.2.8 Estimation of risk assessments of trace elements

In this study two different estimated parameters were used, the Provisional Maximum Tolerable Daily Intake (PMTDI) which estimates the maximum daily intake of a toxic element from individual food or more than one types of food and the unit that is used for this scale is mg of element per day (see Table 2-8). However, recently the United States Environmental Protection Agency (USEPA) has provided another scale for risk assessment, the Target Hazard Quotients (THQ). This is one of the important methods that can give a good idea of the health risk assessment. It is based on non-carcinogenic effects (USEPA, 2000). It gives the ratio of exposure dose to the reference dose, which simply gives the meaning that a value of less than 1 is not of concern. In this study, THQs were estimated for different types of food, and for the first time the THQ for betel quid was estimated.

The following equation shows the model for estimating THQ:

$$THQ = \frac{EFr\ x\ EDtot\ x\ IFR\ x\ C}{RFD\ x\ BWa\ x\ ATn}\ x\ 10^{-3}$$

Where THQ is the target hazard quotient; EFr is the exposure frequency (365 days/year); EDtot is the exposure duration (years); IFR is the food ingestion rate (g/day); C is the concentration (µg/g); RfD is the oral reference dose (mg/kg.day); BWa is the adult body weight (kg); and ATn is the averaging time for non-carcinogens (days x years). The average adult body weight was assumed to be 68 kg for Bangladeshis (Smith *et al.*, 2006).

Table 2-7: PMTDIs of trace elements in foods defined by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FSA, 2009).

Element	**PMTDI (µg/kg .bw. day)**
As (inorganic form)	2.1
Cd	1
Mn	3.6
Pb	200
Se	5
Zn	300-1000

Quantities of different foods consumed by the Bangladeshi population were taken from Zablotska *et al.* (2008). Bangladeshis consume on average 500 g of uncooked rice per day and this was used for calculating daily intakes of elements from rice consumption. The volume of water consumed by the Bangladeshi population has been reported to be 2.7 litres per day (Kile *et al.* 2007). For estimating intakes of elements from tea infusions, three cups of tea per day per person was assumed, with a volume of 237 ml per cup (Peters et al 2001).

3 BANGLADESHI RICE AS A SOURCE OF TOXIC AND ESSENTIAL ELEMENTS AND ITS ARSENIC SPECIATION

Summary

Exposure to arsenic through drinking water in Bangladesh is well known, however, water is not the only source of arsenic, and foods also can be a key source of arsenic contamination. Rice is the staple food in south Asian countries, including Bangladesh and it is known to contain high levels of arsenic. No previous studies have focused on arsenic levels in rice from regions in Bangladesh that contains low groundwater arsenic levels. Sylhet region has lower arsenic levels in groundwater compared to other areas in Bangladesh. Therefore, in this chapter, 98 samples of different varieties of rice (including boro, aman, parboiled and aromatic) were analysed from this region. Levels of arsenic and other elements in Bangladeshi rice were determined. Sylheti rice showed lower arsenic levels compared to other regions in Bangladesh. Furthermore, aromatic rice from Sylhet has the lowest arsenic content (10.1 µg/kg) and the highest selenium content (341 µg/kg) compared to non-aromatic rice.

Arsenic specitaion of Sylhet rice was achieved for some samples, the mean inorganic arsenic species was approximately 70% of the total arsenic which is similar to previous studies for Bangladeshi rice from other regions. Aromatic rice also showed much lower As:Se ratio compared to non-aromatic rice which is important as it has been reported that

Se levels in the Bangladeshi diet is low. In general aromatic rice contains lower levels of toxic elements (apart from Cd which is almost similar to non-aromatic rice) and higher levels of essential elements (especially Se & Zn) compared to non-aromatic rice.

For someone consuming 500 g of non-aromatic and aromatic rice from Sylhet, the daily intake of arsenic can be reduced by 48% and 69%, respectively compared to non-aromatic rice from other parts of Bangladesh. In addition, the daily intake of the essential elements, selenium and zinc will be increased by 46% and 23%, respectively for someone consuming aromatic rice compared to non-aromatic rice from Sylhet. This is also important since recent studies reported selenium and zinc deficiency among Bangladeshis. The data presented in this chapter shows that rice grown in a relatively arsenic unaffected area of Bangladesh contains lower levels of arsenic. Furthermore, the study reveals that aromatic rice has lower arsenic and higher levels of essential elements compared to non-aromatic rice. These two findings can be used by policymakers to modify rice farming and consumption in Bangladesh.

3.1 Introduction

Rice is a staple food for a large number of people in the world. Rice is also becoming popular within Europe. In the UK, rice consumption is particularly popular due to the historical connection between India and Britain. There are many Asian takeaway shops which are distributed throughout many UK cities that sell rice based products. Rice continues to be the main staple for Asian people who live in the UK (Kassam-Khamis *et al.*, 2000). Different types of rice are imported from Asian countries, the USA and from other European countries such as France, Spain, and Italy.

Bangladeshis in Bangladesh are exposed to high doses of arsenic and other harmful elements from different sources. Groundwater and rice are the main sources of arsenic exposure in Bangladesh. It is therefore necessary to monitor human exposure to arsenic and other toxic trace elements present in the food chain, and a number of studies have reported the total arsenic content of foodstuffs from different countries (Tsuda et al., 1995; Dabeka *et al.*, 1993; Schoof *et al.*, 1999; Alam *et al.*, 2003; Roychowdhury *et al.*, 2003; Al-Rmalli *et al.*, 2005; FSA, 2009). Since rice is the main staple food for Bangladeshis, the determination of arsenic and other trace elements in rice is necessary for estimating the human health impact of rice consumption amongst Bangladeshis.

Food chain aspects of arsenic contamination in Bangladesh have recently received attention (Meharg and Mazibur, 2003). Some studies have reported arsenic content in rice grown in different areas of Bangladesh (Duxbury *et al.*, 2003; Das *et al.*, 2004; Williams *et* *al.*, 2005; and Norton *et al.*, 2009). Some of these studies have shown that arsenic contamination of Bangladeshi rice is very high (as much as 1700 µg/kg) (Meharg and Mazibur, 2003). The high levels of inorganic arsenic in Bangladeshi rice have been attributed to irrigation of rice crops using contaminated water. Groundwaters in Bangladesh contain high levels of arsenic and this arsenic is mainly in the inorganic form.

In a study conducted in West Bengal (India), an area known to have high levels of arsenic in the groundwater, high levels of arsenic in rice <0.04 – 605 µg/kg were reported (Roychowdhury *et al.*, 2003). More recently, Meharg *et al.* (2009) determined total arsenic in 901 polished rice samples collected from Aberdeen city markets. These rice samples originated from 10 different countries. The data showed that the lowest arsenic content was in Egyptian rice (0.04 mg/kg) and the highest arsenic was in USA rice (0.25 mg/kg). Although, Bangladeshi rice contained 0.13 mg/kg of total arsenic, 61% of this was present as inorganic arsenic (Meharg *et al.*, 2009).

Rice as the main staple food of the diet in Bangladesh, it is a key source of calories and nutrients intake for Bangladeshis. It contributes about 80% of dietary energy and is a source of many minerals, including selenium and zinc (Tetens *et al.*, 2003). Many varieties of rice are consumed in Bangladesh such as aman (cultivated in waterlogged, lowland rice grown in the wet season during June to November); boro (cultivated in waterlogged, low-lying or medium lands with irrigation during November to May); and aromatic rice (such as Biron rice), which are fine rice consumed in relation to cultural heritage of Bangladeshis during different festivals (Sarker, 2002). Also, both parboiled and non-parboiled rice are consumed in Bangladesh.

Ahmed *et al.* (2011) reported that aromatic rice contained low arsenic levels compared to other types of rice from different region of Bangladesh (Ahmed *et al.*, 2011). It has been reported that non-aromatic rice (aman) gives a higher yield than aromatic rice (fine rice). The average yield of non-aromatic rice is 1,350 kg per acre while the average yield of aromatic rice is 800-920 kg per acre (Sarker, 2002). However, some farmers in Bangladesh prefer to grow aromatic rice to take advantage of higher revenue returns. Aromatic rice requires much less fertilizer, pesticides and irrigation compared to non-aromatic rice; aromatic rice production costs are low compared to other rice (Sarker, 2002).

As mentioned in introduction chapter, selenium deficiency can lead to some diseases, a very low selenium intake from diet (lower than 10 μgSe/day) results in the development of a type of osteoarthritis called Kashin–Beck disease (Li *et al.*, 2007). Selenium was reported to have a role in reducing arsenic toxicity, because the two elements act as metabolic antipodes (Hsueh *et al.*, 2003). A recent study showed that Bangladeshi people may have selenium deficiency in their diet (Spallholz *et al.* 2008). Zinc is a trace micronutrient for humans, more than 200 enzymes require zinc for their activity and also it is necessary for a healthy immune system (Harper *et al.*, 1990). Many studies mentioned Zn deficiency in soils and humans in Bangladesh. It has been reported that children and women in Bangladeshi villages have low serum zinc concentrations (Kongsbak *et al.*, 2006;

Li *et al.*, 2008). Rice is the main source of zinc for rural Bangladeshis and recent studies have suggested increasing zinc levels in Bangladeshi rice to improve the zinc status amongst Bangladeshis (Arsenault *et al.*, 2010, Mayer, 2010). Another study suggests that high levels of arsenic in soils decreases selenium and zinc levels in rice (Norton *et al.*, 2010).

The aim of the research reported in this chapter was to investigate total concentrations of arsenic and of other trace elements in Bangladeshi rice originating mainly from the north-eastern region of Bangladesh collectively called in this thesis as the Sylhet region (specifically samples from Sylhet, Moulvibazar and Habiganj were investigated). The study also included the analysis of a number of Bangladeshi aromatic rice. Previous studies have not focused on rice from the Sylhet region, which generally has lower levels of arsenic in the groundwater compared to other areas in Bangladesh (see Figure 3-1). Speciation of arsenic in selected groups of rice samples were conducted in order to determine the distribution of species in Sylheti rice and for comparison with data published in the literature for Bangladeshi rice.

'Groundwater Studies of Arsenic Contamination in Bangladesh'
DPHE/BGS/DFID (2000)

Figure 3.1: Arsenic levels in groundwater in different districts of Bangladesh. Source of diagram: British Geological Survey (BGS, 2011).

3.2 Materials and Methods

3.2.1 Sampling and sample preparation

Bangladeshi rice samples (98 samples) from Bangladesh, originating mainly from the North-east region of Bangladesh (Sylhet, Moulvibazar and Habiganj) (see Figure 3-1). This region will be referred to collectively as Sylhet in this thesis. Some rice samples from Dhaka market during 2009 were also studied. Other rice samples were bought from ethnic shops in the UK, different types of rice such as long grain (polished and parboiled), aromatic and Basmati which are more consumed amongst Bangladeshi and Asian communities in the UK. Majority of the rice studied were polished rice, most of them were non-parboiled rice (Atap) although a few parboiled samples were also analysed. Furthermore, a few brown Basmati rice were also analysed.

Rice samples were ground with a coffee grinder, and then kept in a clean place for digestion and elemental analysis. Duplicate sub-samples were taken from each sample studied. Rice flour (NISE SRM No.10) was used as a certified SRM. To investigate the moisture content of the rice, 5 g of rice samples (n = 25) chosen to reflect all rice samples, were dried in an oven (Gallenkamp, Hotbox oven) overnight at 80 °C. The average water content of the rice samples was 9.8 ± 0.3%, which agreed well with previous studies (Williams *et al.* 2005).

For measuring the total concentrations of arsenic and other elements, all rice samples were digested using microwave assisted digestion in ultra pure 70% nitric acid (HNO_3) and 30% hydrogen peroxide (H_2O_2) (see Chapter 2 section 2.2.3.1.).

To speciate arsenic in rice, selected samples of Bangladeshi rice were extracted using microwave digestion and were then analysed using a HPLC-ICP-MS instrument. These samples (0.5 g) were weighed into digestion tubes for subsequent speciation analysis (see Chapter 2 section 2.2.7.1.).

3.2.2 Element measurement

Concentrations of As, Cd, Mn, Pb, Se and Zn in the digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (see chapter 2, section 2.2.4.)

3.2.2.1 Arsenic speciation

HPLC-ICP-MS was used to analyse arsenic species in selected Bangladeshi rice samples. A PRP-X100, 10 µm anion-exchange column (150 x 4.1 mm) from Hamilton was used for the speciation of the arsenic species. Retention time for the arsenic species was determined using a species mix comprising of standards of 50 µg/L As^{III}, As^{V}, DMA^{V} and MMA^{V} (see chapter 2, section 2.2.7.1.)

3.2.3 Quality Control and standard reference material

See Table 2-5 and section (2.2.5.) in methodology chapter.

3.3 Results

Different varieties of Bangladeshi rice from Sylhet region (n=90), and Dhaka market (n=8) were investigated. Furthermore, Basmati rice samples (n=8), originating from either India or Pakistan, were also analysed. Arsenic and other trace elements in these samples were detected.

3.3.1 Totals of arsenic and of other elements in Bangladeshi rice

For rice from the Sylhet region (Table 3-1), the mean concentrations of arsenic (± SD) of boro variety was 71.7 ± 33 µg/kg and for aman variety was 85.7 ± 44 µg/kg. The mean concentration of arsenic in parboiled rice was found to be 78.8 ± 22 µg/kg, with a range of 37.8 – 119.2 µg/kg. This is lower than that for rice samples collected from Dhaka market (mean 121.9 ± 58 µg/kg; range 55.4 – 182.7 µg/kg). Interestingly, aromatic rice showed the lowest mean arsenic concentration among all varieties of rice studied. Mean arsenic concentration in aromatic rice from Sylhet region was 48.5 ± 43 µg/kg with a range of 10.1 – 125 µg/kg. Relatively low arsenic levels for aromatic rice (Table 3-2) were associated with Mou Biron T amon (10.1 µg/kg), Changri Aush (12.8 µg/kg), Kalapur bhuna aman (12.8 µg/kg), Kalijira T amon (13.2 µg/kg) and Rata Boro (13.8 µg/kg). The Biron variety of aromatic rice, which is widely consumed in Sylhet region, contained average arsenic levels of 53.3 of µgAs/kg with a range of 25 – 125 µg/kg (Table 3-2). Figure 3-2 shows total arsenic (mean µg/kg) from different studies of Bangladeshi rice. Sylheti rice (in this thesis) contained lower arsenic levels compared with other studies.

Table 3-1: Total arsenic (µg/kg) from different studies of Bangladeshi rice.

Region	Type of rice	As levels (µg/kg)		Reference
		Mean±SD	*Range*	
VARIOUS	AMAN	125	72 - 170	Duxbury *et al.* 2003
VARIOUS	BORO	183	108 - 331	Duxbury *et al.* 2003
GAZIPOR	11 CULTIVARS	92	43 - 209	Meharg and Rahman 2003
BOGRA	4 CULTIVARS	-	158 - 194	Meharg and Rahman 2003
DINAJPUR	BR11	203	-	Meharg and Rahman 2003
MYMENSINGH	BR8	78	-	Meharg and Rahman 2003
DHAKA MARKET	AMAN	130	30 – 300	Williams *et al.*, 2005
DHAKA MARKET	AMAN	-	180 – 310	Williams et al 2006
DHAKA MARKET	BORO	-	210 - 270	Williams *et al.*, 2006
FARIDPUR	BRRI	-	160 - 740	Norton *et al.*, 2009
SONARGAON	BRRI	-	70 - 280	Norton *et al.*, 2009
8 DIFFERENT AREAS	18 CULTIVARS	154	[a]127 – 215	Ahmed *et al.*, 2011
9 DIFFERENT AREAS	20 CULTIVARS	288	[a]217 – 338	Ahmed *et al.*, 2011
ALL OTHERS RICE		***156.6***	***30 - 740***	All previous studies
8 DIFFRENET AREAS	AROMATIC	140	[a]127 – 146	Ahmed *et al.*, 2011
DHAKA MARKET	DIFFERENT	121.9	55.4 – 182.7	This study
SYLHET	BORO (non-aromatic)	71.7	27.1 – 148.1	This study
SYLHET	AMAN (non-aromatic)	85.7	32.6 - 161	This study
SYLHET	PARBOILED (non-aromatic)	78.8	37.8 – 119.2	This study
SYLHET	[b]OTHER RICE	69.7	33.5 – 171.4	This study
ALL SYLHET RICE		***81***	***27.1 – 171.4***	This study
SYLHET	AROMATIC	48.5	10.1 – 125	This study

[a] Geometric means of different areas were used for the range.

[b] Different unknown varieties of rice collected from Sylhet region.

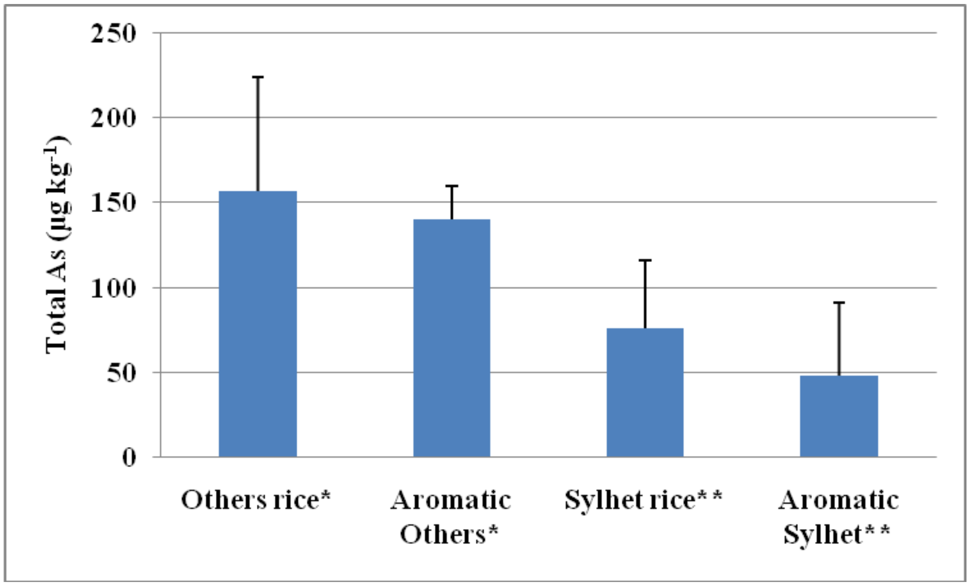


Figure 3.2: Total arsenic (µg/kg) from different studies of Bangladeshi rice, the error bars represent standard deviations. *Analysis of Bangladeshi rice samples taken from the literatures but not from Sylhet. **Rice samples analysed in this thesis.

Tables 3-2, 3-3, 3-4 showed As, Cd, Pb, Mn, Se and Zn concentrations in Bangladeshi rice. For Sylheti rice, the mean concentrations of As, Se, Cd and Pb were 72.6, 43.1, 37.2, 18.1 µg/kg respectively. The levels of Mn and Zn were 15.9 and 10.5 mg/kg respectively. However, for rice collected from Dhaka market, the mean concentrations of As, Se, Cd and Pb were 99.1, 43.6, 36.4, 14.6 µg/kg respectively and of Mn and Zn were 6.7 and 7.8 mg/kg respectively. As mentioned earlier, the mean concentration of arsenic in rice from Sylhet region was lower than that from Dhaka market. Rice samples from Dhaka market are likely to have been grown in other regions of Bangladesh. Selenium and cadmium content are similar whilst lead, manganese and zinc levels for rice from Sylhet region were higher (Tables 3-2, 3-3, 3-4). Individual comparison between Sylheti rice varieties reveals that the highest arsenic level was present in vadro month BRI-28 rice (171.4 µg/kg) followed by atap T aman rice (160.8 µg/kg). As a group, the highest mean arsenic concentration was seen in aman rice (non-aromatic). It is noteworthy that the lowest arsenic level (10.1 µg/kg) (Mou T Biron aman) and the highest selenium level (314.1 µg/kg) were detected in aromatic rice (Tepi boro rice).

The highest Cd and Pb levels were detected in boro rice (non-aromatic) (184.7 and 109.5 µg/kg, respectively). The mean concentrations of Cd and Pb in the five groups of rice from Sylhet are as follows: boro (non-aromatic) (42.2 and 22.1 µg/kg), aman (non-aromatic) (22.4 and 16.0 µg/kg), parboiled (non-aromatic) (29.4 and 33.2 µg/kg) and aromatic (41.7 and 16.9 µg/kg), Basmati (17.8 and 19.5 µg/kg) respectively (Table 3-3).

The highest Mn concentration was detected in Zarnia (32.2 mg/kg). The mean concentrations of Mn and Zn for rice samples from Sylhet are as follows: boro (non-aromatic) (11.3 and 10.2 mg/kg), aman (non-aromatic) (12.1 and 11.4 mg/kg), parboiled (non-aromatic) (12.1 and 9.1 mg/kg) and Biron (aromatic) (14.9 and 13.3 mgkg) for Mn and Zn, respectively. In contrast, parboiled rice from Dhaka market including lota, guti, minikat and shonia types contained low levels of Mn and Zn compared to atap rice from the same market (Table 3-4). These levels were also lower than rice from Sylhet region. Interestingly, minikat rice from Dhaka market contained high levels of As and Pb (182.7 and 24.2 µg/kg, respectively) and low levels of Se, Mn and Zn (22.1 µg/kg, 2.1 and 5.0 mg/day, respectively). Basmati rice from India and Pakistan contained high levels of Se, Mn and Zn (mean 87.9 µg/kg, 8.7 and 9.5 mg/day, respectively), and low levels of As, Cd and Pb (mean 65.4, 17.8 and 19.5 µg/kg, respectively).

Table 3-2: Concentration of As and Se in different types of Bangladeshi rice (µg/kg)

			As			**Se**		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Sylhet	Boro (non-aromatic)	19	71.7	27.3	148.1	46.2	17.8	167.5
	Aman (non-aromatic)	11	85.7	32.6	160.8	40.3	9.1	130.8
	Parboiled (non-aromatic)	13	78.8	37.8	119.2	53.3	14.9	213.7
	Biron (aromatic variety)	9	53.3	25	125	33.1	11.5	67.7
Sylhet								
Non-aromatic	Shail, Shoino moshuri Alo	1	91.1	-	-	24.7	-	-
	Baisher month Atashakh Ala	1	80.6	-	-	46.5	-	-
	Ala shail	1	60.7	-	-	21.2	-	-
	Vadro month 28	1	171.4	-	-	36.1	-	-
	Mukhta	1	33.5	-	-	54.5	-	-
	Aijing	1	68.7	-	-	30.3	-	-
	Shorna	1	57.4	-	-	64.1	-	-
	Binni	1	37.1	-	-	28.4	-	-
	Zarnia	1	58.6	-	-	30.5	-	-
	Irri	1	78.7	-	-	17.8	-	-
	Katari	1	126.7	-	-	17.2	-	-
	Konna shail	1	37.3	-	-	34.6	-	-
	Unknown sample	1	119	-	-	130.8	-	-
Aromatic	Aromatic boro	1	98.5	-	-	42.1	-	-
	Tepi boro	2	39	32.5	45.3	215	117	314
	Gorsi sail boro	1	10.9	-	-	197	-	-
	Khoya boro	1	25.8	-	-	137	-	-
	Damandor mukh boro	1	29.7	-	-	236	-	-
	Baingon bichi boro	1	24.2	-	-	28.9	-	-
	Rata boro	1	13.8	-	-	209.4	-	-
	Changri Aush	1	12.8	-	-	40	-	-
	Tulsi mala	1	58.8	-	-	30.5	-	-
	Murali aush	1	104	-	-	29.3	-	-
	Shona brion	1	49.2	-	-	15.9	-	-
	Fragrant rice Srimongol	1	57.7	-	-	33.4	-	-
	Fragrant rice Kachua	1	16.1	-	-	31.6	-	-

Table 3.2 continued.

			As			Se		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Aromatic	Gondi Biron T aman	1	77.2	-	-	76.1	-	-
	Puti Biron T amon	1	37.6	-	-	44.4	-	-
	Mou Biron T amon	1	10.1	-	-	101.4	-	-
	Chinigura T amon	1	38.6	-	-	52.5	-	-
	Mala T aman	2	85	58	109.6	44.5	32	57
	Joria amon	1	26.7	-	-	66.7	-	-
	Kalijira T amon	1	13.2	-	-	51.6	-	-
	Kalapur bhuna amon	1	12.8	-	-	207.1	-	-
	Goar chora T amon	1	22.9	-	-	67.2	-	-
	Katari bhog T amon	1	27.3	-	-	42.5	-	-
All aromatic rice	*from Sylhet*	25	38.7	10.1	109.6	87	15.9	314
All sylhet rice	(*non-aromatic and aromatic*)	90	72.6	10.1	171.4	43.1	9.1	314
Dhaka market	*ATAP*							
	BR-28	1	116.6	-	-	71.0	-	-
	Nazir	1	92.7	-	-	38.2	-	-
	Unknown	1	54.7	-	-	30.0	-	-
	Chinigura (Aromatic)	1	41.2	-	-	41.4	-	-
	PARBOILED							
	Lota	1	156.8	-	-	36.0	-	-
	Guti	1	55.4	-	-	45.7	-	-
	Minikat	1	182.7	-	-	22.1	-	-
	Shorna	1	92.5	-	-	64.5	-	-
All Dhaka rice		8	99.1	41.2	182.7	43.6	22.1	71.0
India/Pakistan	Basmati (Pakistan)	1	61.3	-	-	52.2	-	-
	Basmati (India)	1	54.9	-	-	68.3	-	-
	Basmati brown (India)	1	80.5	-	-	137.9	-	-
	Basmati brown (India)	1	56.2	-	-	83.4	-	-
	Basmati (Pakistan)	1	41	-	-	49.3	-	-
	Basmati (Pakistan)	1	49.4	-	-	139	-	-
	Basmati (India)	1	78.6	-	-	73.1	-	-
	Basmati (Parboiled)	1	101	-	-	100	-	-
All Basmati rice		8	65.4	41	101	87.9	49.3	139

Table 3-3: Concentration of Cd and Pb in different types of Bangladeshi rice (µg/kg)

			Cd			Pb		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Sylhet	Boro (non-aromatic)	19	42.2	6.9	184.7	22.1	7.1	109.5
	Aman (non-aromatic)	11	22.4	2.3	79.9	16.0	7.5	42.8
	Parboiled (non-aromatic)	13	29.4	5.3	64.5	33.2	7.5	106.8
	Biron (Aromatic variety)	9	25.2	5.2	96.2	20.6	6.2	88.6
Sylhet market								
Non-aromatic	Shail, Shoino moshuri Alo	1	19.9	-	-	12.4	-	-
	Baisher month Atashakh Ala	1	5.7	-	-	7.0	-	-
	Ala shail	1	17.6	-	-	15.5	-	-
	Vadro month 28	1	5.2	-	-	25.4	-	-
	Mukhta	1	65.4	-	-	8.8	-	-
	Aijing	1	52.2	-	-	8.4	-	-
	Shorna	1	172.4	-	-	15.2	-	-
	Binni	1	48.8	-	-	27.1	-	-
	Zarnia	1	37.9	-	-	26.0	-	-
	Irri	1	46.4	-	-	34.0	-	-
	Katari	1	2.6	-	-	18.5	-	-
	Konna shail	1	49.7	-	-	5.9	-	-
	Unknown sample	1	12.4	-	-	42.8	-	-
Aromatic	Basmati Boro	1	33.9	-	-	9.5	-	-
	Tepi boro	2	66.0	52.9	79.6	12	5.8	18.4
	Gorsi sail boro	1	35.4	-	-	25.1	-	-
	Khoya boro	1	41.4	-	-	16.5	-	-
	Damandor mukh boro	1	70.6	-	-	26.7	-	-
	Baingon bichi boro	1	27.6	-	-	11.4	-	-
	Rata Boro	1	62.4	-	-	19.1	-	-
	Changri Aush	1	75.6	-	-	12	-	-
	Tulsi mala	1	28.5	-	-	9.4	-	-
	Shona brion	1	41.2	-	-	19.2	-	-
	Murali aush	1	29.2	-	-	13.4	-	-
	Fragrant rice Srimongol	1	9.6	-	-	5.0	-	-
	Fragrant rice Kachua	1	23.2	-	-	3.6	-	-

Table 3.3 continued.

			Cd			Pb		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Aromatic	Gondi Biron T Aman	1	29	-	-	19.4	-	-
	Puti Biron T amon	1	15.8	-	-	10.6	-	-
	Mou Biron T amon	1	46.1	-	-	15.2	-	-
	Chinigura T amon	1	14	-	-	16.1	-	-
	Mala T aman	2	44	14.8	72.2	10.5	8.4	13.2
	Joria amon	1	48.3	-	-	5.2	-	-
	Kalijira T amon	1	54	-	-	14.5	-	-
	Kalapur bhuna amon	1	78.5	-	-	45.5	-	-
	Goar chora T amon	1	54.6	-	-	5.4	-	-
	Katari bhog T amon	1	41.1	-	-	14.2	-	-
All aromatic rice	*from Sylhet*	25	42.2	14	79.6	14.8	5.2	45.5
All sylhet rice	*non-aromatc and aromatic*	90	37.2	2.6	172.4	18.1	3.6	109.5
Dhaka market	*ATAP*							
	BR-28	1	55.6	-	-	13.6	-	-
	Nazir	1	47.3	-	-	12.3	-	-
	Atap	1	62.9	-	-	12.3	-	-
	Chinigura (Aromatic)	1	29.6	-	-	15.3	-	-
	PARBOILED							
	Lota	1	17.8	-	-	13.1	-	-
	Guti	1	36.1	-	-	18.5	-	-
	Minikat	1	15.3	-	-	24.2	-	-
	Shorna	1	26.2	-	-	7.2	-	-
All Dhaka rice		8	36.4	15.3	62.9	14.6	7.2	24.2
India/Pakistan	Basmati (Pakistan)	1	18.5	-	-	21.7	-	-
	Basmati (India)	1	22.4	-	-	32.5	-	-
	Basmati brown (India)	1	12.1	-	-	36.3	-	-
	Basmati brown (India)	1	17.2	-	-	22.1	-	-
	Basmati (Pakistan)	1	25.1	-	-	10.1	-	-
	Basmati (Pakistan)	1	7.3	-	-	8.1	-	-
	Basmati (India)	1	24.8	-	-	10.3	-	-
	Basmati (Parboiled)	1	15.3		-	14.4	-	-
All Basmati rice		8	17.8	7.3	25.1	19.5	8.1	36.3

Table 3-4: Concentration of Mn and Zn in different types of Bangladeshi rice (mg/kg)

			Mn			Zn		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Sylhet	Boro (non-aromatic)	19	11.3	3.5	21.8	10.2	3.5	21.3
	Aman (non-aromatic)	11	12.1	3.7	19.3	11.4	4.4	15.2
	Parboiled (non-aromatic)	13	12.1	4.2	27.0	9.1	6.3	14.3
	Biron (Aromatic variety)	9	14.9	8.3	28.5	13.3	7.6	19.5
Sylhet market								
Non-aromatic	Shail, Shoino moshuri Alo	1	11.4	-	-	6.5	-	-
	Baisher month Atashakh Ala	1	7.4	-	-	9.5	-	-
	Ala shail	1	19.6	-	-	9.9	-	-
	Vadro month 28	1	12.9	-	-	8.2	-	-
	Mukhta	1	13.4	-	-	10.1	-	-
	Aijing	1	15.8	-	-	11.2	-	-
	Shorna	1	19.5	-	-	12.0	-	-
	Binni	1	32.2	-	-	11.1	-	-
	Zarnia	1	30.7	-	-	13.3	-	-
	Irri	1	23.3	-	-	12.0	-	-
	Katari	1	15.4	-	-	15.2	-	-
	Konna shail	1	12.6	-	-	9.3	-	-
	Unknown sample	1	11.5	-	-	11.9	-	-
Aromatic	Basmati Boro	1	14.3	-	-	17	-	-
	Tepi boro	2	15.3	14.9	15.7	16.3	15.1	17.6
	Gorsi sail boro	1	16.5	-	-	19.5	-	-
	Khoya boro	1	17.4	-	-	16.9	-	-
	Damandor mukh boro	1	28.6	-	-	17.2	-	-
	Baingon bichi boro	1	20.2	-	-	10.8	-	-
	Rata Boro	1	18.9	-	-	17.5	-	-
	Changri Aush	1	8.4	-	-	13	-	-
	Tulsi mala	1	18.9	-	-	17.3	-	-
	Shona brion	1	8.5	-	-	10.6	-	-
	Murali aush	1	12.3	-	-	15.7	-	-
	Fragrant rice Srimongol	1	13.8	-	-	9.1	-	-
	Fragrant rice Kachua	1	12.3	-	-	7.0	-	-

Table 3.4 continued.

			Mn			Zn		
Region	**Rice cultivar**	**n**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Aromatic	Gondi Biron T Aman	1	18.2	-	-	13.6	-	-
	Puti Biron T amon	1	13.3	-	-	12.3	-	-
	Mou Biron T amon	1	9.9	-	-	12.6	-	-
	Chinigura T amon	1	20.1	-	-	9.6	-	-
	Mala T aman	2	14.1	13.4	14.8	13.1	12.2	14.3
	Joria amon	1	11.3	-	-	9.2	-	-
	Kalijira T amon	1	17.7	-	-	14.4	-	-
	Kalapur bhuna amon	1	22.3	-	-	13.3	-	-
	Goar chora T amon	1	8.3	-	-	10.2	-	-
	Katari bhog T amon	1	20.4	-	-	9.2	-	-
All aromatic rice	*from Sylhet*	25	15.7	8.3	28.6	13.3	9.2	19.5
All sylhet rice	*non-aromatic and aromatic*	90	15.9	3.5	32.2	10.5	4.4	21.3
Dhaka market	*ATAP*							
	BR-28	1	9.4	-	-	10.3	-	-
	Nazir	1	7.1	-	-	9.7	-	-
	Atap	1	11.2	-	-	11.2	-	-
	Chinigura (Aromatic)	1	13.3	-	-	11.2	-	-
	PARBOILED							
	Lota	1	4.0	-	-	6.0	-	-
	Guti	1	5.2	-	-	5.0	-	-
	Minikat	1	2.1	-	-	5.0	-	-
	Shorna	1	1.3	-	-	3.9	-	-
All Dhaka rice		8	6.7	1.3	13.3	7.8	3.9	11.2
India/Pakistan	Basmati (Pakistan)	1	8.9	-	-	10.1	-	-
	Basmati (India)	1	7.6	-	-	8.7	-	-
	Basmati brown (India)	1	12.1	-	-	10.7	-	-
	Basmati brown (India)	1	16.9	-	-	11.9	-	-
	Basmati (Pakistan)	1	6.9	-	-	10.2	-	-
	Basmati (Pakistan)	1	6.2	-	-	8.2	-	-
	Basmati (India)	1	7.2	-	-	10.3	-	-
	Basmati (Parboiled)	1	4.1	-	-	5.9	-	-
All Basmati rice		8	8.7	4.1	16.9	9.5	5.9	11.9

3.3.2 As speciation of selected Bangladeshi rice

Arsenic speciation of selected Bangladeshi rice samples (which were measured for total arsenic in this study) was determined. These samples were chosen from different types of rice including atap (non-parboiled), parboiled and aromatic rice. Figure 3-3 showed that the chromatogram of the blank solution that was used for arsenic speciation of Bangladeshi rice. Figure 3-4 showed a typical chromatogram of Sylheti rice. The main arsenic species seen were As^{III}, DMA and As^{V}. Table 3-5 and Figure 3-5 showed the arsenic species concentrations in Bangladeshi rice from Sylhet region. High percentages of inorganic arsenic were detected in rice from this region, which ranged from 77.5 to 100%. Generally, atap rice showed lower extraction percentage compared with parboiled rice. Alo rice (atap and sticky rice) has the lowest arsenic extraction (41%), followed by Biron rice 46% (Table 3-5 and Figure 3-5. A variety of rice (Alo) also contained lowest DMA levels amongst the rice group analysed. Interestingly, the percentag of As^{III} to As^{V} in some of the rice samples such as Katari, Vadro month (BRI 28) were around 50:50. However, in other rice samples As^{III}, which was the most toxic of arsenic species, was the main species present with the ratio of As^{III}: As^{V} ranging between 96:04 and 66:34. The extraction efficiency of arsenic from aromatic rice was lower compared with parboiled rice. The main species detected in aromatic rice was mainly As^{III} and MMA was not detected, except for one rice sample (red Biron). The latter rice contained a small quantity of MMA (0.9 µg/kg) (Table 3-5). Average inorganic arsenic in all Sylhet rice (non-aromatic and aromatic) was 69.7%. Atap long grain rice from USA, which is very popular amongst Bangladeshis residing in the UK, and the corresponding parboiled variety were investigated in this study for comparison with

Bangladeshi rice. The USA long grain rice showed that 33% and 56% of the arsenic species detected was inorganic arsenic, for atap and parboiled rice, respectively (Figure 3-5).

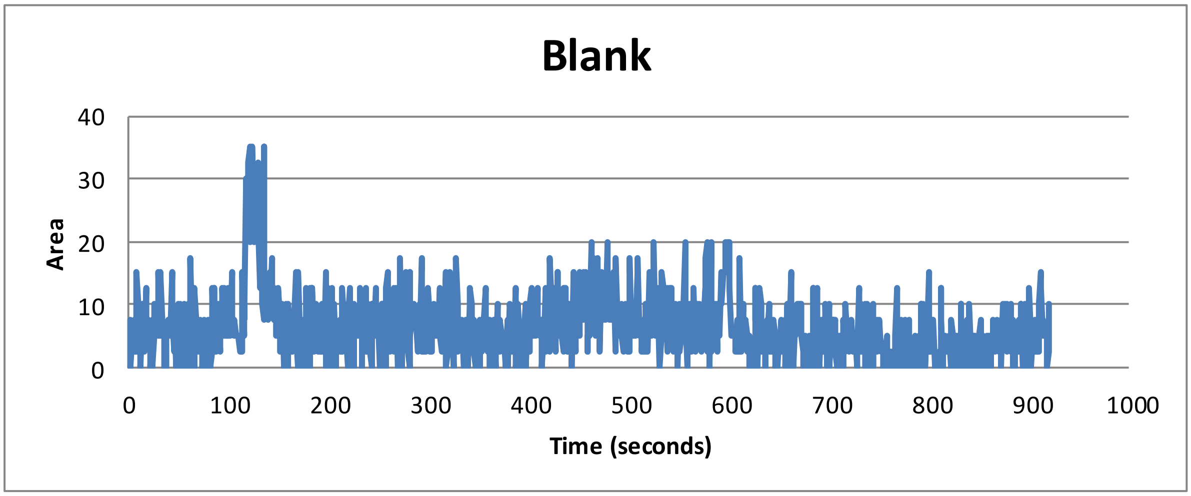


Figure 3.3: Chromatogram of the blank solution (mixture of ammonium phosphate and water) was used for arsenic speciation of Sylheti rice using HPLC-ICP-MS.

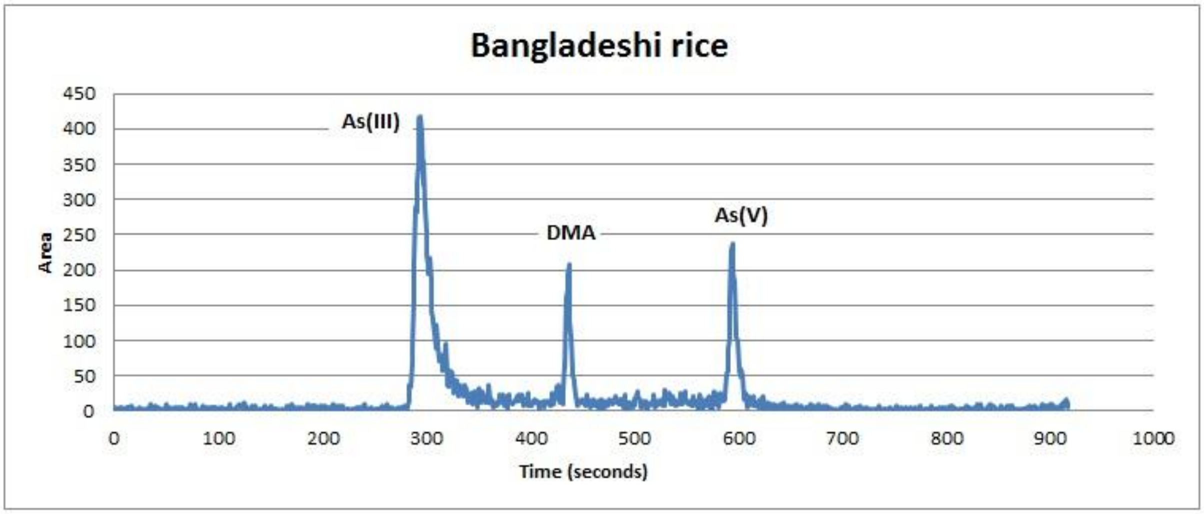


Figure 3.4: Chromatogram of arsenic speciation of Sylheti rice using HPLC-ICP-MS.

Table 3-5: Concentrations and percentages of total As and As species in Bangladeshi rice and contribution of i-As to PMTDI assuming a body weight of 60 kg and a rice consumption rate of 0.5 kg per day.

Rice type	**Total µg As/kg dry wt.**	**Sum of As species µg/kg d. wt.**	**Extraction Efficiency %**	**As^{III} µg/kg d. wt.**	**As^{V} µg/kg d. wt.**	**DMA µg/kg d. wt.**	**MMA µg/kg d. wt.**	**Inorganic As %**	**Contribution of %PMTDI of iAs**
Boro (Unknown)	84.4	63.5	75	53.5	8.2	1.8	nd	73	24.4
Badal boro	100.7	84.0	83	77.35	3.35	3.29	nd	80	32
Aman (Unknown)	101.4	70.7	70	35.09	33.11	2.46	nd	67	27
Atap T aman	160.8	122.3	76	111.84	7.20	3.25	nd	74	47.2
Lucky aman	81.0	70.8	87	64.18	3.91	2.70	nd	84	27
Katari	126.7	86.9	68	37.30	38.72	4.0	nd	60	30.2
Vadro month 28	171.4	135.1	79	66.09	58.83	10.19	nd	73	49.7
Atap rice (Unknown)	115.3	113.7	99	70.69	35.88	7.09	nd	92	42.1
Mala T amon (Aromatic)	109.6	77.2	67	63.2	6.08	7.57	nd	63	27.4
Tulsi mala (Aromatic)	58.8	39.5	67	32.09	4.70	2.71	nd	63	14.7
Murali aush (Aromatic)	104.0	67	66	53.25	5.5	4.2	nd	56	23.1
Srimangal Biron (Aromatic)	117.5	62.5	53	53.53	2.49	5.39	nd	48	22.4
Ulwail Biron (Aromatic)	56.1	25.8	46	24.27	1.23	0.30	nd	45	10

nd: not detectable; d. wt.: dry weight; PMTDI: the provisional maximum tolerable daily intake

Table 3-5 continued.

Rice type	**Total µg As/kg dry wt.**	**Sum of species µg/kg d. wt.**	**Extraction Efficiency %**	**As^{III} µg/kg d. wt.**	**As^{V} µg/kg d. wt.**	**DMA µg/kg d. wt.**	**MMA µg/kg d. wt.**	**Inorganic As %**	**Contribution of %PMTDI of iAs**
Agrain month (Shail)	76.4	47.6	62	33.26	3.47	10.87	nd	48	14.6
Alo shoino moshuri (Shail)	91.1	37.7	41	33.59	3.15	0.96	nd	40	14.5
Alo baisher month	80.6	32.8	41	30.20	2.61	nd	nd	41	13.1
Parboiled rice (Shail)	74.4	64.6	87	39.10	20.47	5.03	nd	80	23.6
Parboiled rice (Sylhet)	85.7	87.0	101	38.90	37.29	10.81	nd	89	30.3
Parboiled rice (Shail)	70.4	73.3	104	54.37	7.69	1.23	nd	88	24.6
Parboiled rice (Kachua)	105.3	98.9	94	77.56	15.15	6.29	nd	88	36.8
Aman shirmoin shiddo	105.9	106.2	100	78.63	21.31	6.24	nd	94	39.5
Amon red Biron (Aromatic)	131.6	120.6	92	65.76	46.72	8.08	0.9	85	44.4
Average of all rice	-	-	***75.3***	-	-	-	-	***69.7***	***28.1***
Atap long grain (USA)	202	131.3	65	66	nd	65	nd	33	26.5
Parboiled rice LG (USA)	190	162	85	107	nd	56	nd	56	42.2

nd: not detectable; d. wt.: dry weight; PMTDI: the provisional maximum tolerable daily intake

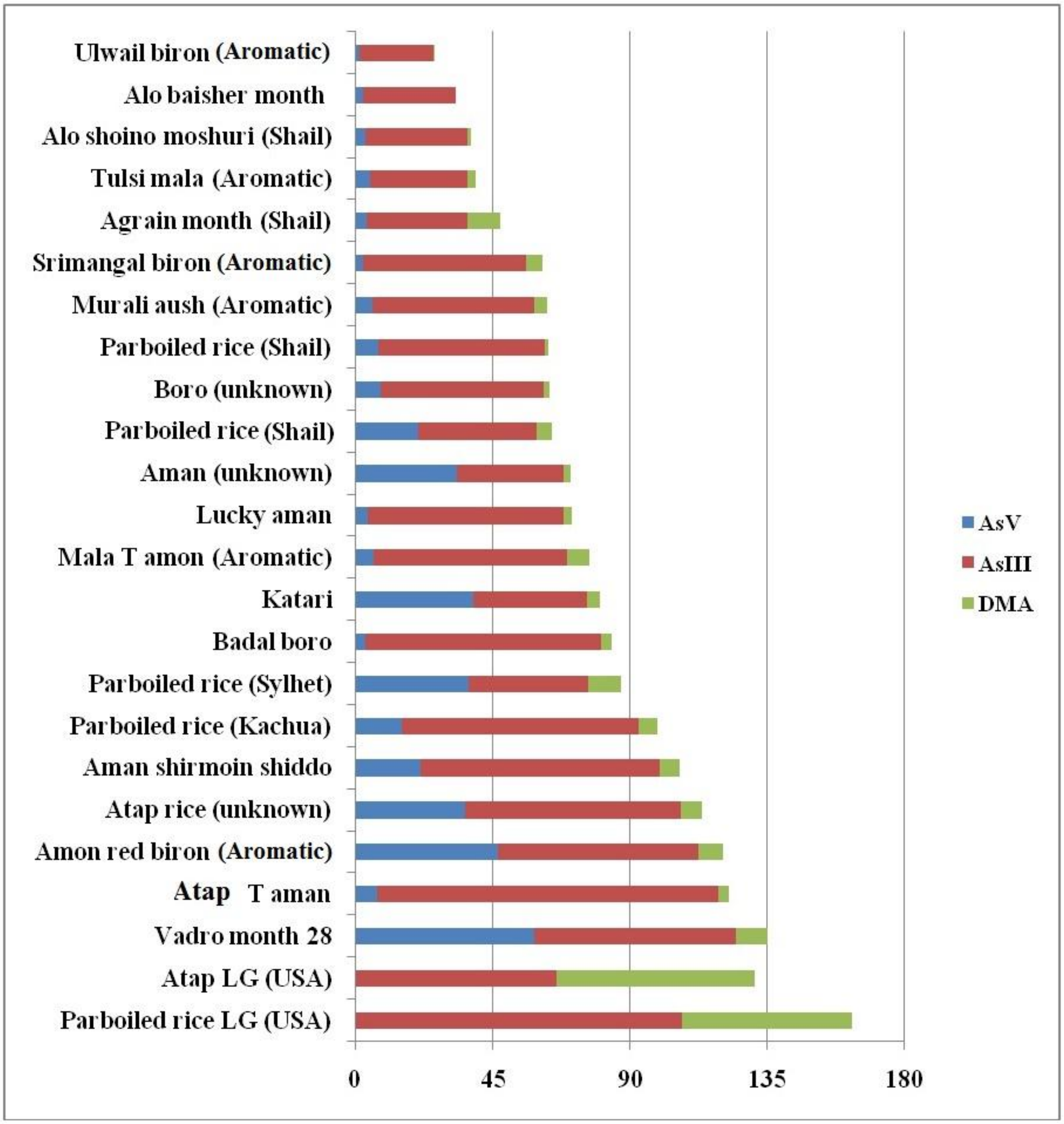


Figure 3.5: Distribution of arsenic species in Bangladeshi rice (Sylhet region). For comparison USA long grain (LG) data is also presented.

Inorganic arsenic as a contribution of PMTDI was calculated for Bangladeshi rice (see Table 3-5). The mean PMTDI of Bangladeshi rice (grown in Sylhet region) was 28.1 ± 11% with a range of 10 – 49.7%.

Figure 3-6 showed that there is a significant correlation between arsenic and the sum of total arsenic in Bangladeshi rice. Inorganic arsenic showed a strong correction (R^2 = 0.990) with sum of total arsenic in rice. In contrast, lower correlation was found for DMA (R^2 = 0.309).

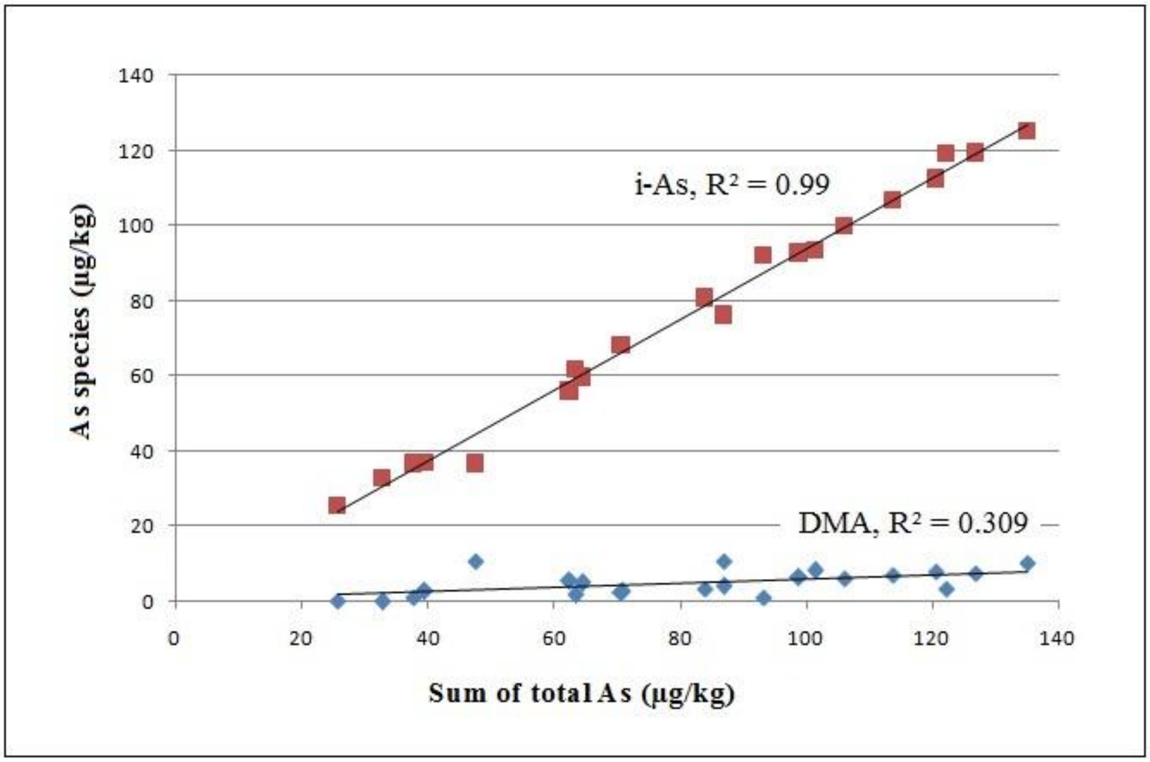


Figure 3.6: Correlation between arsenic species in Bangladeshi rice as a function of sum of species.

3.3.3 Ratios of toxic: essential trace elements in Bangladeshi rice

Ratios of trace elements (toxic:essential) for all Bangladeshi rice and the Basamati rice were presented in Figures 3-7, to 3-18. Data from Figures 3-7 to 3-18 are summarised in Table 3-6. Figure 3-7 and Table 3-6 showed As:Se ratio of 98 different types of Bangladeshi rice. Generally, aromatic rice has low As:Se ratios (Bangladeshi aromatic median: 0.84 and basamati aromatic median: 0.82) compared with non-aromatic rice (boro median; 1.87, aman median: 2.10 and parboiled median: 2.14). Figure 3-8 and Table 3-6 show As:Zn ratio of 98 different types of Bangladeshi rice. The As:Zn ratios for the Bangladeshi aromatic was 0.003 (median), for Basamati aromatic it was 0.006, for non-aromatic rice 0.006, 0.005 and 0.012 (boro, aman and parboiled, respectively). Figure 3-11 and Table 3-6 showed Pb:Se ratio of 98 different types of Bangladeshi rice. Generally, aromatic rice has low Pb:Se ratios (Bangladeshi aromatic median: 0.26 and basamati aromatic median: 0.23) compared with non-aromatic rice (boro median; 0.46, aman median: 0.59 and parboiled median: 0.45).

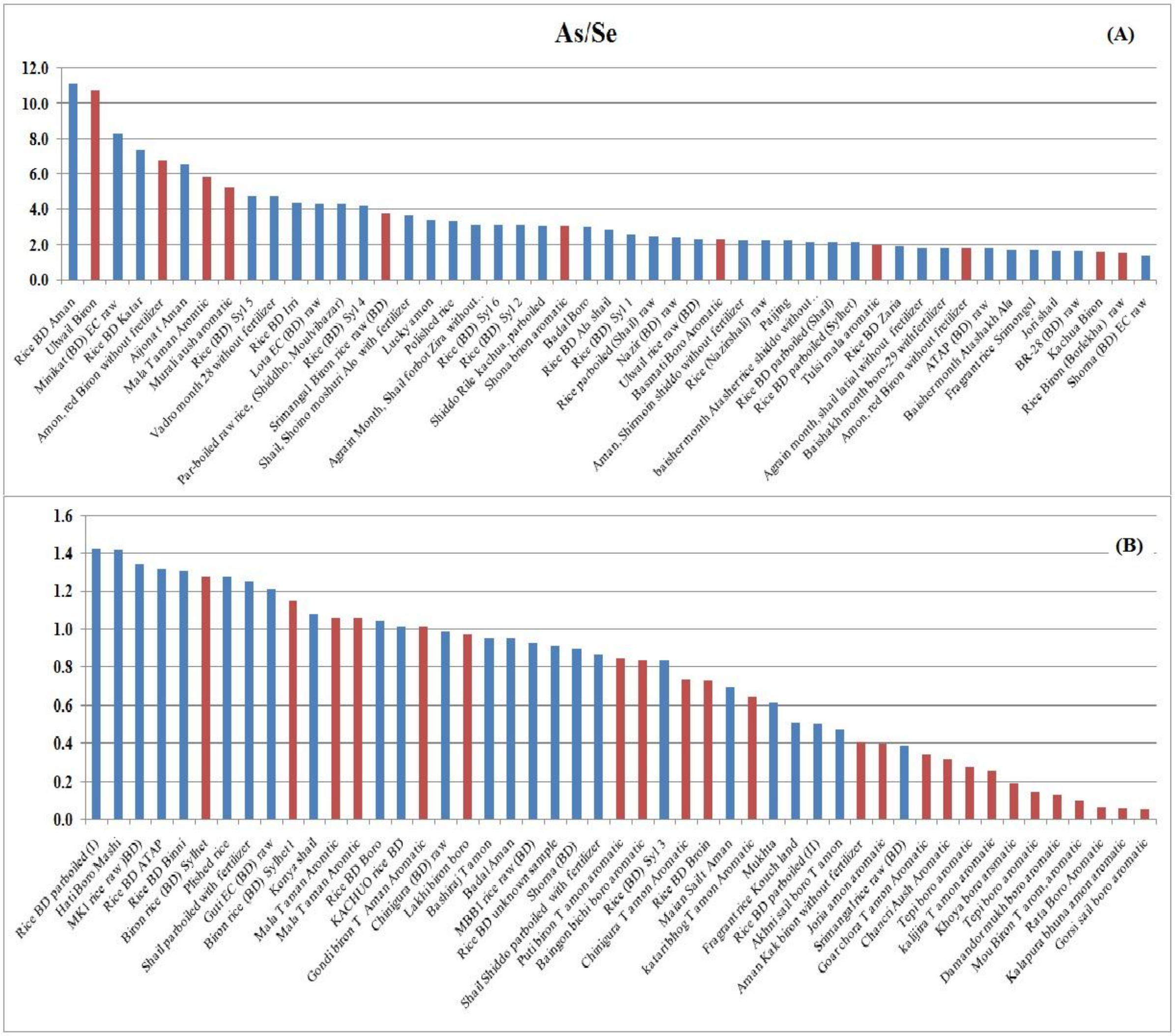


Figure 3.7: As:Se ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 12.0, (B) low scale of figure ranged from 0.0 to 1.6.

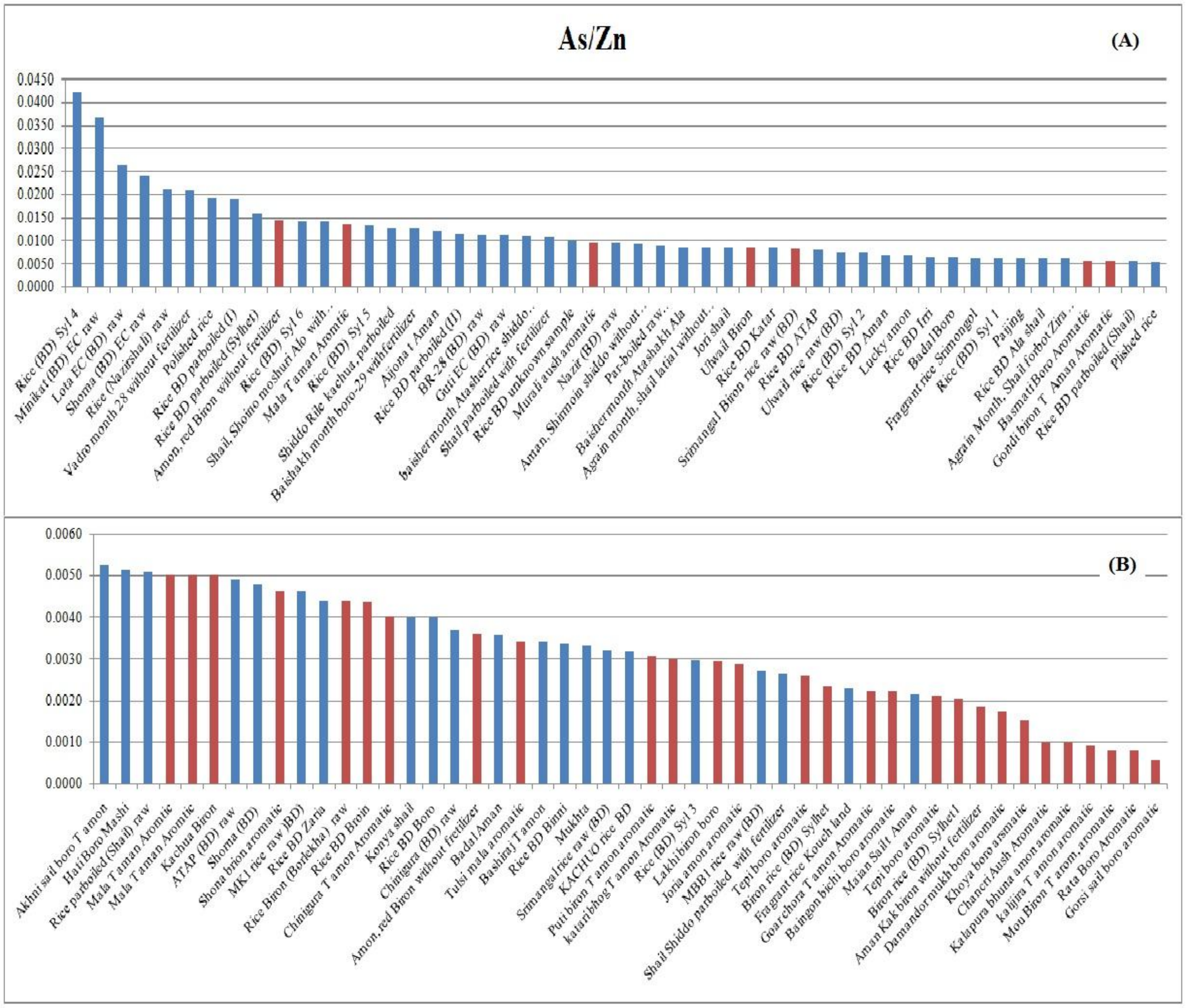


Figure 3.8: As:Zn ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 0.045, (B) low scale of figure ranged from 0.0 to 0.006.

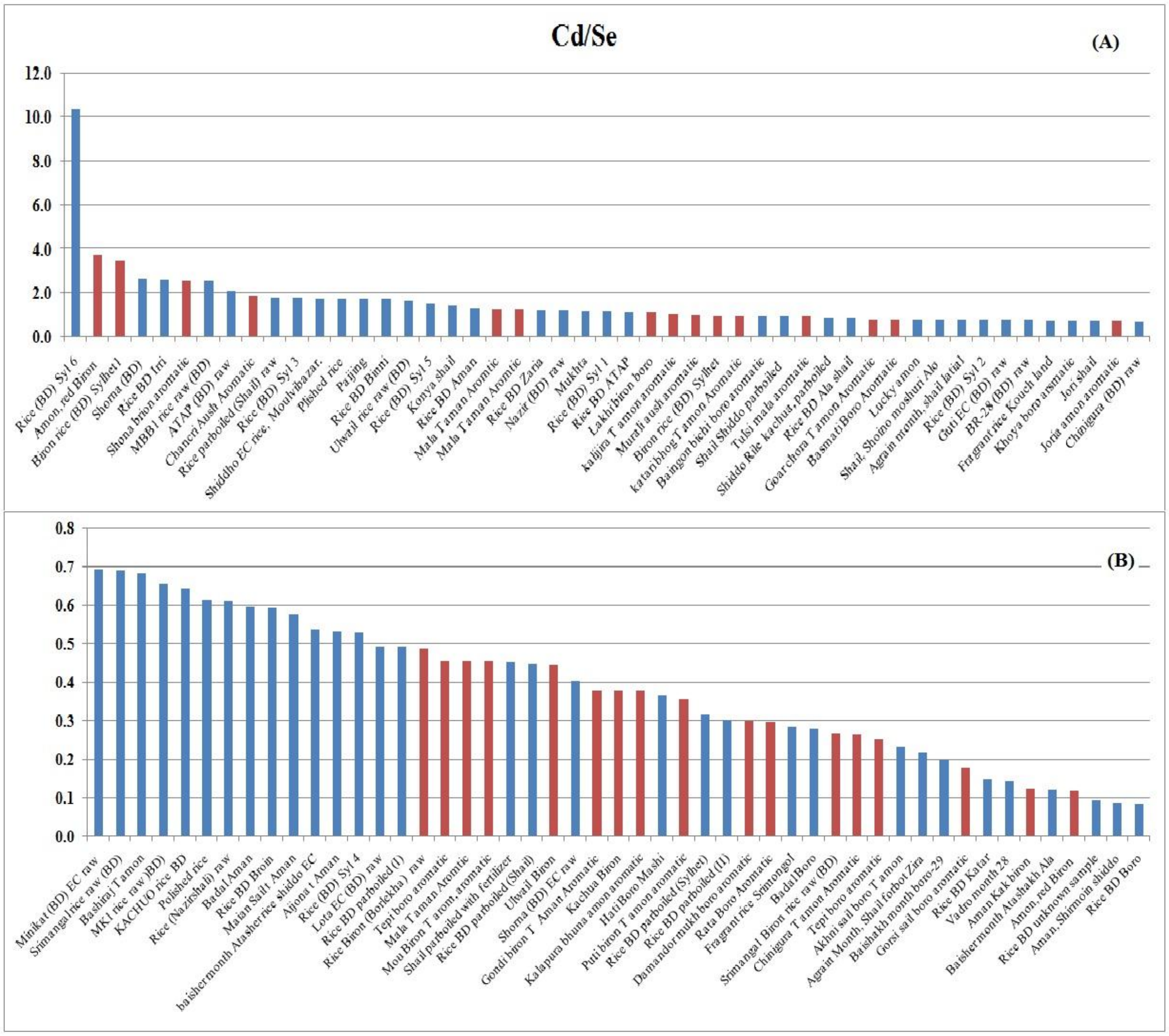


Figure 3.9: Cd:Se ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 12.0, (B) low scale of figure ranged from 0.0 to 0.8.

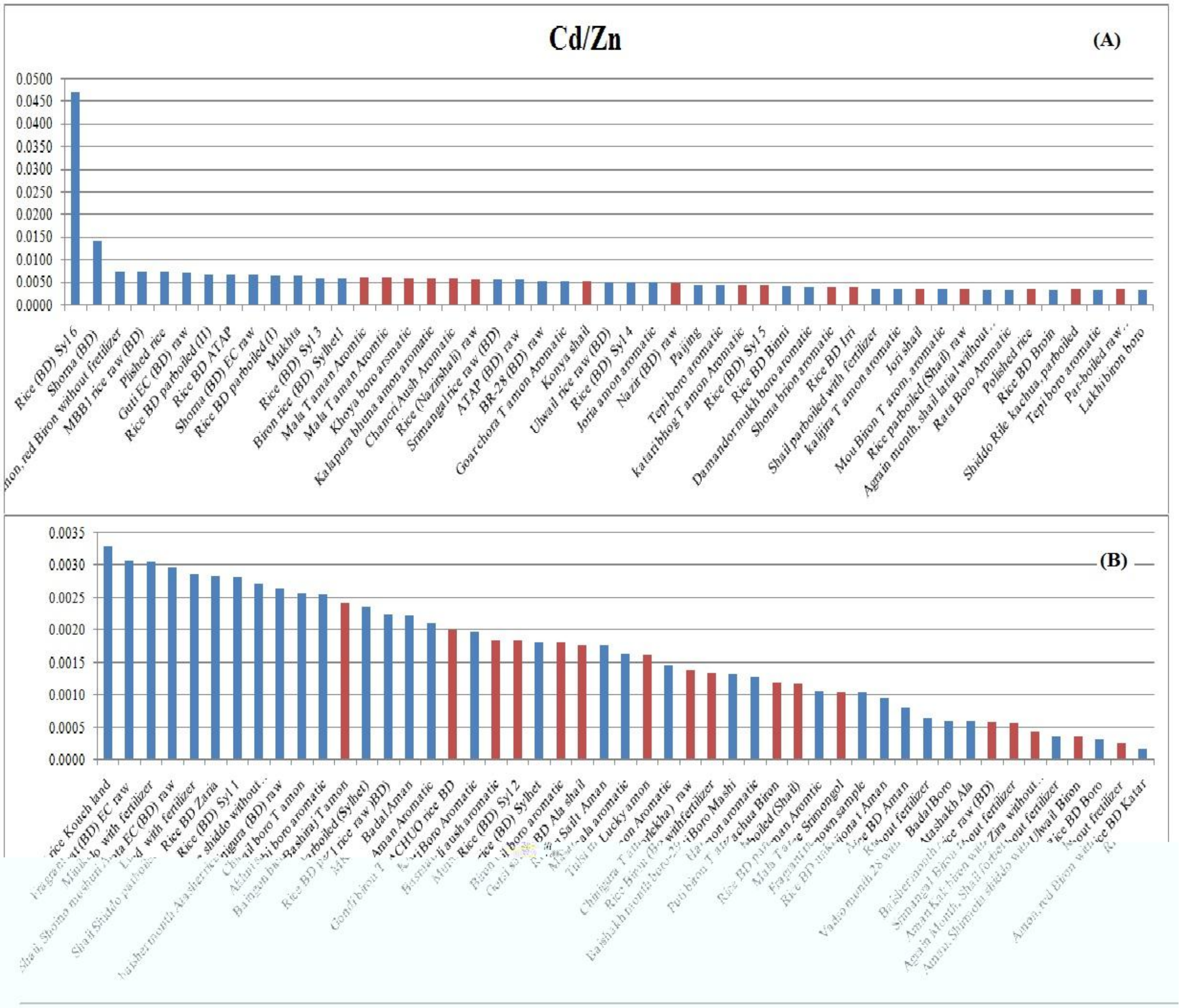


Figure 3.10: Cd:Zn ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 0.05, (B) low scale of figure ranged from 0.0 to 0.0035.

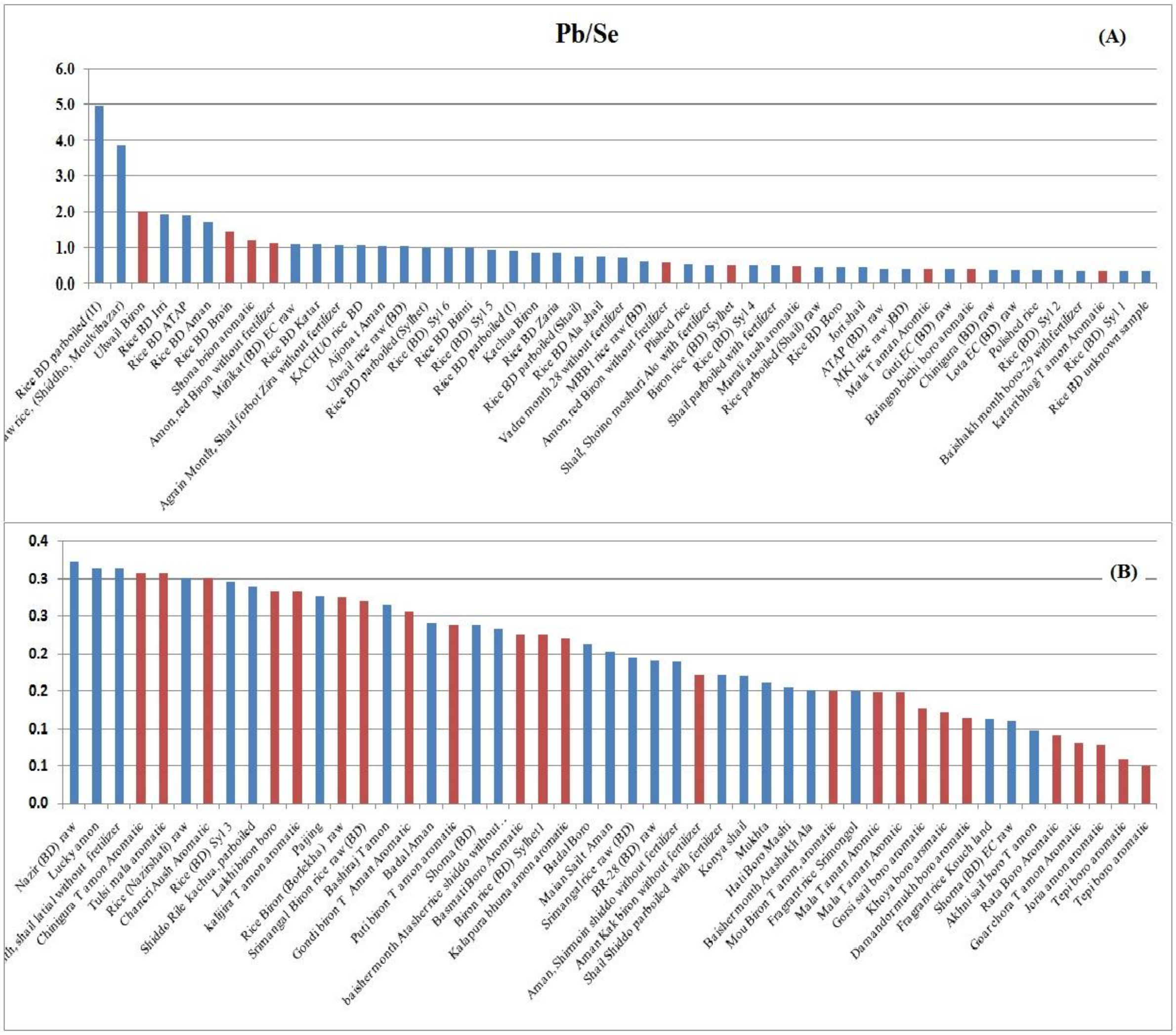


Figure 3.11: Pb:Se ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 6.0, (B) low scale of figure ranged from 0.0 to 0.4.

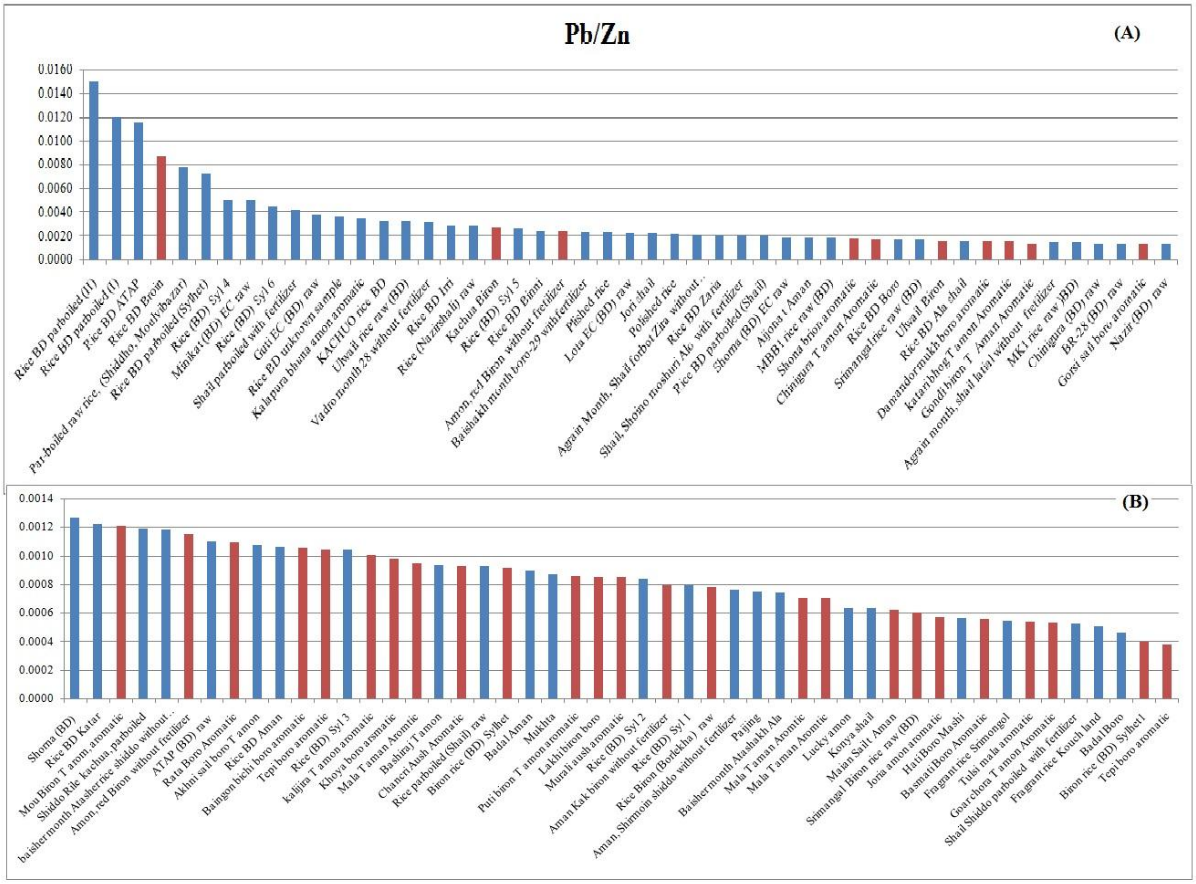


Figure 3.12: Pb:Zn ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 0.016, (B) low scale of figure ranged from 0.0 to 0.0014.

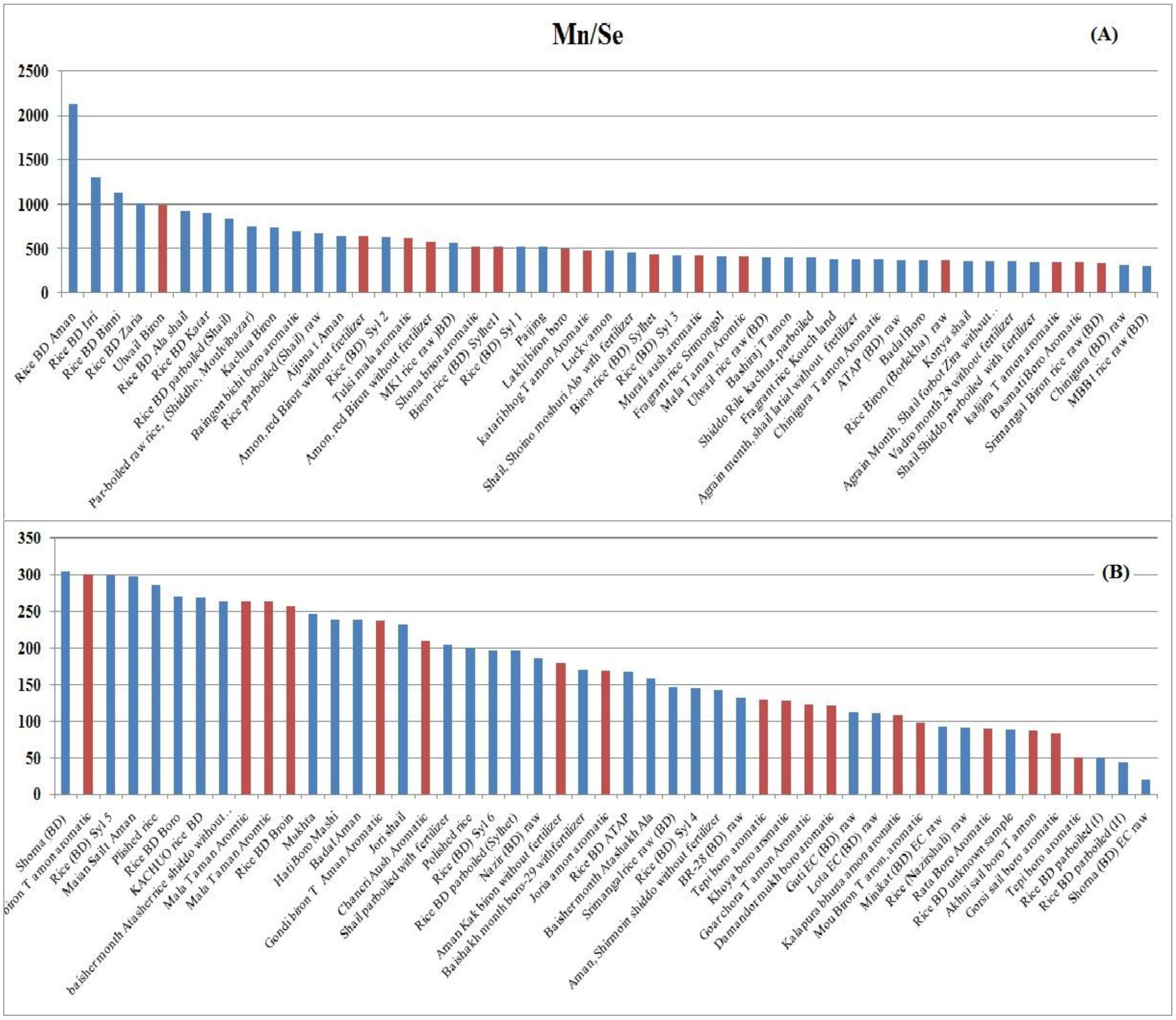


Figure 3.13: Mn:Se ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 2500, (B) low scale of figure ranged from 0.0 to 300.

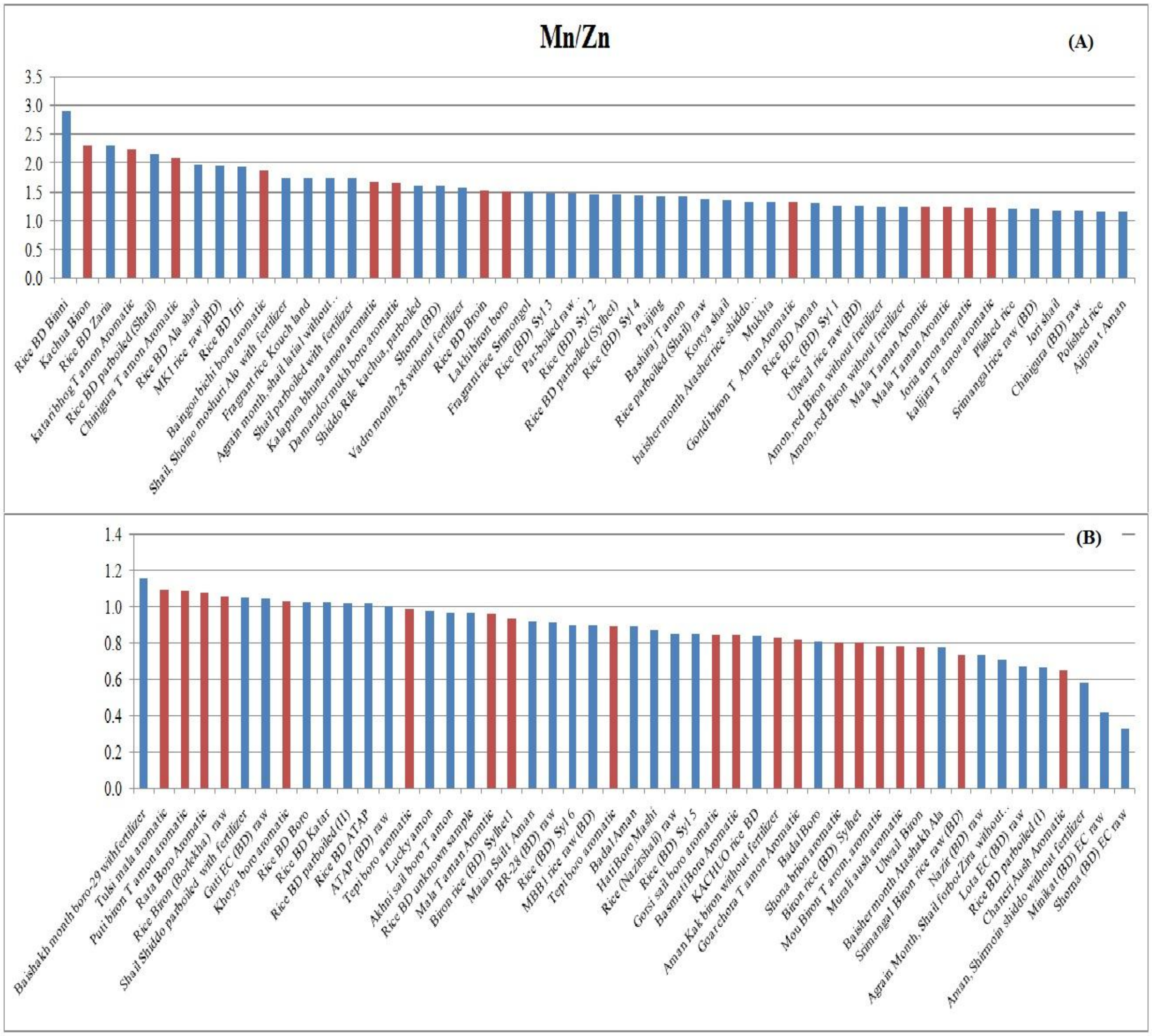


Figure 3.14: Mn:Zn ratio of 98 samples of Bangladeshi rice, blue columns represent non-aromatic rice, red columns represent aromatic rice. (A) high scale of figure ranged from 0.0 to 3.5, (B) low scale of figure ranged from 0.0 to 1.4.

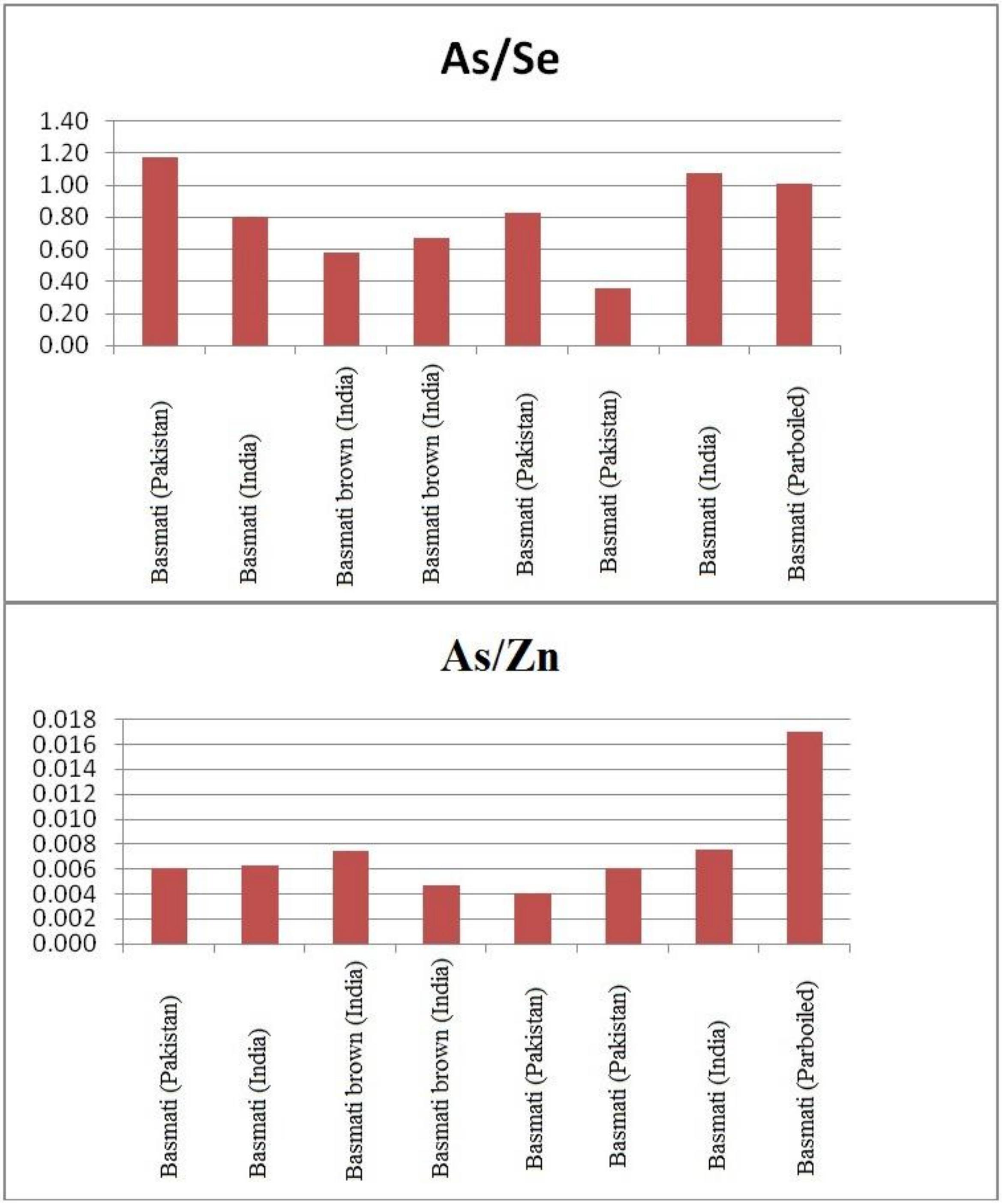


Figure 3.15: As:Se and As:Zn ratio of aromatic rice (Basmati) grown in Pakistan and India but not Bangladesh.

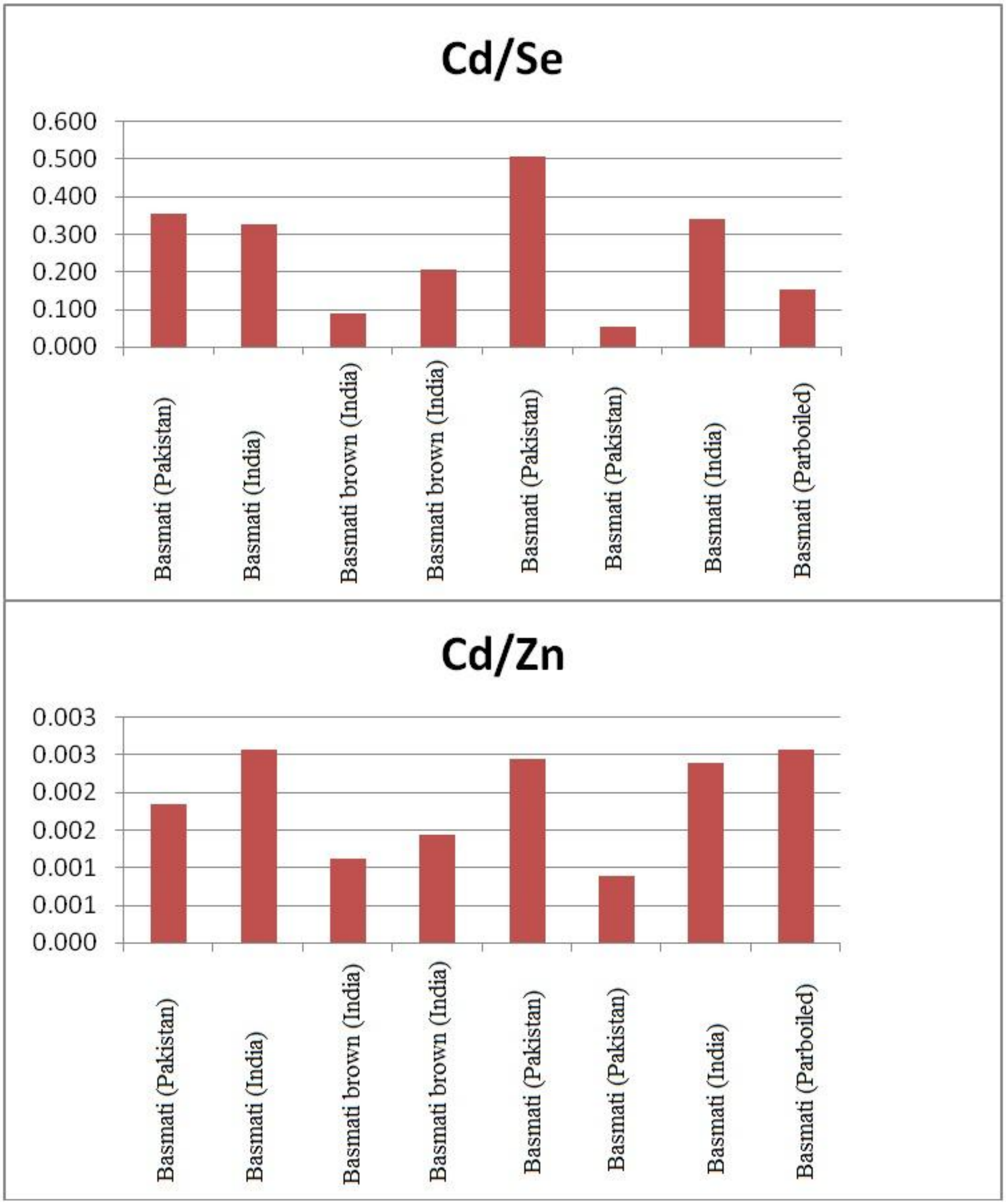


Figure 3.16: Cd:Se and Cd:Zn ratio of aromatic rice (Basmati) grown in Pakistan and India but not Bangladesh.

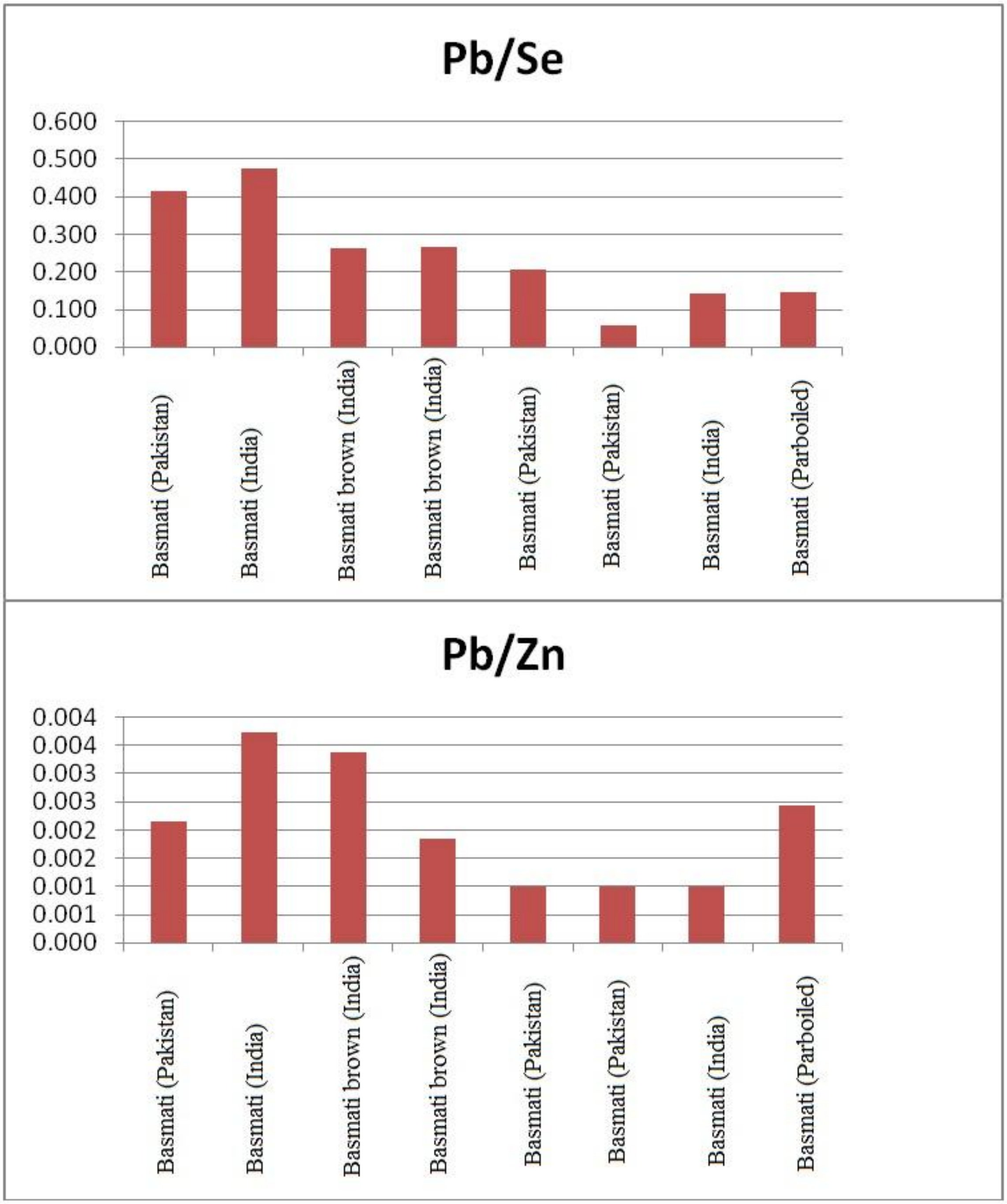


Figure 3.17: Pb:Se and Pb:Zn ratio of aromatic rice (Basmati) grown in Pakistan and India but not Bangladesh.

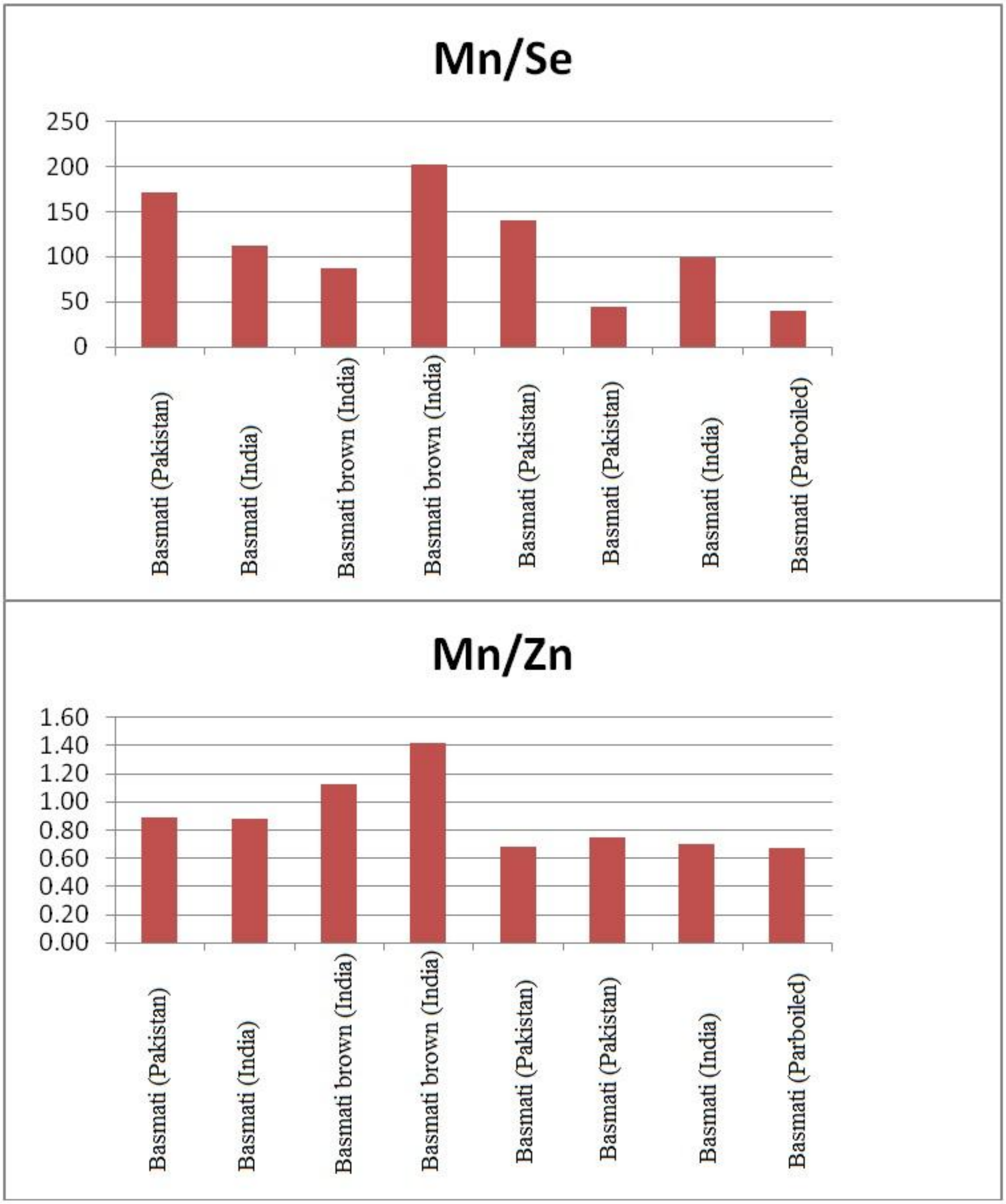


Figure 3.18: Mn:Se and Mn:Zn ratio of aromatic rice (Basmati) grown in Pakistan and India but not Bangladesh.

Table 3-6: Summary of the ratio of toxic:essential data that were presented in figures 3-5 to 3-16 for all rice groups including Sylheti rice and Basmati (Indian/Pakistan).

Ratio	**Aromatic**	**Basmati**	**Boro** **non-aromatic**	**Aman** **non-aromatic**	**Parboiled** **non-aromatic**
As:Se					
mean ± *SD*	1.33 ± 1.92	0.81 ± 0.27	2.31 ± 1.59	3.4 ± 3.4	2.44 ± 1.93
median	0.84	0.82	1.87	2.09	2.14
As:Zn					
mean ± *SD*	0.004 ± 0.002	0.007 ± 0.004	0.008 ± 0.008	0.006 ± 0.004	0.015 ± 0.009
median	0.003	0.006	0.006	0.005	0.012
Cd:Se					
mean ± *SD*	0.81 ± 0.68	0.25 ± 0.16	1.47 ± 1.98	0.82 ± 0.10	0.67 ± 0.46
median	0.66	0.27	0.99	0.62	0.49
Cd:Zn					
mean ± *SD*	0.003 ± 0.002	0.005 ± 0.009	0.005 ± 0.009	0.002 ± 0.002	0.004 ± 0.002
median	0.003	0.004	0.004	0.001	0.004
Pb:Se					
mean ± *SD*	0.36 ± 0.42	0.25 ± 0.14	0.65 ± 0.49	0.84 ± 1.03	1.00 ± 01.38
median	0.26	0.23	0.46	0.59	0.45
Pb:Zn					
mean ± *SD*	0.001 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.001 ± 0.001	0.011 ± 0.028
median	0.001	0.002	0.002	0.001	0.004
Mn:Se					
mean ± *SD*	328 ± 215	112 ± 58	468 ± 333	562 ± 548	245 ± 264
median	311	106	373	441	113
Mn:Zn					
mean ± *SD*	1.07 ± 0.41	0.89 ± 0.26	1.32 ± 0.55	01.06 ± 0.25	1.09 ± 0.48
median	1.18	0.82	1.11	1.07	1.04

SD: Standard deviation

3.3.4 Correlations of arsenic and other elements in Bangladeshi rice

Correlations of arsenic in Bangladeshi rice, grown in Sylhet region, with other elements in different types of rice were investigated. SPSS software was used for the analysis of the rice data. Paired elements correlation was used. Arsenic showed significant correlation (negative correlation) with Cd for all types of rice except parboiled rice for which there was no correlation (see Appendix 2 section 9.2). The As/Cd correlations (r value) were -0.455 (p = 0.017), -0.322 (p = 0.050) and -0.466 (p = 0.006) for boro, aman and aromatic, respectively. Interestingly, selenium has significant correlations with all the elements except Pb (in aromatic rice), Se/As (r = - 0.349, p = 0.046), Se/Cd (r = 0.551, p = 0.001), Se/Mn (r = 0.491, p = 0.004) and Se/Zn (r = 0.552, p = 0.001). A strong positive correlation was detected for Mn/Zn in parboiled and boro rice (r = 0.704 and 0.599, p = 0.034 and 0.001, respectively). In parboiled rice, corrections were found for Se with both Cd and Pb, also strong correlation was found for Cd versus Pb in the same variety. Negative correlation of As to Mn and Zn were also evident for parboiled rice, whereas, no correlations for other rice varieties were detected (see Appendix 2 section 9.2).

3.3.5 Daily intakes of arsenic and other elements

Table 3-7 showed the daily intakes of arsenic and other elements (Cd, Pb, Mn,Se and Zn). Generally, consumption of aromatic rice can result in low daily intakes of arsenic and high daily intakes of Se and Zn compared to consumption of non-aromatic rice (including boro and anam varieties). Bangladeshi aromatic rice has the lowest arsenic intake (24.2 µg/day) for someone consuming 0.5 kg of rice, however, high Se (39.6 µg/day) and Zn (6.6

mg/day) intake. Consumption of Basmati rice also results in low daily intake of arsenic (32.7 µg/day) and the highest daily intake of Se (43.9 µg/day) and Zn (6 mg/kg).

Figure 3-19 showed the daily intakes of arsenic (means of total arsenic were used) from consumption of different quantities of rice (boro, aman, aromatic and Basmati rice). Clearly, aromatic rice can result in the lowest daily intake of arsenic compared to other types of Bangladeshi rice followed by Basmati rice (an aromatic rice from Indian/Pakistan). Daily intake of arsenic from consumption of aromatic rice was 32 and 43% lower than non-aromatic rice (boro and aman varieties, respectively) (from other studies on Bangladeshi rice), respectively. Figures 3-20 and 3-21 showed daily intake of selenium and zinc for aromatic and non-aromatic rice grown in Sylhet (from the current study).

Table 3-7: Daily intake of arsenic and other elements for someone consuming 0.5 kg of rice per day.

Rice	**As*** **(µg/day)**	**Se (µg/day)**	**Zn (mg/day)**	**Cd (µg/day)**	**Pb (µg/day)**	**Mn (mg/day)**
Boro	35.8	23.1	5.1	21.1	11.1	5.7
Aman	42.9	20.2	5.7	11.2	8.1	6.1
Parboiled	39.4	26.7	4.6	14.7	16.6	6.1
Aromatic	**24.2**	**39.6**	6.6	12.6	8.3	7.5
Basmati	32.7	43.9	6.0	8.9	9.7	4.4

*total mean concentrations were used to calculate daily intake (assuming 100% of the total arsenic is present as inorganic arsenic.

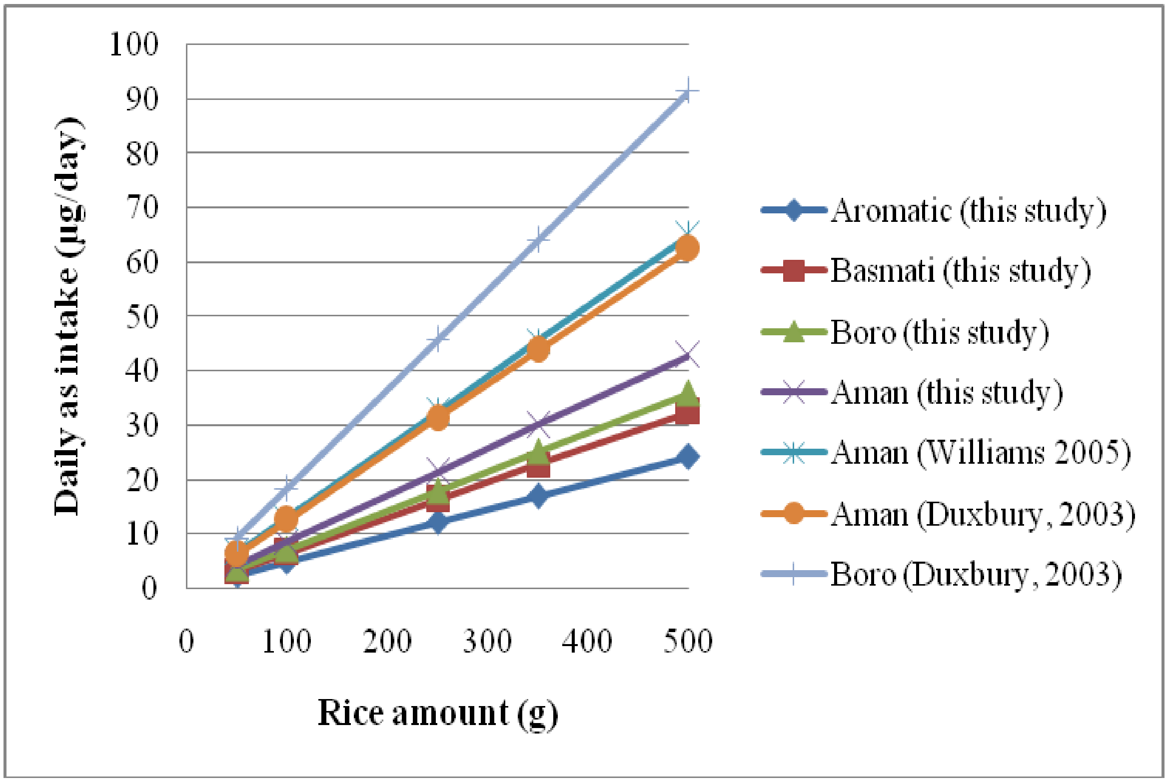


Figure 3.19: Daily intakes of total arsenic (µg /day) from consumption of different types and quantities of rice.

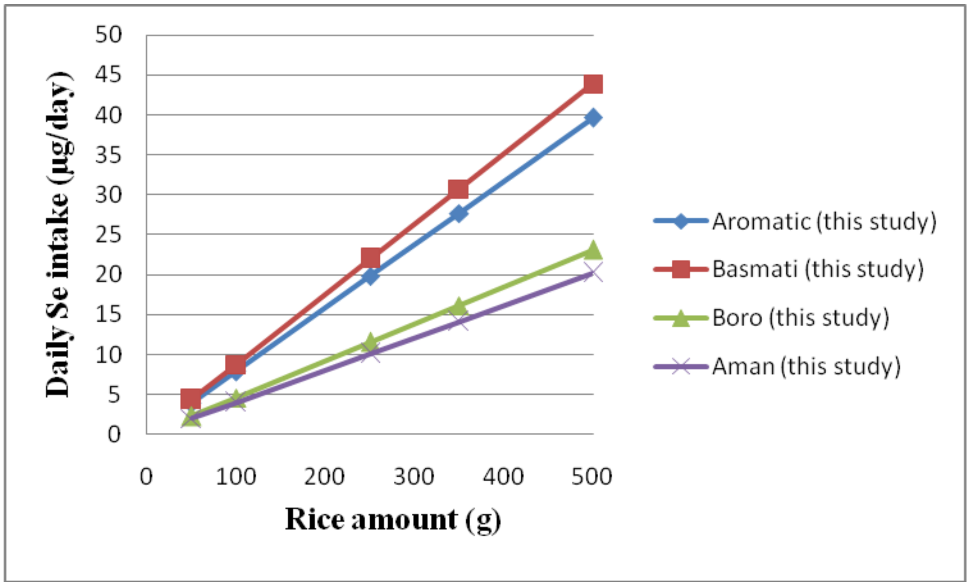


Figure 3.20: Daily intakes of total selenium (µg /day) from consumption of different types and quantities of rice.

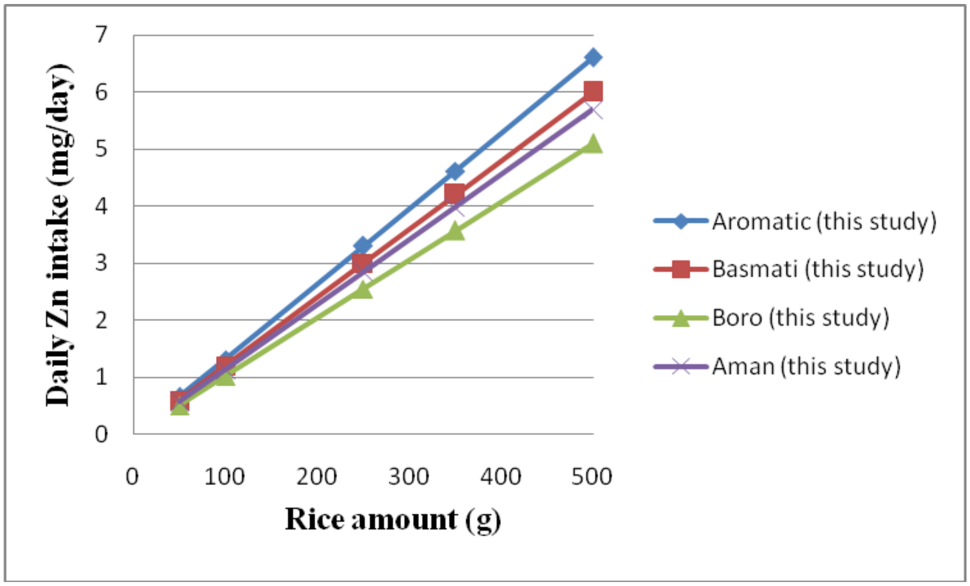


Figure 3.21: Daily intakes of total zinc (µg /day) from consumption of different types and quantities of rice.

3.4 Discussion

Rice is a staple food for Bangladeshis and it can be the main source of micronutrients in rural Bangladesh. This chapter focused on total concentrations of arsenic and other trace elements in Bangladeshi rice originating mainly from the north-eastern region of Bangladesh (Sylhet, Moulvibazar and Habiganj). Previous studies have not focused on rice from this region of Bangladesh. This region generally has lower arsenic levels in groundwater compared to other areas in Bangladesh (see Figure 3-1). Furthermore, for the first time arsenic levels in a large number of aromatic rice from Bangladesh was investigated. For comparison, an aromatic variety of rice (Basmati variety) that originated from India/Pakistan was analysed. This was done in order to see if aromatic rice is different from non-aromatic rice, in terms of arsenic content, irrespective of the region where they are grown. This aspect was further addressed by comparison with data from previous studies on few aromatic rice samples from other regions of Bangladesh that has higher groundwater arsenic levels than Sylhet.

Speciation of arsenic on selected groups of rice samples from Bangladesh was also conducted to evaluate the distribution of arsenic species in rice from Sylhet and especially aromatic rice.

3.4.1 Total concentration of arsenic in Bangladeshi rice

No previous study has focused on the analysis of rice from Sylhet region of Bangladesh although one or two samples from this region were analysed by others (Williams *et al.*, 2007). Table 3-1 provided a comparison of the total arsenic concentrations in Bangladeshi rice from previous studies (from contaminated and non-contaminated regions) and the current study. Aman (grown during the wet season), boro (grown during the dry season), atap (dry dehusking process), parboiled (wet dehusking process) and aromatic (nut-like aroma and taste) and non-aromatic rice cultivars from Sylhet region were investigated (in this thesis). Total arsenic concentrations in rice analysed by all the previous studies (mean ± SD) was 135 ± 49 µg/kg. In contrast, all rice from the Sylhet region in Bangladesh (in the current study) was 81 ± 40 µg/kg, which was 40% less than the total arsenic in previous studies. Meharg and Rahman (2003) reported the lowest arsenic content of Bangladeshi rice (non-aromatic) to be 43 µg/kg, whereas Williams *et al.* (2005) (market samples) reported that the lowest arsenic in Bangladeshi rice was 30 µg/kg. Compared to these two published studies, the lowest arsenic content in (non-aromatic) rice from Sylhet region (27.1 µg/kg) was about 37% less than the Meharg and Rahman (2003) study and 10% less than that reported by Williams *et al.* (2005). Thus the data presented in this thesis has identified non-aromatic rice from Sylhet region with the lowest arsenic levels in Bangladeshi rice thus far reported in the scientific literature.

Total arsenic in aman rice was measured by Duxbury *et al.* (2003) (from various regions in Bangladesh) and Williams *et al.* (2005) (from Bangladeshi market). The mean concentrations (and range) of arsenic in this type of rice were 125 (30 to 300 µg/kg) and 130 (30 to 300 µg/kg) for Duxbury *et al.* (2003) and Williams *et al.* (2005), respectively. The arsenic level in aman rice from the Sylhet region (mean 85.7 µg/kg, with a range of 32.6 to 161 µg/kg) was 31% and 34% lower than that reported by Duxbury *et al.* (2003)

and Williams *et al.* (2005), respectively (Table 3-1). From this it is clear that rice from the Sylhet region has lower arsenic levels compared to other parts of Bangladesh thus far investigated.

Aman rice in Bangladesh has been studied previously by different groups (Duxbury *et al.*, 2003; Williams *et al.*, 2006). The mean concentration of arsenic in aman rice was 125 µg/kg with range 72 - 170 µg/kg (Duxbury et al 2003), and the range of arsenic levels in Williams *et al.* (2006) study was 180 – 310 µg/kg for aman rice (Table 3-1). Aman rice grown in the Sylhet region (specifically studied here) showed lower mean arsenic levels (85.7 µg/kg) with a range of 32.6 – 160.8 µg/kg. The mean arsenic concentration in aman rice from Sylhet was thus 26.7% lower than that reported by Duxbury *et al.* (2003). In general, lower arsenic content has seen for aman rice than in boro rice (Duxbury *et al.*, 2003). However, it has also reported that aman rice from arsenic affected areas have higher arsenic content than aman and boro rice from low arsenic affected areas (Williams *et al.*, 2006, Roberts *et al.*, 2011). As mentioned above, it is possible that the soil characteristics and farming practice - including the use of fertilizers - in this region may be different to other parts of Bangladesh which may explain the lower arsenic level in boro rice from Sylhet.

Boro rice in Bangladesh has been studied previously and high levels of arsenic in this type of rice were detected by Duxbury *et al.* (2003) and William *et al.* (2006). The mean concentration of arsenic in boro rice was 183 µg/kg with a range of 108 - 331 µg/kg

(Duxbury et al 2003), and the range of arsenic levels in Williams *et al.* (2006) study was 210 – 270 µg/kg. In contrast, boro rice grown in the Sylhet region (specifically studied here) showed lower mean arsenic levels (71.7 µg/kg) than boro rice from other regions of Bangladesh, with a range of 27.1 – 148.1 µg/kg. The mean arsenic concentration in boro rice from Sylhet is thus 61% lower than that reported by Duxbury *et al.* (2003). The current study also showed that the lowest arsenic concentration, for non-aromatic rice, was detected in this variety (27.1 µg/kg). All of these comparisons lead to the conclusion that the Sylhet region might be a less arsenic contaminated area in Bangladesh compared to other areas. It is important to mention that the arsenic levels in groundwater from Sylhet region have been reported to be about 20 µg/L (see Figure 3-1). However, other factors may also play a role for the lower arsenic levels in rice from Sylhet, including soil properties, type of fertiliser used etc.

There are no systematic studies in the literature that have compared arsenic levels in aromatic and non-aromatic rice. Data presented in this thesis is the first study to address this. Results obtained showed that, in general, the total arsenic level in Bangladeshi aromatic rice was around 40% lower compared to non-aromatic rice. One aromatic variety showed the lowest level of arsenic (10.1 µg/kg), which was the lowest level reported for any Bangladeshi rice thus far reported in the literature. Previous studies with aromatic rice, from Bangladesh and elsewhere, is very limited (only a few samples analysed) and these have reported low total arsenic levels. Thus for example, Williams et al (2005) measured arsenic content in Chinigura variety (aromatic rice) from Dhaka market, this variety had the lowest arsenic level (30 µg/kg) compared to other rice analysed by the authors. Williams *et al.* (2005) also measured one variety of aromatic rice from Sylhet area (Zamir variety) and the arsenic content was found to be high (170 µg/kg). Recently, Ahmed et al. (2011) reported that aromatic rice contained low arsenic levels compared to other types of rice from different region of Bangladesh (Ahmed *et al.*, 2011). The important finding of the current study was that aromatic rice, from Sylhet, contained lower levels of arsenic than any previous studies of Bangladeshi rice thus far reported in the literature. The notion that aromatic rice have lower arsenic levels, irrespective of the region where they are grown, is supported by data presented in this thesis as it was found that Basmati rice samples (from India and Pakistan) have low arsenic levels that are combatable to levels seen in Bangladeshi aromatic rice. It has been suggested (Ahmed *et al.* 2011) that the genetic makeup of aromatic rice may be an important factor that results in less arsenic being accumulated in their grains compared to non-aromatic rice. Another reason that may explain the lower arsenic levels in aromatic rice is that they are normally grown during the wet season (aman season) and therefore do not require irrigation using groundwater. Furthermore, the cultivation of aromatic rice does not require the use of much fertilizer (Sarker, 2002).

Besides low arsenic levels, the other important benefit of aromatic rice identified in this study is that they also contain high levels of essential elements such as Se and Zn (see discussion below). The only drawback of aromatic rice is that their yield is lower (800-920 tons per hectare) compared to non-aromatic rice (1,350 tons per hectare) (Sarker, 2002).

Generally, it has been reported that the most of high quality rice cultivars are low yielding (Shakeel *et al.*, 2005).

Bangladeshis consume both atap and parboiled rice. Parboiling of rice involves soaking the rice in water, followed by lightly boiling the water. The rice is then dried and mechanically dehusked (Signes *et al.*, 2008b). It was reported that parboiled rice absorbs some arsenic from water and the husk during the parboiling process (Signes *et al.*, 2008b). In the current study, arsenic content of parboiled rice from the Sylhet region was analysed (mean 78.8 µg/kg). This level is also low, compared to other studies, confirming the general finding of this study, which shows low arsenic level in rice from Sylhet. The entry of arsenic into rice, during the parboiling process, could be lower if groundwater or surface water is used in the parboiling process.

3.4.2 Arsenic speciation of selected Bangladeshi rice

Arsenic speciation was carried out for selected samples of rice varieties from Sylhet region of Bangladesh which has low groundwater arsenic levels. The results obtained show that the arsenic in all varieties of rice from the Sylhet region, investigated in this study, was predominantly inorganic arsenic (69.7% of the total arsenic). This is similar to what has been reported for Bangladeshi rice from arsenic affected regions of Bangladesh. For example, Meharg *et al.* (2009) reported 61% inorganic arsenic in Bangladeshi rice. Also the findings are similar to Williams *et al.* (2005), who reported 80% inorganic arsenic for Bangladeshi rice and 81% for Indian rice. However, Williams *et al.* (2005) did not distinguish between As^{III} and As^{V}; both inorganic arsenic species was measured as total inorganic arsenic. In contrast, in the current study both As^{III} and As^{V} were detected in Bangladeshi rice, which can provide a better measure of arsenic toxicity associated with rice consumption. This is because; As^{V} is known to be less toxic than As^{III} (Naidu *et al.*, 2006). Some varieties of Bangladeshi rice, the percentage of As^{III} and As^{V} are similar species, for example Katari rice contains 50% of inorganic as As^{V} and 50% of inorganic as As^{III} (Figure 3-2). Parboiled rice contained the highest inorganic arsenic species compared to the other varieties of rice analysed (about 90%). This might be explained as being due to absorption of arsenic from water (containing mainly inorganic arsenic) during the parboiling process. This has been previously suggested by Signes *et al.* (2008b) for Indian rice. It could also be due to transfer of arsenic from the bran layer and the husk.

3.4.3 Daily intake of arsenic from Bangladeshi rice

The mean %PMTDI of arsenic for Bangladeshi rice (grown in the Sylhet region) was 28.1 ± 11% with a range 10 – 49.7%. This calculation uses the data from the arsenic speciation study and assumes an individual consuming of 500 g of rice per day. In this study, rice samples which contain high levels of arsenic were used for arsenic speciation, and the %PMTDI percentages were calculated for these samples. However, for rice containing the lowest arsenic levels, the %PMTDI values were considerably lower. For example, for aromatic rice containing 10.1 µg/kg with an assumed mean inorganic arsenic content of 69.7%, the %PMTDI will be 2.8%. The %PMTDI of arsenic is therefore lower for aromatic rice. This is expected since; aromatic rice has the lowest levels of arsenic compared to non-aromatic rice (Figure 3-19). The daily intake of arsenic from aromatic rice is 32 and 43% lower than boro and aman rice (from other Bangladeshi studies), respectively. This clearly supports the idea that aromatic rice (especially those grown in Sylhet) is potentially a healthier alternative for Bangladeshis than any other Bangladeshi rice (at least as far as what has been reported in the literature).

3.4.4 Other elements in Bangladeshi rice

Aromatic rice contained higher levels of the essential elements Se and Zn compared to non-aromatic rice. This finding is important as consumption of aromatic rice can improve Se and Zn intake in Bangladeshis. Recently, some experts have recommended zinc-biofortified rice as a solution to Zn deficiency amongst Bangladeshis (Arsenault *et al.*, 2010, Mayer, 2010; Lindstrom *et al.*, 2011). It has been reported that children and women in Bangladeshi villages have low serum zinc concentrations (Kongsbak *et al.*, 2006; Li *et al.*, 2008). In this context, rice from Sylhet especially aromatic rice can provide approx. 60% of the Recommended Daily Allowance (RDA) of zinc. Thus existing aromatic rice from Bangladesh can be used to address zinc deficiency without having to produce new varieties of genetically modified rice or hybrid rice with higher grain zinc levels. In general aromatic rice, irrespective of where they are grown, has not only lower arsenic levels but also higher Zn and Se levels. This is supported by the fact that Basmati rice, an aromatic rice from India/Pakistan, also contains similar levels of As, Se and Zn as Bangladeshi aromatic rice.

Surprisingly, no previous studies reported Mn levels in Bangladeshi rice. This is the first study to explore Mn concentrations in different types of Bangladeshi rice. The mean concentrations of Mn and Zn in rice from Sylhet were higher than that was detected in Dhaka market rice samples. Parboiled rice from Dhaka market contained low levels of

Mn and Zn compared to atap rice from the same market. These levels were also lower than rice from Sylhet region. Manganese is an essential element, but in excess it can be harmful for human health (Lucchini *et al.*, 2009). Recently, high levels of Mn were detected in Bangladeshi groundwater; Bangladeshis may be exposed to high levels of Mn through a combination of exposure from drinking water (Frisbie *et al.*, 2009) and consumption of certain types of foods and non-foods such as tea infusions and betel quid, respectively. From the current study, rice can contribute approximately 50% of the PMTDI (daily intake was calculated to be 5.5 mg/day) of Mn for Bangladeshis. Williams et al. (2007) measured Mn and other elements in US rice. Mn levels in US rice were found to be 17 ± 1 and 27 ± 3 mg/day for two different regions in USA. The former value is very similar to Mn levels in Bangladeshi rice from Sylhet region.

Recently, deficiency of zinc in soils and humans in Bangladesh has been reported (Mayer, 2010) and as mentioned earlier, children and women in Bangladeshi villages have low serum zinc concentrations (Kongsbak *et al.*, 2006; Li *et al.*, 2008, Lindstrom *et al.* 2011). Zinc-biofortified rice has been suggested as a way towards increasing zinc intake in Bangladeshis (Arsenault *et al.*, 2010, Mayer, 2010). It was recently reported that high levels of arsenic in Bangladeshi soils decreases Se and Zn levels in rice (Norton *et al.*, 2010). However, this finding appears to contradict the work of Williams *et al.* (2009) who reported that mean concentration of Se and Zn in three places in Bangladesh were rather high (Gazipur (0.12 and 16.85 mg/day); Jessore (0.05 and 13. 01 mg/day) and Faridpur (0.06 and 14.94 mg/day), respectively. These regions generally have high soil arsenic levels and yet the levels of selenium and zinc appear to be rather high. The arsenic levels reported by Williams *et al.* (2009) were higher than in the rice samples analysed in the current study. The mean arsenic concentrations in rice from the regions (contaminated area) investigated by Williams *et al.* ranged from 0.260 to 0.420 mg/day. However, the mean concentrations of Se and Zn in the current study were 0.043 and 10.5 mg/day (Table 3-3 and 3-5). It is clear that Zn contents in Bangladeshi rice for both studies indicate the presence of significant levels of Zn in rice. The levels present can provide more than 50% of the PMTDI of Zn (assuming someone consumes 0.5 kg per day). Selenium content in Bangladeshi rice (in this study) was similar to Williams *et al.* (2009) study. Aromatic varieties of rice, grown in Sylhet area contained high Se and Zn levels and lowest levels of toxic elements compared with non-aromatic rice. This data suggest that aromatic rice can be a healthier variety of rice for consumption by Bangladeshis.

The toxic elements Cd and Pb were also investigated in the current study. Levels of Cd in aromatic rice (43.1 µg/kg) were similar to non-aromatic rice (34.8 µg/kg). However, Pb levels in aromatic rice were lower (15.8 µg/kg) than non-aromatic rice (36.4 µg/kg). In this respect, aromatic rice contains low levels of toxic elements (arsenic and lead) compared to non-aromatic rice. This could be due to the fact aromatic rice is generally cultivated during the wet (aman) season and therefore is less dependent on the use of groundwater for irrigation. Furthermore, fertilisers use is less than rice grown during boro season (Basak, 2011).

Rice can be a major source of dietary intake of Cd and Pb. As already mentioned, average daily intake of rice can be 500 g per day (Duxbury *et al.*, 2003) which can provide an estimated Cd and Pb intake of 18.6 µg/day (31% of the PMTDI) and 9.1 µg/day (4.3% of the PMTDI), respectively. Means of Cd and Pb content in Bangladeshi rice measured in the current study were 37.2 and 18.1 µg/kg, respectively for the Sylhet region. The levels are similar to what was seen for samples from the Dhaka market. Furthermore, similar results for Cd levels in Bangladeshi rice were published by others. For example, Cd levels in Bangladeshi rice were found to be 33.1 µg/kg (mean value) (Khan *et al.*, 2010) and 47 µg/kg (median value) (Kippler *et al.*, 2010), respectively. High intake of rice is clearly a major factor for the elevated exposure to Cd and Pb in the Bangladeshi population.

Of the different types of rice analysed, one variety of boro rice contained the highest levels of Cd and Pb (184.7 and 109.5 µg/kg, respectively). Boro rice is grown during the dry season and is highly dependent on the use of fertilisers and irrigation (Basak, 2011). Therefore, it is possible that the higher levels of Pb and Cd in this variety of rice could be due to use of fertilisers and contaminated irrigation water. The PMTDI of Cd for a Bangladeshi person eating 500 g of rice can be as high as 92 µg/day. However, the PMTDI of Cd should be 60 µg/day (FSA, 2009) and therefore someone eating this type of boro rice will exceed the PMTDI for Cd by 53%. For Pb, the daily intake was 54.7 µg/day which is equivalent to 26% of the PMTDI [the PMTDI of Pb is 210 µg/day (FSA, 2009)] for this element. No previous studies have reported Pb content in Bangladeshi rice, and as such this is the first study to provide data for this heavy metal which is highly toxic. Recently, in a

Chinese market survey, Pb content in rice was determined and the mean concentration of Pb in Chinese rice was found to be 62 µg/kg (Qian *et al.*, 2010). This value is much higher than the levels detected (in this thesis) for Bangladeshi rice (mean 18.6 µg/kg). The reason for this difference could be due to the possibility of the Chinese rice growing in soil with higher lead levels. This is possible since China is much more industrialised compared to Bangladesh.

In terms of toxic elements:essential elements ratio, aromatic rice show much lower As:Se and Pb:Se ratios compared to non-aromatic rice (Table 3-6). This is beneficial since the levels of the toxic elements are lower relative to the essential element. In general, aromatic rice contains lower levels of toxic elements (apart from Cd which is almost similar to non-aromatic rice) and higher levels of essential elements (especially Zn and Se) compared to non-aromatic rice. All this makes aromatic rice more attractive for the diet of Bangladeshis who are already exposed to high levels of arsenic and have deficiency in the intake of Zn (Mayer, 2010) and Se (Spallholz *et al.*, 2008). If a person consumes 250 g of aromatic rice containing the lowest arsenic level detected in this study (10.1 µg As/kg) and assuming that 100% of the arsenic is present as inorganic arsenic, the PMTDI will be 2%. Obviously, if they consumed 500 g (which is the consume rice intake in Bangladesh) it will be 4%. Thus, the PMTDI for arsenic can be reduced from 28% (the mean PMTDI% calculated in this study) to 4% (7-fold lower arsenic intake) by substituting 500 g of non-aromatic rice with 500 g of aromatic rice. Furthermore, the intake of Zn and Se will be increased by 31.4%, and 125%, respectively. So overall, benefits of eating aromatic rice are a reduction in arsenic intake and an increase in the intake of essential elements.

It is known that the yield of aromatic rice is about 800-920 kg per acre compared to 1,350 kg per acre for non-aromatic (aman) rice (Sarker, 2002). However, some farmers in Bangladesh prefer to grow aromatic rice to take advantage of higher revenue. Furthermore, aromatic rice does not require much fertilizer, pesticides and irrigation compared to non-aromatic rice. Therefore, the overall cost of production of aromatic rice is lower compared to other rice (Sarker, 2002). The price of aromatic rice in Bangladesh is about double that of non-aromatic rice at most retail markets in the capital city Dhaka (The Kushtia Times, 2011). However, considering the benefits (low arsenic intake and increased intake of zinc and selenium), policymakers in Bangladesh can encourage increasing the cultivation of aromatic rice so that the price is affordable to most consumers.

3.4.5 Correlations of arsenic and other elements in Bangladeshi rice

Arsenic showed significant negative correlation versus Cd for all types of rice with the exception of parboiled rice, which showed no correlation. The negative correlation between arsenic and cadmium in rice grains is interesting. It may be related to their similar toxicology and sequestration machineries in plants (Verbruggen *et al.*, 2009). It has been reported previously that arsenic in soils could facilitate cadmium uptake in rice plants (Khan *et al.*, 2010). Cadmium in Bangladeshi paddy fields can come from the use of fertilizers such as triple superphosphate, and cadmium based pesticides (Sun *et al.*, 2008). Further studies are necessary to understand the precise reason underlying the negative correlation. No As/Cd correlation was found for parboiled rice. This could be due to the fact that the relationship between these two elements is disturbed by the additional processing of the rice grain through the parboiling process. The parboiling process can result in uptake and/or loss of certain elements from the rice or from water, it is not surprsing that As:Cd correlation is not seen in parboiled rice.

3.4.6 Limitations of the study and future directions.

A limitation of the present study is that the number of Sylheti rice samples was rather low (98 in total) including aromatic rice (34 rice samples). These samples were collected from the UK shops, some brought for personal consumption from Sylhet and others were purchased from Dhaka market. Hence information regarding soil and water properties in the paddy fields in which they were grown are not known. There is therefore a need for further studies on Bangladeshi rice samples collected from well defined fields in Sylhet and other regions so that the effect of soil characteristics, method of irrigation, fertiliser and pesticide use can be taken in to consideration.

3.5 Conclusion

Sylhet region is a relatively low arsenic contaminated area in Bangladesh; different varieties of rice that were grown in this area have been analysed. This revealed low arsenic levels compared to similar types of rice from other regions of Bangladesh. Bangladeshi rice (from both Sylhet and other regions studied) mainly contain inorganic arsenic (approx. 70%) which is similar to what has been reported in previous studies for Bangladeshi rice. Aromatic rice contained the lowest levels of arsenic and high levels of essential elements such as selenium and zinc, suggesting their potential as healthier alternatives to non-aromatic rice which is more commonly consumed in Bangladesh. Significant levels of cadmium and lead were detected in Bangladeshi rice. Since the Bangladeshis consume large quantities of rice per day, this can lead to a high daily intake of cadmium and lead from rice. An important finding of the study is that aromatic rice contained the lowest levels of toxic elements and higher levels of essential elements compared to non-aromatic rice. The %PMTDI of arsenic can be reduced by 33.7% and 19.2% if someone switches from eating non-aromatic rice (aman and boro rice, respectively) to aromatic rice.

Since rice from Sylhet region contains lower levels of arsenic compared to any other region of Bangladesh thus far studied, this region can be used for growing different cultivars of rice. This needs further investigation in the future.

4 LEVELS OF ARSENIC, CADMIUM, MANGANESE, LEAD, SELENIUM AND ZINC IN BANGLADESHI VEGETABLES AND FISH, WITH ARSENIC SPECIATION FOR FISH

Summary

Human exposure to toxic elements (As, Cd and Pb) is associated with various diseases and high levels of these elements have been detected in the Bangladeshi population warranting further research to identify the source of this exposure. In this chapter As, Cd, Mn, Pb, Se and Zn levels in 400 samples of Bangladeshi food including vegetables, cereals (rice) and fish were determined using ICP-MS. Vegetables contained high levels of Cd and Pb, especially leafy vegetables; daily intakes of Cd and Pb from vegetables consumption were estimated to be 13.14 and 40.7 µg/day, respectively. In contrast, the daily intake of Mn and Zn from vegetables consumption were 4.82 and 3.1 mg/day, respectively, whereas, the daily intakes for As and Se were 5.16 µg/day and 8.66 µg/day, respectively. Of the different vegetables, lal shak (*Amaranthus tricolor*) contained the highest Cd level [303 µg/kg (fresh weight); mean 100.5 (SD 95) µg/kg].

Bangladeshi rice also showed significant concentration of arsenic and cadmium (mean 68.6 and 37.2 µg/kg, respectively) and it is also the main source of dietary intakes of Mn and Zn. Clearly a more balanced diet is necessary to reduce toxic elements intake in the

Bangladeshi population, especially by reducing the very high intake of rice and certain vegetables.

Bangladeshi fish (Hilsha species) contained high levels of total arsenic (mean ± SD was 2.55 ± 1.3 mg/day), and for the first time speciation analysis of this popular Bangladeshi fish was conducted. Species detected in this fish included DMA (69% of total As), arsenobetaine (11% of total As) and arsenosugars (20% of total As). Although inorganic arsenic was not detected in Hilsha, this fish contains high amount of DMA which is known to be more toxic than arsenobetaine.

4.1 Introduction

Bangladeshis mainly consume rice, and large quantities of vegetables and some fish. These can be a source of high exposure to toxic elements (As, Cd and Pb). Toxic elements such as As, Cd and Pb can accumulate in the body for a long time. For example Cd remain in the body with a half-life 10-30 years (Jarup *et al.*, 1998) and it can disrupt a number of biological processes in human organs such as in the kidneys and the lungs (Bernard, 2008). Cadmium exerts its toxic effects mainly on the kidney. It has been reported that over 20 million people in Bangladesh suffer from kidney disease, especially chronic kidney disease (UNB, 2011). Certain populations (in Bangladesh) are exposed to high levels of Cd. For example, Cd concentrations in breast milk of Bangladeshi women have been shown to be the highest (median 0.14 µg/L) compared to other countries (median < 0.1 µg/L) (Kippler *et al.*, 2009). These researchers also found high Cd exposure among children in rural Bangladesh (Kippler *et al.*, 2010). The precise reason for the high Cd levels remains unknown although intake from food is considered to play an important role. Pb can also accumulate in the human body for long periods and an association between Pb exposure and neurotoxicity has been reported (Verstraeten *et al.*, 2008).

As discussed in the introductory chapter, Mn, Se and Zn are essential elements for human health. However, both excess exposure and deficiency of these elements can affect human body functions and cause different diseases (Fergusson, 1990, Li, 2007 and Santamaria, 2008).

As levels in some Bangladeshi vegetables in different districts of Bangladesh have been reported (Alam *et al.* 2003, Das *et al.* 2004 and Karim *et al.* 2008). Very few studies have focused on other trace elements such as Cd, Pb, Mn and Zn in Bangladeshi foods, although some recent studies have determined toxic elements in foods including vegetables (Alam wt al. 2003, Roychodhury *et al.* 2003 and Karim *et al.* 2008). Concentrations of Cd in vegetables were found to be significantly high, especially in leafy vegetables. Many studies have reported Cd levels in vegetables (Cui *et al.*, 2004, Kachenko and Singh, 2006, Karavoltsos *et al.*, 2008, Mor and Ceylan, 2008, Muchuweti *et al.*, 2006, Yan *et al.*, 2009, Zheng *et al.*, 2007). However, the number of studies analysing Cd and other toxic elements focusing on Bangladesh is limited. In one such study, heavy metal levels in different leafy and non-leafy vegetables from Samta village (Jessore district, Bangladesh) were measured (Alam *et al.*, 2003). More recently, vegetables from three different areas (contaminated and non-contaminated) in Bangladesh have been investigated to determine Cd levels (Naser *et al.*, 2009). In the most recent study, Khan *et al.* (2010) measured Cd levels in Bangladeshi rice and some vegetables, and they suggested that significant levels of Cd have entered the food-chain of Bangladeshis.

Manganese, selenium and zinc are essential elements, their levels in Bangladeshi vegetables and fish needs more studies to estimate the daily intakes of these elements through consumption of Bangladeshi foods. A recent study showed that Bangladeshi people may have Se deficiency in their diet (Spallholz *et al.* 2008). Zinc and other elements were determined in Bangladeshi foods by Alam *et al.* (2003); high levels of Zn were detected in leafy and non- leafy vegetables (4.8 mg/kg). Despite the fact the Bangladeshi population depend mainly on fish as the main animal product in their diet, few studies in the literature have determined the arsenic content of Bangladeshi fish (Das *et al.*, 2004) and no arsenic speciation studies for fish has been reported. Many studies have reported arsenic levels in sea fish from other parts of the world (Baeyeans *et al.*, 2009, Vieira *et al.*, 2011, Storelli *et al.*, 2004). Recently, Baeyeans *et al.* (2009) measured As in different types of sea fish from the North Sea and reported mean ± SD of total As as 12.83 ± 12.01 µg/kg (wet weight); organic arsenic species were the major form of arsenic in these fish types and the percentage of inorganic arsenic was 1.03%.

In this chapter, total concentrations of six elements - three toxic (As, Cd, Pb) and three essential (Mn, Se, Zn) - in 400 Bangladeshi products (vegetables and fish) that are consumed by humans were determined. Speciation of arsenic in one of the most popular Bangladeshi fish (Hilsha) was conducted. Daily intakes of the six elements from Bangladeshi foodstuffs were estimated.

4.2 Materials and Methods

4.2.1 Sample collection

Different types of Bangladeshi foods (including rice, leafy and non-leafy vegetables and fishes) (n = 400 samples), which are widely consumed amongst Bangladeshis, were purchased from Bangladeshi shops and UK markets between September 2008 to November 2009. Vegetables and fish samples were of Bangladeshi origin and are popular with the

Bangladeshi communities living in the UK and in Bangladesh. Furthermore, foods such as rice brought into the UK from Bangladesh for personal consumption were also analysed.

4.2.2 Sample preparation

4.2.2.1 Food preparation

See section (2.2.2.) in methodology chapter.

4.2.2.2 Sample digestion

See section (2.2.3.1.) in methodology chapter.

4.2.2.3 Elemental Determination

Concentrations of As, Cd, Mn, Pb, Se and Zn in the digested sample solutions were determined by ICP-MS (see section 2.2.4.).

4.2.2.4 As speciation in Hilsha fish samples

Dried (0.2 g) Hilsha fish (see Figure 4-1) samples (flesh and eggs) were extracted in aqueous solution using a microwave digester (see chapter 2). Subsequently, the arsenic species were detected by HPLC-ICP-MS (see section 2.2.7.1.).

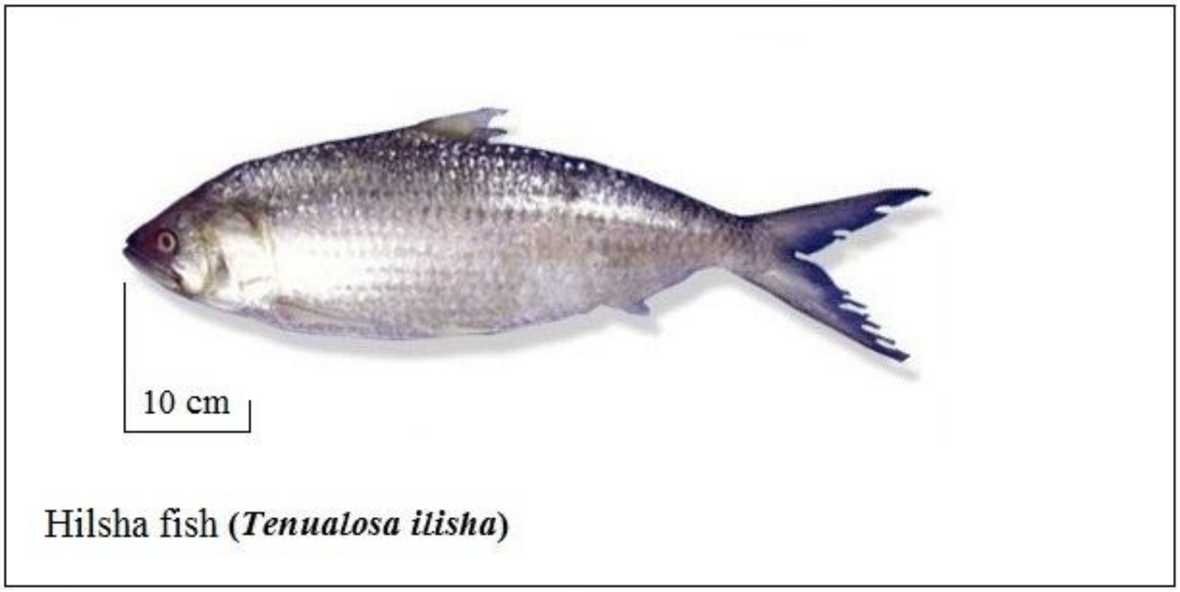


Figure 4.1: Hilsha ilisha (*Tenualosa ilisha*) is one of the popular fish in Bangladesh.

4.2.3 Quality Control and standard reference material

See Table 2-5 in methodology chapter Section (2.2.5.).

4.3 Results

4.3.1 Arsenic and other elements levels in fish

4.3.1.1 Total levels of arsenic in fish

Fish is the major animal product consumed in Bangladesh, and here in this study, 11 varieties of freshwater fish and one variety of sea fish (Hilsha type) sold in ethnic Bangladeshi shops in the UK were analysed (Table 4-1). Concentrations of arsenic in fish were determined. The highest arsenic was detected in Hilsha eggs (*Tenualosa ilisha*) 6.14 mg/kg. Mean concentrations of arsenic ranged from 0.005-3.4 mg/kg for big fish flesh (size was 40 to 75 centimetres) and 0.1-2.5 mg/kg for small fish flesh (size less than 15 centimeters) (Table 4- 1).

4.3.1.2 Total levels of cadmium and lead in fish

Concentrations of Cd and Pb in fish were determined. The highest Cd was detected in Hilsha eggs (*Tenualosa ilisha*) 1725 µg/kg, and the highest Pb level (1160 µg/kg) was detected in Rhui fish (*Labeo rohita*). Mean concentrations of Cd and Pb ranged from 0.6 – 22.6 and 19 – 250 µg/kg for big fish flesh and 1.8 – 188 and 15.8 – 124.5 µg/kg for small fish flesh, respectively. High Cd level was detected in Shoal fish (*Micropterus cataractae*) (in liver) 121 µg/kg compared with 1.1 µg/kg in Shoal fish (flesh), whereas, low Cd levels were 0.7 to 1.5 µg/kg for Rhui fish and Mrigal fish (*Cirrhinus cirrhosus*), respectively (Table 4- 2).

4.3.1.3 Total levels of manganese, selenium and zinc in fish

High levels of manganese in fish were detected. The manganese levels were 2.93, 2.84 and 1.76 mg/kg for Rhui fish, silver fish (small fish) and Ayer fish, respectively. Hilsha egg contains the highest mean concentration of Mn (3.47 mg/kg) (Table 4-3). Generally, Se and Zn concentrations in small fish are higher than in big fish (Table 4-1, 4-3). Se levels were 51.91 and 23.15 mg Se/kg (mean value) in Keski fish and sliver coloured fish (small fish), respectively. In contrst, Se levels in big fish ranged between 0.08 – 1.66 mg/kg (Table 4-1). Zinc levels were 140.6 and 45 mg/kg (mean value) in Keski fish and Nodoi fish, respectively, compared with 14 and 25.5 mg/kg in Hilsha fish and Shoal fish (liver), respectively (Table 4-3).

Table 4-1; As and Se (mg/kg dry weight) levels in different types of fish consumed by Bangladeshis.

Sample	***n***	**As**			**Se**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Big fish							
Hilsha (*Tenualosa ilisha*) (BD)	15	2.55 ± 1.3	0.77	4.44	0.90 ± 1.31	0.21	1.25
Hilsha (Myanmar)	3	4.94 ± 0.7	4.36	6.15	0.43 ± 0.03	0.41	0.46
Rhui (*Labeo rohita*) (BD)	14	0.03 ± 0.03	0.006	0.153	0.26 ± 0.02	0.05	0.35
Rhui (Myanmar)	1	0.105	-	-	0.54	-	-
Ayre (*Sperata aor*)	5	0.014 ± 0.01	0.004	0.032	0.14 ± 0.13	0.03	0.36
Shoal (*Micropterus cataractae*)	1	0.012	-	-	3.06	-	-
Shoal fish (liver)	1	0.016	-	-	1.66	-	-
Mrigala (*Cirrhinus cirrhosus*)	1	0.005	-	-	0.08	-	-
Small fish							
Puti (*Puntius puntio*)	5	0.14 ± 0.04	0.02	0.18	4.16 ± 2.52	3.8	7.4
Silver fish (Unknown type)	2	2.55 ± 0.6	2.2	3.1	23.15 ± 6.31	21.5	30.5
Keski (*Carica soborna*)	2	1.4 ± 0.2	1.2	1.5	51.91 ± 0.43	51.4	52
Chapica (*Gudusia chapra*)	2	0.39 ± 0.2	0.21	0.49	5.69 ± 2.72	4.8	8.6
Taki (*Channa punctata*)	2	0.09 ± 0.007	0.08	0.09	0.89 ± 0.48	0.81	1.5
Nodoi (*Nandus nandus*)	2	0.15 ± 0.04	0.12	0.18	7.63 ± 0.89	6.9	8.2
Tit punti (*Puntius ticto*)	2	0.103 ± 0.02	0.09	0.12	1.72 ± 1.26	1.5	3.3
Eggs							
Fish egg (*Hilsha*) (BD)	10	3.2 ± 1.4	1.3	4.8	2.04 ± 0.98	0.71	2.80
Fish egg (*Hilsha*) (Myanmar)	1	4.8	-	-	1.48	-	-
Chicken egg (white) (UK)	4	0.002 ± 0.002	0.001	0.004	0.64 ± 0.47	0.45	0.87
Chicken egg (yolk) (UK)	4	0.0005	0.0003	0.0007	0.95 ± 0.31	0.65	1.12

BD: Bangladesh.

Table 4-2: Cd and Pb levels (µg/kg dry weight) in different types of fish consumed by Bangladeshis.

Sample	***n***	**Cd**			**Pb**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Big fish							
Hilsha (*Tenualosa ilisha*) (BD)	15	22.6 ± 31	5.7	173	62 ± 61	22.4	240
Hilsha (Myanmar)	3	11.2 ± 3.2	8.7	14.9	36.5 ± 18.7	15.9	52.2
Rhui (*Labeo rohita*) (BD)	14	4.1 ± 4.7	0.3	13.1	250 ± 328	42.6	1160
Rhui (Myanmar)	1	0.6	-	-	21.6	-	-
Ayre (*Sperata aor*)	5	4.2 ± 3.5	1.5	10.4	67.6 ± 15	28.8	74.7
Shoal (*Micropterus cataractae*)	1	1.1	-	-	32.8	-	-
Shoal fish (liver)	1	121	-	-	88.7	-	-
Mrigala (*Cirrhinus cirrhosus*)	1	1.5	-	-	19	-	-
Small fish							
Puti (*Puntius puntio*)	5	8.1 ± 4.4	4	13.7	240 ± 211	81	608
Silver fish (Unknown type)	2	188 ± 54	172	250	114 ± 35	95	145
Keski (*Carica soborna*)	2	22 ± 2.7	20	24	78 ± 14	65	85
Chapica (*Gudusia chapra*)	2	7.8 ± 0.7	7.2	8.2	32.4 ± 5.3	29	37
Taki (*Channa punctata*)	2	1.8 ± 0.7	1.4	2.4	15.8 ± 11.2	10	26
Nodoi (*Nandus nandus*)	2	2.3 ± 0.3	2.1	2.5	32 ± 14.6	21	41
Tit punti (*Puntius ticto*)	2	1.9 ± 0.4	1.6	2.2	22.4 ± 11	16	31
Eggs							
Fish egg (*Hilsha*) (BD)	10	278 ± 518	7.4	1725	62.1 ± 39	9.4	138
Fish egg (*Hilsha*) (Myanmar)	1	7.4	-	-	9.4	-	-
Chicken egg (white) (UK)	4	0.5 ± 0.4	0.3	0.7	15.9 ± 9.1	11	21
Chicken egg (yolk) (UK)	4	0.9 ± 0.4	0.5	1.2	15.3 ± 11	10	22

BD: Bangladesh.

Table 4-3: Mn and Zn levels (mg/kg dry weight) in different types of fish consumed by Bangladeshis.

Sample	***n***	**Mn**			**Zn**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Big fish							
Hilsha (*Tenualosa ilisha*) (BD)	15	0.7 ± 0.5	0.1	7.2	13.4 ± 11.3	2.7	22.5
Hilsha (Myanmar)	3	0.5 ± 0.2	0.2	0.6	4.3 ± 1.2	3.5	5.7
Rhui (*Labeo rohita*) (BD)	14	2.9 ± 3.9	0.4	1.1	6.9 ± 5.7	1.8	6.3
Rhui (Myanmar)	1	0.5	-	-	11	-	-
Ayre (*Sperata aor*)	5	1.7 ± 1.4	0.4	3.2	3.3 ± 0.2	3.0	3.4
Shoal (*Micropterus cataractae*)	1	0.13	-	-	4.2	-	-
Shoal fish (liver)	1	1.5	-	-	25.5	-	-
Mrigala (*Cirrhinus cirrhosus*)	1	0.34	-	-	2.9	-	-
Small fish							
Puti (*Puntius puntio*)	5	6.4 ± 4.8	2.2	7.3	16.4 ± 8.3	7.3	27.7
Sliver fish (Unknown type)	2	2.8± 0.8	2.3	3.4	25 ± 3.7	21	26
Keski (*Carica soborna*)	2	0.8 ± 0.07	0.7	0.8	140 ± 1.1	140	141
Chapica (*Gudusia chapra*)	2	0.6 ± 0.2	0.4	0.7	26.9 ± 16.4	21	44
Taki (*Channa punctata*)	2	0.2 ± 0.007	0.19	0.20	6.4 ± 1.3	6.1	7.9
Nodoi (*Nandus nandus*)	2	0.4 ± 0.2	0.2	0.5	45 ± 9.4	41	54
Tit punti (*Puntius ticto*)	2	0.3 ± 0.007	0.29	0.30	16.2 ± 0.3	16	16.4
Eggs							
Fish egg (*Hilsha*) (BD)	10	3.5 ± 1.7	2.0	5.6	26.5 ± 20	16.1	37.1
Fish egg (*Hilsha*) (Myanmar)	1	3.4	-	-	10.6	-	-
Chicken egg (white) (UK)	4	0.09 ± 0.05	0.08	0.09	1.2 ± 0.5	0.9	1.4
Chicken egg (yolk) (UK)	4	1.5 ± 0.05	1.48	1.51	46.3 ± 13.3	31	56

BD: Bangladesh.

Figures 4-2 to 4-9 shows the ratio of As:Se, As:Zn, Cd:Se, Cd:Zn, Pb:Se, Pb:Zn, Mn:Se and Mn:Zn for different types of Bangladeshi fish and fish eggs. The ratio between toxic and essential elements can be used as a guide for identifying the healthy fish species. Fish with lower ratio can be considered to be less harmful and healthier species for human consumption. Hilsha fish and its eggs showed the highest ratios of As and Cd with both Se and Zn. However, small fish group have the lowest ratios, especially Keski fish. Rhui and Ayer fish have the highest ratios of Pb and Mn with Se and Zn compared with other types of fish. The latter ratios are also low for the small fish.

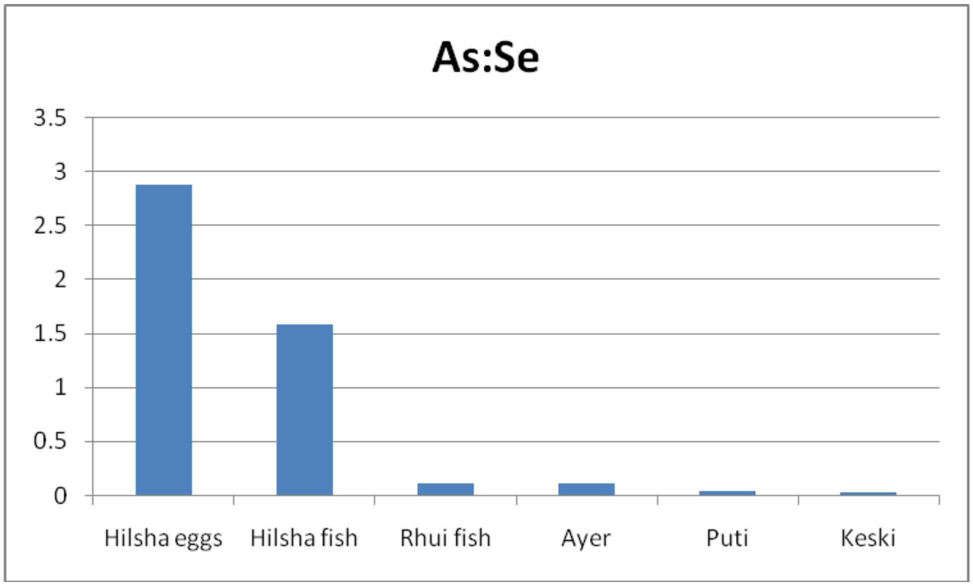


Figure 4.2: Arsenic and selenium ratio of Bangladeshi fish.

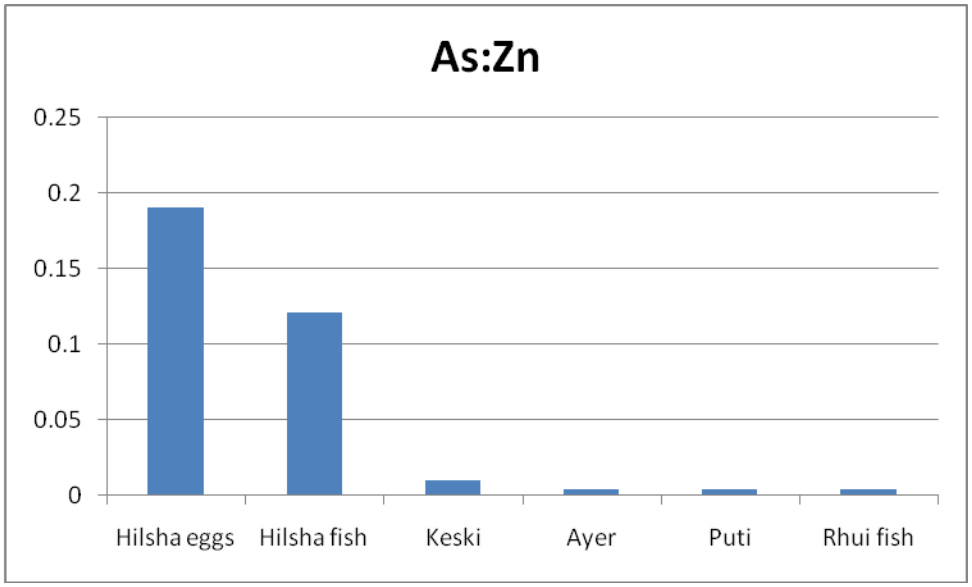


Figure 4.3: Arsenic and zinc ratio of Bangladeshi fish.

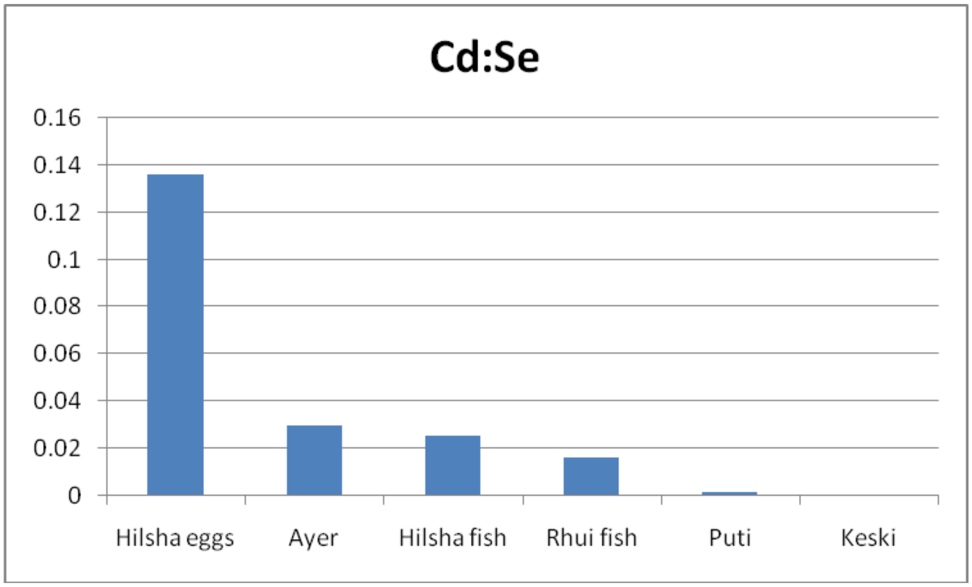


Figure 4.4: Cadmium and selenium ratio of Bangladeshi fish.

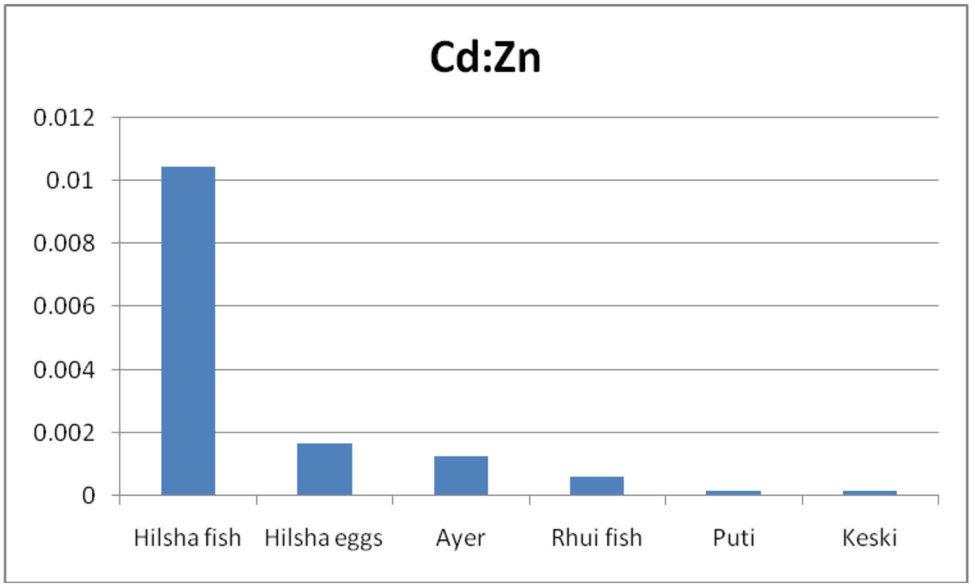


Figure 4.5: Cadmium and Zinc ratio of Bangladeshi fish.

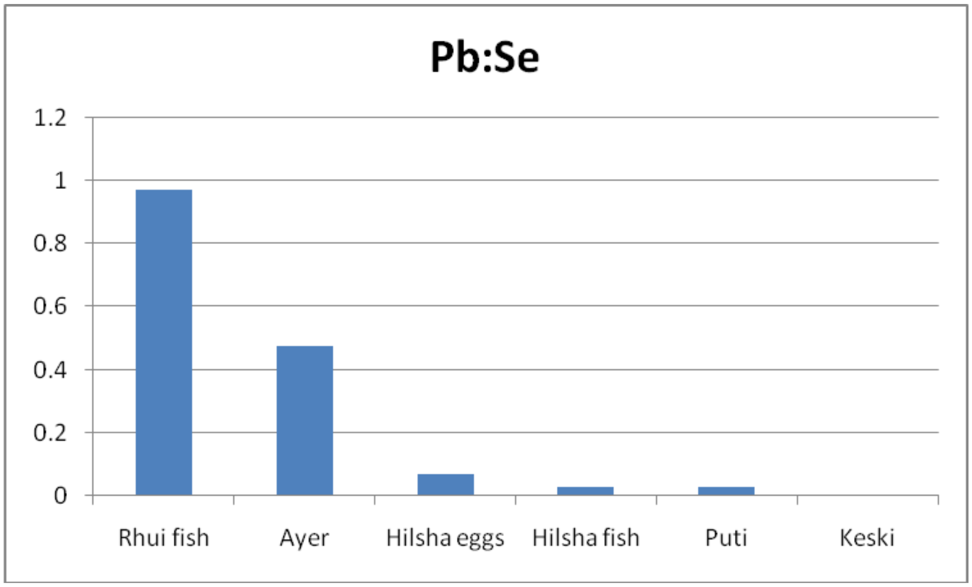


Figure 4.6: Lead and selenium ratio of Bangladeshi fish.

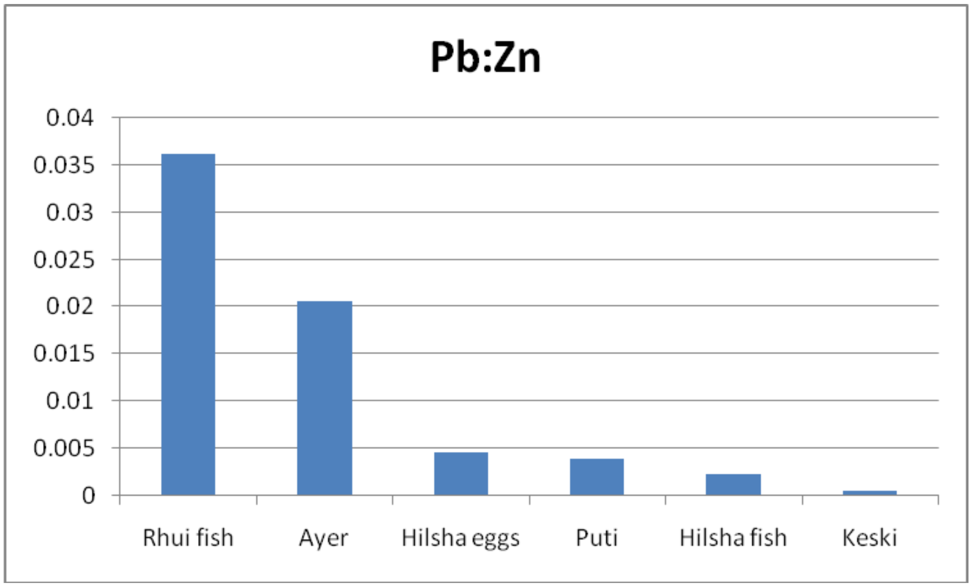


Figure 4.7: Lead and zinc ratio of Bangladeshi fish.

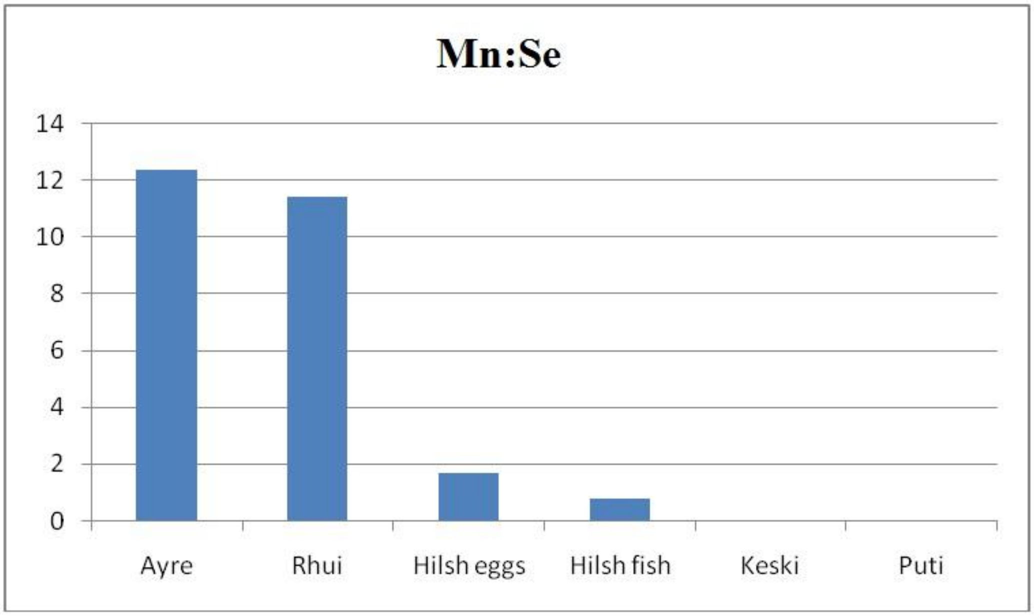


Figure 4.8: Manganese and selenium ratio of Bangladeshi fish.

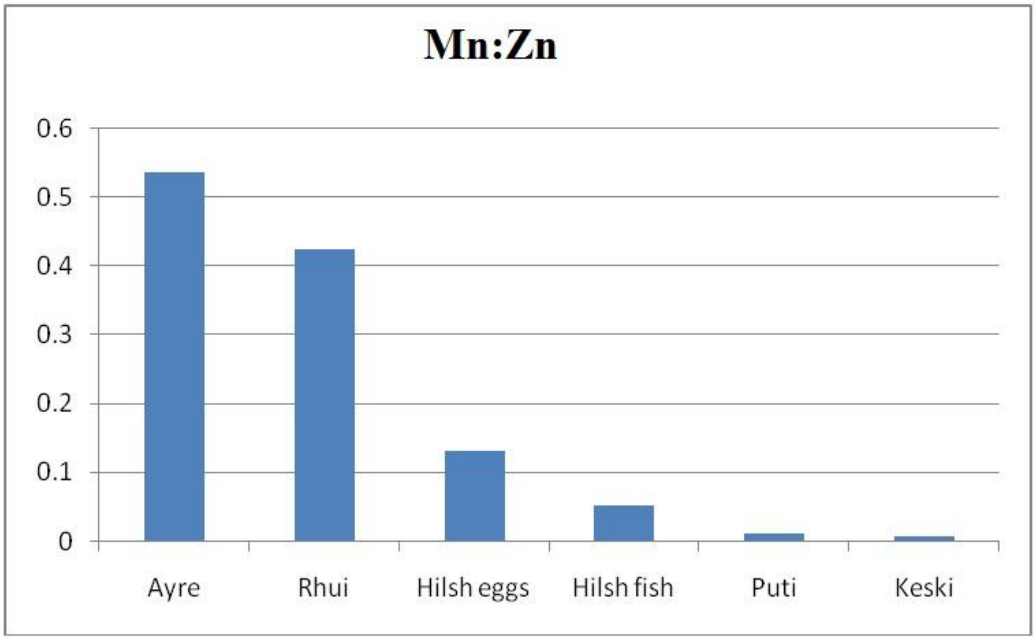


Figure 4.9: Manganese and zinc ratio of Bangladeshi fish.

4.3.1.4 As species in Bangladeshi fish (Hilsha species)

From this study of Bangladeshi fish bought from ethnic shops in the UK, the highest arsenic concentration was measured in a big fish type named Hilsha fish and its eggs. This fish species is very popular amongst Bangladeshis. This study is the first to determine arsenic species in a Bangladeshi fish and its eggs. Eight samples of fish flesh and four samples of eggs were used for arsenic speciation study with tuna fish being used as a reference material (BCR-627). AsBet, DMA and arsenosugars species were detected in Hilsha fish (flesh and eggs) (see Figure 4-10 and 4-11). Extraction efficiencies (%) were 59 – 89 for fish flesh and 36 – 50 for fish eggs. In contrast, for tuna fish tissue (BCR-627) the extraction efficiency was 99%. DMA species was higher than AsBet species in majority of the fish samples analysed, whereas arsenosugars was significant in some samples and in some cases was similar to levels of AsBet species (Table 4-4). Figure 4-12 shows that the percentage of arsenic species including AsBet, DMA and arsenosugars in both Hilsha fish flesh and eggs, DMA is the main species in Bangladeshi Hilsha fish extracts. No AsChol from Hilsha fish was detected.

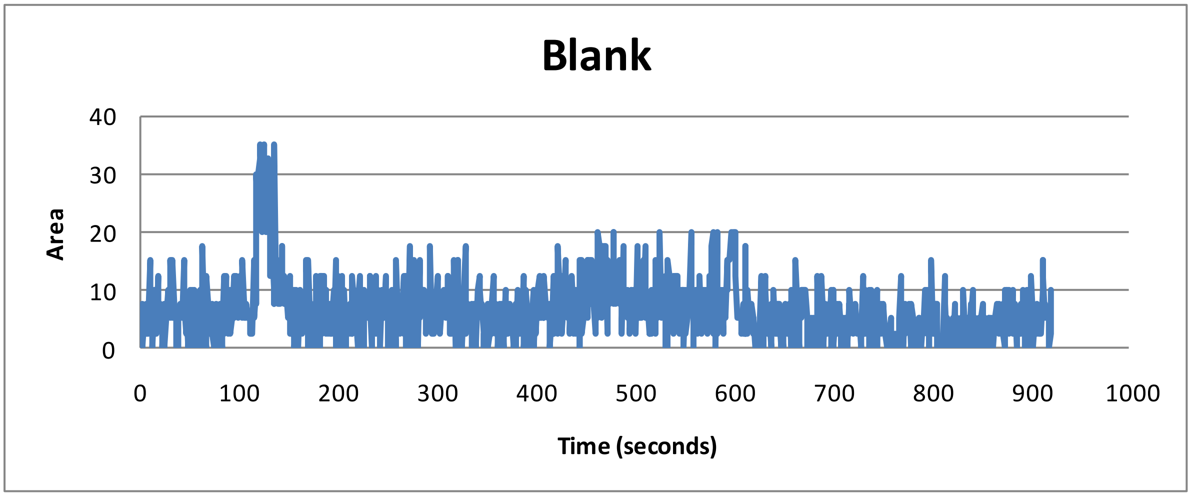


Figure 4.10: Chromatogram of the blank solution was used for arsenic speciation in Hilsha fish using HPLC-ICP-MS.

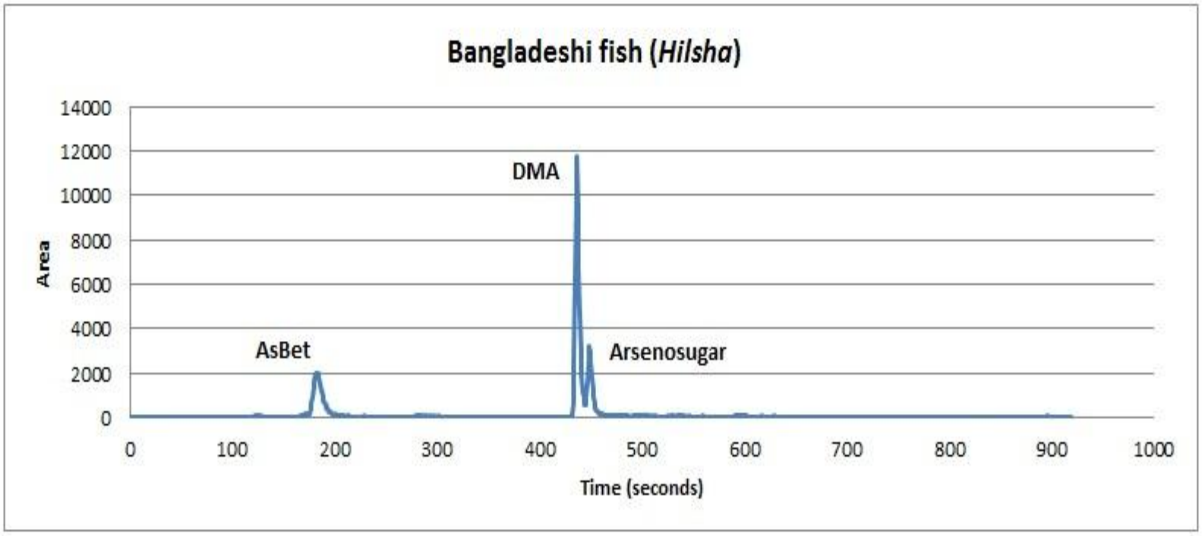


Figure 4.11: Chromatogram of arsenic speciation in Hilsha fish using HPLC-ICP-MS.

Table 4-4: Concentrations and percentages of arsenic species in Bangladeshi Hilsha fish.

Sample	**Total mg As/kg d wt.**	**Extraction efficiency %**	**AsChol mg/kg d wt.**	**AsBet mg/kg d wt.**	**DMA mg/kg d wt.**	**AsSugar mg/kg d wt.**	**Unknown mg/kg d wt.**	**Sum of speices mg/kg d wt.**	**Column recovery %**
Fish flesh									
S1	2.65 ±0. 97	85	nd	0.217	0.883	0.270	nd	1.38 ± 0.068	62
S2	3.12 ± 0.87	89	nd	0.317	1.096	0.393	nd	1.81 ± 0.075	65
S3	3.42 ± 1.39	59	nd	0.025	1.087	0.283	nd	1.39 ± 0.056	69
S4	4.01 ± 0.5	60	nd	0.035	1.248	0.357	nd	1.64 ± 0.066	69
S5	3.32 ± 1.11	67	nd	0.041	1.121	0.288	nd	1.45 ± 0.034	65
S6	2.45 ± 0.65	66	nd	0.086	0.704	0.170	nd	0.95 ± 0.033	59
S7	1.36 ± 0.54	76	nd	0.167	0.427	0.075	nd	0.66 ± 0.045	66
S8	2.27 ± 0.43	74	nd	0.285	0.671	0.195	nd	1.15 ± 0.073	69
Fish eggs									
S1	3.52 ± 0.86	50	nd	0.201	0.652	0.142	nd	0.99 ± 0.074	57
S2	2.98 ± 0.73	36	nd	0.057	0.293	0.046	nd	0.39 ± 0.056	36
S3	2.83 ± 0.34	39	nd	0.075	0.153	0.006	nd	0.23 ± 0.045	21
S4	3.45 ± 0.56	40	nd	0.095	0.069	0.006	nd	0.17 ± 0.043	12
Tuna tissue BCR-627 (n=3)	4.80 ± 0.30	99	7.4	3.277	0.168	0.008	0.014	3.47 ± 0.30	73

Hilsha fish (flesh and eggs) were used for arsenic speciation, for quality control Tuna tissue BCR-627 was used

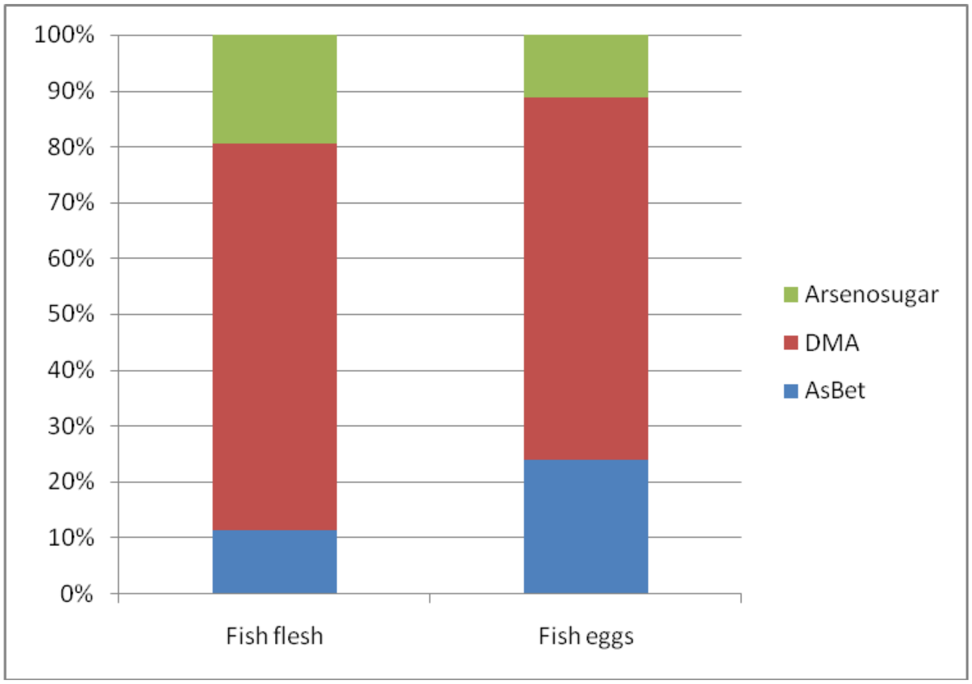


Figure 4.12: Percentages of arsenic species in *Hilsha* fish, means of eight samples of fish flesh (2.55 mgAs/kg) and four samples of fish eggs (3.15 mgAs/kg) were used in this figure.

4.3.2 Total levels of different elements in vegetables and rice

4.3.2.1 Total levels of As, Cd, Pb and Se in vegetables and rice

Generally, low mean levels of arsenic were detected in non-leafy vegetables, 0.3, 0.5 0.7 and 0.9 µg/kg (in aubergine, okra and peas). The highest mean arsenic concentration was in data shak (33.5 µg/kg). However, the content of Cd in leafy and non-leafy vegetables varied between 0.7 and 0.8 µg/kg (in ivy gourd and peas, respectively) followed by 4 and 4.5 µg/kg for lentils and beans, respectively. The highest Cd levels were detected in leafy vegetables (303 µg/kg) and puffed rice (303 µg/kg) followed by polished white rice (184.7 µg/kg). Pb levels ranged from 1.3 and 1.7 µg/kg in onion and aubergine as lower levels, rising to 364 and 505 µg/kg in data shak and tea leaves, respectively. Generally, leafy vegetables contain higher toxic elements compared to non-leafy vegetables (Table 4-5, 4-6 and Figure 4-13). Different varieties of shak (leafy vegetables) were found to have particularly high levels of Cd, with mean concentrations ranging from 46.4 to 100.5 µg/kg (fresh weight). The highest Se levels were 573 and 152 µg/kg in cumin and lentils, respectively.

Rice is a staple food for Bangladeshis, and this was found to contain significant levels of As, Cd and Pb. Of the different types of rice grains analysed, the highest As, Cd and Pb levels detected were 160.8, 184.7 and 109.5 µg/kg and the means were 68.5, 37.2 and 18.5 µg/kg, respectively. However, puffed rice, which is widely consumed by Bangladeshis, has the highest As (173 µg/kg), Cd (303 µg/kg) and Pb (372 µg/kg) content (see Table 4-5, 4-6).The moisture content in the different food samples analysed ranged from ~10% for rice to 92% for leafy vegetables.

Table 4-5 : As and Se levels (µg/kg wet weight) of Bangladeshi foods sold in UK shops.

Sample	***n***	**As**			**Se**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Cereal and cereal products							
Raw rice	98	68.5 ± 41	10.1	160.8	54.9 ± 53	9.1	314.1
Puffed rice	10	119 ± 35	77.1	173.3	26.6 ± 6	14.1	34.5
Wheat flour[a]	2	4	2.8	4.9	10.9	7.6	13.9
Chapatti flour[b]	2	14.5	9.9	18.7	102	34	156
Barley flour[a]	1	14.5	-	-	7.6	-	-
Corn flour[a]	1	nd	-	-	0.3	-	-
Millet flour[a]	3	12.9 ± 6	7.3	21.8	86.7 ± 76	32	178
Bread	1	4	-	-	95.1	-	-
All cereals	**118**	**29.7 ± 42**	**2.8**	**173.3**	**48 ± 42**	**0.3**	**314.1**
Roots vegetables (peeled)							
Potato	2	1.4	0.1	2.6	29	3.1	54.1
Arum Kuchi Mukhi (Taro)	9	10 ± 11	0.9	28.2	11 ± 4	4.8	16.6
Onion	3	1.1± 1	0.1	2.1	22.4± 9	16	29
Carrot	2	3.6	2.0	5.2	17	16.1	17.5
Radish	4	19.7 ± 27	2.0	60.5	14 ± 10	5.0	28.7
Ginger	1	5.3	-	-	3.8	-	-
Garlic	2	5.3	2.6	8.0	7.8	6.5	9.2
Non-leafy vegetables							
Beans	26	2 ± 2	0.1	11.4	33 ± 50	1.8	242
Peas	4	0.9 ± 0.4	0.2	1.4	19.3 ± 31	1.7	87
Lentil	7	4.4 ± 3	1.7	9.8	152 ± 178	18.4	498
Papaya	1	2	-	-	1.3	-	-
Cucumber	2	2.1	2.0	2.3	8.3	5.1	11.6
Sweet pumpkin	3	1.2 ± 0.5	0.7	1.7	8.6 ± 8	1.2	17.0
Karela (Bitter gourd)	6	3.1 ± 4	0.1	10.8	11.2 ± 7	0.8	24.7
Ivy gourd	1	4.1	-	-	12	-	-
Snake gourd	1	6.2	-	-	2.4	-	-
Okra	5	0.7 ± 0.5	0.2	1.8	4.7 ± 4	1.0	13.1
Aubergine	3	0.5 ± 0.1	0.4	0.6	8 ± 7	0.9	15.7
Jhinga slices	6	1.7 ± 0.9	0.5	2.8	9.2 ± 12	1.4	30.9
Jack fruit seeds	3	0.8 ± 0.5	0.2	1.2	10.1 ± 1.4	8.7	11.4
Olive	3	1.6 ± 0.9	1.1	2.7	5.8 ± 4	2.0	9.7
Kachur Mura	4	3.2 ± 1	2.1	4.8	10 ± 7	5.0	18.0
Potal	4	6.7 ± 1	4.5	7.7	6.9 ± 5	3.7	13.8
Hoilfa	2	1.4	0.8	2.2	0.9	0.7	1.1
All non-leafy vegetables	**104**	**3.4 ± 4**	**0.1**	**60.5**	**19 ± 31**	**0.7**	**498**

Table 4-5 continued.

Sample	***n***	**As**			**Se**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Leafy vegetables							
Lal shak (*Amaranthus*)	33	11 ± 7	1.0	28	21 ± 34	0.2	144
Pui shak	4	12 ± 11	6.2	28.7	4.4 ± 2	2.1	6.7
Nali shak	8	12 ± 10	6.1	32.5	31 ± 50	7.4	133
Data shak	9	12 ± 12	1.3	33.5	29 ± 22	5.9	77.1
Lao stem	2	4.7	4.1	5.3	14	11.2	17.3
Dala stem	1	3.5	-	-	77	-	-
Methi[b]	1	6.2	-	-	13	-	-
Curry leaves fresh[b]	1	4.2	-	-	3.2	-	-
All leafy vegetables	**59**	**12.1 ± 14**	**0.9**	**33.5**	**28 ± 30**	**0.2**	**144**
Fruit							
Raw mango	1	4.7	-	-	9.5	-	-
Shatkora (type of citrus fruit)	6	2.4 ± 2	0.9	5.7	3.6 ± 3	0.1	9.0
Banana	1	1.2	-	-	75.6	-	-
Banana stem	1	0.5	-	-	0.7	-	-
Boroi (type of fruit)	3	1.5 ± 0.8	0.4	1.9	2.1 ± 1	0.8	2.9
Date sugar(Khejurer gur)	5	15 ± 14	3.4	34	30 ± 28	17	69
All fruits	**17**	**3.9 ± 4.7**	**0.4**	**34**	**22 ± 28**	**0.1**	**98**
Spices and condiments[b]							
Curcuma (Haldi)	8	31 ± 15	12	48	65 ± 22	41	101
Coriander	8	27.5 ± 10	11	119	112 ± 83	33	268
Cumin	5	29.4 ± 16	16.3	139	573 ± 181	338	781
Cinnamon	5	48 ± 37	27	121	36 ± 10	25	52
Chilli	9	36 ± 21	4.2	80	116 ± 50	10	909
Curry powder	4	23.5 ± 9	16.4	38.3	171 ± 66	72.2	342
Fish powder	1	2775	-	-	1656	-	-
Bay leaves powder	1	69.1	-	-	27	-	-
All spices	**41**	**380 ± 968**	4.2	2775	**345 ± 558**	10	1656
Beverages							
Tea infusions	6	0.2 ± 0.1	0.05	0.3	0.2 ± 0.1	0.04	0.3
All categories	**346**	**58.7 ± 357**	0.05	2775	**67.8 ± 223**	0.04	1656

[a] these products were from UK shops and were used for comparison and daily intake estimation

[b] Indian vegetables and spices from different countries which consumable from Bangladeshis

Table 4-6: Cd and Pb levels (μg/kg wet weight) of Bangladeshi foods sold in UK shops.

Sample	***n***	**Cd**			**Pb**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Cereal and cereal products							
Raw rice	98	37.2 ± 30	2.3	184.7	18.5 ± 16	3.6	109.5
Puffed rice	10	67.9 ± 102	3.8	303	98 ± 117	4.7	372
Wheat flour[a]	2	19.5	16.7	21.9	11.9	9.4	13.7
Chapatti flour[b]	2	23.5	20.5	25.3	65	33.4	122
Barley flour[a]	1	11.2	-	-	5	-	-
Corn flour[a]	1	0.7	-	-	41	-	-
Millet flour[a]	3	21.7 ± 3	17.9	24.8	38.6 ± 32	12.9	54.5
Bread	1	12.4	-	-	204	-	-
All cereals	**118**	**24.3 ± 21**	**0.7**	**303**	**60.3 ± 65**	**3.6**	**372**
Roots vegetables (peeled)							
Potato	2	6.7	2.2	18.6	12.4	9.9	15.0
Arum Kuchi Mukhi (Taro)	9	**15.8 ± 6**	**7.1**	**25.4**	**55.1 ± 50**	**7.3**	**136**
Onion	3	2.7± 0.9	1.4	6.0	1.3± 0.1	1.2	1.4
Carrot	2	10	8.3	12.4	6.4	4.0	8.8
Radish	4	23.7 ± 9	10.8	32.0	12 ± 12	1.8	29.3
Ginger	1	1.8	-	-	5.2	-	-
Garlic	2	6.8	3.7	9.9	2.4	2.2	2.6
Non-leafy vegetables							
Beans	26	4.9 ± 4	0.8	18.3	11 ± 6	1.3	26.3
Peas	4	0.8 ± 0.4	0.2	3.2	8 ± 5	2.8	18.2
Lentil	7	4 ± 2	0.9	9.8	11.2 ± 19.6	1.3	62.6
Papaya	1	0.7	-	-	4.0	-	-
Cucumber	2	5.4	3.9	6.9	4.3	3.2	5.4
Sweet pumpkin	3	8.7 ± 6	3.6	15.2	9.2 ± 4	5.7	14.5
Karela (Bitter gourd)	6	2.6 ± 1	0.6	12.1	25.3 ± 36	1.0	89.7
Ivy gourd	1	0.7	-	-	6.1	-	-
Snake gourd	1	3	-	-	2.5	-	-
Okra	5	4.2 ± 1	2.0	11.5	3.3 ± 2	0.8	6.5
Aubergine	3	18 ± 27	1.6	49.2	1.7 ± 0.2	1.5	1.9
Jhinga slices	6	8.3 ± 6	3.2	19.1	10 ± 6	5.6	20.8
Jack fruit seeds	3	7.6 ± 2	5.5	9.9	4.4 ± 2.1	2.0	5.9
Olive	3	1.7 ± 0.4	1.4	2.3	9.3 ± 5	5.9	15.4
Kachur Mura	4	8.7 ± 4	4.3	12.0	26 ± 25	9.2	56.4
Potal	4	1.6 ± 0.8	1.0	2.4	5.4 ± 2	2.5	7.0
Hailfa	2	19.7	17.3	20.4	12.2	10.1	15.6
All non-leafy vegetables	**104**	**7.6 ± 8**	**0.2**	**49.2**	**8.6 ± 6**	**0.8**	**136**

Table 4-6 continued.

Sample	***n***	**Cd**			**Pb**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Leafy vegetables							
Lal shak (*Amaranthus*)	33	100.5 ± 95	4.1	303	105 ± 84	15	282
Pui shak	4	56.8 ± 16	37.3	92	34.6 ± 18	23	61
Nali shak	8	40.3 ± 22	8.3	71	72.1 ± 96	26	269
Data shak	9	46.4 ± 37	4.7	165	364 ± 504	10	1279
Lao stem	2	7.3	5.9	8.7	59.3	51.2	63.2
Data stem	1	20.4	-	-	21	-	-
Methi[b]	1	2.2	-	-	12.4	-	-
Curry leaves fresh[b]	1	17.6	-	-	12.6	-	-
All leafy vegetables	**59**	**31 ± 29**	**2.2**	**165**	**115 ± 163**	**7.3**	**1279**
Fruit							
Raw mango	1	2.2	-	-	17	-	-
Shatkora (type of citrus fruit)	6	1.9 ± 2	0.3	7.5	11 ± 6	4.5	20.2
Banana	1	0.4	-	-	15	-	-
Banana stem	1	4.3	-	-	4.8	-	-
Boroi (type of fruit)	3	1.7 ± 0.5	1.1	2.6	7.5 ± 3	3.5	9.8
Date sugar (Khejurer gur)	5	2.9 ± 2	1.8	4.9	37 ± 41	8.4	65.7
All fruits	**17**	**2.3 ± 1.2**	**0.3**	**7.5**	**17.4 ± 12**	**3.5**	**65.7**
Spice and condiments[b]							
Curcuma (Haldi)	8	22.7 ± 17	2.8	49	244 ± 222	5.9	5588
Coriander	8	37.6 ± 23	9.1	99.6	193 ± 242	61	684
Cumin	5	49.5 ± 3	46.4	52.3	116 ± 12	103	402
Cinnamon	5	290 ± 30	53	527	2623 ± 2761	126	3161
Chilli	9	61.2 ± 31	13.4	206	208 ± 120	15	4375
Curry powder	4	48.9 ± 27	26	80	129 ± 54	76	207
Fish powder	1	132	-	-	122	-	-
Bay leaves powder	1	71.8	-	-	526	-	-
All spices	**41**	**89.2 ± 87**	**2.8**	**527**	**520 ± 860**	**5.9**	**4375**
Beverages							
Tea infusions	6	0.07 ± 0.05	0.02	0.1	0.5 ± 0.3	0.04	0.7
All categories	**346**	**24 ± 44**	**0.02**	**527**	**105.8 ± 351**	**0.04**	**5588**

[a] these products were from UK shops and were used for comparison and daily intake estimation

[b] Indian vegetables and spices from different countries which consumable from Bangladeshis

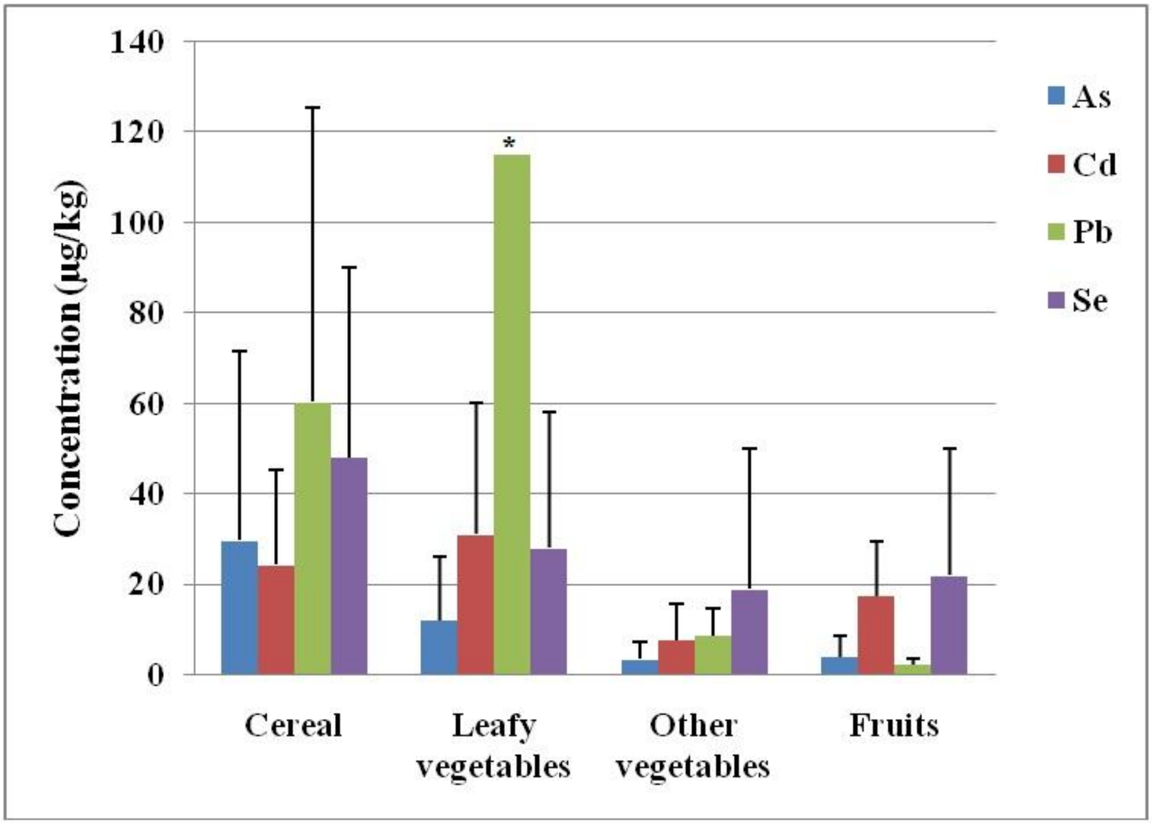


Figure 4.13: Concentrations of As, Cd, Pb and Se in 306 samples of Bangladeshi foods including cereal, vegetables and fruits. Bars represent standard deviation (SD). *Pb in leafy vegetables has high (SD 163) and therefore the bar is omitted.

4.3.2.2 Total levels of Mn, Se and Zn in vegetables and rice

The mean concentrations of essential elements (Mn and Zn) in various foods originating from Bangladesh are presented in Table 4-7 and Figure 4-14. The highest mean concentration of Mn was 564 mg/day (fresh weight) for cinnamon. Leafy vegetables also have the highest content of Mn. Low Mn levels were detected in corn flour (0.016 mg/kg) and papaya (0.62 mg/kg). The highest Zn levels were 25.92 and 17.66 mg/kg for coriander and lentils, respectively (Table 4-7). Different varieties of shak (leafy vegetables) were found to have particularly high levels of Mn and Zn, with mean concentrations ranging from 9.1 to 17.30 mg/kg for Mn and 3.29 to 7.64 mg/kg for Zn.

Table 4-7: Mn and Zn levels (mg/kg wet weight) of Bangladeshi foods sold in UK shops.

Sample	***n***	**Mn**			**Zn**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Cereal and cereal products							
Raw rice	98	13.19 ± 5.6	1.26	32.2	11.04± 3.1	3.5	21.3
Puffed rice	10	8.31 ± 4.4	0.7	16.8	9.01 ± 1.8	6.6	11.7
Wheat flour[a]	2	6.74 ± 2.4	4.9	7.7	5.96 ± 2.0	4.2	8.1
Chapatti flour[b]	2	17.31 ± 3.2	14.8	19.5	23.5 ± 2	20.8	25.1
Barley flour[a]	1	9.15	-	-	9.31	-	-
Corn flour[a]	1	0.016	-	-	0.24	-	-
Millet flour[a]	3	11.89 ± 1.4	9.8	13.8	22.90 ± 4.1	17.6	26.2
Bread	1	5.25	-	-	4.53	-	-
All cereals	**117**	**8.98 ± 5.2**	**0.7**	**32.2**	**7.8 ± 7.3**	**0.24**	**26.2**
Roots vegetables (peeled)							
Potato	2	3.86 ± 4.4	3.6	4.2	8.27 ± 1.7	7.0	9.5
Arum Kuchi Mukhi (Taro)	9	34.3 ± 31.8	5.9	87.6	9.16 ± 7.9	2.0	25.8
Onion	3	0.81± 0.15	0.7	0.92	1.50± 0.3	1.3	1.8
Carrot	2	1.19 ± 0.19	1.1	1.3	2.18 ± 0.7	1.6	2.7
Radish	4	6.26 ± 7.91	1.9	18.1	6.30 ± 2.39	4.2	9.3
Ginger	1	42.32	-	-	3.84	-	-
Garlic	2	1.51 ± 0.22	1.49	1.53	2.27 ± 0.6	1.78	2.75
Non-leafy vegetables							
Beans	26	7.26 ± 5.5	2.6	31.2	5.94 ± 2.1	1.4	11.9
Peas	4	3.94 ± 0.6	3.3	5.1	4.97 ± 1.3	3.7	7.8
Lentil	7	7.73 ± 2.8	5.2	13.5	17.66 ± 5.1	11.8	27.3
Papaya	1	0.62	-	-	2.7	-	-
Cucumber	2	2.93 ± 1.6	1.7	4.1	3.06 ± 0.4	2.8	3.3
Sweet pumpkin	3	1.04 ± 0.5	0.5	1.7	1.74 ± 0.3	1.3	2.0
Karela (Bitter gourd)	6	5.04 ± 4.2	0.9	14.1	4.12 ± 2.6	1.8	9.0
Ivy gourd	1	1.25	-	-	2.29	-	-
Snake gourd	1	0.69	-	-	1.21	-	-
Okra	5	1.85 ± 0.7	1.1	3.2	3.31 ± 0.8	2.1	4.5
Aubergine	3	4.30 ± 4.7	1.2	9.8	1.33 ± 0.3	1.0	1.7
Jhinga slices	6	2.77 ± 5.1	2.1	3.4	5.07 ± 0.9	3.7	5.8
Jack fruit seeds	3	1.57 ± 0.4	1.2	1.9	2.38 ± 0.6	1.7	3.0
Olive	3	3.49 ± 2.2	1.1	5.6	0.41 ± 0.02	0.40	0.43
Kachur Mura	4	**5.72 ± 8.5**	**0.6**	**15.5**	**6.55 ± 4.3**	**2.5**	**11.1**
Potal	4	1.37 ± 0.3	1.2	1.9	2.33 ± 0.2	2.1	2.5
Hoilfa	2	6.33 ± 0.1	6.2	6.5	2.47 ± 0.2	2.2	2.6
All non-leafy vegetables	**104**	**5.20 ± 8.5**	**0.5**	**31.2**	**4.12 ± 3.6**	**0.4**	**11.9**

Table 4-7 continued.

Sample	***n***	**Mn**			**Zn**		
		Mean ±SD	**Min**	**Max**	**Mean ±SD**	**Min**	**Max**
Leafy vegetables							
Lal shak (*Amaranthus*)	33	9.33 ± 8.9	0.7	41.8	8.41 ± 5.3	1.1	29
Pui shak	4	17.30 ± 5.9	13.1	26.1	4.24 ± 0.5	3.5	4.7
Nali shak	8	9.11 ± 4.6	5.7	16.6	3.29 ± 1.1	2.6	5.7
Data shak	9	14.27 ± 35.1	1.3	131	7.64 ± 6.0	1.5	19.2
Lao stem	3	8.33 ± 1.4	7.3	8.9	6.01 ± 0.02	5.9	6.1
Dala stem	1	5.76	-	-	2.47	-	-
Methi[b]	1	8.82	-	-	1.98	-	-
Curry leaves fresh[b]	1	2.81	-	-	1.99	-	-
All leafy vegetables	**59**	**11.57± 8.9**	**0.6**	**131**	**5.17 ± 2.7**	**1.1**	**25.8**
Fruit							
Raw mango	1	21.08	-	-	3.03	-	-
Shatkora (type of citrus fruit)	6	1.55 ± 0.7	0.6	2.8	2.20 ± 0. 9	1.1	3.5
Banana	1	12.88	-	-	8.13	-	-
Banana stem	1	11.56	-	-	7.22	-	-
Boroi (type of fruit)	3	2.90 ± 1.4	1.6	3.8	0.94 ± 0.2	0.78	1.3
Date sugar(Khejurer gur)	5	0.87 ± 0.6	0.4	1.3	1.84 ± 1.3	0.6	2.4
All fruits	**17**	**7.23 ± 7.2**	**0.4**	**1.3**	**3.58 ± 2.6**	**0.6**	**8.1**
Spice and codiments[b]							
Curcuma (Haldi)	8	80.69 ± 51.7	12.8	150	10.03 ± 4.2	4.3	15.4
Coriander	8	25.05 ± 5.4	15.9	56	25.92 ± 5.9	19.6	35.3
Cumin	5	31.94 ± 6.5	24.4	43.5	24.18 ± 3.7	21	28.2
Cinnamon	5	337.6 ± 134	174	564	7.51 ± 2.2	4.4	11.3
Chilli	9	31.65 ± 18.4	12.7	30.3	15.92 ± 4.0	10.2	21.6
Curry powder	4	50.7 ± 7	40.8	60	19.2 ± 1.7	17.6	21.2
Fish powder	1	9.70	-	-	21.21	-	-
Bay leaves powder	1	70.29	-	-	30.06	-	-
All spices	**41**	**79.7 ± 107**	**12.7**	**564**	**19.26 ± 7.7**	**4.3**	**35.3**
Beverages							
Tea infusions	6	5.52 ± 2.6	2.5	10.9	0.23 ± 0.1	0.1	0.4
All categories	**346**	**28.46 ± 99**	**0.4**	**564**	**6.92 ± 7**	**0.1**	**35.3**

[a] these products were from UK shops and were used for comparison and daily intake estimation

[b] Indian vegetables and spices from different countries which consumable from Bangladeshis

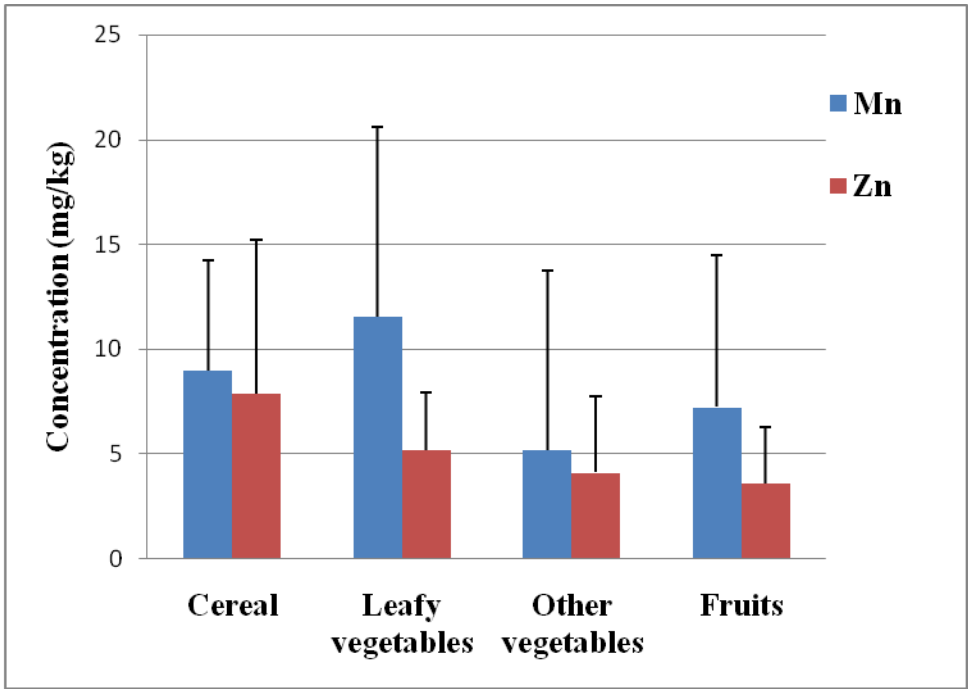


Figure 4.14: Concentrations of Mn and Zn in 306 samples of Bangladeshi foods including cereal, vegetables and fruits. Bars represent standard deviation.

Ratios of toxic elements (As, Cd, Pb and Mn) to essential elements (Se and Zn) in cereal, vegetables and fruits were determined. Figures 4-15 and 4-16 showed that As:Se and As:Zn ratio of ginger, radish, rice and leafy vegetables were higher than other categories. However, lentil, beans and okra showed the lowest ratios. Figures 4-17 and 4-18 showed the Cd:Se and Cd:Zn ratios; the ratios for root and leafy vegetables were the highest amongst all the categories of foods analysed. Figures 4-19, and 4-20 showed the Pb:Se, Pb:Zn ratios for different foods; the ratio for leafy vegetables and ginger are the highest, with rice and lentil having the lowest. Figures 4-21 and 4-22 showed the Mn:Se and Mn:Zn ratios for different foods; the ratio for ginger and leafy vegetables are the highest, with okra and lentil having the lowest.

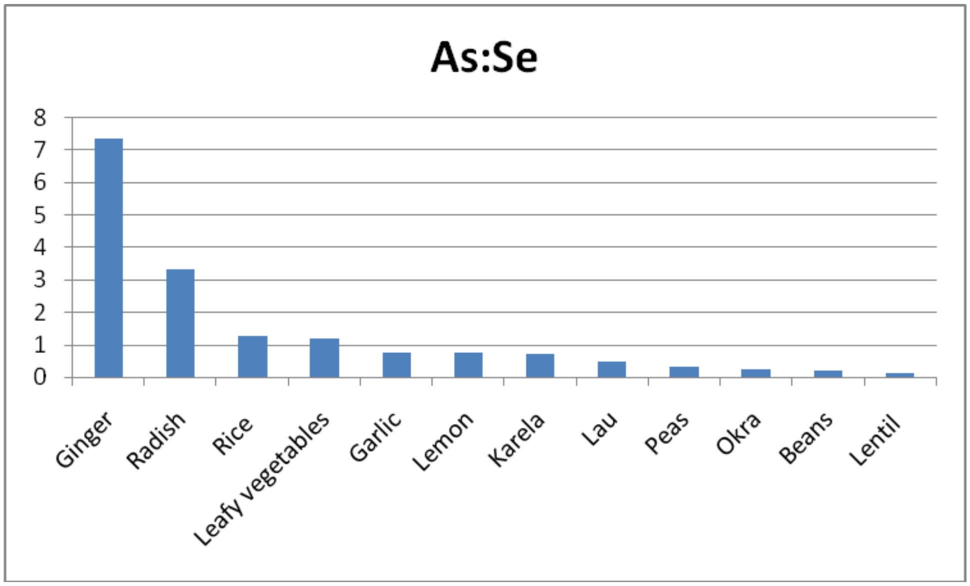


Figure 4.15: Arsenic and selenium ratio of Bangladeshi foods.

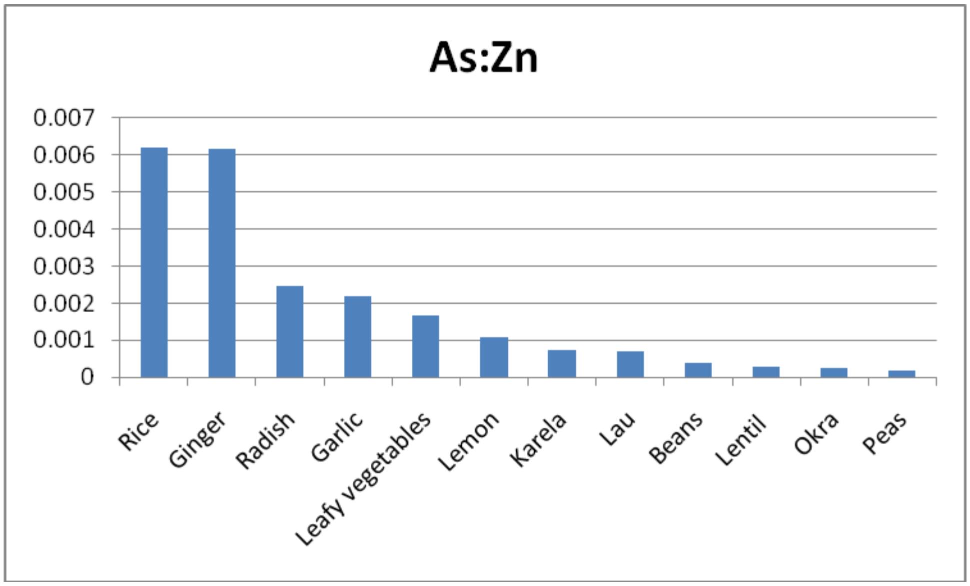


Figure 4.16: Arsenic and zinc ratio of Bangladeshi foods.

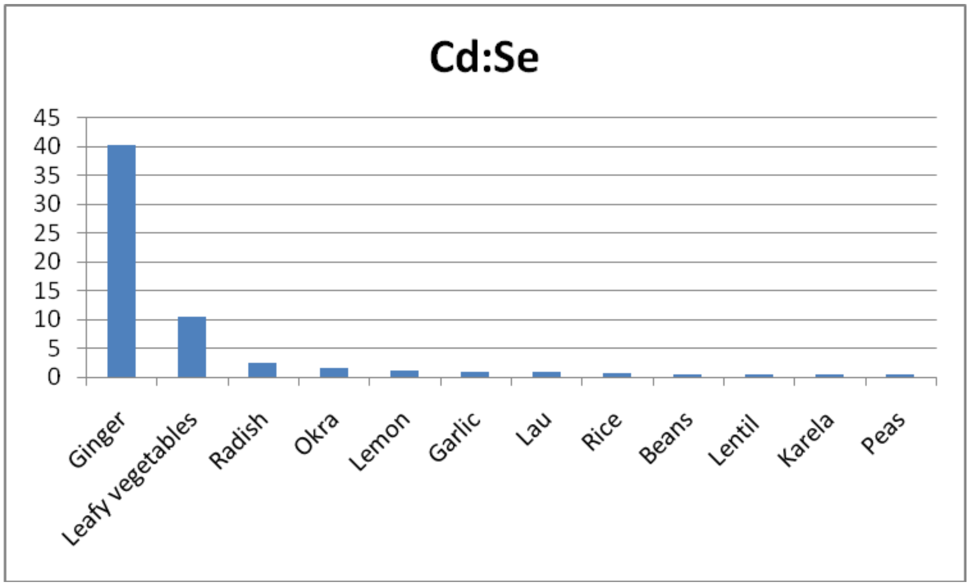


Figure 4.17: Cadmium and selenium ratio of Bangladeshi foods.

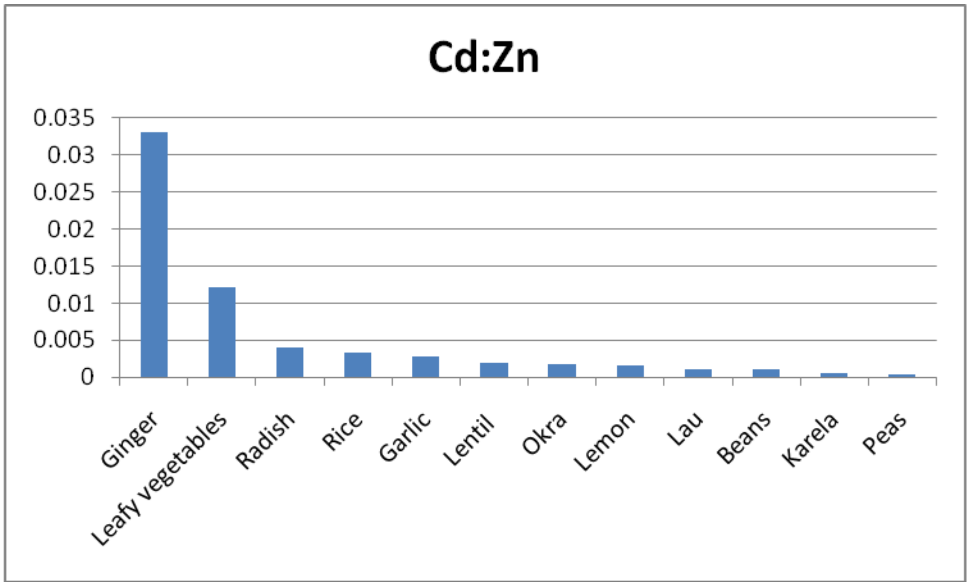


Figure 4.18: Cadmium and zinc ratio of Bangladeshi foods.

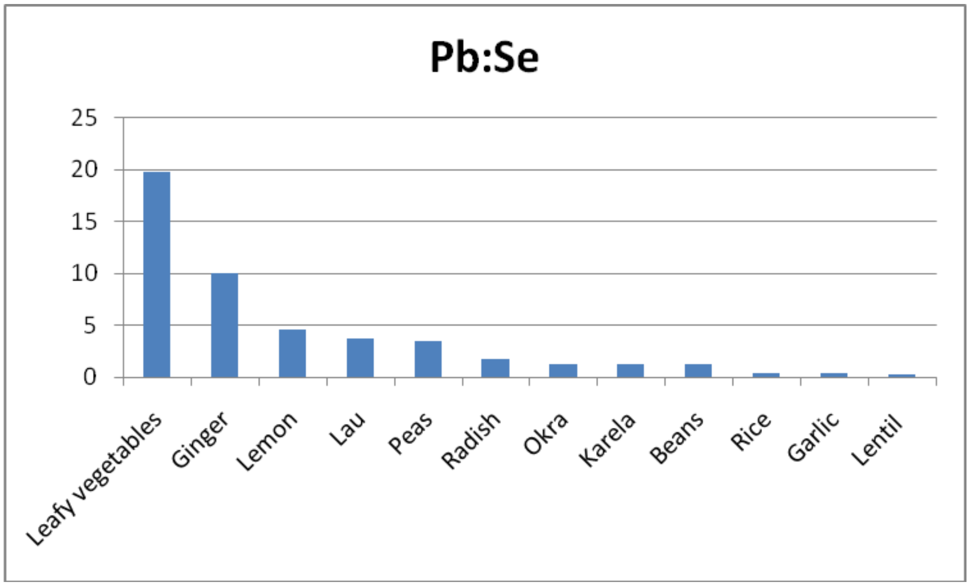


Figure 4.19: Lead and selenium ratio of Bangladeshi foods.

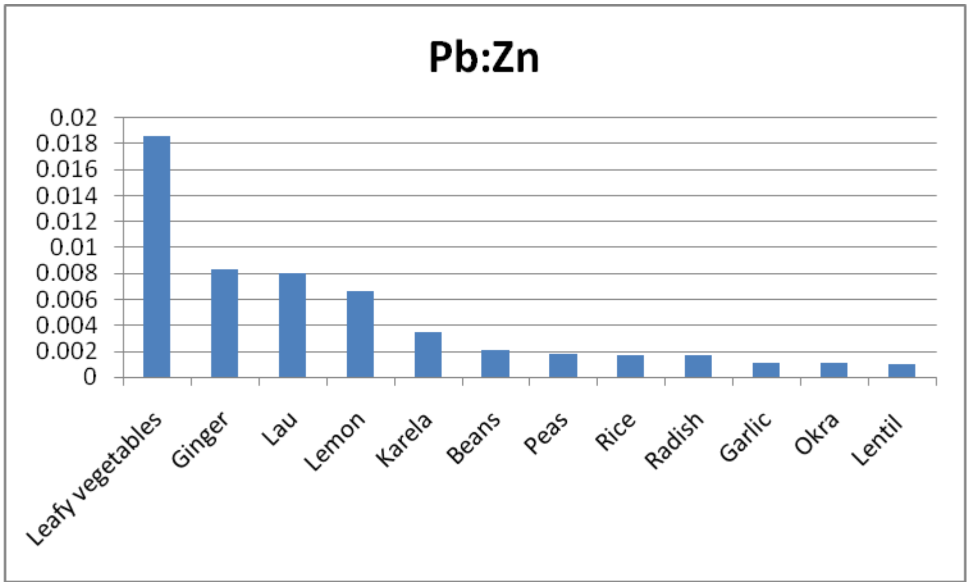


Figure 4.20: Lead and zinc ratio of Bangladeshi foods.

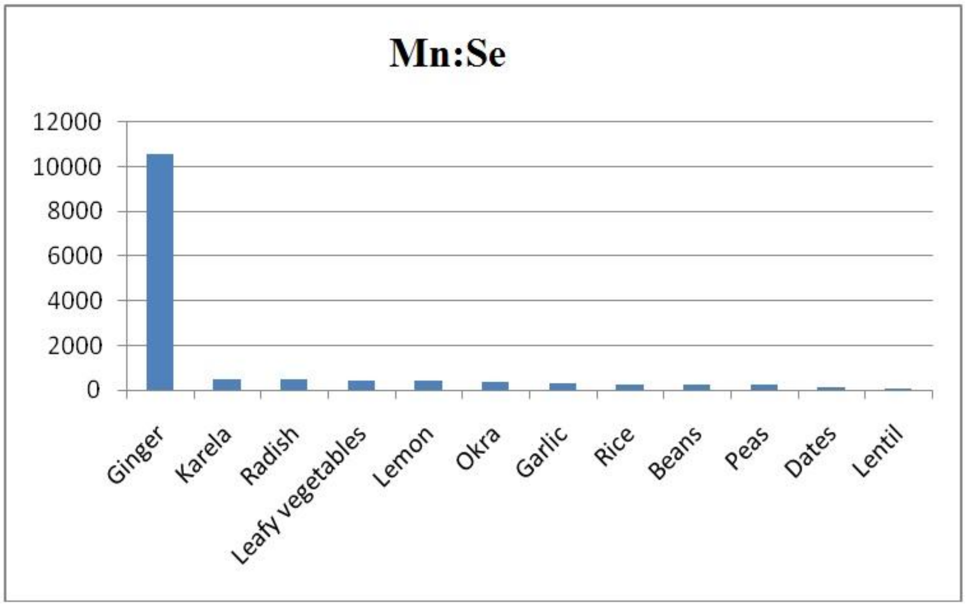


Figure 4.21: Manganese and selenium ratio of Bangladeshi foods.

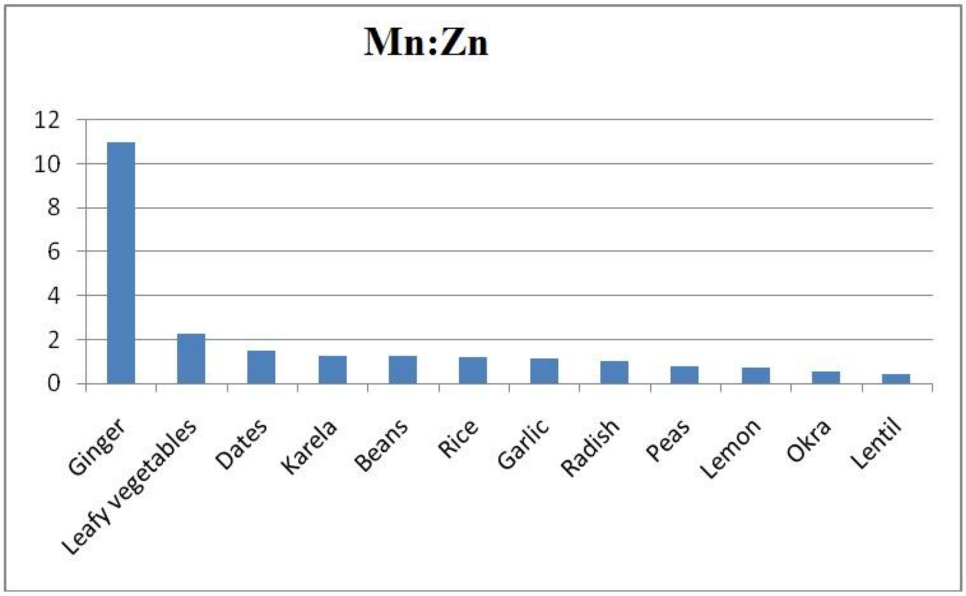


Figure 4.22: Manganese and zinc ratio of Bangladeshi foods.

4.3.3 Correlations between different elements in Bangladeshi leafy vegetables

Table 4-8 showed the correlations between trace elements in Bangladeshi leafy vegetables, including lal shak and pui shak. Arsenic has correlations with all the elements except cadmium. Strong significant correlation was found between arsenic and lead (r = 0.709, *P* = 0.007). Manganese showed significant correlation with arsenic and zinc, and correlation with other elements. Zinc has correlations with all the elements.

Table 4-8 : Correlations between different elements in Bangladeshi leafy vegetables.

	As	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
As						
r	1.000					
P	-					
Cd						
r	0.245	1.000				
P	0.057	-				
Mn						
r	0.506^{**}	0.228	1.000			
P	0.000	0.078	-			
Pb						
r	0.709^{**}	0.349^{**}	0.227	1000		
P	0.007	0.006	0.078	-		
Se						
r	0.329^{**}	0.209	0.118	0.415^{**}	1.000	
P	0.010	0.107	0.367	0.001	-	
Zn						
r	0.562^{**}	0.597^{**}	0.329^{**}	0.562^{**}	0.322^{**}	1.000
P	0.000	0.000	0.010	0.000	0.012	-

r: correlation coefficient, *P*: probability value, **: correlation is significant at the 0.01 level.

4.3.4 Daily intakes of arsenic and other elements

Estimation of daily intakes of elements provides a better understanding of the relationship between food consumption and health impact. Daily intakes of As, Cd, Mn, Pb, Se and Zn around the world has been reported to be 2.1, 1, 150, 3.6, 5 and 300 µg/kg.bw/day (WHO, 2004). Bangladeshis consume different quantities of foods with vegetables (leafy and non-leafy) and rice being dominant. For the calculation of daily intakes of these elements from vegetables and rice, data from a previously published food frequency questionnaire (Zablotska *et al.*, 2008) was used. These authors obtained this information based on data from over 10,628 people in Bangladesh. They estimated that 225 g of vegetables are consumed per person per day. Using their data, the estimated daily intakes of As, Cd and Pb from vegetables were found to be 2.58, 12.03 and 38.3 µg/day for leafy vegetables respectively, and 2.58, 1.11 and 2.42 µg/day for lentils and beans respectively (Table 4-9).

Table 4-9: Daily intakes[a] of elements (µg/day) for Bangladeshis.

Food type	**As**	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
Fish	31.76	0.37	140	4.76	35.21	927
Green vegetables	2.58	12.03	3130	38.30	4.80	1700
Lentil and beans	2.58	1.11	1690	2.42	3.86	1398
Puffed rice	2.18	1.40	130	1.70	1.12	164
Steamed rice	34.31	18.6	6580	14.8	27.40	5510
Tea infusions	0.15	0.05	3930	0.38	0.14	165

[a] Daily intakes of elements were calculated by multiplying the concentration of element (µg/g) in food with the amount of the same food consumpted per day (g/day).

The daily intakes of different elements from rice (assuming 500 g is consumed per day) were estimated to be 34.31, 18.6 and 14.8 µg/day for As, Cd and Pb, respectively.

Daily intakes of trace elements from fish consumption were estimated to be 31.76, 0.37 and 4.67 µg/day for As, Cd and Pb, respectively (Table 4-9).

Highest daily intakes of Mn were 6.58, 3.93 and 3.13 mg/day for rice, tea infusions and green vegetables, respectively. Levels of Se was estimated in fish and rice (35.21 and 27.4 µg/day), respectively. Also daily intake of Zn from rice was high (5.51 mg/day) but low from puffed rice and tea infusion consumption (0.16 and 0.17 mg/day, respectively) (Table 4-9).

4.4 Discussion

Very little information is available in the literature about the toxic element contents of Bangladeshi fish. Bangladeshis consume high quantities of fish and they may be exposed to high levels of toxic elements from fish consumption. Besides fish and rice, vegetables constitute an important part of the Bangladeshi diet. Consumption of different quantities of vegetables has been reported in the literature. Alam *et al.* (2003) has reported that the amount of vegetables consumed in Bangladesh was 130 g per person per day (fresh weight) and it represented 16% of their total food intake (Alam *et al.* 2003). However, in another survey of a very large number of Bangladeshi people (10,628 persons), the quantity of vegetable consumed was estimated to be 225 g per person per day (fresh weight) (Zablotska *et al.* 2008). In this thesis, large number of vegetables and fish were analysed, and trace elements in these samples were determined.

4.4.1 Trace elements in Bangladeshi fish

In this chapter, ratios of toxic elements (As, Cd, Pb and Mn) to essential elements (Se and Zn) in different Bangladeshi fish were calculated (Figures 4-2 to 4-9). These ratios may provide a guide to identify fish that have lower toxic elements. Results show that *Hilsha* species has higher As and Cd levels than other fish species. However, Rhui and Ayer species have high levels of Pb and Mn.

4.4.1.1 Total of As, Cd and Pb in fish

The highest As and Cd levels were detected in fish eggs (Hilsha fish) 6146, 1727 µg/kg, respectively and the highest levels of Pb (1160 µg/kg) was detected in Rhui fish (freshwater fish) (see Table 4-1). In a UK study, As, Cd and Pb were detected in fish samples collected from UK markets in 1999. The levels of these elements were found to be 4300 (As), 20 (Cd) and 20 (Pb) µg/kg (Ysart *et al.* 1999). Compared to the data for Bangladeshi fish presented in this thesis, the arsenic content was similar for some types of fish such as Hilsha species. This species of fish can be very common in the Bangladeshi diet. It is a type of sea fish that migrates from the sea to the river for spawning. For Bangladeshi freshwater fish, the data reported by Ysart *et al.* (1999) are higher than those presented in the current study. In contrast, Cd and Pb levels in Bangladeshi fish were very high compared with the UK study of Ysart *et al.* (1999). However, for the case of arsenic, it is likely the majority of the arsenic species in the fish and seafood samples is present as non-toxic arsenobetaine (Ebisuda *et al.*, 2002). Results presented in this thesis show that Hilsha fish contained high levels of arsenic and for the first time, data on arsenic speciation of Hilsha fish is presented. Daily intakes of As, Cd and Pb from fish consumption were estimated to be 31.76, 0.37 and

4.76 µg/day, respectively. Low Cd intake from fish consumption was estimated compared with much higher intakes of As and Pb. The main source of Cd exposure in the Bangladeshi population is from rice and vegetables, with very little contribution from fish. This is consistent with the observation by Krajcovicova-Kudlackova *et al.* (2006) who reported that the human blood Cd levels decreases with increasing animal food consumption. The lower Cd intake from animal products compared to vegetables explains this observation.

4.4.1.2 Total of Mn, Se and Zn in fish

High levels of manganese in fish were detected; Mn levels were 2,934, 2,838 and 1,762 µg/kg for Rhui fish, small silver coloured fish and Ayer fish, respectively. Selenium and zinc concentrations in small fish were found to be higher than in big fish (Table 4-2). In a study from the UK, Mn, Se and Zn were detected in fish samples collected from UK markets in 1999. Levels of these elements were 1,100, 390 and 9,100 µg/kg, respectively (Ysart *et al.* 1999). The Mn content in Bangladeshi fish was similar to the UK fish study. However, both Se and Zn contents were higher for the Bangladeshi fish.

4.4.1.3 As species in Bangladeshi fish (Hilsha variety)

Arsenic species in Bangladeshi fish (Hilsha type), which contained high levels of arsenic, were determined using HPLC-ICP-MS. The species were detected in an aqueous fish extract. Results obtained showed that the main arsenic species in all the samples were AsBet, DMA and arsenosugar (phosphate) (Table 4-5 and Figure 4-3). AsBet species was less than DMA species in fish extraction; it could be that AsBet species is less soluble in water. AsBet species is considered to be a non-toxic species for humans (Ebisuda *et al.*, 2002). However, DMA is a more toxic form (Vega *et al.*, 2001) and no information about the toxicity of arsenosugar is currently available. In a recent study, investigating arsenic levels in urine of Bangladeshis residing in the UK (Cascio *et al.* 2011), high levels of DMA species in the urine was detected for the Bangladeshis compared to Caucasians. Bangladeshis residing in the UK consume similar types of Bangladeshi fish that are consumed in Bangladesh. Hilsha fish is available in ethnic Bangladeshi shops in the UK and is popular amongst UK Bangladeshis. Thus it is possible that DMA species detected in urine from Bangladeshis residing in the UK (Cascio *et al.* 2011) and Bangladesh (Lindberg *et al.*, 2008 and Hall *et al.*, 2009) can come from Hilsha fish species and rice consumption. Urinary DMA levels in Bangladeshi population drinking arsenic contaminated groundwater are generally attributed to methylation of inorganic arsenic present in water. However, the findings of the current study reveals that consumption of fish such as Hilsha species may complicate the urinary arsenic species by altering the percentage of DMA in urine samples in Bangladeshis. Since DMA levels are used to indicate the methylation capacity of individuals, it is important to take into consideration the role of fish consumption in future risk assessment studies.

4.4.2 Trace elements in Bangladeshi vegetables and rice

Ratios of toxic elements (As, Cd, Pb and Mn) to essential elements (Se and Zn) in different Bangladeshi foods including vegetables and rice were calculated (Figures 4-15 to 4-22). Results showed that root vegetables contain more arsenic followed by leafy vegetables and rice. However, ginger and leafy vegetables contain more Cd, Pb and Mn levels.

4.4.2.1 Arsenic in vegetables and rice.

In Bangladesh, arsenic contaminated groundwater is often used for irrigation of crops and vegetables paving the way for entry of arsenic and other toxic elements in vegetables grown in these arsenic contaminated areas. Some studies of arsenic concentration of Bangladeshi vegetables have been reported (Alam *et al.*, 2003; Das *et al.*, 2003; Karim *et al.*, 2008). In these studies, various vegetables were sampled from regions of Bangladesh that are known to have high levels of arsenic in the ground water.

The highest levels of arsenic were detected in different types of vegetables including ghotkol, taro (loti from Arum plant) and snake gourd with values of 446, 440 and 489 µg/kg (dry weight), respectively, with mean 225 µg/kg (dry weight) for all vegetables (Alam *et al.*, 2003). The mean arsenic concentration in leafy vegetable investigated in the current study was lower compared with Alam *et al.* (2003) study (96 µg/kg dry weight). However, arsenic concentrations for some specific vegetables were similar. In another study, leafy vegetables from Bangladesh were found to contain between 90 to 3990 µg/kg (dry weight) of arsenic (Das *et al.*, 2004). In contrast, in the current study the values of arsenic in leafy vegetables were between 17 – 278 µg/kg (dry weight). Das *et al.* (2004) also reported high levels of arsenic in potatoes (70 – 1360 µg/kg dry weight). These levels are much higher compared to what was detected in the current study [5.2 (1.4) µg/kg dry weight (fresh weight)]. In both of the previous studies (Alam *et al.*, 2003; Das *et al.*, 2004) vegetables were selected from regions of Bangladesh that are known to contain very high levels of arsenic in the groundwater. However, here in this study, one cannot be sure that if the vegetables, which were sold in the UK markets, came from an arsenic affected region of Bangladesh or not.

Rice contained high levels of arsenic and Bangladeshis consume large quantities of rice per day (approximately 500 g dry weight). Recently, Meharg *et al.* (2009) measured arsenic in different types of Bangladeshi rice (from different markets in Bangladesh including Dhaka) and found the mean of total aresnic in rice was 130 µg/kg (Meharg *et al.*, 2009). This is much higher than the arsenic level (68.5 µg/kg) found in rice in the current study (Table 4-4). Nevertheless, this value can contribute to a high daily intake of arsenic, which was estimated to be 34.31 µg/day. Daily intake of arsenic from all vegetables was estimated to be 5.16 µg/day. Leafy vegetables can contribute 2.58 µg/day of arsenic (Table 4-7). The data presented in this thesis showed that Bangladeshis are exposed to higher levels of arsenic from rice consumption compared to vegetable consumption.

4.4.2.2 Cadmium in vegetables and rice.

Many studies have determined Cd levels in leafy and non-leafy vegetables in different countries (Karavoltsos *et al.*, 2008; Mor and Ceylan, 2008; Yan *et al.*, 2009; Zheng *et al.*, 2007; Tripathi *et al.*, 1997). However, very little has been reported for Bangladeshi foods (Alam *et al.*, 2003; Khan *et al.*, 2010; Naser *et al.*, 2009); the current study is the most comprehensive investigation of Cd levels in Bangladeshi food and non-food materials that are consumed by humans.

Levels of Cd measured in comparable leafy and non-leafy vegetables in Mumbai city (India) were found to be 14.9 and 3.2 µg/kg (fresh weight), respectively (Tripathi *et al.*, 1997). These Indian results were lower than the findings in the current study for Cd levels in vegetables. In a Bangladeshi study by Alam *et al.* (2003), which measured Cd in similar leafy and non-leafy vegetables from Samta village, in the Jessore district of Bangladesh, they found that Cd levels in the vegetables ranged from 12 to 216 µg/kg (dry weight). The highest Cd content was detected in a leafy vegetable (ghotkol). In the current study, results show that Cd levels in vegetables (sold in UK markets and imported from Bangladesh) were similar to Cd levels in Bangladeshi vegetables Alam *et al.* (2003) study.

Lal shak (*Amaranthus tricolour)* is a leafy vegetable and it is one of the common vegetables consumed by Bangladeshis. This vegetable had the highest Cd concentration at 303 µg/kg (fresh weight). In another Bangladesh study, amaranth vegetables and bitter gourd were analysed and Cd levels were found to be 33 and 21 µg/kg (fresh weight), respectively (Khan *et al.*, 2010). This was similar to the current study. Naser *et al.* (2009) analysed spinach grown in three different conditions in Bangladesh (directly polluted, indirectly polluted and non-polluted) and Cd levels were 1400, 1187 and 559 µg/kg (dry weight), respectively. The results from the current study ranged between the latter workers non-polluted and polluted area data. This suggests that vegetables sold in the UK markets are imported from different areas in Bangladesh, including Cd contaminated and non-contaminated areas.

In a UK total diet survey (Ysart *et al.*, 1999), green vegetables, potatoes and other vegetables grown in the UK were investigated and Cd levels were found to be 6, 30 and 8 µg/kg (fresh weight). These results reveal that Bangladeshi vegetables sold in UK markets have higher Cd concentrations than the vegetables grown in the UK. A recent study in Greece analysed organic foodstuffs and vegetables collected from a Greek market and determined their Cd levels (Karavoltsos *et al.*, 2008), the highest Cd concentration was found in leafy vegetables (15.4 µg/kg fresh weight), which is very low compared to the current study results. Onianwa *et al.* has reported Cd levels in Nigerian leafy vegetables averaged 220 µg/kg (dry weight) with a range of 90 – 620 µg/kg (dry weight) (Onianwa *et al.*, 2000). Although, these values are higher than the Greek study, the Cd levels are still lower than results from the current study which show Cd content for leafy vegetables to range from 204.3 to 2931 µg/kg (fresh weight) (25 to 283 µg/kg dry weight). Dry weight in the current study was calculated after subtracting the measured water content in vegetables.

More recently, Yan *et al.* (2009) measured Cd levels in pak choi (*Brassica chinensis* L.), a widely consumed vegetable in China, grown in a metal contaminated area; high concentrations of Cd were found in the leafy edible part of the vegetable [ranging from 20 to 550 µg/kg (fresh weight), with an average of 170 µg/kg (fresh weight)]. The Cd levels in roots were even higher than that in leafy edible part (averaged 250 µg/kg fresh weight in roots). In the current study, the Bangladeshi leafy vegetables sold in the UK markets show Cd levels in vegetables that were similar to what has been reported for vegetables grown on contaminated land in other countries including Bangladesh. The significantly high levels of

Cd detected in leafy vegetables from Bangladesh may be of concern for human health for Bangladeshis in Bangladesh and UK Bangladeshi communities that consume large quantities of such vegetables.

It has been reported that cadmium accumulates in the leaves of plants (Alloway *et al.*, 1990), which is consistent with the observation in this study that Cd levels are much higher in leafy vegetables. It has also been reported that the accumulation of cadmium in plants, grown in the same soil, decreased in the following order: leafy vegetables > root vegetables > grain crops (He and Singh, 1994).

Rice contains significant levels of Cd and is a major contributor of dietary Cd intake in rice consuming populations. Rice is a staple food for Bangladeshis; it is consumed at least twice a day. Average daily intake can be 500 g (dry weight) which can provide an estimated Cd intake of 18.6 µg/day (Table 4-7). The mean of Cd levels in Bangladeshi rice in the current study was 37.2 µg/kg (Table 4-4). Similar results for Cd levels in rice were published by others. For example, Cd levels in Bangladeshi rice were found to be 33.1 µg/kg (mean value) (Khan *et al.*, 2010) and 47 µg/kg (median value) (Kippler *et al.*, 2010). Kippler *et al.* (2010) reported that rice consumption is the main source of Cd intake. The results presented in this thesis were consistent with this suggestion.

High intake of rice is a major factor for the elevated exposure to Cd in the Bangladeshi population. The use of fertilisers, especially phosphate based fertilisers which are known to contain Cd, is likely to be the main reason for the high Cd levels in rice. Urea and phosphate based fertilisers, such as Triple Super Phosphate, are widely used in rice production in Bangladesh (Barkat *et al.*, 2010). It is well known that phosphate based fertilizers can contain high levels of different toxic elements including Cd (Dissanayake and Chandrajith, 2009). Furthermore, it is possible that urea, which is also widely used as a fertilizer, may be contaminated with heavy metals including Cd and its application may alter the uptake of Cd from soil (Jalloh *et al.*, 2009). What is of particular concern is the very high level of Cd detected in puffed rice. It is widely known in Bangladesh that urea fertiliser is used for whitening puffed rice to increase their appeal to consumers. The danger of this practice has been highlighted by Shykh Seraj in his writings where he mentions about the popularity of puffed rice in Bangladesh and the dangers of human exposure to harmful chemicals due to the use of urea for whitening the rice (Seraj, 2007). Since the level Cd in puffed rice was very much higher compared to levels detected in other rice samples from Bangladesh, analysed in the current study and by others, it can be concluded that this is likely due to the manufacturing process used for producing puffed rice or due to food adulteration (use of urea for whitening puffed rice). The fact that Cd levels vary between different brands, with some showing levels that are similar to raw rice; it is most likely that the use of urea is mainly responsible for the very high levels detected for some samples. It is important that food safety authorities in Bangladesh act urgently to address the entry of heavy metals and other contaminants in puffed rice which is a very popular food in Bangladesh.

For non-occupationally exposed populations, the source of exposure to Cd is mainly through the dietary route. Vegetables were the main source of toxic elements especially Cd from consumption of Bangladeshi food. Alam *et al.* (2003) has estimated daily intake of vegetables in Bangladesh, they found that the daily intake of Cd from vegetables grown in Samta village (Jessore, Bangladesh) was 9.45 µg/day (Alam *et al.*, 2003). This daily intake for vegetables consumption was similar to daily intake of vegetables estimated in the current study (13.14 µg/day see Table 4-7). This similarity between the data from the two studies added further confidence to the estimation in the current study.

In the current study, the finding of a very high daily intakes of Cd from vegetables and rice (13.14 and 18.6 µg/day) for Bangladeshis can be used to explain the high Cd concentrations measured in breast milk of Bangladeshi women (median 0.14 µg/L) compared to other countries around the world (median < 0.1 µg/L) (Kippler *et al.*, 2009). It may also explain the high Cd exposure among children in rural Bangladesh (Kippler *et al.*, 2010).

Daily intake of Cd for Bangladeshis was the highest reported for any non-occupationally exposed populations around the world, with high vegetables and rice consumption being the main reason for this. Therefore it is recommend that every effort is made to reduce the entry of Cd into the food chain in conjunction with a more balanced diet that includes a reduction in the intake of rice and certain leafy vegetables with high Cd content.

The findings (in the current study) suggest that rice and leafy vegetables are the two dominant contributors towards dietary exposure to Cd which is in agreement with the literature on analysis of different food products by others (see for example, EFSA, 2009). It is also consistent with human biomonitoring studies measuring Cd levels in human biofluids. For example, Krajcovicova-Kudlackova *et al.* (2006) reported higher average blood Cd concentration in vegetarians compared to non-vegetarians (Krajcovicova-Kudlackova *et al.*, 2006). As with many other toxic elements, the presence of Cd in the environment can be from both natural and anthropogenic sources. Amongst factors responsible for high Cd levels in agricultural soil are the use of chemical fertilisers, pesticides, sewage sludge and atmospheric pollution. In Bangladesh, application of pesticides and chemical fertilizers in agricultural is very common and may explain the reason for the occurrence of high levels of Cd in vegetables and rice (Alam *et al.*, 2003).

4.4.2.3 Lead in vegetables and rice.

Lead is well known for its toxic effects on human health. Exposure to Pb can lead to different diseases (Fergusson, 1990; Verstraeten *et al.*, 2008). Few studies have focused on Pb levels in vegetables (see for example, Tripathi *et al.*, 1997; Ysart *et al.*, 1999; Alam *et al.*, 2003). Tripathi *et al.* (1997) has reported that Pb content in leafy and non-leafy vegetables were 100 and 4.1 µg/kg (fresh weight), respectively (Tripathi *et al.*, 1997). The findings presented in this thesis are very similar to the results of this Indian study. In a previous study on vegetables from the UK, Pb levels in vegetables and potatoes were found to be 10 and 20 µg/kg, respectively (Ysart *et al.*, 1999).The values for the vegetables are much lower than that detected in the current study for Bangladeshi vegetables. More recently, Pb content of vegetables were determined in Bangladesh by Alam *et al.* (2003). The highest Pb levels were detected in leafy vegetables (1,689 and 831 µg/kg dry weight for ghotkol and shak, respectively), however, lower Pb level was detected in okra (143 dry µg/kg dry weight) (Alam *et al.*, 2003). In contrast, the data presented in this thesis showed that the highest level of Pb is in data shak (10,660 µg/kg dry weight), which is higher than that measured in the Bangladeshi study. Okra contained 33 µg/kg dry weight of Pb as a mean value. Low levels of Pb in Bangladeshi rice (mean 18.5 µg/kg) were detected.

In the work presented in this thesis, the daily intake of Pb from leafy and non-leafy vegetables was estimated to be 40.7 µg/day (see table 4-7). In contrast, the daily intake of Pb from vegetables by a study from Bangladesh was higher at 74.7 µg/day (Alam *et al.*, 2003). Further work needs to be conducted to better understand the exposure to Pb in Bangladesh population. In a previous study, high levels of Pb in blood were determined for five year old primary school children in Dhaka (Bangladesh) (Kaiser *et al.*, 2001).

4.4.2.4 Manganese, selenium and zinc in vegetables and rice.

Manganese, selenium and Zinc are essential elements and they are required by the human body for various functions. However, exposure to high levels of these elements can lead to some diseases (Santamaria, 2008; Li, 2007). Also deficiency of these elements can be harmful for the human body. Selenium is an antioxidant element and it has been suggested to counteract arsenic toxicity (Hsueh *et al.*, 2003). Thus deficiency in the intake of Se could result in an increase in arsenic induced ill health in the Bangladeshi population (Spallholz *et* *al.*, 2004). Hence, it is important to have in the diet foods that are rich in essential elements such as Se and Zn.

In this thesis, Mn levels were measured in different types of edible vegetables from Bangladesh. High levels of Mn were detected in some of vegetables. In a study on Indian vegetables, Mn levels in leafy and non-leafy vegetables were determined by Roychowdhury *et al.* (2003). Manganese levels were 4.60, 4.43, 8.39 and 12.60 mg/kg fresh weight for leafy vegetables, beans, spinach and lentil, respectively (Roychowdhury *et al.*, 2003). These results (except for lentil) are lower than the findings in the current study (Mn content in lentil was 7.73 mg/kg fresh weight). In a study of UK vegetables, Mn in green vegetables, potatoes and other vegetables were 2.00, 1.90 and 1.60 mg/kg fresh weight, respectively (Ysart *et al.*, 1999). These levels are very low compared to the Mn levels in Bangladeshi vegetables from findings in the current study.

Selenium concentrations were measured in different types of edible vegetables in Bangladesh (in the current study). In another study on vegetables from India, Se levels in leafy and non-leafy vegetables were determined (Roychowdhury *et al.*, 2003). Se levels detected by these workers were 0.72, 8.38, 0.2 and 223 µg/kg fresh weights for leafy vegetables, beans, spinach and lentil, respectively. These results, except for lentil, are lower than the findings of the current study for Bangladeshi vegetables (Se concentration in lentil was 152 µg/kg). Generally, lentil contains the highest levels of Se in all the cereals and vegetables analysed. In the UK study, Se in green vegetables, potatoes and other vegetables were 10, 8 and 20 µg/kg fresh weights, respectively (Ysart *et al.*, 1999). The levels are rather low compared with the levels detected for the Bangladeshi vegetables presented in this thesis. Daily intakes of Mn and Se from green vegetables consumption for Bangladeshis were estimated (in this thesis) to be 4820 and 8.66 µg/day, respectively (Table 4-7).

Vegetables are a good source of zinc. High levels of Zn were detected in leafy and non-leafy vegetables in this study. Mean Zn contents were 6.52, 4.12 mg/kg fresh weight for leafy and non-leafy vegetables, respectively. Zinc content in vegetables was similar to that reported from Samta village in Bangladesh (Alam *et al.*, 2003). Zn levels in leafy and non-leafy vegetables from India were 4.81 and 1.93 mg/kg (Tripathi *et al.*, 1997). These levels are lower than Zn contents in vegetables studied in this thesis. Ysart *et al.* has determined Zn levels in different British vegetables. For green vegetables, potatoes and non-vegetables, the Zn levels were 3.40, 4.50 and 2.60 mg/kg (Ysart *et al.*, 1999), respectively. These levels are lower than that measured in Bangladeshi vegetables in the current study (potatoes contained 8.27 mg/kg fresh weight of Zn). High levels of Zn in vegetables were detected in Samta village (in Bangladesh) (Alam *et al.*, 2003). The daily Zn intake of vegetables in Bangladesh was estimated to be 3.57 mg/day (Alam *et al.*, 2003). This value was very similar to daily intake of Zn estimated from the analysis of Bangladeshi vegetables presented in this thesis (3.1 mg/day, see table 4-7). Rice is also a good source of Mn, Se and Zn. Since the Bangladeshis consume large quantities of rice (approximately 500 g per day), Daily intakes of Mn and Zn from consumption of rice were estimated to be 6.58 and 5.51 mg/day, respectively and for Se was 27.4 µg/day (Table 4-7).

4.5 Conclusion

Levels of As, Cd, Mn, Pb, Se and Zn were determined in Bangladeshi vegetables and fish. Rice and vegetables were the main source of these elements. High levels of toxic elements were detected in leafy vegetables such as lal shak; however, non-leafy vegetables can contribute towards intake of low toxic elements and high essential elements (Se and Zn) such as gourds, okra and lentils. Fish is an integral part of Bangladeshi diet. Big fish contained high ratio of toxic and essential elements compared to small fish. This leads one suggest that small fish can be a healthier food than big fish. High levels of arsenic were detected in Hilsha type fish (big sea fish) which is a very commonly consumed fish in Bangladesh. This is the first study to report speciation of arsenic in Bangladeshi fish. Hilsha fish was found to contain a higher proportion of DMA compared to AsBet species. This issue requires further study by analysing other types of fish including freshwater fish. Results showed that Bangladeshis are exposed to high levels of toxic elements from their diet and a multipronged strategy is required to reduce these elements such as Cd exposure in Bangladeshis. This can include changes in agricultural practice to reduce entry of Cd and other toxic elements in the food chain and also appropriate modification of the Bangladeshi diet to reduce the intake of foods that were very high in As, Cd and Pb including rice and leafy vegetables.

5 BETEL QUID CHEWING ELEVATES HUMAN EXPOSURE TO ARSENIC, CADMIUM, LEAD AND MANGANESE

Summary

Increased skin lesion amongst betel quid [a mixture of *Piper betel* leaves, areca nut, tobacco/flavoured tobacco, lime (calcium hydroxide)] chewers compared to non-chewers has been reported, for people exposed to arsenic contaminated drinking water, in Bangladesh and India. Besides As, Mn levels in Bangladeshi groundwater is also very high. As, Mn, Cd and Pb levels of whole betel quid and of its individual components were determined using inductively coupled plasma mass spectrometry (ICP-MS). The potential contribution of betel quid chewing on the daily intakes of these elements from betel quid chewing were estimated for Bangladeshi populations. The highest concentrations of arsenic was detected in lime (4.56 mg/kg) followed by *Piper betel* leaves (0.406 mg/kg) and *Zarda* (flavoured tobacco) (0.285 mg/kg), with a mean concentrations of arsenic in ordinary betel quids of 0.035 mg/kg (SD 0.02). Mean concentrations of Cd, Mn and Pb in ordinary quids were 0.028 (SD 0.07), 41 (SD 27) and 0.423 (SD 1.4) mg/kg respectively, with highest concentrations found in *Zarda* (1.16 and 53.5 mg/kg for Cd and Pb respectively). However, high levels of Mn were detected in *Piper betel* leaves with an overall average of 135 mg/kg (range 26 -518 mg/kg).

Betel quid chewers displayed a significantly higher arsenic (*P* = 0.012) and Mn (*P* = 0.009) levels. Mean As and Mn concentrations in urine were 52.28 and 1.93 µg/L in chewers compared to 31.84 and 0.62 µg/L in non-chewers, respectively. Chewers display a 3.1 fold increased urinary Mn excretion compared to non-chewers. The daily intake of six betel quids can contribute 1.2, 1.9, 18 and 8.5% of the provisional maximum tolerable daily intake (PMDTI) for As, Cd, Mn and Pb respectively.

The habit of chewing betel quids has been maintained for many decades and this constant exposure to As, Cd, Mn and Pb may result in additive or multiplicative adverse effects on the body. This situation is likely to be particularly harmful for those groups that are already exposed to high levels of As, Cd, Mn and Pb through drinking water and consumption of foods that are rich in these elements, especially vegetables and rice. Since betel quid chewing is most prevalent amongst women, especially pregnant women, the finding of the current study raises concern that this group may be harming their health and that of their unborn babies through increased exposure to As, Cd, Mn and Pb. Additional Mn exposure from foods and groundwater may make betel quid chewers in Bangladesh more vulnerable to ill health. Hypothesise of this thesis is that higher prevalence of tremor, and possibly skin lesions, in betel quid chewers can be attributed to Mn toxicity alone or in synergy with other chemicals, especially arsenic.

5.1 Introduction

High levels of arsenic through drinking contaminated groundwater and consuming rice in Bangladesh and West Bengal (India) were reported (Samanta *et al.*, 1999). These populations are exposed to high levels of arsenic which has been linked to the development of various diseases including hyperkeratosis and skin lesions and millions of people are at risk of developing cancer (Hafeman *et al.*, 2006). The arsenic contaminated water is also used for irrigation of different crops including rice (which is the main food in Bangladesh and India). This leads to further problems, as high levels of arsenic were detected in consumable foods (rice and some vegetables) grown in Bangladesh (Al-Rmalli *et al.*, 2005; Alam *et al.*, 2003; Kile *et al.*, 2007). The risk to human health through intake of toxic elements such as As, Cd and Pb from the food chain is also well established (Freitas *et al.*, 2008; Jarup *et al.*, 1998; Mishra 2009). As, Cd and Pb are the most toxic elements in the world; they can interrupt the functioning of human organs such as kidney and lungs. An association between Pb exposure and neurotoxicity has been reported (Verstraeten *et al.*, 2008).

Manganese is an essential element for human health; recently Mn has been identified as another element that may pose health risks in Bangladeshi populations through drinking Mn contaminated groundwater. Manganese levels higher than the World Health Organisation standard (0.400 mg/l) have been detected in Bangladeshi groundwater (Frisbie *et al.*, 2009; Hafeman *et al.*, 2007; Ljung *et al.*, 2009). Exposure to high levels of Mn can induce neurological effects such as manganism which is characterised by movement disturbances similar to that observed in Parkinson's disease (Lucchini *et al.*, 2009). Some studies have reported a relationship between Mn exposure through drinking water in Bangladesh and children's intellectual performance (Brown and Foos, 2009) and infant mortality (Hafeman *et al.*, 2007).

Elements (As, Cd, Mn and Pb) can increase the generation of reactive oxygen species (ROS), which may have harmful effects at the cellular and organ level (Beyersmann and Hartwing, 2008). Human exposure to these elements via different routes such as water and foods can lead to diverse disease processes. However, intake of these elements from non-food sources may be a contributory factor in the development of disease and this requires further investigation. More recently Ljung *et al.* (2009) investigated Mn levels in drinking water and its relationship to various biomarkers during early pregnancy for Bangladeshi women. Although the authors investigated the relationship between Mn intake from water and Mn concentrations in urine and blood, no strong correlation was found. A possible explanation for the absence of a correlation was that the additional Mn intake from foods was not taken into consideration.

In this regard a significant proportion of the Bangladeshi and Indian populations chew betel quid, the habit of betel quid chewing is widely prevalent in Bangladesh (Gupta and Ray, 2004) and the possibility that it may contributes to increased exposure to high levels of toxic elements and Mn has not been previously addressed.

Betel quid chewing has been practised for hundreds of years; it is a very common social habit in East and South Asian countries. More than 200 millions of East and South Asian population chew betel quid daily (Norton, 1998). The main constituent of a betel quid are *Piper betel* leaves and areca nut (the seed of the Areca catechu plant). It is made by wrapping chopped areca nut in a *Piper betel* leaf and it often also includes some lime (calcium hydroxide) and tobacco leaves (called *Shada* in Bangladesh) or *Zarda* (flavoured tobacco) to improve the taste. The betel quid is commonly known as ‘paan’ in South Asian countries including Bangladesh and India. *Zarda* is often used in betel quids instead of untreated tobacco leaves. A relationship between betel quid chewing and increased risk of arsenic induced skin lesions have been reported by several workers (McCarty *et al.*, 2006; Pilsner *et al.*, 2009; Lindberg *et al.*, 2010).

A significant part of the diet of the Bangladeshi community residing in the UK is composed of foods imported from Bangladesh that are sold in ethnic shops. This is particularly the case with fish, vegetables and betel quid components such as *Piper betel* leaves, areca nut and *Zarda*. It has been previous reported that arsenic levels in Bangladeshi food is high and concern was expressed that UK Bangladeshis have increased exposure to arsenic (Al-Rmalli *et al.*, 2005). Recently Lindberg *et al.* (2009) determined the arsenic content in tobacco and *Zarda*, which are often included in betel quid, and highlighted that tobacco chewing, can further increase the risk of arsenic induced skin lesions among Bangladeshi people.

In this chapter, presents data discussing the potential of human exposure to As, Cd, Mn and Pb through the habit of chewing betel quids. Previously it has been suggested that arecoline, the principal organic compound, in areca nut, may induce oxidative stress that explains greater incidence of skin lesions in chewers (Pilsner *et al.*, 2009). However, as yet no studies have addressed the possibility that high levels of As, Mn and other elements in betel quids, and possibly other dietary products, may be a contributory factor for such adverse health outcomes. To obtain information in this area, urinary As, Cd, Mn and Pb levels in betel quid chewers and non-chewers were investigated. It is also relevant for the sizeable Bangladeshi and Indian communities residing in the United Kingdom. Furthermore, since these toxic elements can readily pass from the mother to the foetus, betel quid chewing by pregnant women may be a particular health concern. Therefore, the PMTDI for As, Cd, Mn and Pb associated with chewing betel quid was estimated.

5.2 Materials and Methods

5.2.1 Urine sample collection and study population

Ethical approval from De Montfort University, Faculty of Health & Life Sciences, Ethics committee, was obtained for a study investigating the dietary and life-style habits of different ethnic groups in the United Kingdom including members of the Bangladeshi community see section (2.2.1.2.). Mn levels in urine were adjusted with specific gravity of urine (Nermell *et al.*, 2008). Urine As and Mn levels in non-chewers and in chewer groups were compared using a nonparametric test (Wilcoxon rank sum test) with P = 0.012 and P = 0.009 being considered as significant for As and Mn respectively.

5.2.2 Betel quid samples

Betel quid and its components that are widely consumed in Bangladesh (*Piper betel* leaves, areca nut, lime, tobacco and *Zarda*) were purchased from UK based ethnic shops in the cities of Leicester, Birmingham, London and Luton during the months of September 2008 and November 2009. Products analysed in this study were of mainly of Bangladeshi origin excluding some areca nut and lime, which were of Indian origin. These products are popular with Bangladeshi communities residing in the UK.

5.2.3 Sample preparations

5.2.3.1 *Piper betel* leaves and other food preparation

Piper betel leaves were washed three times with deionised water, dried in an oven at 80 ^{o}C overnight, and then ground with a grinder. Other samples (areca nut, tobacco and *Zarda*) were also ground after drying, but without prior washing.

5.2.3.2 Betel quid preparation

Betel quid samples (ordinary and sweet, see Figure 5-1 and 5-2) were collected from ethnic shops in UK. However, some betel quids were also prepared in the laboratory by combining different chewing components in proportions that are commonly used in commercial preparations and also based on information obtained from betel quid chwers (see Chapter 2 section 2.2.2.1.)

5.2.4 Sample digestion

5.2.4.1 Betel quid samples digestion

Ordinary and sweet betel quids and their individual components were digested by microwave assisted digestion (see Chapter 2 section 2.2.3.2).

5.2.4.2 Urine analysis

Aliquots (3 – 4 ml) of urine samples were digested (see Chapter 2 section 2.2.3.3.).

Figure 5.1: Betel quid and its components (Areca nut, *Piper betel* leave, lime and *Zarda*).

5.2.5 Elemental determination

Elements in the digested solution samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (see Chapter 2, section 2.2.4.).

5.2.6 Correlation betweenTDI of Mn and Mn levels in urine.

The total daily intake (TDI) of Mn (more details about TDI in Chapter 7) and urinary Mn levels were taken from the literature for UK, Germany, USA, Japan, Pakistan and India (Mumbai) (White and Sabbioni, 1998; Greger *et al.*, 1990; Heitland and Koster, 2006; Okashi *et al.*, 2009; Afridi *et al.*, 2009; Tripathi *et al.*, 2000). For the Bangladeshi population, the TDI was calculated in-house. Urinary Mn levels for the Bangladeshi population residing in Bangladesh were taken from Ljung *et al.* (2009). Since several values for Mn intake are available for a particular country, for the TDI graph values were selected values, if available, where both the Mn intake and urinary Mn excretion were reported in the same paper. The correlation between TDI and Mn urinary levels was calculated using SPSS software (version 17).

5.2.7 Quality control and standard reference material

See section (2.2.5.) in methodology chapter.

Figure 5.2: Ordinary and sweet betel quid and its components.

5.3 Results

5.3.1 As, Cd, Mn and Pb levels in betel quids & its components

ICP-MS determined concentrations of As, Cd, Mn and Pb in betel quids and their various components are shown in Table 5-1. The dry weight of betel quids ranged between 5.5 to 11 g with an average of 7 g. Mean concentrations of As, Cd, Mn and Pb present in betel quids were used for calculating the PMTDI for each of these elements. The standard deviation (SD) was also determined.

The mean concentration of arsenic in whole betel quids was higher for ordinary quids (0.035 mg/kg) compared to sweetened quids (0.02 mg/kg) (see Figure 5-2). As for the individual components, the descending order based on mean levels of arsenic were: lime, tobacco leaves, *Zarda*, *Piper betel* leaves and betel nut. Mean levels of arsenic in lime were 23-fold higher than in tobacco leaves. The highest concentration of arsenic was found in the lime (calcium hydroxide) (4.56 mg/kg). The mean concentration of arsenic in *Piper betel* leaves showed a particularly wide range of arsenic concentrations, with the highest level in the *Khasia* variety (0.406 mg/kg) (Table 5-1). Thus the *Piper betel* leaf, which is the main characteristic component of a betel quid and considered by many to be healthy due to its “green” colour, may contribute a significant proportion of ingestible arsenic depending on the variety used.

Table 5-1: Concentrations of As, Cd and Pb, Mn (mg/kg dry weight) of betel quid chewing components.

Component	**n**	**Mean**	**SD**	**Median**	**Range**
Ordinary quid	26				
As		0.035	0.02	0.033	0.011-0.082
Cd		0.028	0.01	0.028	0.007-0.065
Pb		0.423	0.99	0.204	0.100-5.270
Mn		41	27	30	12-110
Se		0.140	0.065	0.132	0.050-0.320
Zn		9.94	3.17	9.2	5.44-16.10
Sweet quid	12				
As		0.020	0.02	0.013	0.007-0.070
Cd		0.014	0.01	0.010	0.006-0.042
Pb		0.630	0.53	0.370	0.140-1.650
Mn		19	10	16	10-40
Se		0.095	0.037	0.091	0.035-0.154
Zn		9.3	3.25	8.56	6.2-18.8
Piper betel leaves	30				
As		0.107	0.08	0.092	0.013-0.406
Cd		0.049	0.04	0.030	0.006-0.155
Pb		0.725	0.46	0.630	0.258-2.259
Mn		136	106	145	26-518
Se		0.080	0.055	0.054	0.018-0.218
Zn		15.7	9.9	12.2	7.7-64.1
Areca (betel) nut	7				
As		0.013	0.01	0.013	0.002-0.030
Cd		0.016	0.01	0.017	0.003-0.045
Pb		0.102	0.05	0.100	0.057-0.153
Mn		12	5	13	6-21
Se		0.098	0.065	0.103	0.014-0.212
Zn		5.7	1.17	5.87	4-7.1

Table 5-1 contained.

Component	**n**	**Mean**	**SD**	**Median**	**Range**
Lime	6				
As		3.470	0.98	0.291	1.863-4.560
Cd		0.131	0.04	0.062	0.073-0.166
Pb		1.551	0.12	0.960	1.371-1.590
Mn		40	8	42	29-46
Se		0.072	0.033	0.072	0.037-0.107
Zn		1.33	0.28	1.45	0.9-1.5
Tobacco leaves	3				
As		0.151	0.05	0.151	0.116-0.186
Cd		0.641	0.01	0.641	0.638-0.645
Pb		1.06	0.32	1.060	0.840-1.290
Mn		367	140	345	268-466
Se		0.122	0.068	0.107	0.037-0.301
Zn		36.6	21	28.6	2.7-40.3
Zarda (flavoured tobacco)	13				
As		0.150	0.07	0.142	0.040-0.285
Cd		0.402	0.32	0.378	0.030-1.160
Pb		4.820 (0.78)*	14.6	0.830	0.220-53.50
Mn		102	61	97	16-207
Se		0.117	0.033	0.072	0.037-1.5
Zn		21.19	10.8	19.4	12.5-29.6

* mean value of Pb in *Zarda* samples excluding one brand contains 53.50 mg/kg

Of the different betel quid components analysed, *Zarda* had the highest concentrations of Cd and Pb, 1.16 and 53.5 mg/kg, respectively. The highest concentrations of Cd and Pb in betel quids were 0.065 and 5.27 mg/kg, respectively. However, the mean concentrations of Cd and Pb in a single betel quid were found to be 0.028 and 0.423 mg/kg, respectively. The highest concentration of Mn was found in *Piper betel* leaves (518 mg/kg). However, with regards to mean concentration of Mn the decreasing order of concentration (mg/kg) was: tobacco leaves (367); *Piper betel* leaves (136); and *Zarda* (flavoured tobacco; 102). The content of Mn in lime (calcium hydroxide) was considerably lower at 40 mg/kg. Ordinary betel quid contained on average 41 mg Mn/kg, compared to 19 mg/kg for sweeten ones. Areca nut had the lowest concentration of Mn (Table 5-1). Low levels of Se were detected in all betel components, Se in betel quid was 0.14 mg/kg with range 0.05-0.32 mg/kg, and however, Zn was 9.94 mg/kg (Table 5-1).

5.3.2 As speciation in betel quid and *Piper betel* leaves

Good extraction efficiencies of betel quid and *Piper betel* leaves were achieved in aqueous solutions; they ranged from 95 to 105% (Table 5-2). High percentage of inorganic arsenic species in betel quid and *Piper betel* leaves were detected (81 – 100%), some samples of betel quids contained AsBet species, which may from some species that may add to the betel quid. However, *Piper betel* leaves contained DMA, no MMA or arseno-sugar was detected in betel quids (Table 5-2).

Table 5-2: Concentrations and percentages of As species in Betel quids.

Sample	**Total µg As/kg d wt.**	**Ext. effic.[1] %**	**Column recovery %**	**AsBet µg/kg d wt.**	**DMA µg/kg d wt.**	**As^{III} µg/kg d wt.**	**As^{V} µg/kg d wt.**	**Inorganic As % µg/kg d wt.**
Betel quid								
S1	33 ± 4	101	99.8	3.8	nd	29.2	nd	89
S2	31 ± 8	95	100.4	2.1	nd	29.0	nd	94
S3	34 ± 1	98	105.7	6.8	nd	29.3	nd	81
S4	32 ± 4	97	101.6	3.3	nd	29.2	nd	90
S5	33 ± 1	96	96.4	nd	nd	31.8	nd	100
S6	36 ± 3	98	88.4	nd	nd	31.7	nd	100
S7	32 ± 5	105	102.8	nd	nd	32.9	nd	100
S8	34 ± 4	103	97.2	nd	nd	32.4	0.7	100
***Piper betel* leaves**								
S1	225 ± 28	99	107.7	nd	17.8	57.1	167.3	93
S2	228 ± 34	101	104.7	nd	15.6	60.0	163.1	93
TJF(n=3)[2]	600 ± 42	98	90	372 ± 6	26 ± 2	55 ± 7	41 ± 2	18 ± 0.3

[1] Ext. Effic.: Extraction efficiency

[2] Typical Japanese food (certified reference material) contained also AsChol (5.4 µg/kg), MMA (14.3 µg/kg) and arseno-sugar (phosphate) (15.4 µg/kg) species. Ext. Effic.: extraction efficiency. d wt.: dry weight. nd: not detectable.

5.3.3 Urinary As and trace elements levels in betel quid chewers and non-chewers

Arsenic levels and other elements in 37 urine samples from ethnic Bangladeshis living in the United Kingdom were analysed (Table 5-3, 5-4, 5-5). Mean concentrations of trace elements in all urine samples collected (chewers and non-chewers combined) were As (52.28 and 32.84 µg/L for chewers and non-chewers, respectively), Cd (0.63 and 0.36 µg/L for chewers and non-chewers, respectively) and Pb (4.12 and 2.54 µg/L for chewers and non-chewers, respectively). The mean Mn concentration for all urine samples was 1.04 µg/L, with a median of 0.45 µg/L and range of 0.08-5.2 µg/L. The mean urinary Mn levels in chewers (1.93 µg/L, SD 1.8) was significantly higher (3.1 fold; $P = 0.009$) compared to non-chewers (0.62 µg/L, SD 0.4) (see Figure 5-3). From the questionnaire, it was found that an average of 3.5 betel quids was chewed by the volunteers, with a range of 1 - 30 per day. Arsenic in chewer urine samples was also significantly higher (1.6 fold; $P = 0.012$) compared to non-chewers. No significant difference was detected for other trace elements.

Table 5-3: Concentrations of As, Cd, Mn and Pb in Bangladeshi chewers' urine (µg/L).

Sample code	**Gender**	**As**	**Cd**	**Mn**	**Pb**
Nx 002	Male	36.60	0.65	0.20	1.29
Nx 005	Female	29.15	0.53	0.70	1.66
Nx 009	Female	101.10	0.59	1.00	1.36
Nx 012	Female	45.60	0.17	0.80	6.13
Nx 013	Female	75.01	1.59	1.45	5.24
Nx 018	Female	70.72	0.19	0.50	1.54
Nx 019	Female	21.32	0.29	3.20	2.29
Nx 025	Female	74.89	0.25	2.30	1.33
Nx 036	Male	77.50	0.23	0.60	2.64
Nx 042A	Male	86.11	2.22	4.20	17.35
Nx 042B	Male	41.34	0.66	5.20	7.54
Nx 042D	Male	25.90	0.16	2.40	1.29
Nx 042E	Male	38.52	0.47	2.40	2.04
PH 001	Male	43.12	1.11	1.50	8.67
PH 002	Male	17.51	0.28	2.30	1.45
Mean		***52.28***	***0.63***	***1.93***	***4.12***
SD.		***26.21***	***0.59***	***1.70***	***4.42***
Median		***43.07***	***0.47***	***1.50***	***2.04***

Table 5-4: Concentrations of As, Cd, Mn and Pb in Bangladeshi non-chewers' urine (µg/L).

Sample code	**Gender**	**As**	**Cd**	**Mn**	**Pb**
TH 001	Female	12.22	0.15	0.50	0.80
Nx 004	Male	24.70	0.24	0.29	1.11
Nx 008	Male	32.50	0.10	0.80	1.29
Nx 011	Male	32.63	0.28	0.34	1.47
Nx 017	Male	71.17	0.53	0.44	0.89
Nx 022	Male	21.20	0.34	0.59	1.82
Nx 023	Male	82.59	0.27	0.70	11.76
Nx 027	Male	45.44	1.29	0.27	1.70
Nx 033	Male	17.50	0.28	0.30	2.35
Nx 033B	Male	32.80	0.35	1.28	2.81
Nx 034	Male	19.41	0.32	0.50	1.61
Nx 042C	Female	35.80	0.23	0.54	4.45
Nx 042F	Female	67.00	0.25	0.24	4.06
Nx 042G	Female	66.21	0.29	0.40	2.91
Nx 048	Male	12.90	0.61	0.78	2.07
Nx 049	Male	10.60	0.23	0.90	2.98
Nx 063	Male	22.10	0.18	1.37	1.74
Nx 075	Male	3.82	0.29	0.50	2.45
Nx 080	Male	28.42	0.16	1.33	2.53
Nx 087	Male	11.80	0.19	0.40	0.74
Nx 088	Male	7.70	0.88	0.41	1.17
Nx 0B	Male	22.00	0.28	0.80	1.44
Mean		***31.84***	***0.36***	***0.62***	***2.54***
SD.		***22.41***	***0.27***	***0.60***	***2.32***
Median		***24.69***	***0.28***	***0.50***	***1.82***

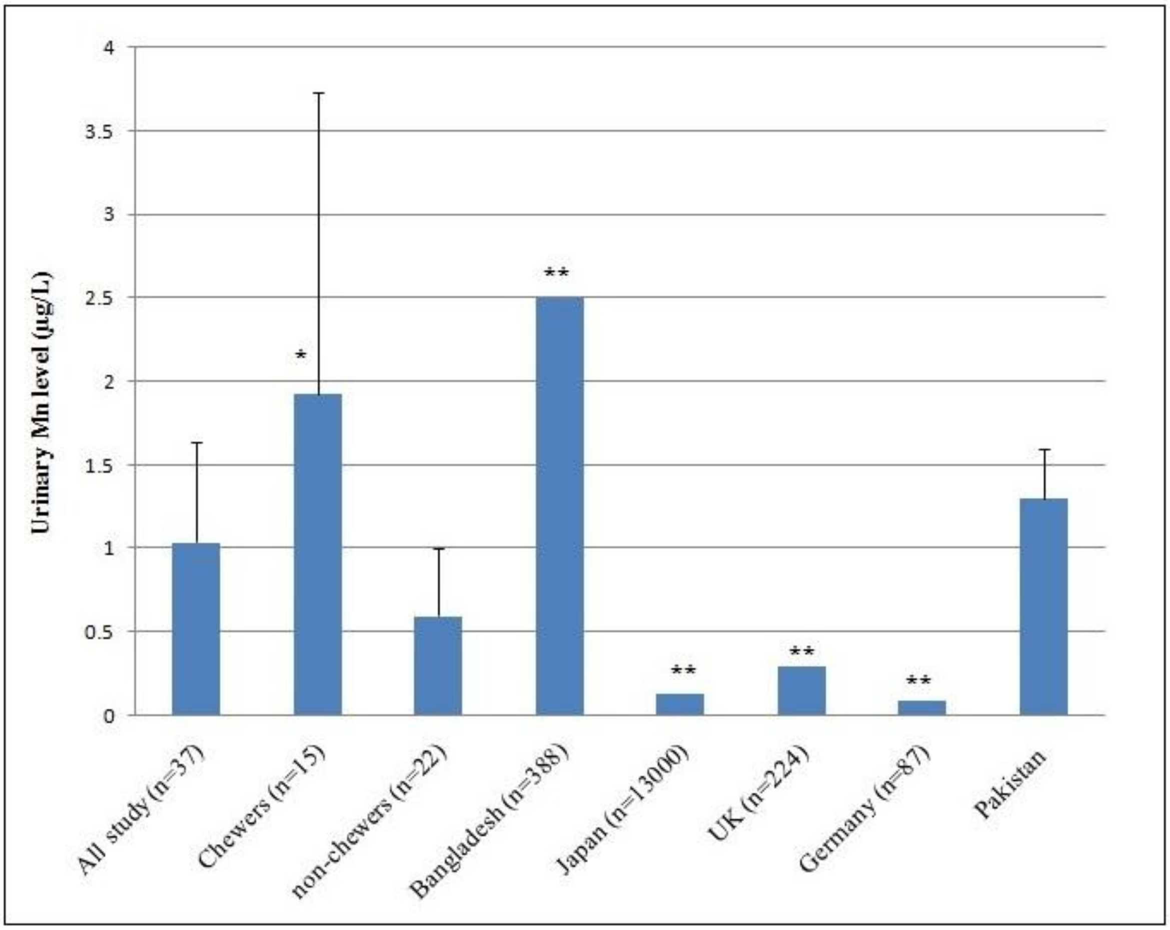


Figure 5.3: Mn levels in urine for populations from different countries in comparison to betel quid chewers and non-chewers from the current study. *Urinary Mn level of Bangladeshi chewers (males and females) residing in the UK (1.93 µg L-1). ** No data published in the letriture.

Table 5-5: Levels of different elements in urine from healthy Bangladeshi subjects classified according to betel quid chewing

	Mean ± SD[a] **(µg/L)**	**95% Confidence intervals (µg/L)**	**Range (µg/L)**
Chewers (n=15)			
As	52 ± 26	38 – 67	17.5 – 101
Cd	0.56 ± 0.58	0.30 – 0.95	0.2 – 2.2
Mn	1.93 ± 1.70	1.4 – 4.3	0.16 – 5.20
Pb	3.5 ± 4.4	1.7 – 6.5	1.3 – 17.3
Se	43.8 ± 40.7	26.2 – 71.2	15 – 170
Zn	462 ± 480	245 – 776	90 – 1803
Non-chewers (n=22)			
As	29.6 ± 22.1	21.1 – 40.8	3.8 – 82.6
Cd	0.31 ± 0.28	0.22 – 0.47	0.1 – 1.3
Mn	0.62 ± 0.60	0.7 – 1.4	0.1 – 2.4
Pb	2.1 ± 2.3	1.4 – 3.5	0.7 – 11.8
Se	35 ± 10	31 – 40	13 – 53
Zn	280 ± 135	222 – 342	91 – 510

[a] No statistically significant difference ($P < 0.05$)

Table 5-6 presented the estimation of the PMTDIs of As, Cd, Mn and Pb through ingestion of individual ordinary betel quids. It has been reported that betel quid consumptions in a Bangladeshi population to be 5.7-6.3 per day (Hafeman *et al.*, 2006). This is higher than that reported for a Bangladeshi population living in the UK, with a range of 2-5 quids per day chewed by 35% and 52% of the males and females respectively (Ahmed *et al.*, 2008). In the current survey of 37 (15 chewers) Bangladeshis an average of 3.5 betel quids per day with a range of 1-30 per day was found. Other studies have also reported that people can chew up to 30 quid per day (Lin *et al.*, 2002; Liu *et al.*, 2000). In the current study, a more modest intake of six quids per day for the various calculations was chosen.

Table 5-6: Percentage of PMTDI for As associated with consumption betel quid chewing.

Elements	**PMTDI (µg/kgb.w) /day** [a]	**PMTDI (µg/day)**[b]	**Percentage of PMTDI in Ordinary betel quid** [c]			
			3 quids	**6 quids**	**10 quids**	**30 quids**
As (inorg.)	2.1	126	0.58	1.2	1.9	5.8
Cd	1.0	60	1	1.9	3.2	9.8
Pb	3.5	210	4.2	8.5	14	42
Mn	160	9600	9	18	30	90
Se	5	300	0.01	0.02	0.03	0.10
Zn	300	18000	1.31	2.32	3.87	11.6

[a] The numerical values shown are the tolerable daily intake for a 60 kg person derived from PMTDIs recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FSA 2009).

[b] Ordinary betel quid is more commonly chewed especially at home.

As shown in Table 5-6 consumption of 6-30 betel quids per day (assuming a Bangladeshi body weight of 60 kg) contributes to 1-6% of the PMTDI, since the PMTDI of inorganic arsenic is 2.1 µg/kg.bw.day (FSA, 2009). For Cd and Pb 1.9 and 8.5% of the PMTDI, respectively, can come from chewing six betel quids per day (Table 5-6). As much as 18% of the PMTDI for Mn can be derived from chewing six betel quids. For an individual chewing 30 betel quids, 90% of PMTDI can be reached. Of particular note is that chewing 30 quids per day was estimated to contribute almost half of the PMTDI for Pb.

5.3.4 Correlation between TDI of Mn and Mn levels in urine

Figure 5-4 shows a strong positive correlation (Pearson correlation, $P = 0.001$) between the total daily intakes (TDI) of Mn (mg/day) and the urinary excretion of Mn (µg/L) for different countries, data derived from the literature are indicated in Figure 5-4. The TDI of Mn for Bangladeshi population (in Bangladesh), calculated in the present study, was estimated to be 18.7 mg per day for non-chewers of betel quid. This is higher than any other country in the world (see chapter 7, Table 7-4). However, the TDI for Bangladeshis in UK was much lower at 8.64 and 9.88 mg/day for all volunteers and chewers, respectively. Urinary Mn levels for Bangladeshi population (2.5 µg/L) were taken from Ljung *et al.* (2009). Urinary Mn levels for UK Bangladeshis are based on this study.

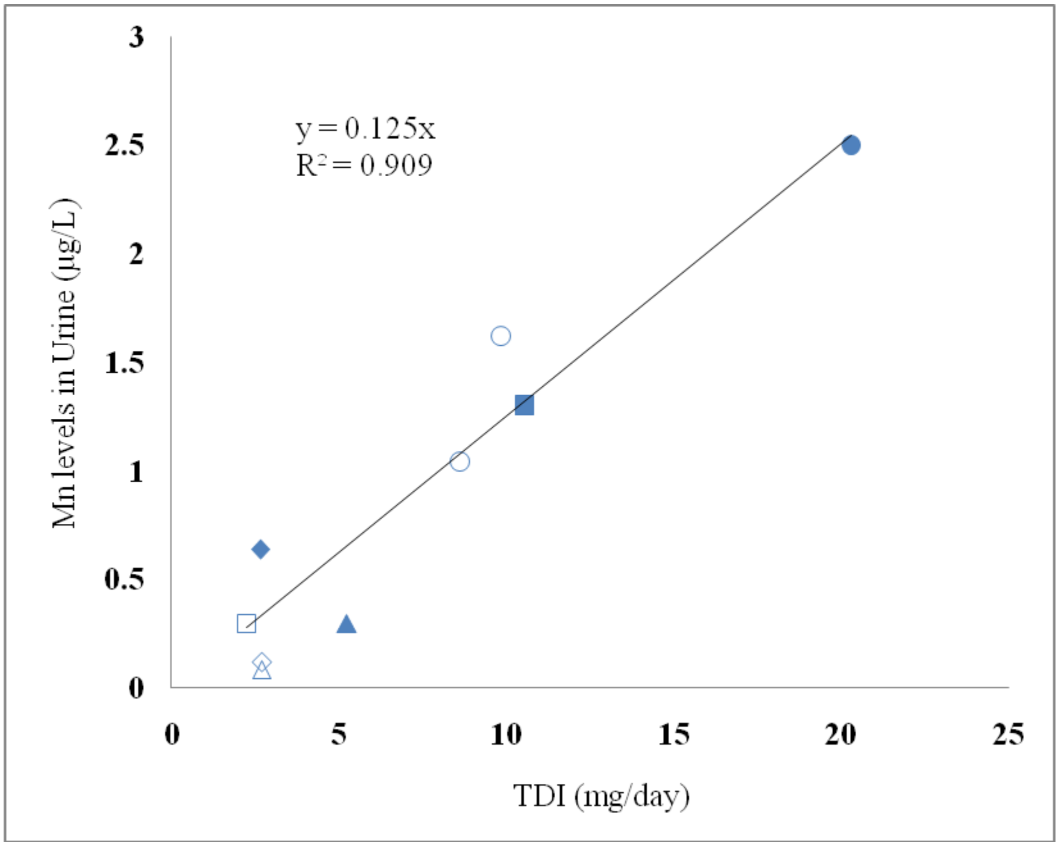


Figure 5.4: Correlation between TDI of Mn and urinary Mn levels for different countries: UK (▲); Germany (Δ); USA (♦); Japan (◊); Pakistan (■); India (□); Bangladeshi residing in Bangladesh (●); Bangladeshi residing in UK, from present study (○).

5.4 Discussion

Very little research has been carried out on the intake of arsenic and other toxic elements, from non-food sources such as betel quid chewing, in populations that are exposed to arsenic through drinking water. Betel quid and their components are widely consumed in arsenic contaminated regions of Bangladesh and India. Exposure to As, Cd, Mn, Pb and other elements from betel quids have not been previously considered despite the fact millions of people in these regions consume it on a daily basis. Only a few studies have investigated arsenic intake from various types of foods consumed in Bangladesh (Alam *et al.*, 2003; Das *et al.*, 2004; Kile *et al.*, 2007). However, these studies did not include contribution from betel quids. Hence, it is very important to monitor the impact of this chewing material on different populations.

Many diseases have been associated with the chewing betel quid including oral cancer (particularly cancer of the bucal and labial mucosa) (Nandakumar *et al.*, 1990; Sankaranarayanan *et al.*, 1998) and oral submucous fibrosis (Auluck *et al.*, 2008). Chewing *Piper betel* leaves and betel nut, either with or without tobacco, can cause cytogenetic changes in the oral epithelium; it is a strong factor for development of oral submucous fibrosis and mouth cancer (Vanwyk *et al.*, 1993). In South Asian communities in the UK, 42% in the 50-80 year age group, chew *Piper betel* leaves and areca nut with and without tobacco and they also use lime (Bedi, 1996 and Shetty and Johnson, 1999). The current study found that lime (calcium hydroxide) has the highest concentration of arsenic amongst the different betel quid components (4.56 mg/kg with mean 3.47 mg/kg). From the discussions with chewers (in this study) some heavy chewers can consume as much as 100 g of lime per week. Furthermore, the level of arsenic in *Piper betel* leaves was high. This leaf along with areca nut are the two key essential components of a betel quid. The intake of arsenic from lime and *Piper betel* leaves has not been previously reported. Previous studies on the relationship between betel quid chewing and health outcomes have been mainly focused on tobacco and areca nut and little attention has been paid to assessment of the harmful effects of *Piper betel* leaves. It has been reported that 78% of Bangladeshi adults chew betel quid with significantly more women than men chewers, and many add tobacco to their quid (Bedi, 1996). Another study reported that Bangladeshi men in the UK have a high prevalence of hyperhomocystinemia, compared to their white counterparts, with betel quid chewing and smoking being strong risk factors (Gamble *et al.,* 2005; Chambers *et al.*, 2000). The fact that betel quid chewers, who consume arsenic contaminated drinking water in Bangladesh, had higher incidence of skin lesions (McGarty *et al.,* 2006) motivated this project to investigate if betel quids contain significant levels of arsenic that may be a contributory factor in the development of adverse health impacts in humans. Fortunately for UK Bangladeshis, exposure to arsenic intake from drinking water is not a problem; unlike the population in Bangladesh where the average content of up to 0.084 mg/kg of arsenic in groundwater have been reported (Frisbie *et al.*, 2009).

Findings in this thesis add further support to the study by Lindberg *et al.* (2010) who investigated the effect of smoking and tobacco chewing on the health of a Bangladeshi population. Although they did not analyse individual components of betel quids, they determined the arsenic content of different types of tobacco and *Zarda* that are included in betel quids. They found significant levels of arsenic in these products although they were 50% lower compared to the values analysed in the current study. This could be due to analysis of different varieties and batches of *Zarda*. Lindberg *et al.* (2010) did not determine the arsenic concentration of *Piper betel* leaf which is the main component, along with areca nut, in betel quid preparations. In this thesis, the highest arsenic concentration was not in tobacco or *Zarda* but in *Piper betel* leaves and lime. Lindberg *et al.* (2010) stated in their paper that chewing tobacco is associated with a greater risk factor for arsenic related skin effects in women compared to men and suggested that this could be associated with poor metabolism of arsenic in women. However, there may be an alternative explanation since women are known to chew a greater number of betel quids per day compared to men (Ahmed *et al.*, 2008). Their exposure to As, Cd, Mn, Pb and other substances including organic compounds are therefore likely to be higher. Possible additive or multiplicative effects of exposure to these toxic elements may provide an explanation for the higher skin related disease amongst betel quid chewers. ROS can be generated by these elements which may cause toxic effects at the cellular and organ level.

Whilst exposure to arsenic and its health impact on the Bangladeshi populations is being extensively studied, very little research has focused on Cd and Pb exposure. Elevated exposure to Cd has been associated with diverse disease processes such as renal effects, bone effects and liver and kidney cancers (Bernard, 2008). It has been reported to mimic estrogen (Johnson *et al.*, 2003) and may increase the risk of hormone related cancer in women (Akesson *et al.*, 2008). Kippler *et al.* (2009) have been addressing the issue of Cd exposure in Bangladesh and found higher Cd levels in breast milk in Bangladeshi women. The mean concentration of Cd in breast milk in Bangladeshi women was found to be 0.14 µg/L which was higher than other countries excluding Japan and India (Kippler *et al.*, 2009). Plant based foods, especially vegetables and rice, are the main source of Cd exposure (see chapter 4). In the current study, the Bangladeshi population with a dominant plant based diet, especially the poor who cannot afford animal products and do not have a balanced and nutritious diet, are likely to be particularly vulnerable to increased Cd exposure. This situation will be further exacerbated for those who chew betel quid. Thus, it is possible, that the high consumption of betel quids by Bangladeshi women is a contributory factor for the elevated Cd exposure resulting in higher levels of this element in their breast milk which can be harmful to their health and that of their breast-fed child.

Although the exposure to Mn from drinking water by Bangladeshi population has been recently highlighted (Frisbie *et al.*, 2009), studies on Mn intake from foods are limited. The first study to address Mn intake from foods in the Bangladeshi diet was reported by Zablotska *et al.* (2008), although this study did not incorporate contributions from betel quids and water. The observation by Hafeman *et al.* (2006) that betel quid chewers, who are likely to be consuming As and Mn contaminated drinking water, had higher incidence of skin lesions and tremor led to determination of the urinary levels of Mn in chewers compared to non-chewers in this thesis and also to carry out a more comprehensive estimation of the dietary intake of Mn that took into consideration contributions from betel quid chewing, tea drinking etc.

In this study, a positive correlation exists between the total daily intake of Mn and urinary excretion of Mn for different populations (Figure 5-4). This is an interesting finding and such a correlation may be used for prediction of either urinary Mn excretion or total daily intake of Mn for other population, provided data on one of these two parameters are available. The positive correlation also suggests that bioaccessibility and bioavailabilty of Mn from different dietary sources, including drinking water, are likely to be similar. Results shows that the average intake of Mn for Bangladeshi population is 18.3 and 18.7 mg/day from foods when betel quids (6 quids) or water (2.7 litres), respectively, are included in the daily intake estimation. When both betel quid chewing and drinking water are included in the calculation, the total daily intake of Mn is 20.3 mg/day for all foods (see chapter 7). Since India is a very large country, there are large variations (2.2 – 9 mg/day) in the daily Mn intake. Similarity in diet and cultural habits of the people of these regions explains the similarities in their daily Mn intake. In contrast, a Mumbai study (Tripathi *et al.* 2000) showed that the daily Mn intake was 2.21 mg/day; these data were used for the Mn TDI and urinary Mn levels graph (Figure 5-4) because the urinary Mn levels were available from this Mumbai study. Daily dietary Mn intakes of different countries were shown in Table 7-5 (Ysart *et al.* 1999, Noel *et al.* 2003, Wilplinger *et al.* 1999, Schafer *et al.* 2004, Marti-Cid *et al.* 2009; Aung *et al.* 2006).

Exposure to elevated levels of Pb has been associated with various diseases including lung cancer (Palma *et al.* 2008), imminuotoxicity (Mishra, 2009) and neurotoxicity (Verstraeten *et al.*, 2008). Due to increased industrialisation and use of Pb based fuels and chemicals, exposure to Pb in Bangladesh is becoming a significant problem and elevated blood Pb levels were detected in primary school children in Dhaka (Bangladesh) (Kaiser *et al.*, 2001). From the current study, 8.5% of PMTDI of Pb can be derived from chewing six betel quids per day, this value can exceed the Pb intake for Bangladesh population if we consider some cities of Bangladesh have high levels of Pb in their air due to traffic pollution in addition to exposure from vegetables and rice which can contain higher levels of Pb compared to animal products. Thousands of economically deprived Bangladeshi's reside in slums of the capital city Dhaka, often adjacent to roads, and are exposed to toxic elements including lead from traffic pollution. This along with absence of a balanced and nutritious diet, are likely to make them more vulnerable to exposure to Pb, especially those who also practice the habit of chewing betel quids.

Since the Bangladeshi community in the United Kingdom have a similar diet to people living in Bangladesh (Kassam-Khamis et al., 2000), the current study determined the level of Mn in urine from 37 volunteers (15 betel quid chewers and 22 non-chewers). As can be seen from Figure (5-3), betel quid chewers have a statistically significant (P = 0.009) higher Mn levels compared to non-chewers, which is likely to be due to the high Mn content in betel quids that they chew. Urinary Mn levels in the Bangladeshi population residing in Bangladesh have been determined by Ljung *et al.* (2010), they analysed urine from 388 Bangladeshi women and reported a mean concentration of 2.5 µg/L with a median value of 1.6 µg/L. Interestingly, this was similar to Mn level in urine for the male betel quid chewers in the current study (2.25 µg/L), but was around 1.5-fold higher than that for the female chewers in the current study (1.62 µg/L). The average urinary Mn level in the UK Bangladeshi community (chewers and non-chewers combined, 1.04 µg/L) was 3.4-fold higher compared to the general UK population (0.3 µg/L) (White and Sabbioni 1998) and was also far higher than those reported for other countries, including Germany, USA, Japan and Pakistan (Greger *et al.*, 1990; Heitland & Koster, 2006; Ohashi *et al.*, 2006; Afridi *et al.*, 2009). Although the higher urinary Mn for population residing in Bangladesh may partly arise from Mn in drinking water, this was not the case for UK Bangladeshis where the Mn in drinking water is relatively low at < 50 µg/L (FSA, 2002). Thus, the similarity between the urinary Mn levels in UK Bangladeshi chewers and Bangladeshi females (residing in Bangladesh) (Ljung *et al.*, 2009), can be mainly attributed to Mn intake from betel quids.

Manganese intake by Bangladeshi females is of particular concern as Mn can be easily transferred from the mother to the foetus via the placenta, and subsequently crossing the blood-brain barrier of the underdeveloped brain affecting neurodevelopment (Schetter, 2001). In this context, it has been reported that children exposed to Mn through drinking contaminated water in Bangladesh displayed poor intellectual function (Wasserman *et al.*, 2004).

Several studies have attributed betel quid consumption with the development of different diseases including oral cancer, diabetes, cardiovascular disease etc (Vanwyk *et al.*, 1993; IARC, 2004). In the UK, Asians have the highest incidence of head and neck cancer which has been attributed to smoking and betel quid chewing (Smith *et al.*, 2003). As pointed out earlier, chewing betel quids was associated with a higher risk of skin lesions (McCarty *et al.*, 2006) and tremor (Hafeman *et al.*, 2006) in populations exposed to high levels of arsenic in their drinking water. Unfortunately, these studies linking betel quid chewing with certain health impacts in arsenic exposed populations did not analyse betel quids. However, a recent study analysed the arsenic content of tobacco used in betel quids (Lindberg *et al.*, 2010) and reported that chewing tobacco was associated with a greater risk factor for arsenic related skin effects in women compared to men. They suggested that that this could be associated with poor metabolism of arsenic in women. However, the current study suggests that there may be an alternative explanation since women are known to chew a greater number of betel quids per day compared to men. For example, a study (Ahmed *et al.*, 2008) on the Bangladeshi population in the UK reported that 52% of the women they surveyed chewed 2-5 betel quids per day compared to 35% in men; over 10 betel quids were chewed by 7% of the women as compared to only 1.5% of men; the study reported that the chewing habit was prevalent amongst 64.1% of the females (Ahmed *et al.*, 2008). Another study from Bangladesh, reported that on average 47% of females and 4% of males chew betel quids (Yunus, 2001). They are also exposed to greater quantities of other chemicals from tobacco/*Zarda*, which may adversely affect their health, even though smoking is far less common in Bangladeshi women compared to men (Bedi, 1996).

Lindberg *et al.* (2010) only focused on arsenic content of tobacco rather than the other substances consumed as part of the chewing material such as *Piper betel* leaves, areca nut, lime etc. In the current study, the arsenic content of individual components of betel quid was determined and the highest arsenic content in the chewing material was detected in *Piper betel* leaves. Lindberg *et al.* (2010) found that different brands of *Zarda* contained arsenic between 0.33-0.54 mg/kg (mean 0.45 mg/kg, n=8), which was about two fold greater compared to that in cigarettes and bidis. As females consume greater number of betel quids, and men are more likely to be smokers, the exposure to arsenic in females is likely to be greater since betel quids contain higher arsenic levels. However, the arsenic levels in cigarettes and betel quids were relatively low compared to what is derived from water and food, therefore it is more likely that the high Mn level present in betel quids, or even other chemicals such as arecoline as suggested by Pilsner *et al.* (2010) is responsible for the greater incidence of skin effects seen in chewers, especially females.

The mechanism underlying Mn induced toxicity in people exposed to high levels of Mn is poorly understood. However, it has been suggested that Mn plays a role in the generation of ROS that may result in neurotoxicity (Ali *et al.*, 1995). More recent studies have provided evidence suggesting that oxidative stress induced by Mn exposure can trigger apoptosis of neural stem cells (Tamm *et al.*, 2008). In light of these studies, this thesis hypothesises that increased Mn exposure through a combination of diet, betel quid chewing and drinking water results in oxidative stress and cellular damage that may result in Mn induced neurotoxicity in certain sectors of the Bangladeshi population. Simultaneous exposure to high levels of both manganese and arsenic may result in increased toxicity which may explain the observation of greater tremor and skin lesions in betel quid chewers. Although, several studies have reported modulating effect of areca nut in As induced skin lesions (McCarty *et al.*, 2006; Pilsner *et al.*, 2009; Lindberg *et al.*, 2010), only Pilsner *et al.* (2009) and Lindberg *et al.* (2010) attempted to provide an explanation for this observation. Pilsner *et al.* (2009) suggested that ROS generated by arecoline, a key compound in areca nut, may be responsible for the higher incidence of tremor and skin lesions in betel quid chewers, whereas Lindberg *et al.* (2010) suggested poorer As metabolism in females rather than any particular substance in betel quids. The current study suggests that the inorganic (Mn and As) and organic (arecoline) and components of betel quids, are jointly responsible for the adverse health outcomes in betel quid chewers who drink contaminated groundwater. This needs to be further investigated in the future.

A limitation of the present study is that the number of volunteers for the urinary elemental analysis was rather low (37 in total) and that it was only possible to conduct studies with UK Bangladeshi population, rather than with participants residing in Bangladesh who drink groundwater often contaminated with high levels of toxic elements. Furthermore, for Mn the absence of information on Mn bioavailability and bioaccessibility from foods and betel quids makes it difficult to obtain an accurate estimation of exposure levels and risk assessments. Finally, this thesis assumed that the entire betel quid is ingested which is very common amongst the Bangladeshi community. However, this is not the case for all individuals as some spit out the juice accumulated in the mouth whilst others spit out the fibrous portion of the betel quid after extensive chewing. Thus, the level of Mn exposure from betel quid chewing for the latter groups will be lower. Future studies needs to address these issues.

Considering the fact that As, Cd, Mn and Pb levels are significant in betel quids, possible health impacts associated with increased exposure to these elements in regular consumers of betel quids needs to be taken in to account in any public health improvement strategies. Millions of people in Bangladesh and India have a diet that is dominated by plant based products especially vegetables and rice and their consumption of animal products is either very limited due to economic reasons or is entirely absent due to religious and/or cultural reasons. This makes them more vulnerable to higher exposure to As, Cd, Mn and Pb as these elements are present in higher levels in plant based foods.

This situation can be further worsened by the habit of chewing betel quids which can contribute significantly towards exceeding the total permitted daily intake of As, Cd and Pb. This may subsequently result in various diseases including adverse neurological effects, kidney disease, oral cancer and submucous fibrosis etc. Although the mechanism underlying the disease needs to be investigated, it is possible that the higher incidence of skin lesions and other adverse health outcomes in betel chewers in arsenic exposed regions may arise due to adverse additive or multiplicative effects, such as oxidative stress, induced by exposure to a cocktail of chemicals including As, Cd, Mn and Pb. The additional daily burden of exposure to As, Pb, Mn and Cd from betel quids may exacerbate the situation for chewers. Although the current study has focused on toxic elements such as As, Cd and Pb, it is possible that other inorganic and organic components may play a role and this aspect needs to be investigated in the future.

5.5 Conclusion

This is the first study to evaluate the total content of arsenic in betel quids, analysing each of its major components, so that its contribution to the daily intake of arsenic can be established. Lime and *Piper betel* leaves were identified to contain the highest arsenic levels and betel quids can provide between 1-6% of the total daily intake of arsenic for someone chewing between 6-30 quids per day. Arsenic speciation of betel quids reveal that the main species is As^{III} which is the most toxic of the arsenic compound. In addition to arsenic, the study in this thesis found significant levels of Cd, Mn and Pb in different components of betel quids. The additive or multiplicative adverse effects, such as generation of ROS, may result from the simultaneous exposure to As, Cd making chewers more vulnerable to ill health. Whilst it cannot be certain that the intake of As, Pb, Cd, Mn from betel quids can explain the increased incidence of skin lesions and other adverse health outcomes in chewers, the increased exposure may be sufficient to tip the balance favouring the development of disease processes.

It is possible that As, Cd, Mn and Pb in synergy with organic substances in betel quids may be responsible. Those with a mainly plant based diet, that often contain higher levels of As, Cd, Mn and Pb compared to animal products, are at greatest risk if they further elevate their exposure to these elements through betel quid chewing. Betel quid chewing is most prevalent amongst women who may be unknowingly harming their health and that of their unborn babies (for those who are pregnant) through increased exposure to As, Cd, Mn and Pb.

Betel quid consumption is concerned, especially those consuming large number of quids per day, are at risk of developing ill health and public health policy makers should seriously consider implementing policies to reduce or eliminate the consumption of betel quids in populations where this practice is prevalent.

6 EARTH-EATING CAN BE A SIGNIFICANT SOURCE OF ARSENIC, LEAD AND OTHER TRACE ELEMENTS EXPOSURE IN BANGLADESHI WOMEN

Summary

Geophagy or earth-eating is common amongst some Bangladeshi women, especially those who are pregnant, both in Bangladesh and in the United Kingdom. A large proportion of the population in Bangladesh is already exposed to high concentrations of arsenic and other toxic elements from drinking contaminated ground water. Additional exposure to arsenic from non-food sources have not been adequately addressed and herein is presented the first study to monitor arsenic levels in baked clay (known as Sikor), the consumption of which can potentially further exacerbate arsenic induced ill health. In the present study, ten Sikor samples from Bangladesh were analysed for As, Pb, Cd, Mn, Fe, Se and Zn using ICP-MS. Of particular concern was the levels of As (3.8-13.1 mg/kg) and Pb (21-26.7 mg/kg) and their possible impact on human health. Modest consumption of 50g of Sikor is equivalent to ingesting 370 µg of arsenic and 1235 µg of lead per day (median value used), most entirely as carcinogenic inorganic arsenic. Such consumption exceeds the permitted maximum tolerable daily intake (PMTDI) of inorganic arsenic by almost 2-fold. The potential exposure to arsenic from ingesting 50 g of Sikor is greater than that from drinking 4.4 litres of arsenic contaminated water (84 µg/L), although research is required to determine the bioaccessibility of As from Sikor. It is concluded that Bangladeshi

population consuming large quantities of Sikor, especially those who are pregnant and are at the same time also drinking arsenic contaminated water, are particularly vulnerable to developing ill health.

6.1 Introduction

The deliberate eating of non-food or non-nutritive substances is known as pica (Woywodt and Kiss, 2002). Many different types of pica have been described in the literature such as ingestion of baby powder, charcoal, calcium hydroxide (lime), ash, uncooked starch and ice (Young *et al.*, 2008). Geophagy is the most common type of pica and involves deliberate eating of earth such as soil and clay. It is an ancient practice that is still widespread in many parts of the world such as in Asia, Africa and South America, North America and parts of Europe (Ghorbani, 2008).

Historically, geophagy was practiced in India, China and Persia for many centuries and it is still widely practiced in these areas. The practice of eating clay has been common in all sectors of the Indian society irrespective of caste and religion (Laufer, 1999). The Greeks and Romans were familiar with geophagy; Hippocrates of Kos (*ca*. 460 - 377 BC) is considered to be the first to provide a description of geophagy in his classical textbook (Woywodt and Kiss, 2002). Ibn Sina (Avicenna) also discussed in detail geophagy in his widely used medical textbook (Woywodt and Kiss, 2002). Also several civilizations have used clays for their therapeutic value (Finkelman, 2006). In the 16th century Daud al-Antaki from Syria, mentioned the use of clays as a medicine to stop haemorrhaging and diarrhea and to treat skin diseases (Lev, 2005).

The precise reasons underlying the practice of geophagy remains unknown, although some suggest consumption for nutritional (Ghorbani, 2008; Yanai, 2009) and medicinal purposes (Ferrell, 2008). Geophagy during pregnancy has often recommended as a means to increase the intake of some essential elements (especially Ca, Mg, Zn, Fe, Cu, Mn, Se). Consumption of clay is also common during times of famine.

In parts of Africa, soil eating is common amongst females, especially children and pregnant women; it is a part of their culture and is a widely accepted practice. They consume a variety of soil types, which are sold in shops and markets. The prevalence of soil eating amongst pregnant women in Kenya, Ghana, Namibia and Tanzania has been reported in the literature (Luoba *et al.*, 2004; Vermeer, 1971; Thomson, 1997; Kawai, 2009). Bangladeshi women residing in Bangladesh (Bangladeshi soil eating, 2008) and also in the UK consume baked clay known as Sikor (Middleton, 1989). Although the overall prevalence of this habit amongst the Bangladeshi community has not been determined, it is known to be mostly practiced by pregnant women. Some have suggested that this practice may help them to counter iron deficiency, which can be very common for pregnant women especially in the first months of pregnancy. Excess intake of even an essential element can result in disease states (Salvador, 2010).

Besides trace elements, soils contain different types of organisms including bacteria (such as *Escherichia coli, Salmonella* and *Shigella*) and fungi which may cause human health problems from earth eating (Ishii, 2010). Several stomach diseases have been related to ingestion of soil including stomachaches, acid indigestion, nausea, and diarrhea. Both toxoplasma and hookworm infection from soil eating have been reported (Hough, 2007;

Brand and Ekosse, 2009). Recent studies have suggested that geophagy may be associated with an increased risk of developing anemia (Thomson, 1997; Kawai, 2009; Young, 2010). The practice of geophagy amongst the Bangladeshi and West Bengal (India) population is of particular concern, as people from these regions are already exposed to high levels of arsenic from drinking contaminated groundwater and rice consumption (Kile *et al.*, 2007). With respect to toxic elements, especially arsenic, the practice of geophagy amongst the Bangladeshi and West Bengal (India) population is particularly worrying as people from these regions are already exposed to high levels of arsenic from drinking contaminated groundwater and rice consumption. Despite the high vulnerability of pregnant women and their unborn babies to this practice, the toxic element intake through geophagy and associated potential adverse health implications has not been explored. The exposure to arsenic through drinking water has been linked to the development of various diseases including hyperkeratosis and skin lesions and millions are at risk of developing cancer (Zablotska *et al.*, 2008).

The presence of high levels of other elements such as Pb, Cd and Mn in drinking water can act in synergy with arsenic in an additive or multiplicative manner to increase the negative health impact. These elements can increase the generation of reactive oxygen species (ROS) which may induce harmful effects at the cellular and organ level (Beyersmann and Hartwig, 2008). A further increase in the intake of these elements through non-food sources, especially the practice of Sikor consumption, has not been addressed although they may be a contributory factor in exacerbating diverse disease processes. However, exposure of Bangladeshi pregnant women and unborn babies to toxic elements through drinking water, atmospheric pollution and diet has been reported by various workers. For example, Kile *et al.* (2008) measured the maternal and umbilical cord blood levels of arsenic, cadmium, manganese, and lead in rural Bangladesh and reported that exposure to mixtures of these elements is widespread amongst pregnant women.

As yet no study has been reported in the literature that investigated arsenic content in Sikor from Bangladesh although it has been reported that some women may consume as much as 50 to 60g of Sikor per day (Abrahams *et al.*, 2006). Although exposure to arsenic from accidental ingestion of soil by children and adults have been extensively studied for various populations (Ljung *et al.*, 2006; Kaiser *et al.*, 2001; Chiang *et al.*, 2008; Shivoga and Moturi, 2009) no work has previously investigated this in relation to geophagy or accidental soil ingestion in Bangladesh or West Bengal (India) even though this is particularly vital for these regions as they are already exposed to high levels of arsenic. Only one previous study (Abrahams *et al.*, 2006) determined the content of trace elements in Sikor from Bangladesh, which focused primarily on Pb and did not present any data for arsenic.

The aim of this Chapter was to determine the content of arsenic in Sikor and estimate if the intake levels could be high enough to be a risk to human health and if the exposure from this source can further exacerbate the arsenic induced ill health in exposed populations in Bangladesh. The arsenic intake is already very high in Bangladeshi diet (both in the UK and especially in Bangladesh) and additional intake from Sikor may explain the observation of greater arsenic-induced ill health. Also in the present study, arsenic speciation of Sikor samples was studied. Besides arsenic, the levels of Pb, Cd and Mn in Sikor samples from Bangladesh were determined, and the PMTDIs for the elements were estimated and potential health risks discussed.

6.2 Materials and Methods

6.2.1 Sample collection

Eight bags of Sikor imported from Bangladesh, openly sold in ethnic Bangladeshi shops in the United Kingdom, were purchased from two cities (Birmingham and Leicester) and one town (Luton) during January to April 2010. These bags contain 250 g of Sikor as small tablets, the weight of these tables (see Figure 6-1) ranged between 12.3-18.5 g with the mean of 15 g.

6.2.2 Sample preparations

All glassware and plastic were cleaned by soaking them in 10% HNO_3 for least 12 hours and then rinsed several times with double distilled water. Sikor samples were ground and dried in an oven at 80 ^{o}C overnight.

6.2.3 Samples digestion

See section (2.2.3.4.) in methododlogy chapter.

Figure 6.1: Typical example of Sikor tablets from Bangladesh purchased from shops in the United Kingdom. Shikor Mati can be translated as Sikor Earth.

6.2.4 Sample extraction

See section (2.2.7.2.) in methodology chapter.

6.2.5 Instrumentation

6.2.5.1 Total As and other elements analysis

6.2.5.1.1 Using ICP-MS instrument

Elements in the digested Sikor samples were determined by inductively coupled plasma mass spectrometry (ICP-MS) (see section 2.2.4.2.). For Sikor extracted samples, the arsenic concentrations were determined using an Agilent 7500 ICP-MS (Agilent Technologies, UK) (see section 2.2.7.3.).

6.2.5.1.2 Using XRFS instrument

See Chapter 2.

6.2.5.2 Arsenic speciation analysis

For arsenic speciation analysis, HPLC pump (GP50-2 pump, Dionex, USA) was coupled with ICP-MS (Agilent Technologies, UK) was used (see section 2.2.7.3.).

6.2.6 Methodology for risk estimation

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), (FSA, 2009) method was used to determine the PMTDIs of toxic elements from baked clay. The unit used for this scale is mg of element per day. The average adult body weight of the Bangladeshi women was taken to be 60 kg (FSA, 2009).

6.2.7 Quality control and standard reference material

Sample masses were measured to an accuracy of ± 0.1 mg. Trace elements concentrations obtained by ICP-MS technique were evaluated by the use of certified reference materials and were found to be in good agreement with the certified values of the references material. The analytical procedure and the reliability of the digestion process were validated by analysis with each measurement blank, soil reference material (Montana I 2710a) was used, the average recovery of trace elements from the reference material ranged from 75 and 101% of the certified values (Table 6-1). However, soil reference material (BCSS-1) was used for measuring arsenic in both extracted solutions and separated arsenic species.

Table 6-1: Selected certified mean values for Montana I (2710a) soil reference material, and the mean concentrations (mg/kg) determined from this sample following *aqua regia* digestion.

Element	**As**	**Cd**	**Fe**	**Mn**	**Pb**	**Zn**
Certified soil (SRM)	154	12.3	4320	214	552	418
Concentration determined (n=3)	143	11.3	3450	160	558	376
Recovery (%)	93	90	80	75	101	80
Detection limits [1]	0.1	0.002	3.9	0.1	0.3	1.3

[1] Detection limits were calculated as 3 × standard deviation (3 × Std. Dev.) of blank values.

6.3 Results

Typical Sikor samples, originating from Bangladesh, and sold in ethnic shops in the UK are shown in Figure 6-1. Concentrations of elements in Sikor purchased from shops in Leicester, Birmingham and Luton (UK) are given in Table 6-2, for three toxic elements (As, Pb, Cd) and for three relatively non-toxic essential elements (Fe, Mn, Zn). The increasing order of these elements (Cd<As<Pb<Mn<Zn<Fe) was the same regardless of whether mean, median or maximum concentration was applied as a criterion.

Table 6-2: Concentrations (mg/kg unless otherwise indicated) of As, Cd, Fe, Mn, Pb and Zn in Sikor samples.

Element	**Mean**	**SD**	**Median**	**Range**
As	7.8	2.5	7.4	3.80-13.1
Cd	0.32	0.1	0.34	0.09-0.4
Fe (g kg^{-1})	35.4	4	33.8	29.5-42.9
Mn	57.2	3.2	32.2	25.8-80
Pb	23.2	0.9	24.7	21-26.2
Se	1.43	1.8	1.46	1.19-1.66
Zn	69.6	15	67.5	51.4-100

Number of samples (n) = 10. All the data presented were obtained for duplicate samples. SD: standard deviation.

Major compounds and trace elements in five Sikor samples were analysed using X-Ray Fluorescence Spectroscopy (XRFS). Table 6-3 shows the different compounds and trace elements in Sikor samples detected using this technique. Elements contents (mean value) were 5, < 0.5, 36, <3.0 and 76 mg/kg for As, Cd, Pb, Se and Zn, respectively (Table 6-3). However, Fe_2O_3 and Mn_3O_4 content in Sikor were 6.21% (62.1 g/kg) and < 0.01% (< 100 mg/kg), respectively. These data are agreement with the ICP-MS data (Table 6-2).

Content of arsenic in clay from Bangladesh has been reported to vary from 3.525 to 6.639 mg/kg (Chowdhury *et al.*, 2003). In another study, clayey sediments from Bangladesh have been investigated and concentrations of As, Pb and Zn in the clay were measured, the concentrations of these elements were ranged 4-18, 13-32 and 35-111 mg/kg for As, Pb and Zn, respectively (Yamazaki *et al.*, 2003). However, in the Bengal Delta in India, the arsenic content in clay ranged between 4-10 mg/kg (Sengupta *et al.*, 2004). These results are not too different from what was found in the analysis of Sikor samples in the current study.

Table 6-3: XRFS data of Sikor samples (n =5).

Component	**Mean ± SD**	**Range**
Major data (%)		
SiO_2	56.3 ± 0.9	55 – 57
Al_2O_3	23.9 ± 0.5	23 – 24
Fe_2O_3	6.2 ± 1	5.3 – 7.6
Mn_3O_4	< 0.01	< 0.01
Trace data (mg/kg)		
As	5 ± 1	3 – 6
Cd	< 0.5	< 0.5
Pb	36 ± 3	33 – 40
Se	< 3.0	< 3.0
Zn	76 ± 5	72 - 85

Speciation of arsenic from Sikor samples by HPLC-ICP-MS showed that the main species was inorganic arsenic (As^V) at up to 100% of the total extractable arsenic (Table 6-4, Figure 6-2). The recoveries for arsenic extraction from Sikor samples ranged from 31 to 37%; it is not uncommon for low recoveries in soil samples (Gallardo et al, 2002, Georgiadis *et al.*, 2006; Ellwood *et al.* 2003). In the current study, H_3PO_4 (1M) and four hours contact time was employed, which is widely used for soil and clay extraction. Similar results (48% recovery) were obtained in a previous study (Giacomino *et al.*, 2009) for arsenic speciation in soil where H_3PO_4 (1.6M) and six hours contact time was used for extraction. However, other studies (Watts *et al.*, 2008; Alam *et al.*, 2007) have found that percentage extraction can reach up to 97% using H_3PO_4. The low recovery in the present study could be due to the nature of the material such as the high Fe content. It is possible that Fe binds arsenic strongly, preventing its release under the conditions used for the extraction. Previous study reported that different exchangeable phases can play important factors for the low solubility of arsenic from soils such as bound to carbonates, bound to Fe-Mn oxide and bound to insoluble organics and sulphides phases (Carbonell-Barrachina *et al*., 1999).

Table 6-4: Determination of arsenic in Sikor samples.

Sample	**Total As (mg/kg)**	**Extracted As (mg/kg)**	**Extraction Efficiency (%)**	**Speciated As^{V} (mg/kg)**	**Column Recovery (%)**
S1	7.2	2.31	32	1.91	83
S2	6.7	2.07	31	1.57	76
S3	6.3	2.07	33	1.12	54
S4	7.4	2.71	37	1.21	45
S5	5.5	1.87	34	1.03	55
S6	7.1	2.18	31	1.64	75
S7	8.1	2.78	34	1.87	67
S8	7.4	2.28	31	1.88	82

All Sikor samples contain only As^{V} species.

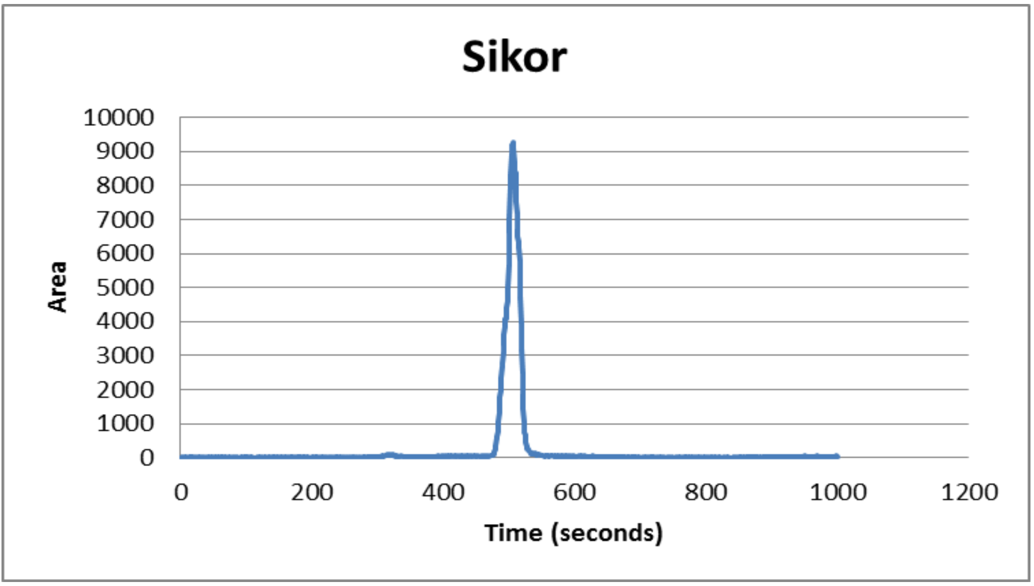


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Figure 6.2: Chromatogram of arsenic speciation in Sikor sample using HPLC-ICP-MS, the peak in this figure reflects As^{V} species.

Abraham *et al.* (2006) reported that Sikor, equivalent to 3–4 tablets of their Birmingham sample (i.e. ca. 48.6–64.8 g), can be consumed per day by the pregnant Bangladeshi women. Similar levels of soil ingestion were reported for pregnant Kenyan women, with a median daily intake of 41.5 g from soil sample (Geissler *et al.*, 1998). In light of these studies, in the current study 50 g of Sikor consumption per day was selected as a modest for the estimation of the PMTDI of different elements. Figure 6-3 presents daily arsenic intake for different amount of Sikor consume per day, minimum, median and maximum arsenic contents were used in the figure 6-3. It is clear that consumption of one tablet of Sikor (about 15 g) can be covered the PMTDI of arsenic (median value).

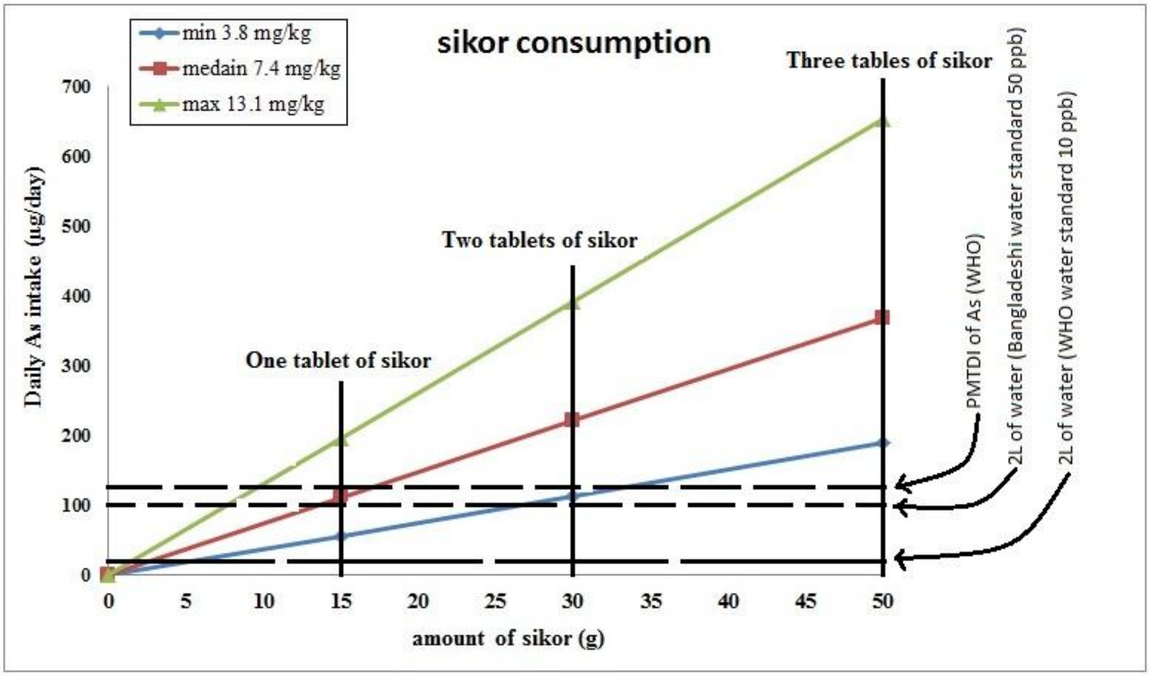

Figure 6.3: Daily aresnic intake from Sikor consumption using minimum, median and maximum of arsenic content in the Sikor.

Table 6-5 presents the estimation of the PMTDI of As, Cd, Mn and Pb through ingestion of 50 g of Sikor; arsenic content was 370 µg (median value used) in this quantity of Sikor. The data (in this thesis) shows that ingestion of 50 g of Sikor can exceed the PMTDI for arsenic by almost 2-fold and for Pb by almost 5-fold. For both Cd and Mn, consumption of this amount of Sikor contributed only a proportion of the PMTDI: 28.3 and 13.4% of the PMTDI for Cd and Mn, respectively.

Table 6-5: Percentage of PMTDI for different elements (median values were used) associated with consumption of Sikor (asumming 50 g of Sikor per day).

Element	**PMTDI (µg/kg.bw.day)** [a]	**PMTDI (µg/day)** [a]	**Percentage of PMTDI from 50 g of Sikor**
As	2.1	126	293
Cd	1.0	60	28.3
Mn	200	12000	13.4
Pb	3.5	210	588
Se	5	300	23.8
Zn	300	18000	19.3

[a] The numerical values shown are the tolerable daily intake for a 60 kg person derived from PMTDIs recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FSA, 2009).

6.4 Discussion

High human exposure to arsenic through drinking water in Bangladesh and West Bengal (India) is well known. The practice of earth-eating is also prevalent in these regions. It is therefore surprising that there are no published reports of the level of arsenic intake from this non-food material for people from these regions, despite many studies focusing on arsenic intake from water and rice. Geophagy is especially common amongst pregnant women and their resultant increased exposure to arsenic may not only be harmful to themselves but also to their unborn babies. Such concern prompted this study to analyse Sikor samples imported from Bangladesh to assess the potential dangers of elevated exposure to arsenic. Not surprisingly, the content of arsenic is high and eating 50 g of Sikor (this corresponds to approximately three tablets, see Figure 6-1) is equivalent to consuming 4.2 litres of arsenic contaminated water containing 84 µg/L (Frisbie *et al.*, 2009) of arsenic (the Bangladeshi average for arsenic, exceeding the WHO standard of 10 µg/L) (WHO, 2001).

In this thesis, speciation analysis revealed that Sikor consists primarily of inorganic arsenic. The relatively modest level of daily Sikor consumption alone (excluding arsenic intake from water and foods) exceeds the PMTDI for inorganic arsenic by almost 1.9-fold. This finding has relevance not only to the arsenic exposed population in Bangladesh whose total daily arsenic intake from all sources can exceed PMTDI by 4-fold, but also to Sikor consumers in the UK who do not normally exceed PMTDI for inorganic arsenic. Pregnant women are of particular concern in this context since arsenic, lead (Rudge *et al.*, 2009), cadmium (Sorkun *et al.*, 2007), and manganese (Rossipal *et al.*, 2000) can transfer from the mother to the foetus, placing the health of the unborn baby at risk. Research is required to determine the bioaccessibility of arsenic within Sikor; due to its complex mineral composition not all the arsenic is likely to be bioaccessible. A previous *in vitro* study has shown that the bioaccessibilities of arsenic and cadmium from soils were higher than those for Fe, Mn and Pb; the mean percentages range was 9.7-28.7 and 12.9-27.2% for As and Cd, respectively (Ljung *et al.*, 2007). Recently, another study has shown that 17- 46.9% of arsenic from soil is bioaccessible with a mean of 27.3% (Girouard and Zagury, 2009). If 27.3% of the arsenic from Sikor is bioaccessible, it could adversely influence the health of people already overexposed to high levels of this element from multiple sources; the present study estimates that 50 g of Sikor consumption would lead to 101 µg of bioaccessible inorganic arsenic (equivalent 80% of the PMTDI).

Adverse health effects associated with consumption of non-food substances, such as chewing tobacco, has been reported including greater skin lesions in some women which have been attributed to their lower ability to metabolise As (Lindberg *et al.*, 2008). In light of such findings, even moderate Sikor consumption (15 g or one piece per day) amongst Bangladeshi women can significantly contribute in further increasing their PMTDI for aresnic which can further exacerbate arsenic-induced ill health such as higher prevalence of skin lesions amongst some women.

Abrahams *et al.* (2006) determined some elements in Sikor from Bangladesh including Fe, Mn, Pb and Zn but not As and Cd. The latter study found the mean concentrations of Fe, Mn, Pb and Zn in the Sikor from Birmingham were 58800, 69, 80 and 38 mg/kg, respectively. The values for Pb are higher compared with the current study results (21-26.7 mg/kg), however, the current study reference material gave 101% recovery of Pb, whereas, the results for Abrahams *et al.* (2006) showed more than 264% recovery for Pb. Thus the difference between the current study and that of Abrahams *et al.* could be due to differences in the recovery of Pb after the digestion procedures used by the two studies. It is possible, that the elevated recovery (264%) may result in an overestimation of the Pb content by Abrahams *et al.* (2006). In this context, it is noteworthy that the Pb content determined in the current study is within the range previously reported for Bangladeshi clay by other workers 13-32 mg/kg (Yamazaki *et al.*, 2003). In any case, both studies reveal high Pb content in Sikor samples which is a health concern for the consumers of this material. Exposure to elevated levels of Pb has been associated with various diseases including lung cancer (Laurer *et al.*, 1993), immunotoxicity (Mishra, 2009) and neurotoxicity (Verstraeten, 2008). Due to increased industrialization and use of Pb based fuels and chemicals, exposure to Pb in Bangladesh is becoming a significant problem and elevated blood Pb levels were detected in primary school children in Dhaka (Bangladesh) (Kaiser *et al.*, 2001). From the current study, the PMTDI of Pb can exceeded by 6-fold through ingestion of 50 g of Sikor per day. Such a high level of exposure can present health problem to all women and pregnant women are also at risk of harming their unborn child.

Various diseases including neurological effects, skin disease, kidney disease, lungs cancer, immunotoxicity (Mishra, 2009) etc may arise from consumption of Sikor.

Recently, Kippler *et al.* (2009) reported the effect of Cd on human health in Bangladesh. They found higher Cd levels in breast milk in Bangladeshi women. Similarly, Kile *et al.* (2009) also found high levels of Cd in maternal and umbilical cord blood from rural Bangladeshi women. The quantity of Cd detected in 50 g of the Sikor samples analysed in the current study is quite high (28.3% of the PMTDI) and if one takes in to account the consumption of leafy vegetables which also contain high levels of Cd, the danger from excess exposure to Cd is obvious. For women living in rural Bangladesh, the daily calorie intake is dominated by vegetables and rice since animal products, which have lower Cd content, are often beyond their reach due to high costs. Moderate intake of fish is the main animal based product in the diet of many Bangladeshis who cannot readily afford to purchase poultry and meat. Thus a diet already rich in Cd, due to high rice and vegetable intake, is further worsened by the consumption of Sikor. In addition, the authors have recently found that betel quid chewing can be an additional source of Cd exposure in Bangladeshi women (Al-Rmalli *et al.*, 2011). In light of these studies, it is plausible to state that some of the excess Cd detected in the breast milk Bangladeshi women may come from the consumption of non-food materials such as Sikor.

Very little data is available on the prevalence of Sikor consumption amongst Bangladeshi women in Bangladesh and in the UK. Due to the stigma associated with the practice, many women are too ashamed to admit that they consume Sikor. This makes it difficult to determine the prevalence of this habit and its potential impact on human health. However, the commercial availability of Sikor in both the UK and Bangladesh clearly indicates there is a demand for the substance in both countries, although it is not possible to translate this information into number of consumers. The fact that pregnant women consume Sikor in large quantities has been recently highlighted by a gynecologist working in a Bangladeshi hospital (Bangladesh soil eating, 2008). In parts of Africa earth-eating is openly practiced and is an acceptable social habit and, consequently, the scientific research on earth-eating in African women is much more extensive compared to Bangladesh and India. Its prevalence among pregnant women ranged from 65% in Kenya, 46% in Ghana, 42% in Namibia, to 29% in Tanzania (Luoba *et al.*, 2004; Vermeer, 1971; Thomson, 1997; Kawai, 2009).

Previously it has been reported that the major minerals in Sikor from Bangladesh are kaolin, illite and quartz. It was found to be very low in organic C (0.8%) and is essentially salt free (Abrahams *et al.*, 2006). Sikor is considered to be a source of nutrients due to its high concentrations of essential elements. The current study reveals that the level of the essential elements (Fe, Zn and Mn) can either contribute towards the recommended daily intake of these elements or can result in an excess intake for those with already high intake of Fe, Mn and Zn. However, at the same time the current study shows that it may also be harmful to human health due to the presence of toxic elements namely As, Pb and Cd. Thus depending on the nutrient status of the consumer, ill health can result from either exposure to toxic elements and/or overexposure to essential elements from ingestion of

Sikor. How Sikor consumption can influence the uptake of different elements (essential or toxic) in vivo needs to be determined to determine its impact on human nutrition and health.

The current study has shown that Sikor can be a good source of essential elements such as Fe, Zn and Mn but at the same time it can also provide potentially toxic doses of As and Pb. Just consuming 50 g of Bangladeshi Sikor per day can result in exceeding the PMTDI for As and Pb by 3 and 6-fold, respectively. This is potentially harmful to consumers in the United Kingdom who can purchase this material from ethnic shops with a sizeable Bangladeshi community. In the UK, the Bangladeshi community have a diet which is very similar to their country of origin and consume foods imported from Bangladesh (Smith *et al.*, 2003). The situation is likely to be far worse for Sikor consumers in Bangladesh, especially those who are already exceeding their daily intake of arsenic from consumption of contaminated water and foods such as rice. Furthermore, presence of microbes in the Sikor samples could be an additional health risk that requires further investigation.

The present study postulates that women displaying higher prevalence of arsenic-induced ill health in Bangladesh may well be those who are further elevating their arsenic exposure through consumption of Sikor. This needs to be urgently investigated and public health policy makers need to act urgently to discourage the practice of eating Sikor in both UK and Bangladesh, and indeed other communities elsewhere. Although the mechanism underlying the toxic trace-element induces ill health needs to be investigated, it is possible that the higher incidence of skin lesions and other adverse health outcomes in women, especially pregnant women, in arsenic exposed regions, may arise due to adverse additive or multiplicative effects, such as oxidative stress, induced by exposure to a mixture of elements present in Sikor including As, Cd and Pb. The current study recommends that these women are advised to reduce or stop consuming non-food substances such as Sikor and betel quid and spend the money saved on having a balanced diet that also includes animal products and thereby reducing their intake of Cd from plant based foods.

6.5 Conclusion

Exposure to arsenic from baked clay, known as Sikor, has not been previously considered despite the fact many women in these regions (Bangladesh and India) consume it on a daily basis. This study reveals for the first time that baked clay can be an additional source of arsenic exposure in women which can be particularly serious, especially for women who are already exposed to high levels of arsenic through drinking water and food consumption. This increased exposure may explain higher prevalence of arsenic-induced skin lesions in some Bangladeshi women. This thesis also showed that the content Cd in this material is very high and may explain previous studies that reported high levels of Cd in the blood, urine and breast milk of Bangladeshi women. Since Sikor is consumed more often and in higher quantities during pregnancy, by Bangladeshi women both in Bangladesh and in the UK, the potential adverse health and developmental effects to the unborn baby is of particular concern.

Arsenic, cadmium, manganese and lead can generate reactive oxygen species in biological systems which can adversely affect the cells and organs in the mother and the foetus. Since the PMTDI of As and Pb, can be exceeded by 2- to 5-fold by just ingesting 50 g of Sikor per day, consumers in the UK and Bangladesh are in danger of unknowingly harming their health. This thesis recommends that those responsible for public health act to reduce or eliminate the consumption of baked clay in populations where this practice is prevalent. Consumers should be provided with the choice of purchasing Sikor that do not have the elevated levels of As, Cd, Mn and Pb that the current study found in samples imported into the UK from Bangladesh and sold in local ethnic markets. Meanwhile, more studies needs to be carried to evaluate arsenic exposure from Sikor consumption in Bangladeshi and Indian women, including the bioaccessibility of arsenic and other elements from Sikor.

7 TOTAL DAILY INTAKES OF ARSENIC AND OTHER TRACE ELEMENTS IN BANGLADESHI POPULATION

7.1 Introduction

Exposure to arsenic through drinking water has been linked to the development of various diseases including hyperkeratosis and skin lesions and millions are at risk of developing cancer in Bangladesh (Hafeman *et al.*, 2006). Use of arsenic rich groundwater for irrigation of crops is adding to this problem as high levels of arsenic has been detected in rice and vegetables grown in Bangladesh (Alam *et al.*, 2003, Duxbury *et al.*, 2003, Kile *et al.*, 2007; Al-Rmalli *et al.*, 2005; Meharg *et al.*, 2009). The arsenic crisis in Bangladesh is well known, especially inorganic arsenic consumption from water. However, intake of arsenic and other toxic elements from foods may be a contributory factor in the development of disease that requires further investigation.

Intake of toxic elements such as As, Cd and Pb from foods can cause potential risk to human health due to the transfer of toxic elements into the food chain (Freitas *et al.*, 2008). It has been reported that As, Cd and Pb can increase the generation of reactive oxygen species (ROS) which may have harmful effects at the cellular and organ level (Beyersmann and Hartwing, 2008). The fact that intake of arsenic and other toxic elements from the food chain is a risk for human health is well established (Freitas *et al.*, 2008; Jarup *et al.*, 1998; Mishra, 2009).

Bangladeshi populations in Bangladesh and those residing in the UK have some similarities in their diet, since many Bangladeshi foods including vegetables and fish are imported into the UK and are widely consumed by the UK Bangladeshis (Kassam-Khamis *et al.*, 2000). Certain diseases including cancers and diabetes have been linked to exposure to toxic elements (Bernard, 2008; Gunter *et al.*, 2006; Hafeman *et al.*, 2006; Haque *et al.*, 2003). UK Bangladeshis have a high prevalence of diabetes compared with other UK communities (Cascio *et al.*, 2011).

Manganese is an essential element for human health; however, exposure to high levels of Mn can induce neurological effects such as manganism, which is characterised by movement disturbances similar to that observed in Parkinson's disease (Lucchini *et al.*, 2009). Mn can accumulate at the cellular level in mitochondria, where it disrupts oxidative phosphorylation and increases the generation of ROS (Gunter *et al.*, 2006). Mn exposure from drinking water for Bangladeshi women has been reported by Ljung *et al.* (2009). The recommended dietary allowance (RDA) for Mn was estimated by WHO to be 2.3 and 1.8 mg per day for male and female, respectively (FSA, 2009). Unlike arsenic, the source of additional Mn exposure through food consumption has not as yet been addressed for the Bangladeshi population.

Selenium and Zinc are essential elements that have many functions in the human body. It has been reported that Se has preventive potency against Keshan disease (Li *et al.*, 2007) and it has important antioxidant activities (Shen *et al.*, 2011). It may protect the body against arsenic toxicity (Sunde and Hoekstra, 1980; Pilsner *et al.*, 2011). Another study reported that Se can have positive role in the immune system functions (Larsen, 1993). The RDA for Se was estimated to be 55 µg per day for women and 70 µg per day for men (it depends on the body weight) (Committee on Dietary Allowances, 1989). However, the maximum daily intake of Se was estimated to be 5 µg/kg.bw.day (FSA, 2009). The maximum daily intake of Zn was estimated to be 300 - 1000 µg/kg.bw.day (FSA, 2009).

Cadmium is a toxic element, and human exposure to high levels of Cd can result in the development of various diseases. It can disrupt a number of biological processes in human organs such as in the kidneys and the lungs (Bernard, 2008). Certain populations are exposed to high levels of Cd. For example, Cd concentrations in breast milk of Bangladeshi women have been shown to be the highest (median 0.14 µg/L) compared to other countries (below 0.1 µg/L) (Kippler *et al.*, 2009). These researchers also found high Cd exposure among children in rural Bangladesh (Kippler *et al.*, 2010). The precise reason for the high Cd levels remains unknown although intake from food is considered to play an important role. Total daily intakes have been estimated for a number of countries. For example, the TDI was reported to be 14 (Ysart *et al.*, 1999) and 9.45 µg Cd per day (Alam *et al.*, 2003) for UK and the Samata region of Bangladesh, respectively. Lead is another highly toxic element, exposure to elevated levels of Pb has been associated with various toxic effects including lung cancer (De Palma *et al.*, 2008), immunotoxicity (Mishre, 2009) and neurotoxicity (Verstraeten *et al.*, 2008). Due to increased industrialisation and use of Pb based fuels and chemicals, exposure to Pb in Bangladesh is becoming a significant problem and elevated blood Pb levels were detected in primary school children in Dhaka (Bangladesh) (Kaiser *et al.*, 2001). The TDI of Pb was estimated to be 24 µg/day for UK (Ysart *et al.*, 1999).

Dietary exposure to these elements (essential and toxic) has been used to assess risk from food consumption, and then compared with recommended safe levels. Different scales are used for risk assessment, such as the Provisional Tolerable daily intakes (PMTDIs) (µg/kg.bw.day) or the Provisional Tolerable Weekly Intakes (PTWIs) (µg/kg.bw.week), both set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FSA, 2009). These scales are used by JECFA in identifying tolerable intakes of foods and non-foods (FSA, 2009). Recently the United States Environmental Protection Agency (USEPA) has provided another scale for risk assessment, the Target Hazard Quotients (THQ), which is based on non-carcinogenic effects and is an important method of health risk assessment (USEPA, 2000).

The aims of this Chapter are as follows:

a) To estimate the total daily intakes of As, Cd, Pb, Mn, Se and Zn for Bangladeshi populations.
b) To determine the risk assessment for the Bangladeshi population by estimating the PMTDIs and THQs for these elements.
c) To correlate the TDI, PMTDI and THQs for the Bangladeshi population in relation to the prevalence of different diseases.

7.2 Materials and Methods

7.2.1 Sample collection

Different types of foods (such as rice, vegetables and fish) and non-foods (betel quid components and baked clay) that are widely consumed in Bangladesh were purchased from UK based ethnic shops in the cities of Leicester, Birmingham, London and Luton. Products analysed in this study were mainly of Bangladeshi origin and are popular with Bangladeshi communities living in the UK and in Bangladesh.

7.2.2 Sample preparations

All food and non-food samples were treated as described in Chapters 4, 5 and 6. Elements in these samples were then determined by ICP-MS, as described in Chapter 2.

7.2.3 Methodology for risk estimation

7.2.3.1 Calculation of Total Daily Intake (TDI)

Total daily intake of each of the trace element was calculated by adding the concentration (mg or µg) of that element present in all the different types of foods and drinking water that is consumed per day. The TDI will therefore depend on the concentrations of these elements present in foods and/or water and quantities consumed.

7.2.3.2 Calculation of toxic:essential trace element ratio

In this study, a Food Toxicity Scale (FTS) for risk assessment of food consumption has been developed which can be a useful tool for identifying foods that have low toxic element and high essential element levels. In this scale, the ratio of toxic element (such as As, Cd, Pb) to essential element (such as Se and Zn) for each individual food (rice, vegetables, fish etc) were calculated. The equation utilises (see below) three important factors that provides information regarding the relationship between the content of toxic element present in a food versus the content of essential elements. The three factors used are as follows:

(i) The concentration of the toxic element in the food under study.

(ii) The toxic element: essential element ratio in the particular food under study (ratio factor).

(iii) The quantity of the particular food consumed per day (kg per day).

Then equation utilising the above factors can be expressed to obtain the FTS value for a particular food.

$$FTS = ln\left[\left(\frac{1}{n}\right)\sum_{i=1}^{n}\left([ToxEi]\ x\left(\frac{[ToxEi]}{[EE1]}+\frac{[ToxEi]}{[EE2]}+\cdots\cdots\right)x\ Q(kg/day)\right)\right]$$

where FTS is the food toxicity scale; ToxE is concentration of toxic element; EE is concentration of essential element; Q is quantity of food consumed (kg per day).

Also FTS can describe as follows:

$$FTS = ln\left(\left(\frac{1}{n}\right)\sum_{i=1}^{n} TDI * ratio\ factor\right)$$

where TDI is the total daily intake of toxic element and ratio factor is the ratio of the toxic element: essential element(s).

An worked example calculating the toxicity associated with two different types of rice (one with a low As level and one with a high As level but both having the same level of the essential elements Se and Zn) is given below:

The FTS equation will be as a following:

$$FTS = ln\left([As]\ x\ \left(\frac{[As]}{[Se]} + \frac{[As]}{[Zn]}\right)\ x\ Q(kg/day)\right)$$

$$FTS = ln\ (TDI * ratio\ factor)$$

(i) Rice Containing [As] = 20 µg/kg, [Se] = 100 µg/kg, [Zn] = 10000 µg/kg, and 0.5 kg of rice consumed per day.

$$FTS = ln\left[\left(\left([20]\ x\ \left(\frac{[20]}{[100]} + \frac{[20]}{[10000]}\right)\right)\ x\ 0.5\right)\right] = 0.70$$

(ii) Rice Containing [As] = 200 µg/kg, [Se] = 100 µg/kg, [Zn] = 10000 µg/kg and 0.5 kg of rice consumed per day.

$$FTS = ln\left[\left(\left([200]\, x \left(\frac{[200]}{[100]} + \frac{[200]}{[10000]}\right)\right) x\ 0.5\right)\right] = 5.31$$

In this thesis, the FTS for different Bangladeshi foods were calculated in order to identify the foods that are likely to be more harmful to human health.

The equation has the advantage of being applied to determine the toxic effects of more than one element in a particular food. Thus for example, for the cumulative toxicity of As, Cd and Pb in rice can be calculated in the following manner:

$$FTS = ln\left[\left(\frac{1}{3}\right) x \left(\left([As]\, x \left(\frac{[As]}{[Se]} + \frac{[As]}{[Zn]}\right)\right) + \left([Cd]\, x \left(\frac{[Cd]}{[Se]} + \frac{[Cd]}{[Zn]}\right)\right) + \left([Pb]\, x \left(\frac{[Pb]}{[Se]} + \frac{[Pb]}{[Zn]}\right)\right) x\ Q\right)\right]$$

(i) Rice Containing [As] = 20 µg/kg; [Cd] = 20 µg/kg; [Pb] =20 µg/kg; [Se] = 100 µg/kg, [Zn] = 10000 µg/kg and 0.5 kg of rice consumed per day.

$$FTS = ln\left[\left(\frac{1}{3}\right) x \left(\left([20]\, x \left(\frac{[20]}{[100]} + \frac{[20]}{[10000]}\right)\right) + \left([20]\, x \left(\frac{[20]}{[100]} + \frac{[20]}{[10000]}\right)\right) + \left([20]\, x \left(\frac{[20]}{[100]} + \frac{[20]}{[1000]}\right)\right) x\ 0.5\right)\right] = 0.70$$

(ii) Rice Containing [As] = 200 µg/kg; [Cd] = 50 µg/kg; [Pb] = 100 µg/kg; [Se] = 100 µg/kg, [Zn] = 10000 µg/kg and 0.5 kg of rice consumed per day.

$$FTS = ln\left[\left(\frac{1}{3}\right) x \left(\left([200]\, x \left(\frac{[200]}{[100]} + \frac{[200]}{[10000]}\right)\right) + \left([50]\, x \left(\frac{[50]}{[100]} + \frac{[50]}{[10000]}\right)\right) + \left([100]\, x \left(\frac{[100]}{[100]} + \frac{[100]}{[1000]}\right)\right) x\ 0.5\right)\right] = 3.92$$

7.2.3.3 Calculation of PMTDIs and THQs scales

The percentage Provisional Maximum Tolerable Daily Intake (PMTDI%) estimates the maximum daily intake of toxic elements from individual food or more than one type of food, and the unit that is used for this scale is mg or µg of element per day. Recently the United States Environmental Protection Agency (USEPA) has provided another scale for risk assessment, the Target Hazard Quotients (THQ) (see chapter 2, section 2.2.8).

7.3 Results

Concentrations of As, Cd, Pb, Mn, Se and Zn in rice, fish, vegetables and non-foods (betel quid and baked clay) imported from Bangladesh and sold in the UK markets were determined. The data are presented as mean, minimum, maximum and median levels (Chapters 4, 5 and 6). Mean concentrations of these elements present in foods and non-foods were used for calculating the total daily intakes for the Bangladeshi population residing in Bangladesh based on the FFQ data of Zablotska et al. (2008). The PMTDIs and THQs for these elements were estimated.

7.3.1 Total daily intakes (TDIs) of As, Se and Mn

Tables 7-1, 7-2 and 7-3 show TDIs of As, Se and Mn around the world. Table 7-5 shows daily intakes of these elements from all foods and non-foods (including drinking water) were 301, 90.36 and 18724 µg/day, respectively. Table 7-4 presents the estimation of daily intakes of As, Se and Mn from consumption of two important Bangladeshi foods, fish (31.76, 35.21 and 141.0 µg/day, respectively) and rice (34.32, 27.40 and 5465 µg/day, respectively). The highest daily arsenic intake in Bangladesh comes from drinking water (226.8 µg/day). However, very low Se and Zn level (<3.0 and <1.0 µg/L, respectively) in Bangladeshi groundwater has been reported (Frisbie *et al.*, 2002). The daily intake of Se from drinking water was 2.7 µg/day (Frisbie et al., 2002). From the current study, beans and lentils have been found to contribute significantly to the daily intake of Se (3.86 µg/day). In contrast to Se, the daily intake of Mn from drinking water in Bangladesh is very high [estimated to be 2,160 µg/day (Frisbie *et al.*, 2008)]. In the current study, high daily Mn intake (4,160 µg/day) can be derived from drinking tea infusion.

Table 7-1: Estimation of daily dietary intake of As around the world.

Country	**TDI of As µg/day**	**Reference**
Austria	27	Woidich and Pfannhauser (1977)
Bangladesh, Food	68	Kile *et al.* (2007)
Bangladesh, Foods + water	150 (43 – 490)	Ohno *et al.* (2007)
Bangladesh, Only vegetables	105	Karim et al. (2008)
Bangladesh, Foods	74	This study
Bangladesh, Foods + water	306	This study
Canada	48.5	Debeka *et al.* (1993)
Croatia	11.7	Sapunar-Postruznik *et al.* (1996)
Denmark, mainly fish consumption	118	Larsen *et al.* (2002)
France	109	Leblanc *et al.* (2000)
Germany	6.9 (0.6 – 98)	Wilhelm *et al.* (2003)
India	801	Roychowdhury *et al.* (2003)
Japan	182 (27 – 376)	Mohri *et al.* (1990)
Japan	160 – 280	Tsuada *et al.* (1995)
Korea	38.5	Lee *et al.* (2005)
Mexico, in summer	69.3	Del Razo *et al.* (2002)
Mexico, in winter	56	Del Razo *et al.* (2002)
Pakistan	83 - 104	Arain *et al.* (2009)
Spain	221	Delgado-Andrade *et al.* (2003)
Spain	51.5	Matos-Reyes *et al.*(2001)
Sweden	60	Jorhem *et al.* (1998)
Thailand	105 – 406	Tupwongse *et al.* (2007)
Thailand	287 (68.2 – 564)	Ruangwises and Saipan (2009)
UK	56 - 67	MAFF, (1998)
UK	63	Ysart *et al.* (1999)
UK	55	FSA, (2000)
UK	99	FSA, (2009)
USA	155.2	Scalon *et al.* (1999)
USA	58.1	Egan *et al.* (2002)
USA	195	Cleland *et al.* (2009)

Total daily intakes of As for different countries around the world and compared with estimation of daily intake of As for Bangladeshis from the current study.

Table 7-2: Estimation of daily dietary intake of Se around the world.

Country	**TDI of Se µg/day**	**Reference**
Australia	57 - 87	Fardy *et al.* (1989)
Bangladesh	90.4	This study
Belgium	28 - 61	Robberecht *et al.* (1994)
Brazil, Sao Paulo	28 – 37	Maihara *et al.* (2004)
Canada	98–224	Gissel-Nielsen (1998)
China	3 - 11	Dumont *et al.* (2006)
Denmark	48	Larsen *et al.* (2002)
Finland, before use Se fertilizer	25	Aro *et al.* (1995)
Finland, after use Se fertilizer	67 - 110	Anttolainen *et al.* (1996)
Greece	39.2	Pappa *et al.* (2006)
India	27–48	Mahalingam *et al.* (1997)
Ireland	50	Murphy *et al.* (2002)
Italy	43	Allegrini *et al.* (1985)
Japan	104 – 199	Miyazaki *et al.* (2001)
Japan	118	Miyazaki *et al.* (2004)
Libya	13 – 44	El-Ghawi *et al.* (2005)
Lithuania	100	Golubkina *et al.* (1992)
México	61 - 73	Valentine *et al.* (1994)
Nepal	23	Moser *et al.* (1988)
Netherlands	67	Foster and Sumar (1997)
Norway	80	Meltzer *et al.* (1992)
Scotland, UK	30 – 60	MacPherson *et al.* (1997)
Spain	72.6	Díaz-Alarcon *et al.*(1996a)
Spain	23	Matos-Reyes *et al.* (2010)
Sweden	38	Dumont *et al.* (2006)
Switzerland	70	Dumont *et al.* (2006)
Thailand	44.1 - 91	Tupwongse *et al.* (2007)
Turkey	30	Dumont *et al.* (2006)
UK	34	Barclay *et al.* (1995)
UK	29 – 39	MAFF (1997)
UK	34	FSA (2000)
UK	48 - 58	FSA (2009)
USA	110 – 126	Egan *et al.* (2002)
USA	60 - 160	Longnecker *et al.* (1991)

Total daily intakes of Se for different countries around the world and compared with estimation of daily intake of Se for Bangladeshis from the current study.

Table 7-3: Estimation of daily dietary intake of Mn around the world.

Estimation method	**TDI of Mn mg day^{-1}**	**References**
UK total diet study, healthy adult consumers (UK)	4.50	Ysart *et al.* (1999)
UK total diet study, healthy adult consumers (UK)	5.24[a]	FSA, (2009)
Duplicate meals, healthy individuals (France)	2.15	Noel *et al.* (2003)
Duplicate diet tech., healthy individuals (Austria)	4.69	Wilplinger *et al.* (1999)
Duplicate portion tech., Healthy men (Germany)	2.70[a]	Schafer *et al.* (2004)
DPTech, Healthy women (Germany)	2.40	Schafer *et al.* (2004)
DPTech, Vegetarian Healthy women (Germany)	5.50	Schafer *et al.* (2004)
Healthy adult (Catalonia, Spain)	2.23	Marti-Cid *et al.* (2009)
Healthy adult (USA)	2.66[a]	Greger *et al.* (1990)
Market basket study, healthy adult (Japan)	2.72[a]	Aung *et al.* (2006)
Healthy women (Punjab, India)	8.98	Kawatra and Bakhetia(2008)
Healthy adult (Mumbai, India)	2.21[a]	Tripathi *et al.* (2000)
Duplicate diet, Healthy adult (Pakistan)	10.54[a]	Iyengar *et al.* (2002)
Daily dietary intake(Murshidabad, India)	8.72	Roychowdhury *et al.* (2003)
Diet study of Bangladesh, US data based	16.51	Zablotska *et al.* (2008)
TDI of Bangladeshi population for non-chewers (BD)	18.7	This study
TDI of Bangladeshi population for chewers (BD)	20.30[a]	This study
TDI of Bangladeshi population for non-chewers (UK)	8.64[a]	This study
TDI of Bangladeshi population for chewers (UK)	9.88[a]	This study

Total daily intakes of Mn for different countries around the world and compared with estimation of daily intake of Mn of Bangladeshis from the current study.

[a] These data were used for correlation between TDI and Urinary Mn levels (Chapter 5).

Table 7-4: Daily intake[a] (µg/day) of As, Se and Zn in foods and water for Bangladeshis.

Food	**As**	**Se**	**Mn**
Meat[b]	0.14	0.64	58
Poultry[b]	0.10	2.70	27
Fish	31.76	35.21	141 (405)[c]
Leafy vegetables	2.58	4.80	2751 (5400)[c]
Lentil and beans	2.58	3.86	1285
Steamed rice	34.32	27.40	5465
Puffed rice	2.18	1.12	168
Tea infusions	0.15	0.14	4168
Others	0.40	11.80	2500
Sum of foods	74.21	87.66	16564
Water [d]	226.80	2.70	2160
Total element intake	301.01	90.36	18724

[a] The quantity of food consumed (g/day) was taken from a previous study (Zoblotska *et al.* 2008)

[b] Animal products measured in this study were purchased from the UK markets.

[c] Mean of element concentration (maximum value).

[d] Estimated using data taken from Frisbie *et al.* (2002)

Figures 7-1 and 7-2 show the percent daily intake of As and Se for different Bangladeshi foods. Figure 7-1 (A) shows that fish and rice are the main source of arsenic from food categories, whereas water (Figure 7-1 B) has the highest arsenic content amongst all the foods/non-foods consumed in Bangladesh. Interestingly, fish and rice were also the main sources of Se in the food category (Figure 7-2 A). Percent daily intake, excluding drinking water, for arsenic and selenium were 46 and 31% for rice and 43 and 40% for fish, respectively (Figure 7-1 A and Figure 7-2 A).

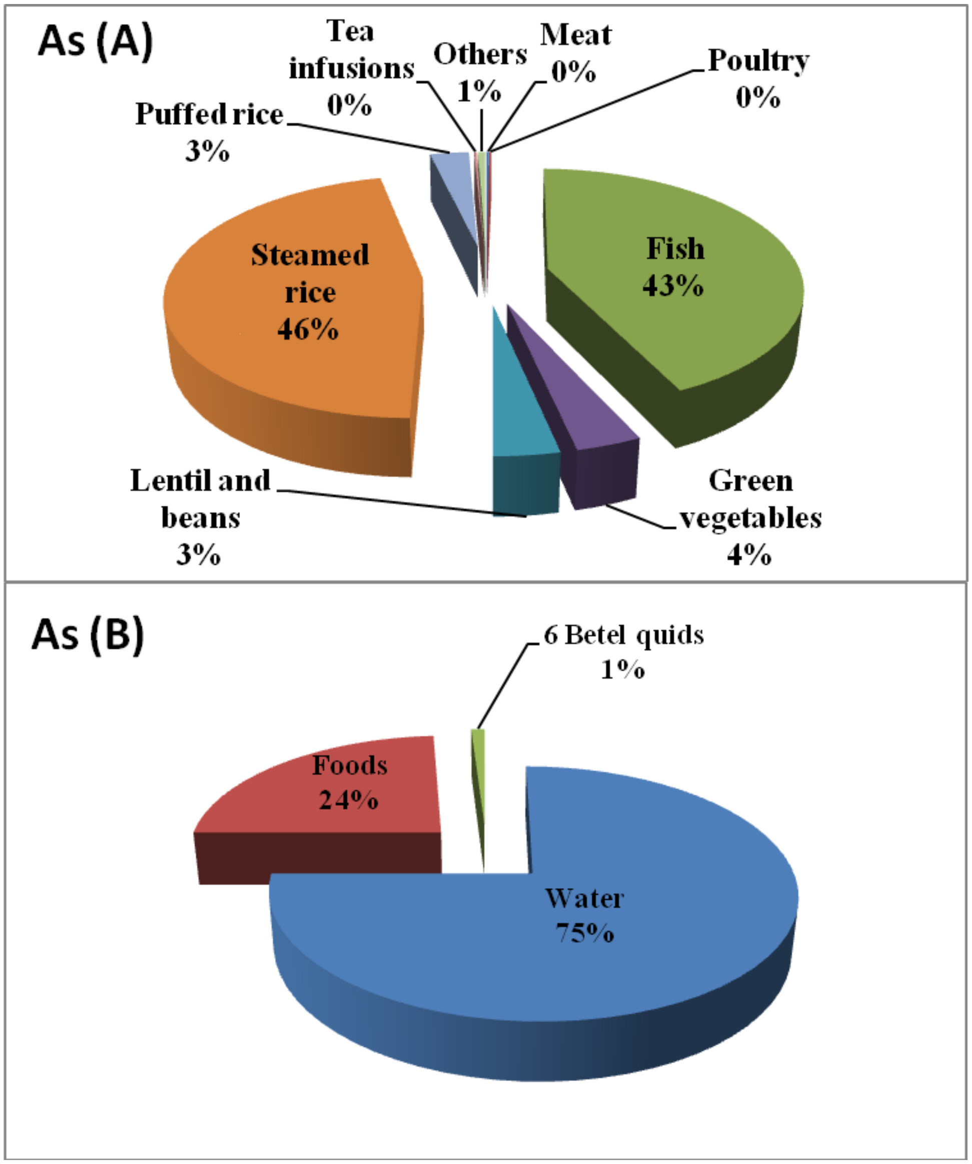

Figure 7.1: Daily intake of As in Bangladeshi foods. (A) Daily As intake (%) through different Bangladeshi foodstuffs (100% = 74.21 µg As per day), (B) Total Daily As intake (%) for all Bangladeshi foods including water (100% = 301.01 µg As per day).

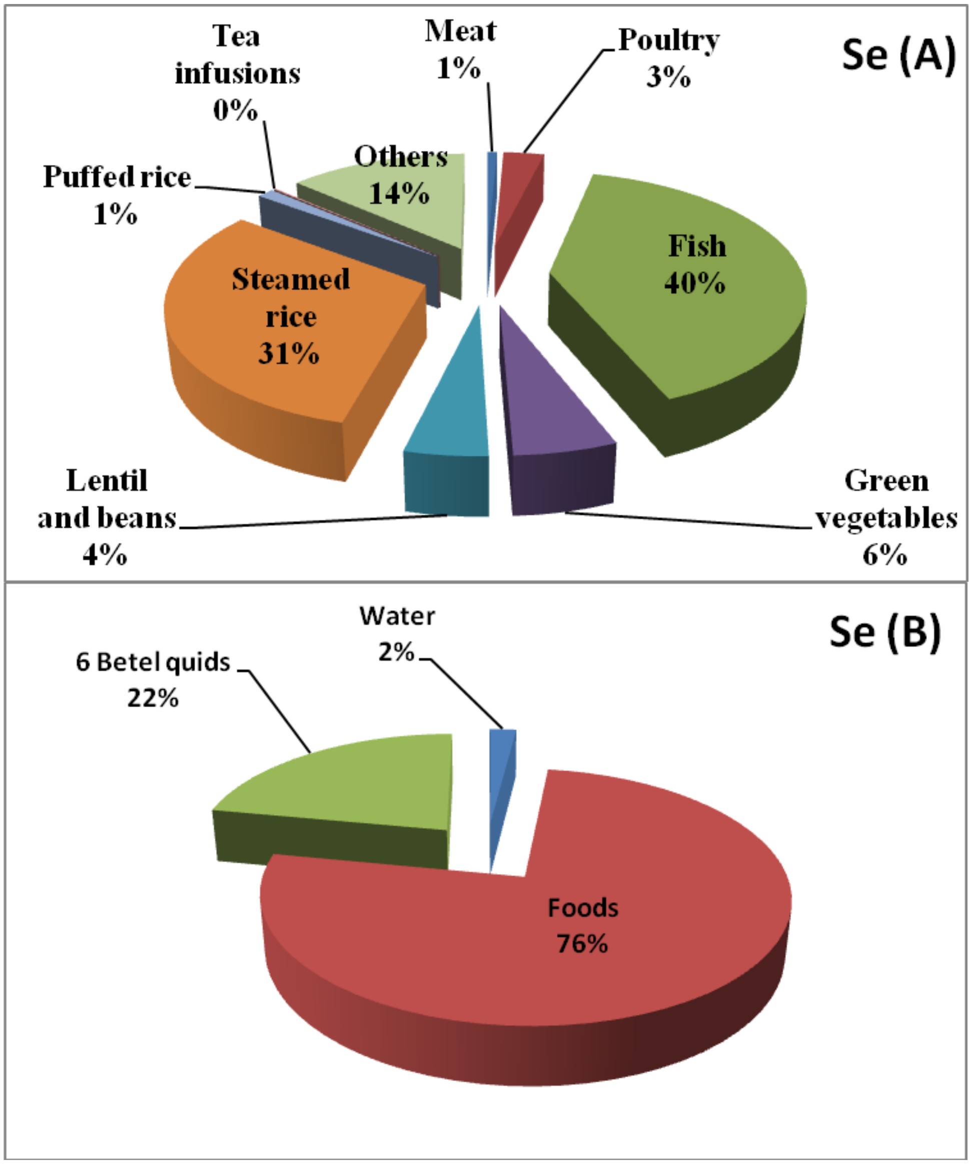

Figure 7.2: Daily intake of Se in Bangladeshi foods including betel quids. (A) Daily Se intake (%) through different Bangladeshi foodstuffs (100% = 87.66 µg Se per day), (B) Total Daily Se intake (%) for all Bangladeshi foods including water (100% = 90 µg Se per day).

Figure 7-3 (A) shows that %TDI of Mn for all types of Bangladeshi foods. Of these, steamed rice, tea infusion and green vegetables contained the major quantities of Mn amongst foods. The %TDI was 33, 28 and 17% for rice, tea infusion and green vegetables, respectively. Betel quid is another source of Mn (which was discussed in chapter 5). Here %TDI of Mn associated with betel quid chewing (for six betel quids per day) was estimated to be 8% of TDI. Drinking water contributed towards 11% of TDI. The %TDI for sum of all foods was 81% (Figure 7-3 B).

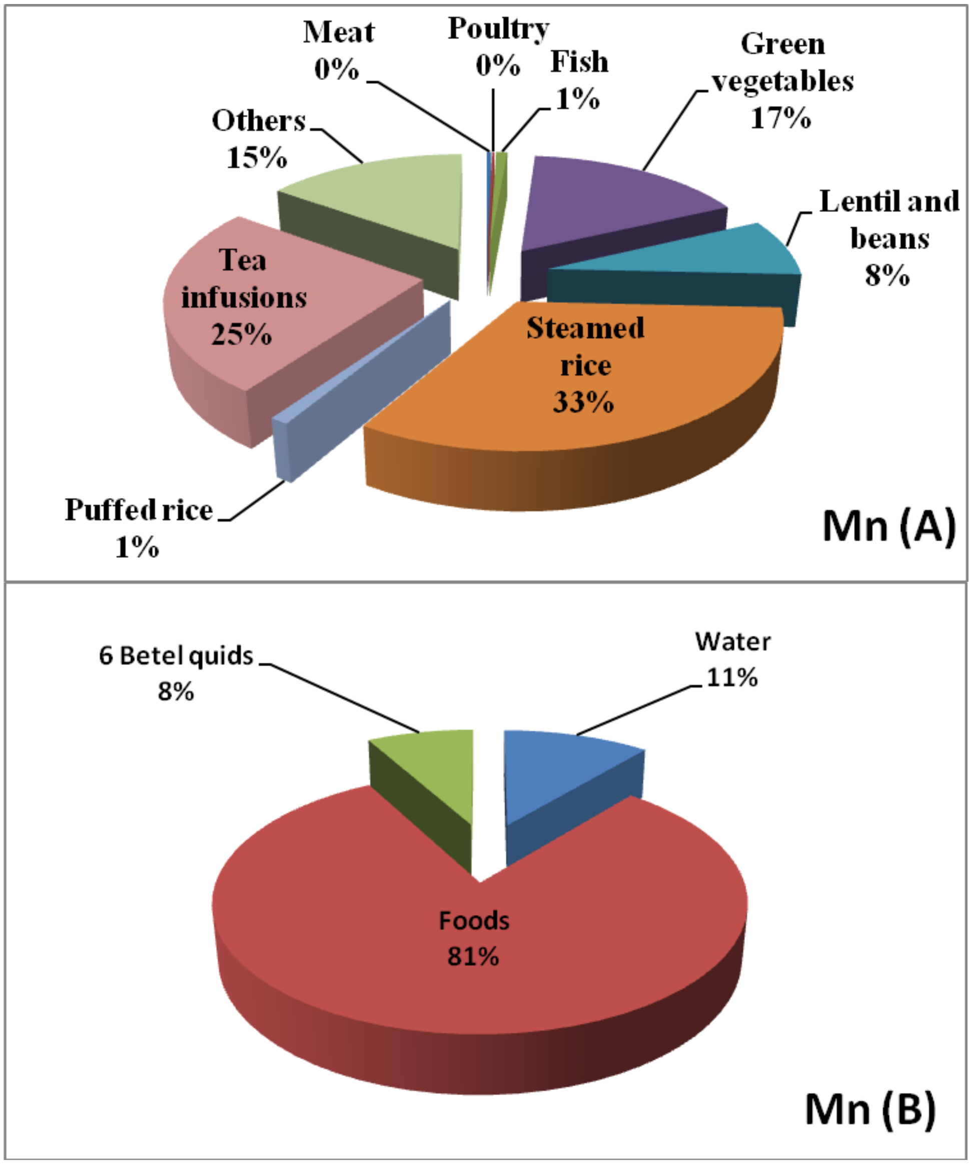

Figure 7.3: Daily intake of Mn from Bangladeshi foods including betel quid chewing. (A) Daily Mn intake (%) through different Bangladeshi foodstuffs (100% = 16.57 mg Mn per day); both meat and poultry are less than 0.5%, (B) Total Daily Mn intake (%) for Bangladesh

7.3.2 Total daily intakes of Cd, Pb and Zn

Tables 7-5, 7-6, 7-7 show the TDI of Cd, Pb and Zn around the world. Table 7-8 presents the estimation of daily intakes for Bangladeshis. Bangladeshi foods that contribute significantly towards the daily intakes of Cd, Pb and Zn were rice (18.61, 14.84 and 5509 µg/day, respectively), green vegetables (12.03, 28.29 and 1700 µg/day, respectively) and fish (0.36, 4.76 and 927 µg/day, respectively). TDI of Cd, Pb and Zn excluding water were 38.55, 74.44 and 11203 µg/day, respectively. Rice and green vegetables are the main sources of Cd and Pb intake. However, fish and animal products contain low Cd levels. Rice contained the highest quantity of Zn followed by vegetables (Table 7-8).

Figures 7-4, 7-5 and 7-6 show that %TDI of Cd, Pb and Zn from Bangladeshi foods. The %TDI for Cd was 49, 31, 14 and 3% for rice, green vegetables, puffed rice, beans and lentil, respectively. The %TDI of Pb was 20, 51, 13 and 8% for rice, green vegetables, beans and lentil, respectively. The %TDI of Zn was 49, 15, 13% for rice, green vegetables and beans and lentil, respectively.

Table 7-5: Estimation of daily dietary intake of Cd around the world.

Country	**TDI of Cd µg/day**	**Reference**
Bangladesh, for foods	34.55	This study
Bangladesh, Samta	9.45	Alam *et al.* (2003)
Brazil, rice	2.4	Ekholm *et al.* (2007)
Denmark	16	Larsen *et al.* (2002)
Finland	7.0	Batista *et al.* (2010)
France	17	Leblanc *et al.* (2000)
France	24.4	Sirot *et al.* (2008)
Greece	11	Vontsa *et al.* (1998)
India, Bamby city	2.5	Tripathi et al. (1997)
India, Mumbai	2.2	Capdevila *et al.* (2003)
Japan, female farmers	27.4 – 33	Horiguchi *et al.* (2004)
Korea	14.3	Lee *et al.* (2006)
Lebanon	15.8	Nasreddine *et al.* (2010)
New Zealand	19	NZFSA, (2004)
Poland	2.81	Falco *et al.* (2005)
Spain, Catalonia	15.7	Llobett *et al.* (2003)
Spain, fish and seafood consumption	1.34	Falco *et al.* (2006)
Spain, Tarragona, edible vegetables	2.02	Capdevila *et al.* (2003)
Thailand	8.4 – 14.7	Tupwongse *et al.* (2007)
UK	9	FSA, (2000)
UK	12	Ysart et al. (2006)
UK	11-13	FSA, (2009)
USA	35.1	Scanlon *et al.* (1999)
USA	11.5 – 14.2	Egan *et al.* (2002)

Total daily intakes of Cd for different countries around the world and compared with estimation of daily intake of Cd for Bangladeshis from the current study.

Table 7-6: Estimation of daily dietary intake of Pb around the world.

Country	**TDI of Pb µg/day**	**Reference**
Bangladesh, Food	74.44	This study
Bangladesh, Foods & water	101.44	This study
Bangladesh, Foods	74.4	Alam *et al.* (2009)
Denmark	18	Larsen *et al.* (2002)
France	52	Leblanc *et al.* (2000)
Korea	24.4	Lee *et al.* (2005)
Netherlands	34	Ellen *et al.* (1990)
New Zealand	33	Vannoort *et al.* (1990)
New Zealand	9	NZFSA, (2004)
Sweden	25	Jorhem *et al.* (1998)
Thailand	7 – 12.6	Tupwongse *et al.* (2007)
UK	24	Ysart *et al.* (1999)
UK	7.4	FSA, (2000)
UK	6	FSA, (2009)
USA	31.2	Scalon *et al.* (1999)
USA	4.2 – 18.2	Egan *et al.* (2002)

Total daily intakes of Pb for different countries around the world and compared with estimation of daily intake of Pb Bangladeshis from the current study.

Table 7-7: Estimation of daily dietary intake of Zn around the world.

Country	**TDI of Zn Mg/day**	**Reference**
Bangladesh, Foods	11.2	This study
Bangladesh, Only vegetables	3.11	This study
Bangladesh, Only vegetables	3.57	Alam *et al.* (2003)
Belgium	13.2	Buchet *et al.* (1983)
Chile for male	7.6 ± 3.4	Olivares *et al.* (2004)
Chile for female	6.4 ± 3.5	
Denmark	11.7	Bro *et al.* (1990)
France	14	Biego *et al.* (1998)
Taiwan	9.5 ± 11.4	Wang and Duo, (1999)
Thailand	8 - 11	Tupwongse *et al.* (2007)
UK	8.4	FSA (2000)
UK	8.83	FSA (2009)
USA	12.7	Egan *et al.* (2002)

Total daily intakes of Zn for different countries around the world and compared with estimation of daily intake of Zn for Bangladeshis from the current study.

Table 7-8: Daily intake[a] (µg/day) of Mn, Cd and Pb in foods and water (Bangladeshis residing in Bangladesh).

Food	**Cd**	**Pb**	**Zn**
Meat[b]	0.12	0.68	220
Poultry[b]	0.13	1.38	208
Fish	0.36	4.76	927
Green vegetables	12.03	38.29	1700
Lentil and beans	1.11	2.42	1400
Steamed rice	18.61	14.84	5509
Puffed rice	5.40	1.69	164
Tea infusions	0.05	0.38	165
Others	0.66	10.10	910
Sum of foods	38.55	74.44	11203
Water [c]	2.70	27.00	3
Total element intake	41.25	101.44	11206

[a] The quantity of food consumed (g/day) was taken from a previous study (Zoblotska *et al.* 2008)
[b] Animal products measured in this study were purchased from the UK markets.
[c] Estimated using data taken from Frisbie *et al.* (2002)

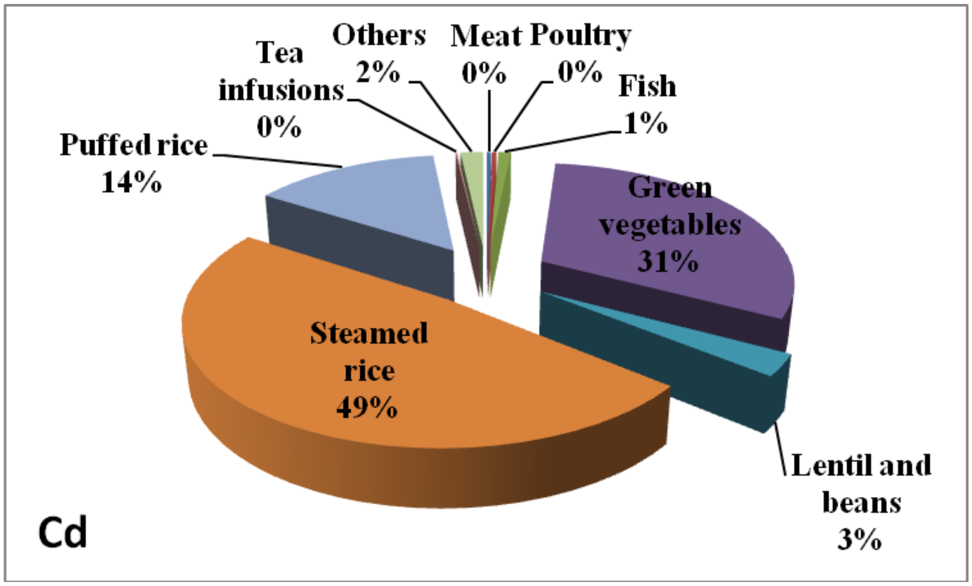

Figure 7.4: Daily intake of Cd in Bangladeshi foods (100% = 38.55 µg Cd per day).

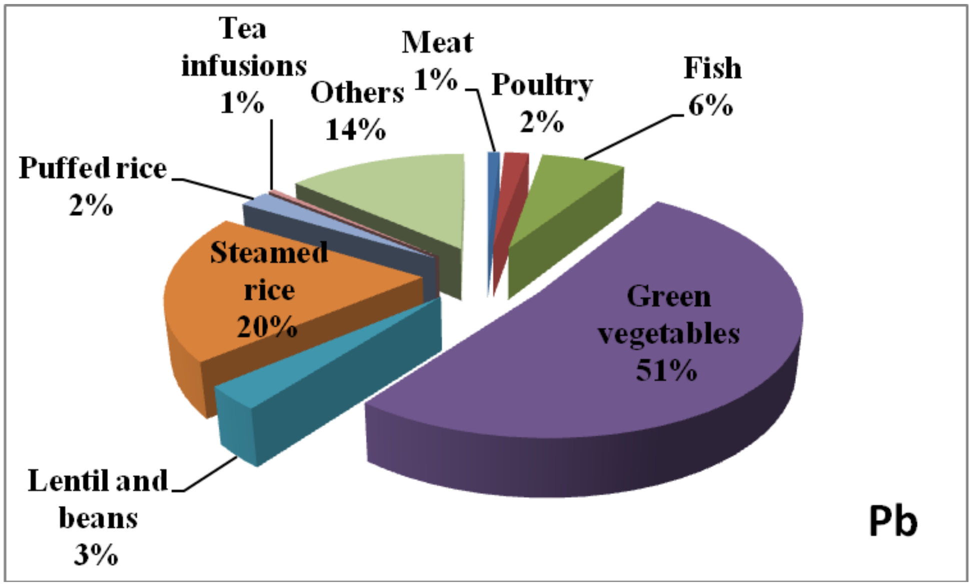

Figure 7.5: Daily intake of Pb in Bangladeshi foods (100% = 75.44 µg Pb per day).

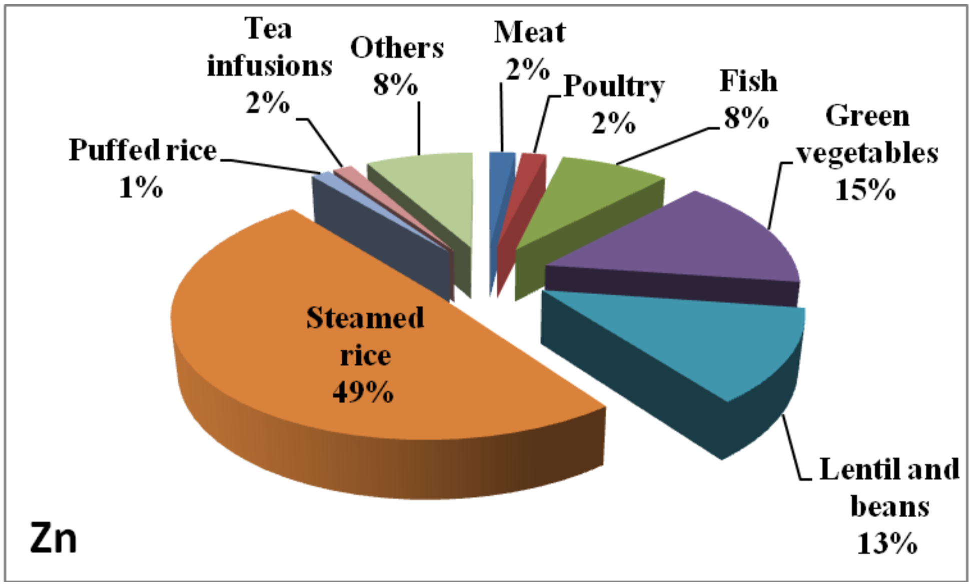

Figure 7.6: Daily intake of Zn in Bangladeshi foods (100% = 11. 203 mg Zn per day).

7.3.3 Daily intakes of trace elements from non-aromatic and aromatic rice

The data from Chapter 3 revealed that aromatic rice has far lower arsenic levels compared to non-aromatic rice and therefore consumption of this variety of rice could reduce the arsenic exposure in Bangladeshis. Therefore, the daily intake of arsenic and selenium was also calculated for aromatic rice. This was done to provide an idea of what would happen if the Bangladeshi people switched from eating non-aromatic rice to aromatic rice. As can be seen from Figure 7-7, the daily intake for arsenic reduces from 39.5 µg/day (non-aromatic rice) to 23 µg/day (aromatic rice) and the daily intake for selenium increases from 21 µg/day (non-aromatic rice) to 39.5 µg/day (aromatic rice).

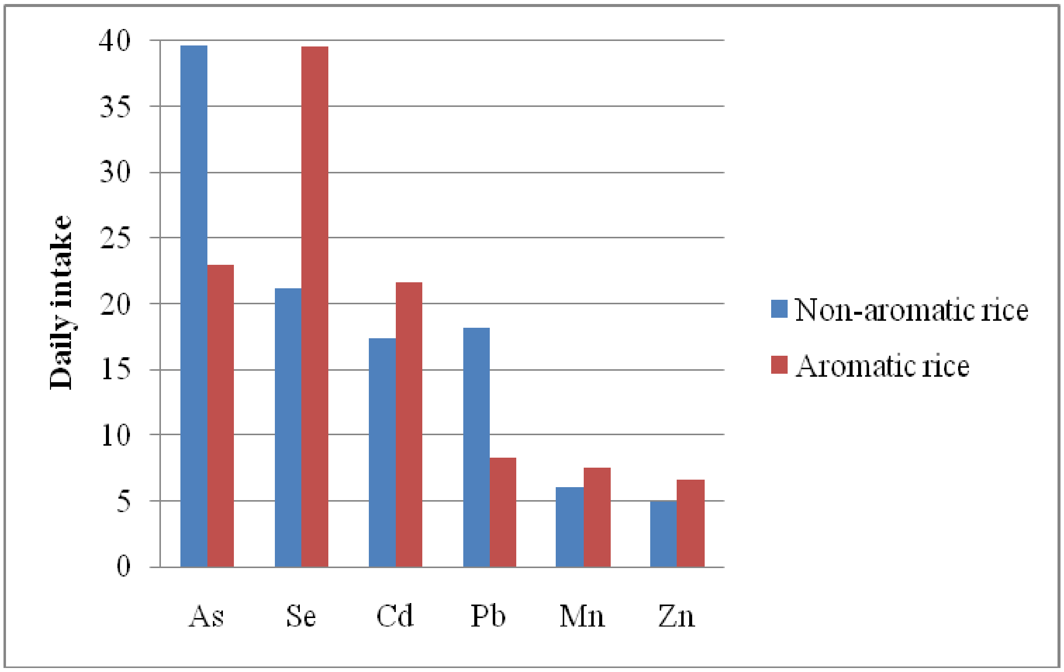

Figure 7.7: Comparison of daily intakes of trace elements from non-aromatic and aromatic rice [or As, Se, Cd and Pb (µg/day) and for Mn and Zn (mg/day)].

7.3.4 Ratios of toxic: essential trace elements in Bangladeshi foods

Ratios of trace elements (toxic:essential) for all Bangladeshi foods, which were determined in this study including rice (for non-aromatic and aromatic Sylheti rice), vegetables and fish are presented in Figures 7-8 to 7-10. Figure 7-8 shows As:Se and As:Zn ratios for different types of Bangladeshi foods. Leafy vegetables have the highest As:Se ratio, followed by puffed rice and raw rice. Low As:Se ratios are seen for milk, eggs and lentils. Generally, rice and leafy vegetables (such as spinach) are the main sources of arsenic whereas lentils, small fish and okra have the lowest ratio amongst all foods analysed.

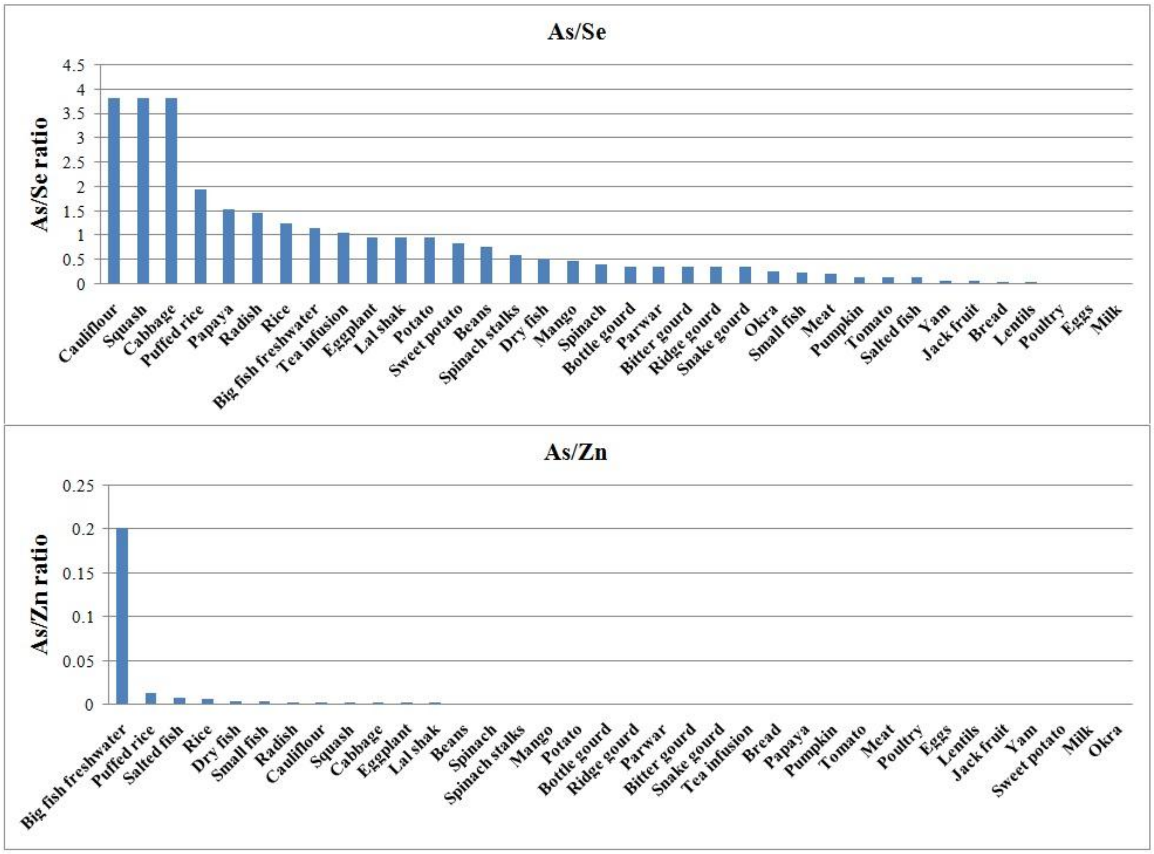

Figure 7.8:As:Se and As:Zn ratios of different Bangladeshi foods.

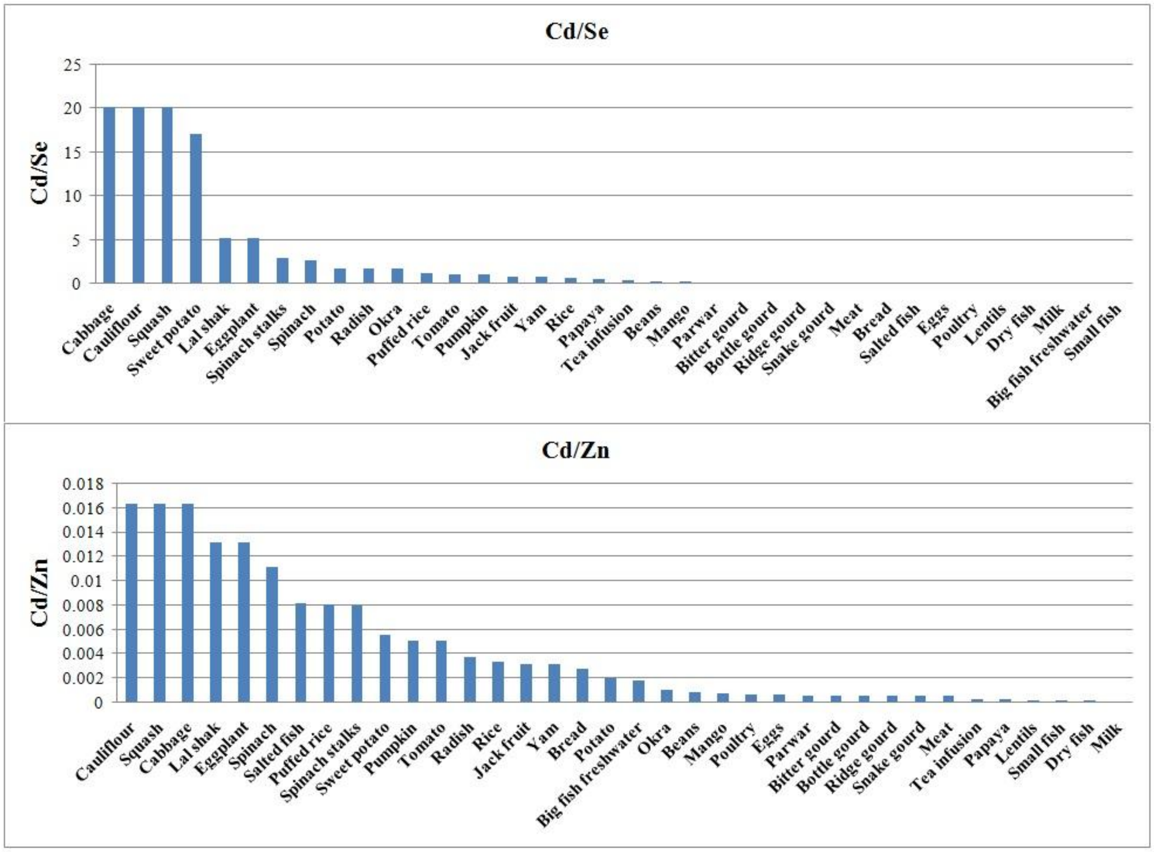

Figure 7.9: Cd:Se and Cd:Zn ratios of different Bangladeshi foods.

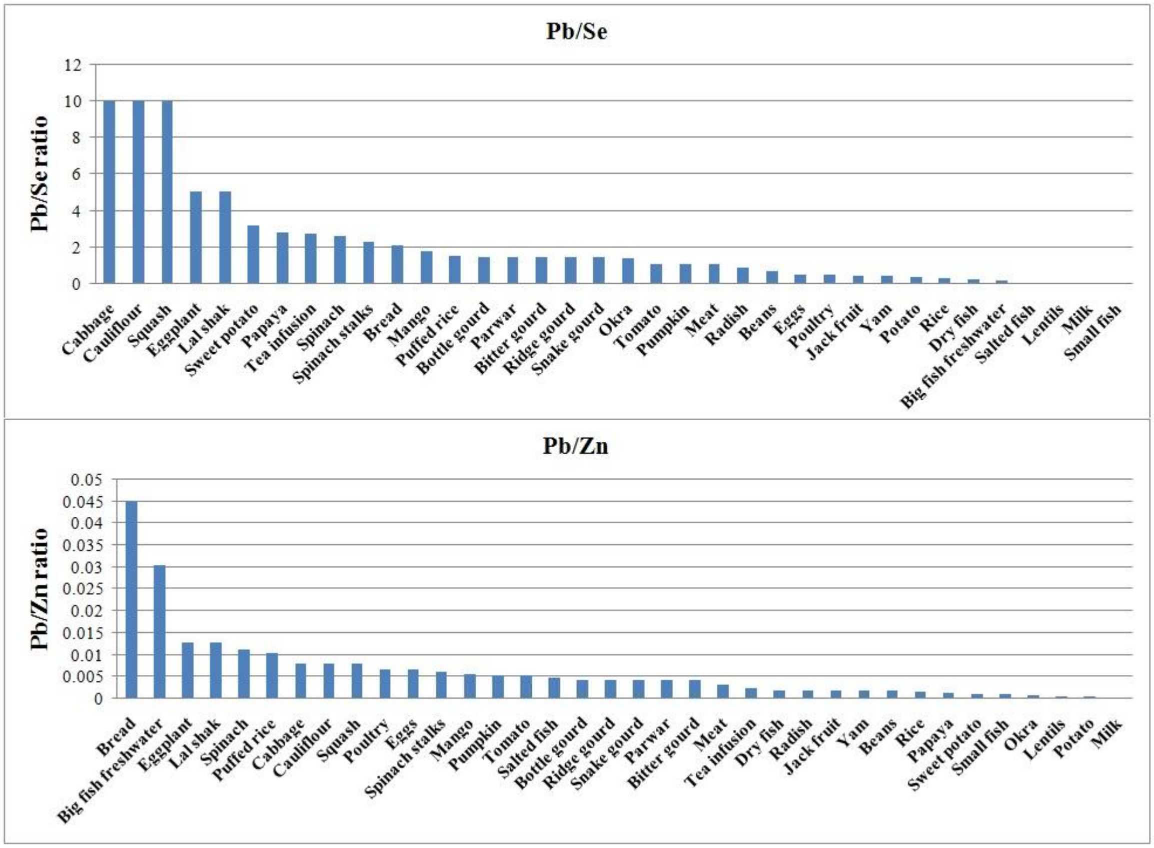

Figure 7.10:Pb:Se and Pb:Zn ratios of different Bangladeshi foods.

Figures 7-11 and 7-12 show the FTS scale for different foods. It presents the data for individual toxic elements (As, Cd, Pb) and for the combination of the three elements (As+Cd+Pb). The equations used for obtaining the data for the single and combined elements are described in the methods section of this chapter (see section 7.2.3.2.). From Figure 7-11 (A), it can be seen that the highest FTS values for arsenic in foods is seen for rice (10.67), followed by big freshwater fish (10.46), puffed rice (8.36). The lowest FTS values for arsenic is seen for milk (-1.16), followed by lentils (-0.13) and okra (0.23). From Figure 7-11 (B), it can be seen that the highest FTS values for cadmium in foods is seen for cabbage (10.32), followed by cauliflower (9.84), spinach stalks (9.51) and rice (9.45). The lowest FTS values for cadmium is seen for milk (-3.09), followed by small fish (-2.63) and lentils (-1.69). From Figure 7-11 (C), it can be seen that the highest FTS values for lead in foods is seen for spinach stalks (9.02), followed by bread (8.96), cabbage (8.93). The lowest FTS values for lead is seen for milk (0.71), followed by salted fish (0.81) and lentils (1.00).

Finally, from Figure 7-12, it can be seen that the highest FTS values for three toxic elements combined (arsenic, cadmium and lead) in foods is seen for rice (9.88), followed by cabbage (9.47), big freshwater fish (9.38). The lowest FTS values for the combined toxic elements are seen for milk (-0.22), followed by lentils (0.23) and salted fish (1.58). Data presented in these Figures can help in the identification of foods that have lower toxic element content and higher essential element content. Thus low FTS values are indicative of foods that can be considered to be safer. The scale is particularly valuable in identifying foods that are potentially harmful to the health of Bangladeshis.

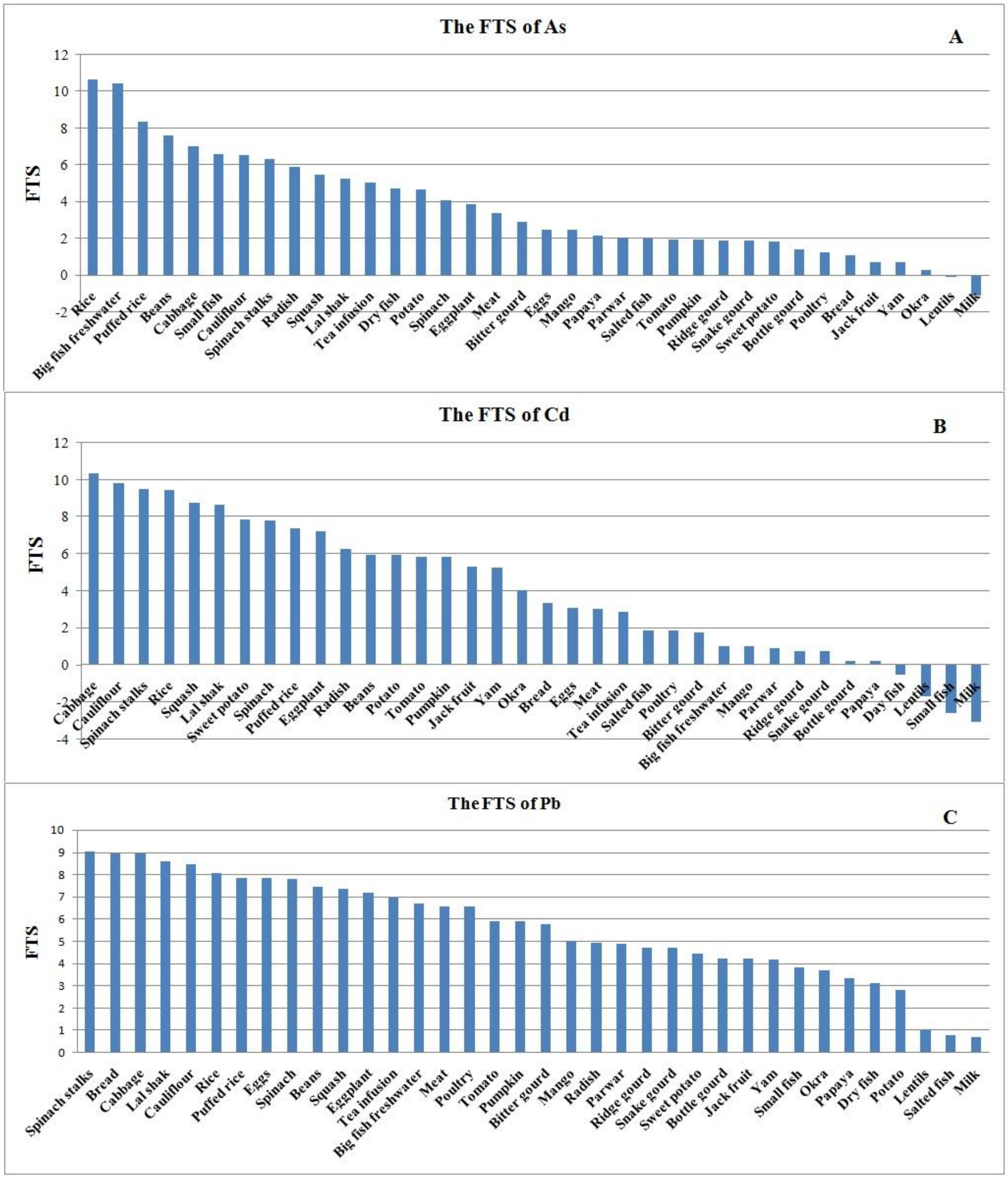

Figure 7.11: The FTS scale of toxic elements for all Bangladeshi foods. (A) the FTS of As versus Se and Zn, (B) the FTS of Cd versus Se and Zn, (C) the FTS of Pb versus Se and Zn.

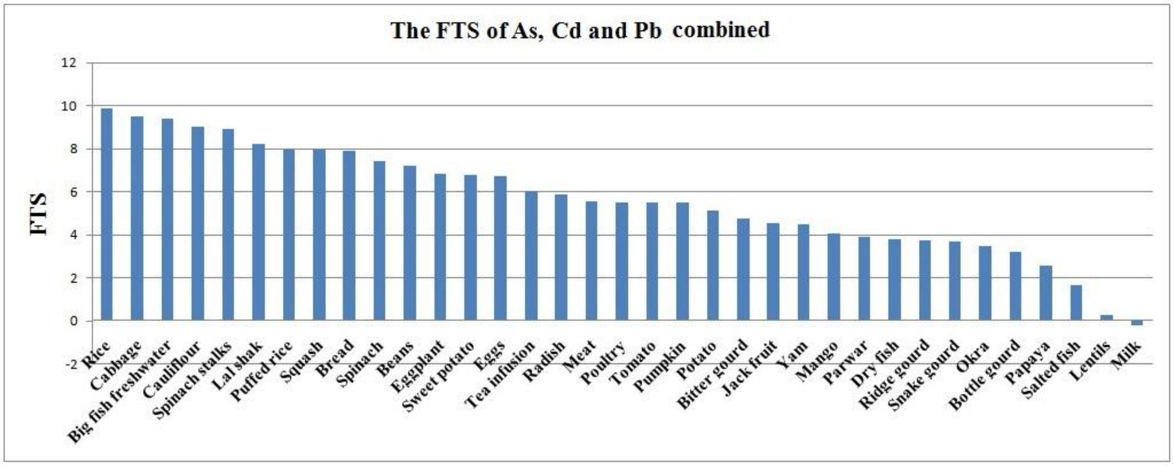

Figure 7.12: The FTS scale of toxic elements combined versus Se and Zn for all Bangladeshi foods

Figure 7-13 shows a comparison of the FTS values for arsenic in 24 different types of rice (aromatic and non-aromatic) for which both total and inorganic arsenic levels are known. It can be seen that the atap LG USA rice has the highest FTS value when total arsenic is used for the calculation. However, when the FTS is calculated using the content of inorganic arsenic, rice Ajona T aman and Aman red Biron have the highest FTS value. Overall, the lowest FTS values are seen for Ulwail Biron (aromatic), Alo baisher and Talsi mala (aromatic).

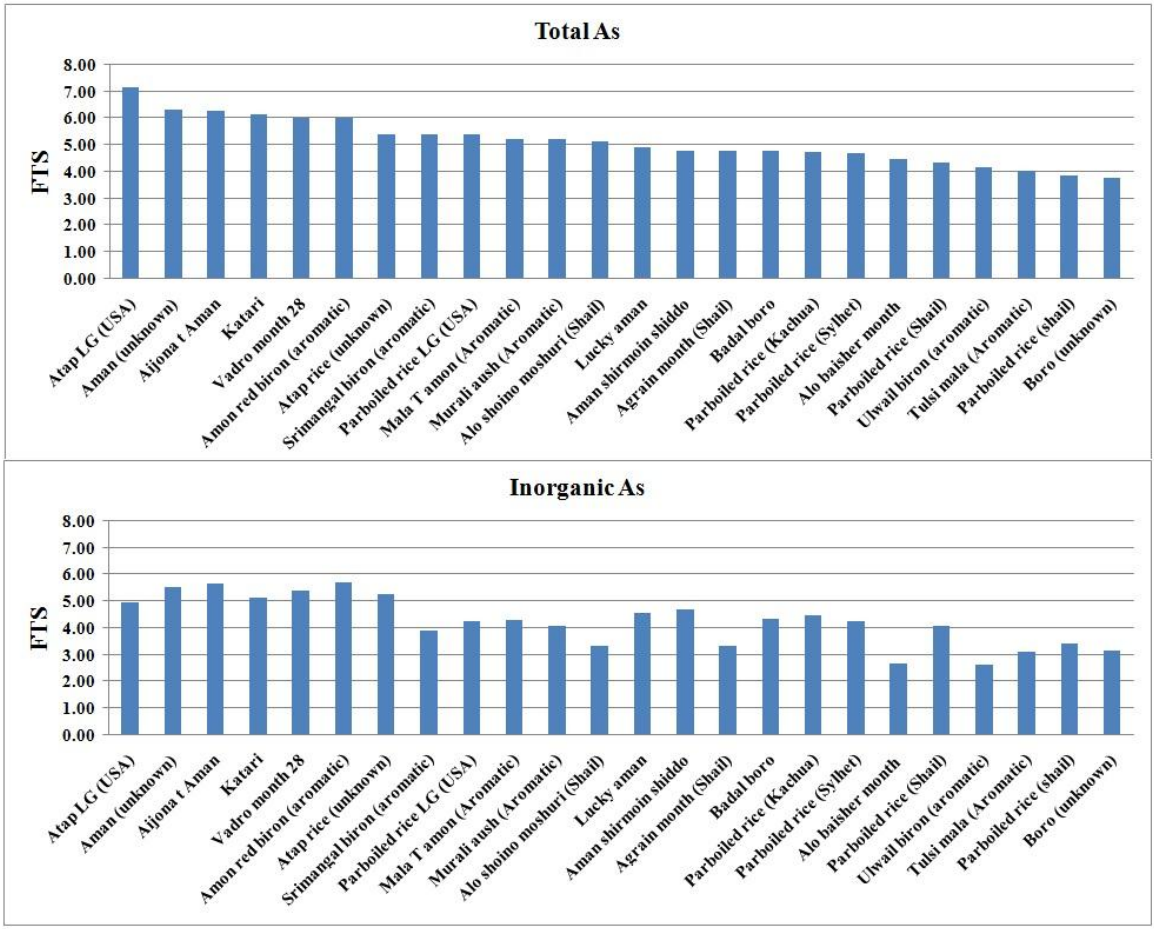

Figure 7.13: Comparison of the FTS values for arsenic in 24 different types of rice (aromatic and non-aromatic) for which both total and inorganic arsenic levels are known

Figure 7-14 shows the FTS of non-aromatic and aromatic Bangladeshi rice for all the elements which were analysed in this thesis. It is very clear that As:Se and Pb:Se ratios of aromatic rice are lower than that for non-aromatic rice. The other ratios for aromatic rice are also lower than non-aromatic rice.

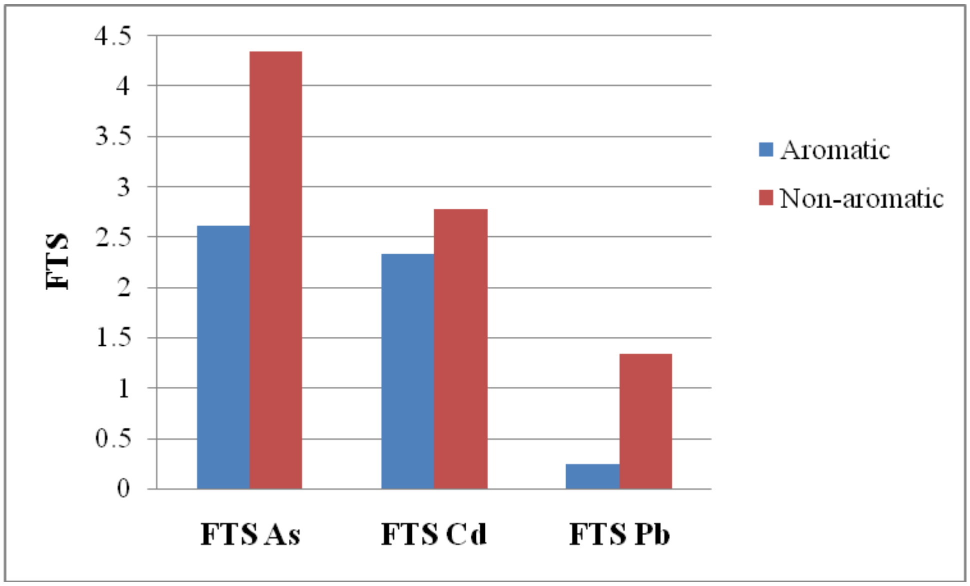

Figure 7.14: Comparison of the FTS scale of aromatic and non-aromatic Bangladeshi rice for As, Cd and Pb versus Se and Zn.

7.3.5 Estimation of risk assessment of trace elements

In order to determine the PMTDIs and THQs of elements for the total diet in the Bangladeshi population, the mean consumption of different Bangladeshi foods (including rice, fish, vegetables and water) were taken partly from analysis in the current study and partly from the literature (Table 7-9). These data were used for estimating the daily intakes (Table 7-4 and 7-8). Contents of elements in different foods (rice, vegetables, fish etc.), tea infusions, betel quids and drinking water were also used for calculating both PMTDIs and THQs for As, Cd, Mn, Pb, Se and Zn.

Quantities of foods (g per day) consumed by the Bangladeshi population were taken from Zablotska *et al.* (2008). In this study, consumption of uncooked rice was assumed to be 500 g (dry weight) per day. Trace elemental levels in Bangladeshi groundwater, reported by Frisbie *et al.* (2002), were used for intake of the different elements from water. This was combined with data from the current study for the intake of trace elements, from consumption of foods and non-foods, to determine the total daily intake of these elements in Bangladesh. Table 7-9 shows the percentage of the PMTDI for As, Cd, Mn, Pb, Se and Zn associated with the consumption of both foods and non-foods. Water and rice provide the highest daily intake of arsenic (159.7 and 27.2% of the PMTDI, respectively). Sikor is the highest daily contributor of arsenic and Pb intakes for Bangladeshi women who eat baked clay (see chapter 6). Sikor also contributes significantly towards the daily intake of Cd (28.3% of the PMTDI) (Table 7-9). However, rice and vegetables are the major source of Cd (30 and 16% of the PMTDI, respectively).

Table 7-9: The percentage of PMTDIs of Bangladeshi food and non-food.[a]

	iAs	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
Rice	27.2	31.0	57.4	7.1	9.1	30.6
Leafy vegetables	1.5	16.0	22.9	16.9	0.8	5.2
Fish	-	0.6	1.5	2.3	11.7	5.2
Other vegetables	2.6	5.9	27.6	2.5	2.1	12.1
Water	159.7	0.8	22.7	12	1.0	0.015
Betel quid chewing [b]	1.0	1.7	18.1	1.7	7.3	2.0
Baked clay [c]	293	28.3	13.4	588	23.8	17

[a] the PMTDI: The Provisional Maximum Tolerable Daily Intake, assuming the body weight for adult is 60 kg.
[b] Six quids of ordinary betel quid are more commonly consumed by chewers.
[C] 50 g of baked clay per day.

Rice provides the highest daily intake of Mn (57.4% of the PMTDI). As much as 18% of the PMTDI for Mn can be derived from chewing six betel quids. For an individual chewing 30 betel quids, 90% of the PMTDI can be reached. High levels of Mn intake also come from other foods including vegetables and tea infusions.

THQ is the other scale that is used for human health risk assessment for trace elements, values that are higher than one are considered to be of health concern. Table 7-10 show that THQs of As, Cd, Mn, Pb, Se and Zn for the Bangladeshi population. Sikor contained the highest levels of arsenic and lead, 2- and 5-fold of the maximum desirable THQ. For Bangladeshis, both drinking water and eating Sikor showed the highest THQ values (1.65 and 2.83, respectively). Rice has significant THQ values for toxic elements including As, Cd and Mn. The THQ for Mn in betel quids was 0.18 (for 6 quids per day), which is a significant value and is similar to the THQ for Mn from drinking water (0.21). The THQs of rice and tea infusions were 0.55 and 0.37, respectively. The THQ for Mn derived from all foods is higher at 1.55. The THQ for the total Bangladeshi diet was estimated to be 1.94 (this includes water and 6 betel quids). This is almost 2-fold of the maximum desirable THQ (Table 7-10).

Figure 7-15 shows a comparison of the percentage of PMTDI of arsenic associated with eating Sikor (50 g), drinking water (2.7 Litres) and rice consumption. It is very clear that 50 g of Sikor contributes towards a higher intake of arsenic than both water (assuming 84 µg iAs/L) and rice (63 µg iAs/L).

Table 7-10: The THQs of Bangladeshi foods and non-foods.

	iAs	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
Rice	0.29	0.28	0.55	0.04	0.08	0.28
Leafy vegetables	0.01	0.05	0.12	0.05	0.008	0.03
Fish	-	0.003	0.02	0.005	0.28	0.02
Other vegetables	0.003	0.014	0.07	0.004	0.007	0.025
Water	1.65	0.02	0.21	0.12	0.008	0.00015
Betel quid chewing	0.01	0.02	0.18	0.08	0.018	0.021
Baked clay	2.83	0.24	0.31	5.06	0.19	0.18

The THQs:The Target Hazard Quotients.

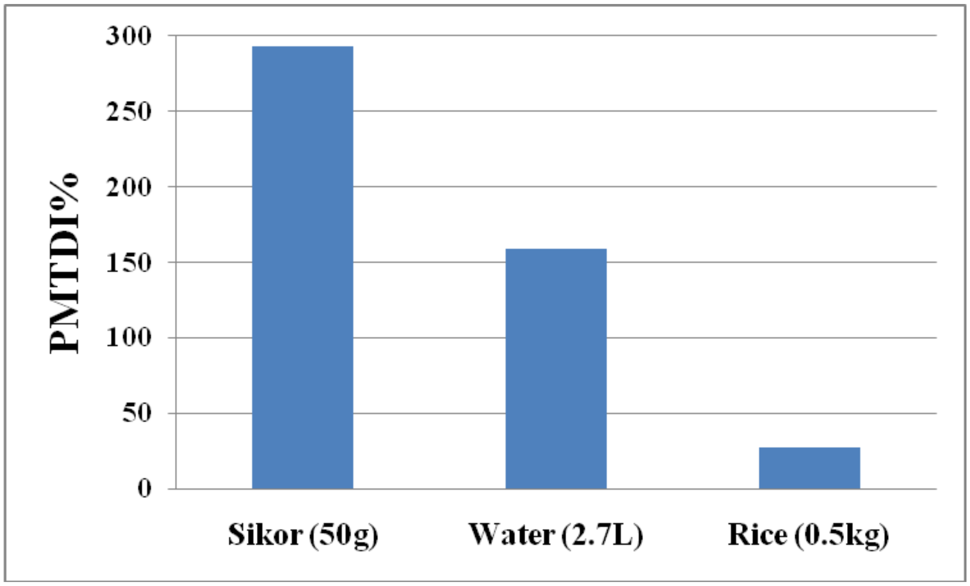

Figure 7.15: Percentage of the PMTDI of arsenic from consumption of Sikor, water (84 µg iAs/L) and rice (63 µg iAs/L) in Bangladesh.

7.3.6 Comparison between the FTS, the PMTDI and the THQ of different food

As can be seen from Figure (7-16) the three different scales used for risk assessment of Bangladeshi foods show the same general trends although the actual values (units) are different.

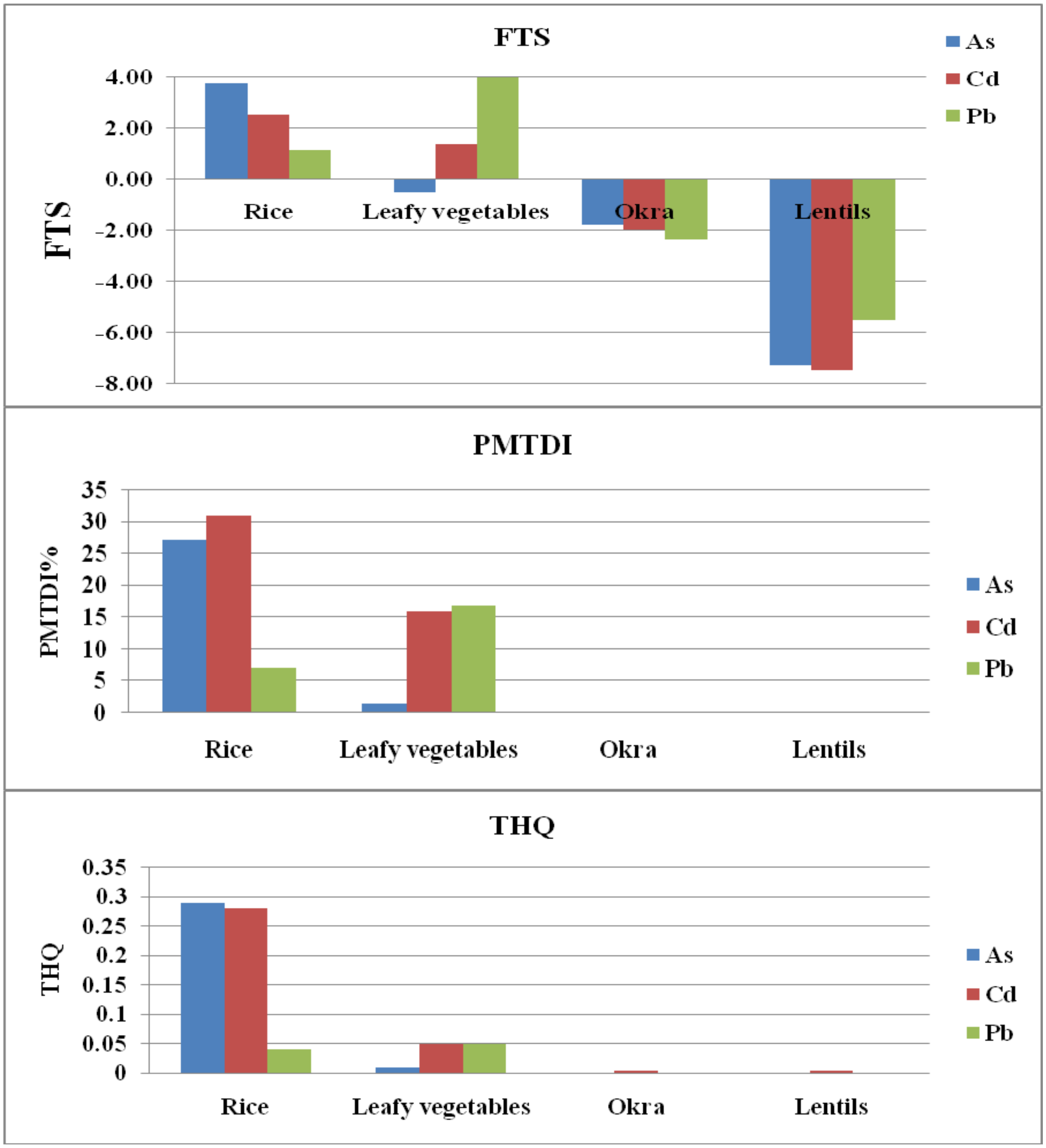

Figure 7.16 Comparison between The FTS to the PMTDI and THQ.

7.4 Discussion

This is the first study to comprehensively determine the TDI of selenium, zinc, cadmium manganese and lead for the Bangladeshi population. Furthermore, the total intake of arsenic was determined through the use of the arsenic content of the largest number and the most diverse range of foods thus far reported in the literature for Bangladeshi foods. For example, this is the first study to consider arsenic intake from betel quids chewing and eating baked clay.

7.4.1 Total daily intakes of trace elements

7.4.1.1 TDI of As, Cd and Pb

The daily intake of arsenic in Bangladesh was found to be 306 µg/day including drinking water (daily intake from foods is 74 µg/day). This value is higher than that for the Japanese population (160-280 µg/day for a two year period) (Tsuda *et al.*, 1995), and is much higher than that reported previously for the daily intake for other countries with the exception of a region in India (West Bengal) where the daily intake of arsenic was reported to be 801 µg/day (Roychowdhury *et al.*, 2003). In contrast to values found in the current study, Kile *et al.* (2007) reported that daily intake of arsenic in Bangladesh, based on a duplicate diet survey (excluding water), was 68 µg/day (median value). Another study in Bangladesh reported that the mean daily intake of arsenic from water and food was 150 µg/day (range 43 – 490 µg/day) (Ohno *et al.*, 2007). TDI from the Kile *et al.* (2007) study was very similar to the finding in the current study for foods (74 µg/day), which shows that the arsenic levels in Bangladeshi foods were similar and the daily consumption are similar for both studies. Kile *et al.* (2007) found that when the arsenic level in the drinking water is low, the main contributor to arsenic intake comes from food. Their study focused on Pabna region of Bangladesh and the situation may be different for other regions. More recently, a value of 105 µg/day, just from consumption of vegetables, was estimated for Bangladeshis (Karim *et al.*, 2008) (see Table 7-1). Furthermore, it is important to keep in mind that in contrast to the current study, previous estimates by other workers did not include arsenic intake from betel quid chewing. Although, there are differences in the estimates of daily intake between different studies, overall the level of arsenic in the Bangladeshi diet is very high and may explain the observation of adverse health outcomes in the Bangladeshi population. Baked clay provides the highest daily intakes of arsenic and lead (370 and 1235 µg/day, respectively).

Rice and vegetables provide some of the highest daily intake of Cd from the food category. Daily intake of Cd from vegetables grown in Samta village (Bangladesh) was estimated to be 9.45 µg/day (Alam *et al.*, 2003). The latter TDI for vegetables consumption is similar to the TDI calculated for vegetables in this thesis (13.14 µg/day see table 7-2). Table 7-6 shows that the TDI of different countries. This reveals that the estimated TDI of Cd for Bangladeshis is higher than almost all the other countries except for the USA (Scanlon *et al.*, 1999) and a group of Japanese female farmers (27.4 – 33 µg/day) (Horiguchi *et al.*, 2004). The latter intake is very similar to the values (34.55 µg/day) for Bangladeshis determined in this study. Very low TDI of Cd, compared to the value determined in this study, was reported for India (Tripathi *et al.*, 1997; Raghunath *et al.* 2006) (see table 7-5). TDI of Cd in food for India was 2.2 µg/day. The researchers may have measured certain types of vegetables (non-leafy vegetables) or their survey underestimated the actual value. Alternatively, Indian vegetables studied by the authors may contain low levels of Cd.

Table 7-6 shows the TDI of Pb around the world. The TDI of Pb by the Bangladeshis estimated in the current study was 101.4 µg/day. This value is very high compared with other TDI values thus far reported in the literature for other countries of the world. The most likely reason for this is the consumption of large quantities of rice and leafy vegetables. These types of food are the main source of Pb in Bangladeshi diet. Excluding water, Bangladeshi food contributed 74 µg/day of Pb which is also very high. This study shows that the intake of Pb by Bangladeshis is 9-fold higher compared to the intake through UK foods (see table 7-6).

Bangladeshis have one of the highest intake of As, Cd and Pb from food consumption. This may have serious health implications for Bangladeshis. Toxicity associated with high intake of arsenic can include different types of cancer including cancer of the lungs, skin etc (Tanabe *et al.*, 2001; Hafeman *et al.*, 2006). Exposure to cadmium is linked with kidney disease, and lung cancer (Waalkes, 2000). Lead exposure was linked with different diseases such as neurotoxicity and cancers (Verstraeten *et al.*, 2008). Nearly, twenty million people suffer from chronic kidney disease in Bangladesh (UNB, 2011) which may be linked to exposure to high levels of heavy metals amongst other exposure to toxic substances.

7.4.1.2 TDI of Mn, Se and Zn

Manganese content in water in Bangladesh is 0.800 mg Mn/L (mean value) (Frisbie *et al.*, 2002), which is higher than the recommended WHO level. The recommended dietary allowance (RDA) for manganese is 2.3 and 1.8 mg per day for adult males and females, respectively (RDA, 2011). However, the oral exposure recommendations and guidelines include USEPA's oral reference concentration of 0.14 mg/kg.b.w.day (USEPA, 2007). The maximum daily intake for Mn is 0.20 mg/kg.b.w.day (FSA, 2009).

The results in this thesis shows that the average intake of Mn for Bangladeshi population is 18.3 and 18.7 mg/day from foods when betel quids (6 quids) or water (2.7 litres), respectively (Table 7-4), are included in the daily intake estimation. When both betel quid chewing and drinking water are included in the calculation, the total daily intake of Mn is 20.3 mg/day for all foods. These results are not too different from the daily Mn intake of 16.51 mg/day calculated by Zablotska *et al.* (2008), despite the fact they did not include water and betel quid intake in their calculation. If 2.7 litres of water and 3 cups of tea per day are included in their calculation then the total intake of Mn for chewers (six betel quids) will be 20.3 mg/day and 18.6 mg/day for non-chewers. It is interesting that the intake calculated in the current study (16.57 mg/day) and Zablotska *et al.* (2008) study (16.51 mg/day) are essentially identical when water and betel quids are excluded. From an international perspective, the total Mn intake by Bangladeshi population is by far the highest compared to non-occupationally exposed groups in any other country for which data has been published so far (Table 7-3). It is noteworthy that the high Mn intake countries are from South Asia, namely Pakistan (Iyengar *et al.*, 2002) with reported dietary intake of Mn of 10.54 mg/day, followed by regions of India with values ranging from 2.21 to 8.72 mg/day (Tripathi *et al.*, 1997; Roychowdhury *et al.*, 2003).

As already mentioned, selenium is an essential element for humans. The total daily Se intake for Bangladeshis estimated in this thesis was 90.4 µg/day. Many studied have reported TDI of Se in foods around the world (Mahalingam *et al.*, 1997; Miyazaki *et al.*, 2004; Moser *et al.*, 1988; FSA, 2009; Egan *et al.*, 2002) (see Table 7-2). TDIs of Se in the UK and India were estimated to be 48-58 and 27-48 µg/day, respectively (Mahalingam *et al.*, 1997; FSA, 2009). These values are half of the Bangladeshi TDI estimated in this thesis. In other studies from Japan and USA, the TDI of selenium were estimated to be 118 and 110- 126 µg/day, respectively for the two countries (Miyazaki *et al.*, 2004; Egan *et al.*, 2002). The TDI of Japanese and American populations were similar to that estimated for Bangladeshis in this thesis. This finding suggests that Bangladeshis do not appear to have a diet that is deficient in selenium. This is in contrast to what has been reported in the literature that there is Se deficiency in Bangladesh (Spallholz *et al.*, 2004). The latter authors suggested that due to Bangladeshi soils containing low levels of Se, Bangladeshi foods are likely to have low levels of this element (Spallholz *et al.*, 2008). However, in another study that measured blood Se levels in Bangladeshis (Chen *et al.*, 2007); the mean of Se level was found to be 150.2 µg/L. This level is rather high and is comparable to levels found in populations that are known to have sufficient selenium in their diet such as the USA (Bleys *et al.*, 2007). Also there is no evidence in the literatures for any disease amongst Bangladeshis related to Se deficiency such as Keshan disease. Selenium can counteract the toxicity of arsenic and some groups are doing clinical trials on arsenic exposed Bangladeshi population by giving them selenium supplements (Spallholz *et al.*, 2004). The impact of such supplementation needs to be carefully followed, including selenium speciation in blood, since the data presented in this thesis suggests that the Bangladeshi diet has sufficient levels of selenium. However, it is important to note that the selenium intake from animal products, other than fish, is very low for Bangladeshis. In this context, the Bangladeshi population could be limited in certain types of selenium species such as selenomethionine that are rich in animal products (Schrauzer, 2000).

As already pointed out zinc is an essential element for human body and it plays vital roles in many important biological processes (Harper *et al.*, 1990). For Bangladeshis, the total daily Zn intake in this thesis was estimated to be 11.2 mg/day. Many studies have reported TDI of Zn in foods around the world (Tupwongse *et al.*, 2007; FSA, 2009; Egan *et al.*, 2002). In a study from Thailand, the TDI of Zn was estimated to be 8-11 mg/day. This value is similar to the TDI of Zn for Bangladeshis estimated in this thesis. This may not be too surprising since the two countries are geographically close to each other and the Thai population also have a similar type of diet to the Bangladeshis although there are important differences. In the UK and USA, TDI of zinc were 8.83 and 12.7 mg day^{-1} (FSA, 2009; Egan *et al.*, 2002). The TDI of Zn for Bangladeshis in the current study was intermediate between that of UK and USA. This suggests that the Bangladeshis consume adequate quantities of Zn per day and therefore Zn deficiency from the diet in Bangladesh is unlikely. However, very recent studies have reported Zn deficiency amongst Bangladeshis and recommended rice biofortfied with zinc as a solution (Mayer *et al.*, 2011; Lindstrom *et al.* 2011). The apparent contradiction between the high TDI (the current study) and low plasma zinc levels (Lindstrom *et al.*, 2011) could be due to the low bioavailability of zinc from rice or the high level of phytic acid in the leafy vegetables that reduces the bioavailibity of zinc. Zinc content in Bangladeshi rice was measured in the current study, and the mean ± SD was 11 ± 3 mg/kg. This is similar to another study which found 12.6 and 13.2 mg/kg zinc in aman and bror rice, respectively (Mayer *et al.*, 2011). Also Mayer *et al.* reported that TDI of Zn for children in Bangladesh ranged from 6.17-7.53 mg/day. In the UK study, TDI of Zn for children was estimated to be 8-9 mg/day (FSA, 2009). This indicates the TDI of Zn for Bangladeshi children are similar to that for UK children. It can be concluded that there is sufficient levels of zinc in the Bangladeshi diet. However, since the Zn is mainly derived from rice in Bangladeshis, it is possible that this along with the high intake of leafy vegetables creates a condition where zinc bioavailibity is reduced. As already mentioned, this could due the presence of plant chemicals such as oxalate and phytic acid which can reduce the bioavailability of Zn in the body (Lonnerdal *et al.*, 2011). For these reasons Bangladeshis may need to alter their diet, such as reducing their intake of certain leafy vegetables (for example, lal shak) and rice and simultaneously increase their intake of zinc from animal products.

7.4.1.3 Daily intake of trace elements from non-aromatic and aromatic rice

An important finding of the thesis reported in chapter 3 is the significantly low level of arsenic in aromatic rice compared to non-aromatic rice. As shown in this chapter, if the

Bangladeshi people consume 0.5 kg of aromatic rice instead of non-aromatic rice, their exposure to arsenic from foods can be reduced by 40%. Furthermore, their intake of selenium and zinc will be increased by 46 and 23% (see Figure 7-7), respectively. Thus aromatic rice may be beneficial for not only reducing the total arsenic intake but the increased selenium intake could be beneficial in terms of countering arsenic toxicity associated with arsenic exposure from drinking water.

7.4.2 Estimation of risk assessment of trace elements

7.4.2.1 Ratio of toxic and essential elements

Ratio of toxic and essential elements were calculated to assess the risk of toxic elements in Bangladeshi foods including rice and vegetables, which are the main classes of foods consumed in Bangladesh. Figures 7-8, 7-9, 7-10 present the As/Se & As/Zn, Cd/Se & Cd/Zn and Pb/Se & Pb/Zn ratios for all types of foods before and after adjustment by the actual quantities (g wet weight per day) of foods consumed. It is very important to know the concentrations of both the toxic and essential elements in the same food for which the toxic:essential element is being calculated. After this, the ratio can provide a meaningful approach for identifying which foods contain low levels of a toxic element and high levels of essential elements. The multiplication of the ratio with quantity of a specific food consumed gives a better reflection of the level of exposure since the concentration of arsenic can vary between samples and also the quantity of food consumed. Thus for example, for a type of rice containing 100 µg/kg of arsenic, 100 µg/kg of selenium and 10000 µg/kg of zinc will give a weight-adjusted As/Se and As/Zn ratios (FTS value) of 2.31 and 3.92 for someone consuming 100 g and 500 g of rice, respectively. If the ratios were not multiplied by the toxic element concentration and the quantity of rice consumed, the value will be the same for both (i.e. 1.01), in which case it will not be possible to say which person will have a higher load of exposure to a toxic element. The multiplication with quantity consumed reveals that consumption of 500 g of rice will result in a higher FTS value since the total intake of arsenic (and selenium) will be higher than consumption of 100 g of rice. Although higher levels of selenium can potentially counteract the toxicity associated with high levels of arsenic, it is safer to have lower levels of exposure to arsenic. It is therefore, preferable to have foods that have low arsenic (10 µg/kg) and low selenium (10 µg/kg) than having a food that has very high levels of arsenic (100 µg/kg) and selenium (100 µg/kg) although in both cases the ratio will be identical. Therefore, it is important to know the original concentration of arsenic (and selenium) in the rice for a reliable assessment of the risk. The FTS of latter cases will be 1.61 and 3.91 for 10 and 100 µg/kg of both arsenic and selenium, respectively.

Aromatic rice has low FTS values compared to non-aromatic rice (see Figure 7-14). Leafy vegetables are widely consumed in large quantities amongst the Bangladeshi population and the FTS scale for this class of food is significantly high. Vegetables with low FTS for As/(Se&Zn) are tomato and gourd vegetables. The foods with the lowest FTS for As/(Se&Zn) are milk, lentil and okra. Thus, it can be recommended that Bangladeshis can reduce their intake of arsenic and at the same time increase their intake of selenium by reducing their consumption of rice and leafy vegetables and increasing their consumption of lentil and animal products such as fish.

This FTS was also used for estimating the toxic elements to zinc ratio in Bangladeshi foods (Figure 7-8, 7-10, 7-12). This revealed that like with FTS for As:Se, rice and leafy vegetables showed the highest FTS. High FTS for As/Zn and Cd/Zn were seen for big fish. In contrast low FTS were seen for gourds, lentils, okra, tomato and small fish. These results suggest that big fish have higher levels of toxic elements (Cd and As) compared to small fish. This could be due to the fact the main big fish analysed in thesis were farmed freshwater fish (apart from Hilsha which is caught in the river). It is possible that these farmed fish are fed contaminated fish food. This issue needs to be investigated in the future as it is known that lead and chromium rich tannery waste are burnt to produce fish feed (The independent, 2011). A whole range of toxic chemicals including chromium is used for processing the leather and it is possible that other heavy metals are also used leading to the entry of toxic chemicals into the food chain. The Bangladeshi diet depends heavily on small fish and it is good to see that the FTS values for this category of fish is low indicating that they have lower levels of toxic elements. Generally, small fish consumed in Bangladesh are from rivers and lakes and are not likely to be fed commercially produced fish feed. This may explain why they have lower levels of toxic elements.

The FTS devised in this thesis provides a simple scale that can be used for risk assessment of toxic elements in foods and can help identify which foods to avoid or include in the diet. For the Bangladeshi diet, rice and leafy vegetables, both of which are consumed in large quantities, are the main sources of toxic elements and have the highest FTS values.

Rice is certainly a nutritious food and contains significant levels of essential elements. However, the large quantity of rice consumed by the Bangladeshi population makes them vulnerable to higher exposure to toxic elements. On the basis of the findings of the FTS analysis, it can be recommended that the Bangladeshi population reduce their intake of rice and leafy vegetables and simultaneously increase their consumption of gourds, lentils, non-leafy vegetables, and small fish (and other animal products). Such a change in the dietary pattern can be healthier for the Bangladeshi population who are already exposed to high levels of toxic elements from drinking water.

7.4.2.2 Estimation of PMTDIs and THQs

The PMTDI and THQ are the other risk assessment scales that are usually used for estimating risk from toxic elements in drinking water and food consumption. For non-occupationally exposed populations, the source of exposure to trace elements is mainly through the dietary route. Through performance of risk assessment analysis, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established the Provisional Tolerable Weekly Intake (PMTDI) of 2.1 (inorganic arsenic), 1 and 3.6 µg/kg body weight for As, Cd and Pb, respectively (FSA, 2009). In this thesis, the PMTDI was used for risk assessment of Bangladeshi foods.

Contaminated groundwater is the main source of arsenic in Bangladesh. The mean concentration of arsenic in water in four districts in Bangladesh was measured by Frisbie *et al.* (2002) and an average content of up to 84 µg/L of arsenic was found. Assuming 2.7 litres of water is consumed per day in Bangladesh, the daily intake of As in Bangladesh from drinking water is 226.8 micrograms (for water As level of 84 µg/L). This value is equivalent to more than 159.6% of the PMTDI per day (assuming Bangladeshi body weight is 60 kg). Rice is the second main of source of arsenic exposure for Bangladeshis. The %PMTDI was estimated for rice to be 27.2%. This result is in agreement with the FTS calculated in section (7.4.2.1.) since both estimations show which foods have the highest values (toxicity/risk) for a given quantity of food intake (see Figure 7-16).

The high Cd levels in Bangladeshi foods are consistent with the high Cd concentrations that were detected in breast milk of Bangladeshi women (median 0.14 µg/L). This level of Cd in breast milk is higher compared to other countries around the world (below 0.1 µg/L) (Kippler *et al.*, 2009). This finding was used to explain the high Cd exposure among children in rural Bangladesh (Kippler *et al.*, 2010). In light of the data presented in this thesis, it is recommended that the Bangladeshis modify their diet to make it more balanced. As already pointed out, this could be done through reducing their consumption of rice and leafy vegetables and simultaneously increasing their consumption of animal products.

Results of the current study (Table 7-9), showed that Mn content in betel quids was high and 18.1% of the PMTDI for Mn can be derived from chewing six betel quids. The highest manganese intake is from rice and other foods including tea fusions. Manganese content was 10.9 mg/kg in Bangladeshi rice (in this thesis). The daily consumption of six ordinary betel quids and two meals of rice and other food can provide more than 145% of the PMTDI for Mn (Table 7-9). This level of exposure is excessive considering that this value was obtained by excluding manganese in water. Manganese consumption through drinking can account for 22.7% of the PMTDI (Table 7-9).

The percentage of PMTDI of Pb from leafy vegetables and rice (16.9 and 7.1, respectively) show that these foods are the main source of Pb. This is in good agreement with the toxic:essential trace element ratio scale that was used in this study. However, other vegetables showed lower PMTDI% of Pb (such as gourd and lentil). For people who chew betel quids and eat Sikor, the exposure to Pb is very high. Eating 50 g of Sikor results in exceeding %PMTDI for lead by 5-fold. Chewing six betel quids can account for 8.5% of the PMTDI (Table 7-9).

Results presented in this thesis show that rice can be an important source of Se and Zn. It provides 9.1% (Se) and 30.6 (Zn) of the PMTDI %, (table 7-9). In the Bangladeshi diet, fish is the main source of Se (11.7% of the PMTDI). In contrast, non-leafy vegetables (such as gourds and lentils) are important sources of Zn, providing 12% of the PMTDI. These observations are in good agreement with the toxic elements:essential elements ratio scale established in this study (section 7.4.2.1. and Figure 7-16).

THQ is the other scale that is used to assess risks associated with trace element consumption from foods and water. Here in this study, THQs of arsenic was estimated to be 0.01, 0.41 and 1.52 for chewing six betel quid, food intake and water consumption, respectively. Also the THQ for all the foods combined was 1.94 which is quite high since THQ values of greater than one are considered to be of concern (Table 7-10). Rice and leafy vegetables again showed the highest THQs of all foods. THQs were 0.28 and 0.05 for rice and leafy vegetables, respectively (Table 7-10). THQs of Pb in rice and leafy vegetables were 0.04 and 0.05, respectively. Highest THQs were associated with eating Sikor. The THQs were 2.83 and 5.06 of arsenic and lead, respectively, for someone eating 50 g of Sikor. As discussed already in chapter (6), this is of concern for Bangladeshi women who eat Sikor.

THQ of manganese in individual betel quid and for all dietary intake in Bangladesh were estimated, and the results show that the THQ of manganese in betel quid was 0.18 (for 6 quid per day), this is a significant value compared with THQ for water (0.23) and other food (1.74) (Table 7-10). However, the THQ for all dietary components in Bangladesh was 2.15. The THQs of manganese were estimated for the Bangladeshi population to be 0.18, 0.55 and 0.21 for betel quids, rice and water, respectively. Although foods shows the highest THQ, the nature of the manganese species present in foods and its bioaccessibility and bioavailability may differ to that derived from betel quids and water. Surprisingly, the THQ of manganese resulting from water and betel quids are very similar, but again the bioaccessibility and bioavaliability could be very different. The issue of manganese species, manganese bioavailability and bioaccssibility needs to be investigated in the future. Nevertheless, the total THQ of manganese from Bangladeshi diet (including food, water and betel quids) is very high (1.94) as the THQ values greater than one are considered to be of concern (FSA, 2000). As already pointed out, the daily intake of manganese by Bangladeshis is the highest compared to other communities thus far reported and the health impact of this requires further investigation. Several studies have attributed betel quid consumption with the development of different diseases including oral cancer, diabetes, cardiovascular disease etc (Vanwyk *et al.*, 1993; IARC, 2004). In the UK, Asians have the highest incidence of head and neck cancer which has been attributed to smoking and betel quid chewing (Smith *et al.*, 2003). As pointed out earlier, chewing betel quids was associated with a higher risk of skin lesions (McCarty *et al.*, 2006) and tremor (Hafeman *et al.*, 2006) in populations exposed to high levels of arsenic in their drinking water. The suggestion of this thesis is that the inorganic (such as arsenic and manganese) and organic (arecoline) components of betel quids, are jointly responsible for the adverse health outcomes in betel quid chewers who drink contaminated groundwater. This needs to be further investigated in the future.

Fish also showed high level of Se and is a significant source of this element in the Bangladeshi diet. The THQ of Se in fish was 0.28, rice had the second highest THQ value for Se (0.08) (Table 7-10). The THQ of Zn from rice consumption (0.28) was the highest compared to all the other foods. The THQ of leafy vegetables was lower (0.03). These findings are in good agreement with the FTS values calculated in this study (see Figure 7-16).

This is the first comprehensive study for Bangladeshi foods and non-foods to estimate risk assessment of six trace elements (As, Cd, Pb, Mn, Se and Zn), and different scales were used for this purpose. In conclusion, the work presented in this chapter not only determined the TDI for six different elements but it also developed a new Food Toxicity Scale (FTS) which can be helpful in identifying risk of individual foods in terms of the levels of a toxic element versus an essential element. The advantage of the FTS scale, compared to the PMTDI and THQ, is that it provides a relationship between two elements (toxic versus essential) rather than the toxicity effects of a single element. A combination of the TDI, PMTDI, THQ and FTS analysis has provided valuable information on the intake of different elements, from a diverse range of foods, and their benefits and risks. Overall, the Bangladeshi population clearly needs to modify their diet to make it more balanced, especially by reducing their reliance on rice and leafy vegetables.

On the basis of the findings of this study the following recommendations are suggested:

(i) reduction in the consumption of rice (by about 50% or more). This will help reduce the intake of not only arsenic but also cadmium, manganese and lead.

(ii) the TDI for arsenic can be reduced from 39.5 µg/day to 23 µg/day by switching from the consumption of non-aromatic rice to aromatic rice.

(iii) since aromatic rice has lower levels of arsenic than non-aromatic rice, the cultivation of aromatic rice should be further increased across the country.

(iv) the Bangladeshis could also further increase the cultivation of rice in areas where rice arsenic levels are low. This study has shown that rice from Sylhet region has much lower arsenic levels and consumption of Sylheti rice (even non-aromatic ones) could reduce the TDI of arsenic by 2-4 fold compared to consumption of rice from arsenic-affected regions.

(v) switching from non-aromatic rice to aromatic rice will increase the TDI of selenium and zinc by 46 and 23%, respectively. These elements can help counter the toxic effects of arsenic

(vi) the consumption of animal products by Bangladeshis is very low. Increasing the consumption of animal products (this could include small fish, meat or poultry) will help increase the intake of essential elements such as selenium and zinc that are present in higher levels in animal products.

(vii) reduce the intake of certain leafy vegetables (especially lal shak, pui shak etc) that are very high in toxic elements (Cd and Pb).

(viii) increase the intake of vegetables with very low FTS values which includes lentils, gourds, tomato, peeled potatoes.

(ix) completely avoid or at least significantly reduce the chewing of betel quids.

(x) the consumption of Sikor by some Bangladeshi women should be avoided. Sikor can be a high source of exposure to As and Pb.

(xi) address issues of entry of toxic elements in certain foods such as big fish, puffed rice, leafy vegetables etc.

(xii) besides the above recommendations, steps should be taken to reduce the levels of toxic elements in the Bangladeshi environment, the food chain and groundwater.

(xiii) establish stronger food safety regulations to prevent the entry of toxic elements through certain practices such as overuse of fertilisers, whitening of puffed rice using urea or making fish feed from toxic tannery wastes and the unregulated use of fertilisers and pesticides.

8 OVERALL CONCLUSION

Bangladeshis are exposed to high concentrations of arsenic from drinking water and eating food. In Bangladesh, many patients with melanosis (blackening of skin), leuco-melanosis, keratosis (hardening of palms and soles), hyperkeratosis, gangrene and skin cancers have been identified (Tanabe *et al.*, 2001; Hafeman *et al.*, 2006). Bangladesh has one of the highest incidences of diabetes around the world. A study has estimated that 2.3 million of Bangladeshis suffer from diabetes (for 2000), however this is likely to reach 11.1 million for 2030 (Wild *et al.*, 2004). Furthermore, it has been reported that exposure to even low levels of arsenic may result in the development of diabetes (Navas-Acien *et al*., 2006) although another study (focusing on an area in Bangladesh) has reported that there is no association between arsenic exposure and diabetes (Chen *et al.*, 2010). The Bangladeshi population in Bangladesh and those residing in the UK have some similarities in their diet, since many Bangladeshi foods including vegetables and fish are imported into the UK and are widely consumed by the UK Bangladeshis (Kassam-Khamis *et al*., 2000; Al-Rmalli *et al*., 2005; Cascio *et al*., 2011). UK Bangladeshis were shown to have a high prevalence of diabetic disease compared with other UK communities (NHS, 2006). Furthermore, a recent study has reported that urinary inorganic arsenic and DMA levels are higher in UK Bangladeshis compared to the general UK population (Cascio *et al*., 2010). The data presented in this thesis reveals that Bangladeshis may be exposed to high levels of arsenic through their diet including rice, vegetables and non-food (such as baked clay, betel quids etc.). Also fish is an integral part of Bangladeshi diet, in this thesis, different types of

Bangladeshi fish including Hilsha were analysed. Hilsha fish was found to contain high levels of arsenic and DMA (70%) was found to be the main species of arsenic. This species could have toxic properties (Craig, 2003).

The total daily intake (TDI) of arsenic for the Bangladeshi population was estimated in the current study to be 306 µg/day (including drinking water in Bangladesh). This is one of the highest daily intakes around the world. Rice and water are the main source of arsenic for Bangladeshis in Bangladesh. High percentage of inorganic arsenic (approx. 70%) was found (in the current study) in Sylhet rice which is compatible to previous studies. This thesis also reveals that aromatic rice contains low arsenic levels compared to non-aromatic rice. Betel quids and baked clay were analysed (in this thesis) and they contained high levels of arsenic. This issue has not been previously considered. Baked clay can be an additional source of arsenic exposure in women which can be particularly serious, especially for women who are already exposed to high levels of arsenic through drinking water and food consumption. The PMTDI of arsenic, can be exceeded by 2-fold by just ingesting 50 g of baked clay per day. This increased exposure may explain higher prevalence of arsenic-induced skin lesions in some Bangladeshi women and their unborn baby.

Exposure to elevated levels of Cd and Pb have been linked with various diseases including kidney disease (Waalkes, 2000; IARC, 1987), lung cancer (De Palma *et al.*, 2008), immunotoxicity (Mishre, 2009) and neurotoxicity (Verstraeten *et al.*, 2008).

Recently, it has been reported that over 20 million people in Bangladesh suffer from chronic kidney disease (UNB, 2011). The rate of kidney disease in UK Bangladeshis was reported to be three to five-folds higher than that of Caucasians (De Brito-Ashurst *et al*., 2011 and other references cited therein). Cadmium and Lead are present in the majority of foods especially vegetables. This thesis reveals that Bangladeshis are also exposed to high levels of Cd and Pb. From the data in the current study, both leafy vegetables and rice were the main sources of Cd and Pb exposure. Interestingly, puffed rice was found to contain the highest levels of Cd amongst foods (303 µg/kg) analysed and the reason for this could be the use of urea in the manufacturing process. TDI of Cd and Pb were estimated (in this study) to be 34.5 and 74.4 µg/day, respectively. Baked clay can contribute the intake of high levels of these elements for some Bangladeshi women. The PMTDI of Pb, can be exceeded by 5-fold by just ingesting 50 g of baked clay per day. A multipronged strategy is required to reduce exposure toxic elements such as Cd and Pb in Bangladeshis.

Bangladeshi water contains high levels of Mn, which is also a matter of concern for Bangladeshis. The consumption of food and non-foods, including betel quids that are high in Mn can be a major source of Mn intake for Bangladeshis. Chewing betel quids has been linked with many diseases including oral cancer (Nandakumar *et al.,* 1990; Sankaranarayanan *et al*., 1998) and oral submucous fibrosis (Auluck *et al.*, 2008) and possibly tremor (Hafeman *et al.* 2006). TDI of Mn was estimated (20.3 mg/day) in this study to be the highest in the world. The additive or multiplicative adverse effects, such as generation of ROS, may result from the simultaneous exposure to As, Cd and Mn, making chewers more vulnerable to ill health. It is possible that As, Cd, Mn and Pb in synergy with organic substances in betel quids may be responsible. Betel quid chewing is most prevalent amongst women who may be unknowingly harming their health and that of their unborn babies (for those who are pregnant) through increased exposure to As, Cd, Mn and Pb.

Finally, the total daily intakes and risk assessment of six trace elements (As, Cd, Mn, Pb, Se and Zn) were estimated, and different scales for this purpose were used such as PMTDI and THQ. A new Food Toxicity Scale (FTS) was developed, which may help towards further improving food risk assessment. This scale can be used to identify foods that are harmful or beneficial so that the Bangladeshis can make appropriate modifications to their diet to reduce their exposure to toxic elements.

Some changes in agricultural practice in Bangladesh is also required to decrease the levels of toxic elements, and increase the levels of essential elements, in common Bangladeshi foods such as rice and vegetables. This is necessary to reduce entry of As, Cd, Pb and other toxic elements in the food chain. Furthermore, appropriate modification of the Bangladeshi diet can be made to reduce the intake of foods that are very high in As, Cd and Pb including rice and leafy vegetables. The study found that increasing the consumption of aromatic rice and reducing the intake of non-aromatic rice could be another solution towards reducing the intake of arsenic and increasing the intake of essential elements such selenium and zinc.

9 REFERENCES

ABRAHAMS, P.W., FOLLANSBEE, M.H., HUNT, A., SMITH, B. AND WRAGG, J. 2006. Iron nutrition and possible lead toxicity: An appraisal of geophagy undertaken by pregnant women of UK Asian communities. Applied Geochemistry, 21, 98-108.

AFRIDI, H.I., KAZI, T.G., KAZI, N.G., JAMALI, M.K., ARAIN, M.B., SIRAJUDDIN, KANDHRO, G.A., SHAH, A.Q., BAIG, J.A. 2009. Evaluation of arsenic, cobalt, copper and manganese in biological Samples of Steel mill workers by electrothermal atomic absorption Spectrometry. Toxicology and Industrial Health, 25, 59-69.

AHMED, S., AKTER, M., MAHZABEEN, R., SAYEED, S., MOMTAZ, H., SAYEED, M.A. & IM-4 MBBS STUDENTS (BATCH B). 2008. Prevalence of tobacco consumption in a rural community of Bangladesh. Ibrahim Medical College Journal, 2, 58-60.

AHMED, Z.U., PANAULLAH, G.M., GAUCH JR, H., MCCOUCH, S.R., TYAGI, W., KABIR, M. S., DUXBURY, J. M. 2011.Genotype and environment effects on rice (Oryza sativa L.) grain arsenic concentration in Bangladesh. Plant Soil, 338, 367–382.

AKESSON, A., JULIN, B. AND WOLK, A. 2008. Long-term dietary cadmium intake and postmenopausal endometrial cancer incidence: A population-based prospective cohort study. Cancer Research, 68, 6435-6441.

AKTER, A. & ALI, M. H. 2011. Arsenic contamination in groundwater and its proposed remedial measures. International Journal of Environmental Science and Technology, 8, 433-443.

ALAM, M.G.M., SNOW, E. T. AND TANAKA, A. 2003. Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. Science of The Total Environment, 308, 83-96.

ALAM, M.G.M., TOKUNAGA, S. AND STAGNITTI, F. 2007. Removal of arsenic from contaminated soils using different salt extractants. Journal Environmental Science and Health Part A-Toxic/Hazardous Substances and Environmental Engineering, 42, 447-451.

ALI, M. A., BADRUZZAMAN, A.B.M., JALIL, M.A., HOSSAIN, M. D., AHMED, M. F., AL MASUD, A., KAMRUZZAMAN, M. D. AND RAHMAN, M. A. 2003. Fate of arsenic extracted with groundwater, in M.F. Ahmed, M.A. Ali and Z. Adeel, (Ed.). Fate of arsenic in the environment, Bangladesh University of Engineering and Technology and United nations University, pp.7-20.

ALI, M., TARAFDAR, S. A. 2003. Arsenic in drinking water and in scalp hair by EDXRF: A major recent health hazard in Bangladesh, Journal of Radioanalytical and Nuclear Chemistry, 256, 297-305.

ALI, S.F., DUHART, H.M., NEWPORT, G.D., LIPE, G.W. AND SLIKKER, W. 1995. Manganese-Induced Reactive Oxygen Species - Comparison between Mn+2 and Mn+3. Neurodegeneration, 4, 329-334.

ALLEGRINI, M., LANZOLA, E., GALLORINI, M.1985. Dietary selenium intake in a coronary heart disease study in northern Italy. Nutrition Research, 5, S-398-S-402.

ALLOWAY, B. J., JACKSON, A. P. AND MORGAN, H. 1990. The accumulation of cadmium by vegetables grown on soils contaminated from a variety of sources. Science of the Total Environment, 91, 223-236.

AL-RMALLI, S. W., HARIS, P. I., HARRINGTON, C. F. & AYUB, M. 2005. A survey of arsenic in foodstuffs on sale in the United Kingdom and imported from Bangladesh. Science of the Total Environment, 337, 23-30.

AL-RMALLI, S.W., JENKINS, R.O., HARIS, P.I. 2011. Betel Quid Chewing Elevates Human Exposure to Arsenic, Cadmium and Lead. Journal of Hazardous Materials 190, 69-74.

AL-RMALLI, S.W., JENKINS, R.O., WATTS, M.J., HARIS, P.I. 2010. Risk of human exposure to arsenic and other toxic elements from geophagy: trace element analysis of baked clay using inductively coupled plasma mass spectrometry. Environmental Health, 9, 79-86.

ANTTOLAINEN M, VALSTA LM, ALFTHAN G, KEEMOLA P. 1996. Effect of extremefish consumption on dietary and plasma antioxidant levelsand fatty acid composition. European Journal of Clinical Nutrition, 5, 741–6.

APOSHIAN, H. V., ZAKHARYAN, R. A., AVRAM, M. D., SAMPAYO-REYES, A., WOLLENBERG, M. L. 2004. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxication of the trivalent arsenic species. Toxicology and Applied Pharmacology, 198, 327– 335

ARAIN, M.B., KAZI, T.G., BAIG, J.A., JAMALI, M.K., AFRIDI, H.I., SHAH, A.Q., JALBANI, N., SARFRAZ, R. A. 2009. Determination of arsenic levels in lake water, sediment, and foodstuff from selected area of Sindh, Pakistan: Estimation of daily dietary intake. Food and Chemical Toxicology, 47, 242–248.

ARAO, T., KAWASAKI, A., BABA, K. & MATSUMOTO, S. 2011. Effects of Arsenic Compound Amendment on Arsenic Speciation in Rice Grain. Environmental Science and Technology, 45, 1291-1297.

ARO A, ALFTHAN G, VARO P. 1995. Effects of supplementation of fertilizers on human selenium status in Finland. Analyst, 120, 841–843.

ARSENAULT, J. E., YAKES, E. A., HOSSAIN, M. B., M. ISLAM, M., AHMED, T., HOTZ, C., LEWIS, B., RAHMAN, A. S., JAMIL,K. M., BROWN, K. H. 2010. The Current High Prevalence of Dietary Zinc Inadequacy among Children andWomen in Rural Bangladesh Could Be Substantially Ameliorated by Zinc Biofortification of Rice. Journal of Nutrition, 140, 1683–1690.

ASHOWORTH, W. 1991. The Encyclopedia of Environmental Studies. Fate on file. New York.

AULUCK, A., ROSIN, M. P., ZHANG, L. W., KN, S. 2008. Oral Submucous Fibrosis, a Clinically Benign but Potentially Malignant Disease: Report of 3 Cases and Review of the Literature. Journal of Canadian Dental Association, 74, 735-740.

AUNG, N.N., YOSHINAGA, J., TAKAHASHI, J.I. 2006. Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan. Food Additives and Contaminants, 23, 883-894.

BANGLADESHI SOIL EATING, 2008. Doctors worried over Bangladeshi soil eating. Link:http://in.reuters.com/article/2008/03/31/us-bangladesh-soil idINDHA16397020080331 (Accessed 26 September, 2011)

BARCLAY MNI, MACPHERSON A, DIXON J. 1995. Selenium content of a range of UK foods. Journal of Food Composition Analysis, 8, 307–18.

BARKAT A, FARIDI R, WADOOD SN, SENGUPTA SK, HOQUES NM E. 2010. A Quantitative Analysis of Fertilizer Demand and Subsidy Policy in Bangladesh. Final Report PR #9/08.http://www.nfpcsp.org/agridrupal/sites/default/files/Barkat.pdf (Accessed 26 September, 2011).

BASAK, J.K. 2011. Fertilizer Requirement for Boro Rice Production in Bangladesh. http://www.unnayan.org/reports/food/Fertilizer_Requirement_for_Boro_Rice_Production_in_Bangladesh.pdf [Accessed 13 October, 2011]

BATISTA, B.L., SOUZA, VCD., DA SILVA, FG., BARBOSA, F. 2010. Survey of 13 trace elements of toxic and nutritional significance in rice from Brazil and exposure assessment. FOOD Additives and Contaminants Part B-Surveillance, 3, 253-262.

BEATY, R. D. AND KERBER, J D. 1993. Concepts, Instrumental and Techniques in Atomic Absorption Spetrophotometry. Perkin Elmer Corporation, Norwalk, CT.

BEDI, R. 1996. Betel-quid and tobacco chewing among the United Kingdom's Bangladeshi community. The British Journal of Cancer. Supplement, 29, S73-77.

BERNARD, A. 2008. Cadmium & its adverse effects on human health. Indian Journal of Medical Research, 128, 557-564.

BEYERSMANN, D. & HARTWIG, A. 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Archives of Toxicology, 82, 493-512.

BGS. 2001. British Geological Survey, Keyworth, UK. http://www.bgs.ac.uk/arsenic/bphase2/maps/nhs/as2.gif [Accessed 26 September, 2011].

BHUMBLA, N.K. AND KEEFER, R.F. Arsenic mobilization and bioavailability in soils, in: J.O. Nriagu, (1994). Arsenic in the Environment, Part I, Cycling and Characterization, Wiley, New York, pp. 51.

BIEGO, G.H., JOYEUX, M., HARTEMANN, P., DEBRY, G. 1998. Daily intake of essential minerals and metallic micropollutants from foods in France. Science of The Total Environment, 217, 27 –36.

BLEYS, J., NAVAS-ACIEN, A., GUALLAR, E. 2007. Serum selenium and diabetes in U.S. adults. Diabetes Care, 30, 829-34.

BOHARI, Y., LOBOS, G., PINOCHET, H., PANNIER, F., ASTRUC, A., POTIN-GAUTIER, M. 2002. Speciation of arsenic in plants by HPLC-HG-AFS: extraction optimisation on CRM materials and application to cultivated samples. Journal of Environmental Monitoring, 4, 596-602.

BORAK, J., HOSGOOD, H.D. 2007. Seafood arsenic: implications for human risk assessment. Regulatory Toxicology and Pharmacology, 47, 204–12.

BOWEN, H.J.M. 1979. Elemental Chemistry of the Elements, Academic Press, London and New York, pp. 60-61.

BRO S, SANDSTROM B, HEYDORN K. 1990. Intake of essential and toxic trace elements in a random sample Danish men as determined by the duplicate portion sampling technique. Journal of Trace Element Electrolytes and Health Diseases, 4, 147 –155.

BROWN, M.T. AND FOOS, B. 2009. Assessing Children's Exposures and Risks to Drinking Water Contaminants: A Manganese Case Study. Human and Ecological Risk Assessment. 15,923-947.

BUCHET, J. P., LISON, D., RUGGERI, M., FOA, V. AND ELIA, G. 1996. Assessment of exposure to inorganic arsenic, a human carcinogen, due to the consumption of seafood. Archives of Toxicology, 70, 773-778.

BUCHET, J.P. AND LAUWERYS, R. 1987. Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicology and Applied Pharmacology, 91, 65-74

BUCHET, J.P. LAUWERYS, R. AND ROELS, H. 1981. Urinary excretion of inorganic arsenic and all its metabolites after repeated ingestion of sodium meta arsenite by volunteers. International Archives of Occupational and Environmental Health, 48, 111-118

BUCHET, J.P., LAUWERYS, R., VANDERWOORDE, A., PYCKE, J.M. 1983. Oral daily intake of cadmium, lead, manganese, copper, chromium, mercury, calcium, zinc and arsenic in Belgium: a duplicate meal study. Food and Chemical Toxicology, 21, 19 –24.

BUTCHER, D. V. AND SNEDDEN, J. 1998. A Practical Guide to Graphite Furnace Atomic Absorption Spectrometry.

BUTTON, M. AND WATTS, M.J. 2008. British Geological Survey, IR/08/050.

CAMPBELL, J.A., STARK, J.H. AND CARLTON-SMITH, C.H. 1985. in Proc. Int. Symp. On Heavy Metals in The Environment, Athens, Vol. 1, 478.

CAPDEVILA F, NADAL M, SCHUHMACHER M, DOMINGO JL. 2003. Intake of lead and cadmium from edible vegetables cultivated in Tarragona Province, Spain. Trace Elements and Electrolytes, 20, 256-261.

CARBONELL-BARRACHINA, A.A.; AARABI, M.A.; DELAUNE, R.D.; GAMBRELL, R.P.; PATRICK, W.H. 1998. Arsenic in wetland vegetation: Availability, phytotoxicity, uptake and effects on plant growth and nutrition. Science of the Total Environment, 217, 189-199.

CARBONELL-BARRACHINA, A., JUGSUJINDA, A., DELAUNE, RD., PATRICK, WH., BURLO, F., SIRISUKHODOM, S., ANURAKPONGSATORN, P. 1999. The influence of redox chemistry and pH on chemically active forms of arsenic in sewage sludge-amended soil. Environment International, 25, 613-618.

CASCIO, C., RAAB, A., JENKINS, R. O., FELDMANN, J., MEHARG, A. A., HARIS, P. I. 2011. The impact of a rice based diet on urinary arsenic. Journal of Environmental Monitoring, 13,257-265.

CATHARINA E BRAND, L. D. J. EKOSSE, GEORGES-IVO E. 2009. Medical Technology SA, 23, 11-13.

CHAKRABORTI, D., RAHMAN, M. M., DAS, B., MURRILL, M., DEY, S., MUKHERJEE, S. C., DHAR, R. K., BISWAS, B. K., CHOWDHURY, U. K., ROY, S., SORIF, S., SELIM, M., RAHMAN, M., QUAMRUZZAMAN, Q. 2010. Status of groundwater arsenic contamination in Bangladesh: A 14-year study report. Water Research, 44, 5789-5802.

CHAMBERS, J. C., IRELAND, H., THOMPSON, E., REILLY, P., OBEID, O. A., REFSUM, H., UELAND, P., LANE, D. A., AND. KOONER, J. S. 2000. Methylenetetrahydrofolate Reductase 677 *C→T* Mutation and Coronary Heart Disease Risk in UK Indian Asians. Arteriosclerosis, Thrombosis, and Vascular Biology, 20, 2448-2452.

CHATTERJEE, A., DAS, D., MANDAL, B.K., ROYCHOWDHURY, T., SAMANTA, G. AND CHAKRABRTI, D. 1995. Arsenic in ground water in 6 districts of West Bengal, India – the biggest arsenic calamity in the world .1. arsenic species in drinking water and urine of the affected people. Analyst, 120, 643-650.

CHEMAT, S., LGHA, A., AMAR, H.A., CHEMAT, F. 2004. Ultrasound assisted microwave digestion, Ultrasonic Sonochemistry, 11, 5-8.

CHEN, Y., HALL, M., GRAZIANO, J. H., SLAVKOVICH, V., VAN GEEN, A., PARVEZ, F., AHSAN, H. 2007. A Prospective Study of Blood Selenium Levels and the Risk of Arsenic-Related Premalignant Skin Lesions. Cancer Epidemiology, Biomarkers and Prevention, 16, 207–13.

CHEN, Y., AHSAN, H., SLAVKOVICH, V., LOEFFLER PELTIER, G., GLUSKIN, R. T., PARVEZ, F., LIU, X., GRAZIANO, J. H. 2010. No Association between

Arsenic Exposure from Drinking Water and Diabetes Mellitus: A Cross-Sectional Study in Bangladesh. Environmental Health Perspectives, 118, 1299-1305.

CHIANG, W.F., YANG, H.J. AND LUNG, S.C. 2008. A comparison of elementary schoolchildren's exposure to arsenic and lead. Journal of Environmental Science and Health Part C Environmental Carcinogenesis & Ecotoxicology Reviews. 26, 237-255.

CHOWDHURY, M.A.I., AHMED, M.F. AND ALI, M.A. 2003. In Fate of Arsenic in the Environment.Edited by: Ahmed MF, Ali MA, Adeel Z. Published by ITN Centre, BUET and United Nations University, 21-37.

CLELAND, B., TSUCHIYA, A., KALMAN, D. A., DILLS, R., BURBACHER, T. M., WHITE, J. W., FAUSTMAN, E. M., MARIEN, K. 2009. Arsenic Exposure within the Korean Community (United States) Based on Dietary Behavior and Arsenic Levels in Hair, Urine, Air, and Water. Environmental Health Perspectives, 117, 632-638.

CONCHA, G. NERMELL, B. AND VAHTER, M. 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicological Science, 44, 185-190.

CONCHA, G., VOGLER, G., NERMELL, B., VAHTER, M. 1998. Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. International Archives of Occupational and Environmental Health, 71, 42-46.

CRAIG, P. J. 2003. Organometallic compounds in the environment. Second editied, ISBN 0471899933.

CUI, Y. J., ZHU, Y. G., ZHAI, R. H., CHEN, D. Y., HUANG, Y. Z., QIU, Y., LIANG, J. Z. 2004. Transfer of metals from soil to vegetables in an area near a smelter in Nanning, China. Environment International, 30, 785-791.

DABEKA, R.W., MCKENZIE, A.D., LACROIX, G.M.A., CLEROUX, C., BOWE, S., GRAHAM, R.A., CONAHER, H.B.S., VERDIER, P. 1993. Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. Journal of AOAC International, 76, 14-25.

DAS, H. K., MITRA, A. K., SENGUPTA, P. K., HOSSAIN, A., ISLAM, F., RABBANI, G. H. 2004. Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environment International, 30, 383-387.

DE BRITO-ASHURST, I. L.P., SANDERS, T.A.B., THOMAS, J.E., YAQOOB, M.M., DOBBIE, H. 2009. Dietary salt intake of Bangladeshi patients with kidney disease in East London: An exploratory case study**.** e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism, 4, e35-e40.

DE BRITO-ASHURST, I., PERRY, L., SANDERS, TAB., THOMAS, JE., YAQOOB, MM., DOBBIE, H. 2011. Barriers and facilitators of dietary sodium restriction amongst Bangladeshi chronic kidney disease patients. Journal of Human Nutrition and Dietetics, 24, 86-95.

DE PALMA, G., GOLDONI, M., CATALANI, S., CARBOGNANI, P., POLI, D.,MOZZONI, P., ACAMPA, O., INTERNULLO, E., RUSCA, M., APOSTOLI, P. 2008. Acta Biomedical Journal, 79, 43-51.

DEL RAZOA, L.M., GARCIA-VARGASB, G. G., GARCIA-SALCEDO, J., SANMIGUELB, M. F., RIVERAB, M., HERNANDEZ, M. C. 2002. Arsenic levels in cooked food and assessment of adult dietary intake of arsenic in the Region Lagunera, Mexico Cebriana. Food and Chemical Toxicology, 40, 1423–1431.

DELGADO-ANDRADE, C., NAVARRO, M., LOPEZ, H., LOPEZ, M. C. 2003. Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation of daily dietary intake. Food Additives and Contaminants. 20, 923–932.

DIAZ-ALARCON, J.P., NAVARRO, M., LOPEZ, H., LOPEZ, M.C. 1996. Determination of selenium in meat products by hydride generation atomic absorption spectrometry—Se levels in meat, organ meat, and sausages in Spain. Journal of Agriculture Food Chemistry, 44, 494–7.

DISSANAYAKE CB. AND CHANDRAJITH, R. 2009. Phosphate mineral fertilizers, trace metals and human health. Peradeniya Journal of National Science Foundation Sri Lanka, 37, 153-165.

DUMONT, E., VANHAECKE, F., CORNELIS, R. 2006. Selenium speciation from food source to metabolites: a critical review. Analytical and Bioanalytical Chemistry, 385, 1304–23.

DUXBURY, J. M., MAYER, A. B., LAUREN, J. G., HASSAN, N. 2003. Food chain aspects of arsenic contamination in Bangladesh: Effects on quality and productivity of rice. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 38, 61-69.

EBISUDA, K., KUNITO, T., KUBOTA, R. AND TANABE, S. 2002. Arsenic concentrations and speciation in the tissues of ringed seals (Phoca hispida) from Pangnirtung, Canada. Applied Organometallic Chemistry 16, 451-457.

EFSA, 2009. European Food Safety Authority. Cadmium in food. Scientific opinion ofthe Panel on Contaminants in the Food Chain (Question No EFSA-Q-2007-138). Adopted on 30 January 2009. The European Food Safety Authority Journal, 980, 1–139.

EGAN, S.K, TAO, S.S, PENNINGTON, J.A, BOLGER, P.M. 2002. US Food and Drug Administration's Total Diet Study: intake of nutritional and toxic elements, 1991-96. Food Additives and Contaminants, 19, 103-25.

EGANY, S. K., TAOY, S., PENNINGTON, J. A. T., BOLGERY, M. P. 2002. US Food and Drug Administration’s Total Diet Study: intake of nutritional and toxic elements, 1991-96. Food Additives and Contaminants, 19, 103-125

EKHOLM, P.,REINIVUO, H., MATTILA, P., PAKKALA, H., KOPONEN, J., HAPPONEN AHELLSTROM, J., OVASKAINEN, M.L., 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland, Journal of Food Composition and Analysis 20, 487-495.

EL-GHAWI, U.M., AL-SADEQ, A.A., BEJEY, M.M., ALAMIN, M.B. 2005. Determination of selenium in Libyan food items using pseudocyclic instrumental neutron activation analysis. Biological Trace Element Research, 107, 61–71.

ELLEN G, EGMOND E, VAN LOON JW, SAHERTHAN ET, TOLSMA K. 1990. Dietary intakes of some essential and non-essential trace elements, nitrate, nitrite and N-nitrosamines, by Dutch adults: estimated via a 24-hour duplicate portion study. Food Additives and Contaminants, 7, 207 –221.

ELLWOOD, M.J., MAHER, W.A. 2003. Measurement of arsenic species in marine sediments by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. Analytica Chimica Acta, 477, 279-291.

EPA, 2000. Risk-Based Concentration Table. Environmental Protection Agency, Philadelphia PA, Washington DC, United States. Risk-Based Concentration Table Environmental Protection Agency, Philadelphia PA, Washington DC, United States.

EPA, 2001. National primary drinking water regulations, arsenic and clarifications to compliance and new source contaminants monitoring, Vol.66, No.14/Monday, January 22 / Rules and Regulations. (Environmental Protection Agency), Federal Register.

ERIKSON, K.M. AND ASCHNER, M. 2003. Manganese neurotoxicity and glutamate-GABA interaction. Neurochemistry International, 43, 475-80.

EUREKA E. ADOMAKO, A.R.M. SOLAIMAN , PAUL N. WILLIAMS , CLAIRE DEACON , G.K.M.M. RAHMAN , ANDREW A. MEHARG. 2009. Enhanced transfer of arsenic to grain for Bangladesh grown rice compared to US and EU. Environment International, 35, 476–479.

EUROPEAN COMMISSION. Scientific Cooperation (SCOOP) reports on tasks 3.2.11. Assessment of dietary exposure to arsenic, cadmium, lead and mercury of the population of EU Members States; 2004.

FALCO G, LLOBET JM, ZAREBA S, KRZYSIAK K, DOMINGO JL. 2005. Risk assessment of trace elements intake through natural remedies in Poland. Trace Elements and Electrolytes, 22, 222-226.

FALCO G., LLOBET JM., BOCIO A., DOMINGO JL. 2006Daily intake of arsenic, cadmium, mercury, and lead by consumption of edible marine species. Journal of Agricultural and Food Chemistry, 54, 6106-6112.

FAO, 2006. Arsenic contamination of irrigation water, soil and crops in Bangladesh: Risk implications for sustainable agriculture and food safety in Asia, RAP PUBLICATION 2006/20. ftp://ftp.fao.org/docrep/fao/009/ag105e/ag105e00.pdf [Last accessed on 12 May 2011].

FARDY, J.J., MCORIST, G.D., FARRAR, Y.J. 1989. The determination of selenium status in the Australian diet using neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry 133, 397-405

FEED ADDITIVE COMPENDIUM, 1975. Minneapolis, The Miller Publishing Company, vol. 13 pp. 330.

FERGUSON, J. F. AND GAVIS. J. 1972. A Review of Arsenic Cycle in Natural Waters. Water Research, 6, 1259-1274.

FERGUSSON, J. E. 1990. The heavy elements: Chemistry, Environmental Impact and Health Effects, Pergamon Press, Oxford, pp.551.

FERRELL, R.E. 2008. Medicinal clay and spiritual healing. Clays and Clay Minerals, 56, 751-760.

FINKELMAN, R. B. 2006. Health benefits of geologic materials and geologic processes. International Journal Environment Research Public Health, 3, 338-342.

FRANCESCONI, K. A., KUEHNELT, D. 2004. Determination of arsenic species: A critical review of methods and applications, 2000-2003. Analyst, 129, 373 – 395.

FRANCESCONI, K. A. AND EDMONDS, S. 1994. Biotransformation of arsenic in the marine environment, in: Nriagu, J. O., (Ed.). Arsenic in the Environment, Part I, Cycling and Characterization, Wiley, New York, pp. 221-262.

FREITAS, M. C., PACHECO, A. M. G., BACCHI, M. A., DIONISIO, I., LANDSBERGER, S., BRAISTED, J., FERNANDES, E. A. N. 2008. Compton suppression instrumental neutron activation analysis performance in determining trace- and minor-element contents in foodstuff. Journal of Radioanalytical and Nuclear Chemistry, 276, 149-156.

FRISBIE, S. H., MITCHELL, E. J., MASTERA, L. J., MAYNARD, D. M., YUSUF, A. Z., SIDDIQ, M. Y., ORTEGA, R., DUNN, R. K., WESTERMAN, D. S., BACQUART, T., SARKAR, B. 2009. Public Health Strategies for Western Bangladesh That Address Arsenic, Manganese, Uranium, and Other Toxic Elements in Drinking Water. Environmental Health Perspectives, 117, 410-416.

FRISBIE, S.H., ORTEGA, R., MAYNARD, D.M., SARKAR, B. 2002. The concentrations of arsenic and other toxic elements in Bangladesh's drinking water. Environmental Health Perspectives, 110, 1147-1153.

FSA. 2002. The Natural Mineral Water, Spring Water and Bottled, Drinking Water (Amendment) (England) Regulations. Food Sandard Agency.

FSA. 2009. Measurement of the concentrations of metals and other elements from the 2006 UK total diet study. Food Sandard Agency.

GALLAGHER, P. A., SHOEMAKER, J. A., WEI, X., BROCKHOFF-SCHWEGEL, C. A., CREED.J. T. 2001. Extraction and detection of arsenicals in seaweed via accelerated solvent extraction with ion chromatographic separation and ICP-MS detection, Fresenius Journal Analytical Chemistry, 369, 71-80.

GALLARDO, M.V., BOHARI, Y., ASTRUC, A., POTIN-GAUTIER, M., ASTRUC, M. 2002. Speciation analysis of arsenic in environmental solids Reference Materials by highperformance liquid chromatography-hydride generation-atomic fluorescence spectrometry following orthophosphoric acid extraction. Analytica Chimica Acta, 441, 257-268.

GAMBLE, M. V., AHSAN, H., LIU, X. H., FACTOR-LITVAK, P., ILIEVSKI, V., SLAVKOVICH, V., PARVEZ, F., GRAZIANO, J. H. 2005. Folate and cobalarnin deficiencies and hyperhomocysteinemia in Bangladesh. The American Journal of Clinical Nutrition, 81, 1372-1377.

GEISSLER, P.W., SHULMAN, C.E., PRINCE, R.J., MUTEMI, W., MNAZI, C., FRIIS, H., LOWE, B. 1998. Geophagy, iron status and anaemia among pregnant women on the coast of Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 92, 549-553.

GEORGIADIS, M., CAI, Y., SOLO-GABRIELE, H.M. 2006. Extraction of arsenate and arsenite species from soils and sediments. Environmental Pollution, 141, 22-29.

GHORBANI, H. 2008. Geophagia, a Soil - Environmental Related Disease. International Meeting on Soil Fertility Land Management and Agroclimatology. Turkey, 957-967.

GIACOMINO, A., MALANDRINO, M., ABOLLINO, O., VELAYUTHAM, M., CHINNATHANGAVEL, T., MENTASTI, E. 2009. An approach for arsenic in a contaminated soil: speciation, fractionation, extraction and effluent decontamination. Environmental Pollution, 158, 416-423.

GIROUARD, E. AND ZAGURY, G. J. 2009. Arsenic bioaccessibility in CCA-contaminated soils: Influence of soil properties, arsenic fractionation, and particle-size fraction. Science of the Total Environment, 407, 2576-2585.

GISSEL-NIELSEN G. 1998. Effects of selenium supplementation of field crops. In: Frankenberger WT, Engberg RA, editors. Environmental chemistry of selenium. New York: Marcel Dekker; p. 99–112.

GLOVER, J., LEVANDER, O., PARLZEK, J., VOUK, V., 1979. Selenium. In: Handbook on the Toxicology of Metals. (L. Friberg, G.F. Nordberg and V.B. Vouk, eds.), Elsevier/North-Holland Biomedical Press, Amsterdam. pp. 555-557.

GOLUBKINA NA, SHAGOVA MV, SPIRICHEV VB. 1992. Selenium intake by the population of Lithuania. Voprosy pitaniia journal, 1, 35–7.

GOYER, R.A. 1986. Toxic effects of metals. In: Cassarett and Doull's Toxicology. The basic science of poisons. Klaassen, C.D., M.O Amdur, and J.Doull, (eds.) 3rd ed. McGraw-Hill, New York.

GREGER, J.L., DAVIS, C.D., SUTTIE, J.W., LYLE, B.J. 1990. Intake, serum concentrations, and urinary-excretion of manganese by adult males. American Journal of Clinical Nutrition. 51, 457-461.

GUNDERSON, E. L. 1995. Dietary intakes of pesticides, selected elements, and other chemicals: FDA total diet study, June 1984 April 1986. Journal of AOAC International 78, 1353-1363.

GUNTER, T.E., GAVIN, C.E., ASCHNER, M., GUNTER, K.K. 2006. Speciation of manganese in cells and mitochondria: A search for the proximal cause of manganese neurotoxicity. Neurotoxicology, 27, 765-776.

GUPTA, P.C, AND RAY, C.S. 2004. Epidemiology of betel quid usage. Annals Academy of Medicine Singapore. 33, 31-36.

GUTHRIE, B.E. AND ROBINSON, M.F. 1977. Daily intakes of manganese, copper, zinc and cadmium by New Zealand women. British Journal of Nutrition, 38, 55 –63.

HAFEMAN, D., AHSAN, H., ISLAM, T., LOUIS, E. 2006. Betel quid: Its tremor-producing effects in residents of Araihazar, Bangladesh. Movement Disorders, 21, 567-571.

HAFEMAN, D., FACTOR-LITVAK, P., CHENG, Z.Q., VAN GEEN, A., AHSAN, H. 2007. Association between manganese exposure through drinking water and infant mortality in Bangladesh. Environmental Health Perspectives, 115, 1107-1112.

HAQUE, R., MAZUMDER, D. N. G., SAMANTA, S., GHOSH, N., KALMAN, D., SMITH, M. M., MITRA, S., SANTRA, A., LAHIRI, S., DAS, S., DE, B. K., SMITH, A. H. 2003. Arsenic in drinking water and skin lesions: Dose-response data from West Bengal, India. Epidemiology, 14, 174-182.

HARPER, H. A., RODWELL, V. W., MAYES, P. A. 1990. “Review of physiological chemistry” 17th edition.

HARVARD. 2011. Chronic arsenic poisoning: history, study and remediation Available:

HE QB AND SINGH BR, 1994. Effect of organic matter on the distribution, extractability anduptake of cadmium in soils. European Journal of Soil Science, 44, 641-650.

HEITKEMPER, D.T., VELA, N. R., STEWART, K. R., WESTPHAL, C. S. 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. Analytical Atomic Spectroscopy, 16, 299-306.

HEITLAND, P. AND KOSTER HD. 2006. Biomonitoring of 30 trace elements in urine of children and adults by ICP-MS. Clinica Chimica Acta, 365, 310-318.

HELGESEN, H. AND LARSEN, E. 1998.Bioavailability and speciation of arsenic in carrots grown in contaminated soil, Analyst, 123, 791.

HIRONAKA, H. AND AHMED, SK.A. 2003. Arsenic concentration of rice in Bangladesh: in M.F. Ahmed, M.A. Ali and Z. Adeel, (Ed.). Fate of arsenic in the environment, ISBN 984-32-0507-3, pp.123-130.

HORIGUCHI H, OGUMA E, SASAKI S, MIYAMOTO K, IKEDA Y, MACHIDA M, KAYAMA F , 2004. Dietary exposure to cadmium at close to the current provisional tolerable weekly intake does not affect renal function among female Japanese farmers. Environmental Research, 95, 20-31.

HOSSAIN, M., NAHER, F., SHAHABUDDIN, Q. 2005. Food Security and Nutrition in Bangladesh: Progress and Determinants. Electronic Journal of Agricultural and Development Economics, 2, 103-132.

HOUGH, R. L. 2007.Soil and human health: an epidemiological review. European Journal Soil Science, 58, 1200-1212.

http://phys4.harvard.edu/~wilson/arsenic/pictures/arsenic_project_pictures2.html [Accessed on: 26 September 2011].

IARC, 1986. The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans. International Agency for Research on Cancer, Lyon.

IARC, 1993. Cadmium and cadmium compounds (Group1). IARC monographs. International Agency for Research on Cancer, Lyon.

IARC. 2004. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 85, Tobacco Habits Other than Smoking: Betel quid and Areca-nut Chewing. IARC, Lyon. IARCMonographs on the Evaluation of Carcinogenic Risks to Humans, Vol 85, Tobacco Habits Other than Smoking: Betel quid and Areca-nut Chewing IARC, Lyon.

INOUE, Y., KAWABATA, K., TAKAHASHI, H., ENDO, G., 1994. Determination of arsenic compounds using inductively coupled plasma mass spectrometry with ion chromatography. Journal of Chromatography A, 675, 149 – 154.

IRGOLIC, K.J. 1994. Determination of total arsenic and arsenic compounds in drinking water, in: Chappell, W. R. Arsenic exposure and health. Science and Technology Letters.

ISHII, S., YAN, T., VU, H., HANSEN, D. L. R., HICKS, E., SADOWSKY, M. J. 2010. Microbes Environment, 25, 8-14.

IYENGAR, G.V., KAWAMURA, H., PARR, R.M., MIAH, F.K., WANG, J-X., DANG, H.S., DJOJOSUBROTO, H., CHO, S-Y., AKHER, P., NATERA, E.S. 2002. Dietary intake of essential minor and trace elements from Asian diets. Food and Nutrition Bulletin, 23 (3 Supplement), 124-128.

JALLOH, MA. CHEN, J., ZHEN, F., ZHANG, G. 2009. Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress. Journal of Hazardous Materials, 162, 1081-1085.

JARUP, L., BERGLUND, M., ELINDER, C. G., NORDBERG, G., VAHTER, M. 1998. Health effects of cadmium exposure: A review of the literature and a risk estimate. Scandinavian Journal of Work Environment and Health, 24, 1-52.

JOHNSON, M. D., KENNEY, N., STOICA, A., HILAKIVI-CLARKE, L., SINGH, B., CHEPKO, G., CLARKE, R., SHOLLER, P. F., LIRIO, A. A., FOSS, C., REITER, R., TROCK, B., PAIK, S., MARTIN, M. B. 2003. Cadmium mimics the in vivo effects of estrogen in the uterus and mammary gland. National Medical, 9, 1081-1084.

JORHEM, L., BECKER, W., SLORACH, S. 1998. Intake of 17 Elements by Swedish Women, Determined by a 24-h Duplicate Portion Study. Journal of Food Composition and Analysis, 11, 32–46.

JUDITH, A., BRISBIN, A. JOSEPH A. CARUSO., A. 2002. Comparison of extraction procedures for the determination of arsenic and other elements in lobster tissue by inductively coupled plasma mass spectrometry, Analyst, 127, 921-929.

KABATA-PENDIAS AND PENDIAS, 1984. Trace elements in soils and plants. CRC Press Inc. Boca Raton, pp. 171.

KACHENKO, A. AND SINGH, B. 2006. Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia. Water Air and Soil Pollution, 169, 101-123.

KAISE, T. AND FUKUI, S. 1992. The chemical form and acute toxicity of arsenic compound in marine organisms. Appled Organometallic Chemistry, 6, 155-160.

KAISE, T., WATANABE, S., ITOH, K. 1985. The acute toxicity of arsenobetaine. Chemosphere, 14, 1327-1332.

KAISER, R., HENDERSON, A. K., DALEY, W. R., NAUGHTON, M., KHAN, M. H., RAHMAN, M., KIESZAK, S., RUBIN, C. H. 2001. Blood lead levels of primary school children in Dhaka, Bangladesh. Environmental Health Perspectives, 109, 563-566.

KARAVOLTSOS, S., SAKELLARI, A., DASSENAKIS, M., SCOULLOS, M. 2008. Cadmium and lead in organically produced foodstuffs from the Greek market. Food Chemistry, 106, 843-851.

KARIM, M. M., BEGUM, Z. R. 1999. Groundwater Arsenic Contamination Inventories and Risk Assessment using Geographic Information System: Case Studies Kishoreganj and Netrokona Districts of Bangladesh. Paper Presented at the 92nd Annual Meeting of Air & Waste Management Association, St. Louis, Missouri, USA, 20 - 24 June 1999.

KARIM, R. A., HOSSAIN, S. M., MIAH, M. M. H., NEHAR, K., MUBIN, M. S. H. 2008. Arsenic and heavy metal concentrations in surface soils and vegetables of Feni district in Bangladesh. Environmental Monitoring and Assessment, 145,417-425.

KASSAM-KHAMIS, T., JUDD, P.A., THOMAS, J.E. 2000. Frequency of consumption and nutrient composition of composite dishes commonly consumed in the UK by South Asian Muslims originating from Bangladesh, Pakistan and East Africa (Ismailis). Journal of Human Nutrition Dietary, 13, 185-196.

KAWAI, K., SAATHOFF, E., ANTELMAN, G., MSAMANGA, G., FAWZI, W. 2009. Geophagy (Soil eating) in Relation to Anemia and Helminth Infection among HIVInfected Pregnant Women in Tanzania. American Journal of Tropical Medicine and Hygiene, 80, 36-43.

KAWATRA, B.L. AND BAKHETIA, P. 2008. Consumption of heavy metal and minerals by adult women through food in sewage and tube - Well irrigated area around Ludhiana city (Punjab, India). Journal of Human Ecology, 23, 351-354.

KHAN, S. I., AHMED, A. K. M., YUNUS, M., RAHMAN, M., HORE, S. K., VAHTER, M., WAHED, M. A. 2010. Arsenic and Cadmium in Food-chain in Bangladesh-An Exploratory Study. Journal of Health Population and Nutrition, 28, 578-584.

KHAN, S. I., AHMED, A.K.M., YUNUS, M., RAHMAN, M., HORE, S. K., VAHTER, M., WAHED, M. A. 2010. Arsenic and Cadmium in Food-chain in Bangladesh—An Exploratory Study. Journal of Health Population Nutrition, 28, 578-584.

KILE, M. L., HOUSEMAN, E. A., BRETON, C. V., SMITH, T., QUAMRUZZAMAN, O., RAHMAN, M., MAHIUDDIN, G., CHRISTIANI, D. C. 2007. Dietary arsenic exposure in Bangladesh. Environmental Health Perspectives, 115, 889-893.

KILE, M., WRIGHT, R., AMARASIRIWARDENA, C., QUAMRUZZAMAN, Q., RAHMAN, M., MAHIUDDIN, G., CHRISTIANI, D. 2009. Maternal and Umbilical Cord Blood Levels of Arsenic, Cadmium, Manganese, and Lead in Rural Bangladesh [Abstract]. Epidemiology, 20, s149-s150.

KIPPLER, M., LONNERDAL, B., GOESSLER, W., EKSTROM, E.C., EL ARIFEEN, S., VAHTER, M. 2009. Cadmium interacts with the transport of essential micronutrients in the mammary gland-A study in rural Bangladeshi women. Toxicology, 257:64-69.

KIPPLER, M., NERMELL, B., HAMADANI, J., TOFAIL, F., MOORE, S., VAHTER, M. 2010. Burden of cadmium in early childhood: longitudinal assessment of urinary cadmium in rural Bangladesh. Toxicological Letters, 198, 20-5.

KLAPEC, T., MANDIC, M.L., GRGIC, J., PRIMORAC, LJ., IKIC, M., LOVRIC, T., GRGIC, Z., HERCEG, Z. 1998. Daily dietary intake of selenium in eastern Croatia. Science of the Total Environment, 217, 127-136.

KOCH., I., WANG, L., REIMER, K. J., CULLEN, W. R. 2000.Arsenic species in terrestrial fungi and lichens from Yellowknife, NWT, Canada. Applied Organometallic Chemistry, 14, 245-252.

KONDO, H., ISHIGURO, Y., OHNO, K., NAGASE, M. TOBA, M., TAKAGI. M.1999. Naturally occurring arsenic in the groundwaters in the southern region of Fukuoka Prefecture, Japan. Water Research, 33, 1967-1972.

KONGSBAK K, WAHED MA, FRIIS H, THILSTED SH. 2006. Acute phase protein levels, T. trichiura, and maternal education are predictors of serum zincin a cross-sectional study in Bangladeshi children. Journal of Nutrition, 136: 2262–8.

KRACHLER, M., ROSSIPAL, E., MICETIC-TURK, D. 1999. Trace element transfer from the mother to the newborn - investigations on triplets of colostrum, maternal and umbilical cord sera. Eureapan Journal of Clinical Nutrition, 53, 486-494.

KRAJCOVICOVA-KUDLACKOVA, M., URSINYOVA, M., MASANOVA, V., BEDEROVA, A., VALACHOVICOVA, M. 2006. Cadmium blood concentrations in relation to nutrition. Central European Journal of Public Health, 14,126 - 129.

L'VOV, B. V. 1997. Interpretation of atomization mechanisms in electrothermal atomic absorption spectrometry by analysis of the absolute rates of the processes. Spectrochimica Acta, Part B, 52B, 1-23.

LAI, V. W. M., SUN, Y., TING, E., CULLEN, W. R., REIMER, K. J. 2004. Arsenic speciation in human urine: are we all the same? Toxicology and Applied Pharmacology, 198, 297-306.

LAPARRA JM, VELEZ D, BARBERA R, FARRE R, MONTORO R. 2005. Bioavailability of inorganic arsenic in cooked rice: Practical aspects for human health risk assessment. Journal of Agricultural Food Chemistry, 53, 8829-8833.

LARSEN, H. J. S. 1993. Relations between selenium and immunity, Norwegian Journal of Agricultural Sciences Supplement, 11, 105-119.

LARSENY, E. H., ANDERSENY, N. L., MULLERY, A., PETERSENY, A., MORTENSEN, G. K., PETERSEN, J. 2002. Monitoring the content and intake of trace elements from food in Denmark. Food Additives and Contaminants. 19, 33-46.

LAUFER, B. 1999. Anthropological Series Volume XVIII, No. 2. http://www.archive.org/stream/geophagy182lauf/geophagy182lauf_djvu.txt [Last Accessed 25 September 2011].

LAURER, G.R., GANG, Q.T., LUBIN, J.H., LI, J.Y., KAN, C.S., XIANG, Y.S., JIAN, C.Z., YI, H., DE, G.W. AND BLOT, W.J. 1993. Skeletal Pb-210 levels and lung-cancer among radon-exposed tin miners in southern china. Health Physics. 64, 253-259.

LE, X. C., CULLEN, W. R. AND REIMER, K. J. 1994. Human urinary arsenic excretion after one-time ingestion of seaweed, crab, and shrimp, Clinical Chemistry, 40, 617-624

LEE, H., CHO, Y., PARK, S., KYE, S., KIM, B., HAHM, T., KIM, M., LEE, J., KIM, C. 2006. Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury Journal of Food Composition and Analysis, 19, S31–S37.

LENNTECH, 2011. Physical and chemical properties of trace elements. http://www.lenntech.com/Periodic-chart-elements/As-en.htm [Last Accessed 25 Septamber 2011].

LEV, E. 2005. Turkiye Klinikleri Journal of Medical Ethics, 13, 74-80.

LI, C. 2007. Selenium deficiency and endemic heart failure in China: A case study of biogeochemistry for human health. AMBIO, 36, 90-93.

LI, L., EKSTROM, E.C., GOESSLER, W., LONNERDAL, B., NERMELL, B. M., RAHMAN, A., EL ARIFEEN, S., PERSSON, L.A., et al. 2008. Nutritional status has marginal influence on the metabolism of inorganic arsenic in pregnant Bangladeshi women. Environment Health Perspective, 116: 315–21.

LIANFANG, W. AND JIANGHONG, H. 1994.In: J.O. Nriagu, (Ed.). Arsenic in the Environment. Part II: Human Health and Ecosystem Effects, Wiley, New York, pp.159-172.

LIM, C.K. 1986. HPLC of small molecules, a practical approach. Edited by C.K. Lim, IRL Press Limited.

LIN, S. H., LIN, Y. F., CHEEMA-DHADLI, S., DAVIDS, M. R., HALPERIN, M. L. 2002. Nephrology Dialysis Transplantation, 17, 708-714.

LINDBERG, A. L., RAHMAN, M., L. PERSSON, A., VAHTER, M. 2008.The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. Toxicological and Appled Pharmacology, 230, 9-16.

LINDBERG, A.L., SOHEL, N., RAHMAN, M., PERSSON, L.A., VAHTER, M. 2010. Impact of Smoking and Chewing Tobacco on Arsenic-Induced Skin Lesions. Environment Health Perspective, 118, 533-538.

LINDSTROM, E HOSSAIN, M.B., LONNERDAL, B., RAQIB, R., EL ARIFEEN, S., EKSTROM, E.C. 2011. Prevalence of anemia and micronutrient deficiencies in early pregnancy in rural Bangladesh, the MINIMat trial. Acta Obstetricia et Gynecologica Scandinavica, 90, 47-56.

LIU, C. J., CHEN, C. L.,CHANG, K. W., CHU, C. H., LIU, T. Y. 2000. Canadian Medical Association Journal, 162, 359-360.

LJUNG, K., OOMEN, A., DUITS, M., SELINUS, O., BERGLUND, M. 2007. Bioaccessibility of metals in urban play ground soils. Journal of Environmental Science and Health, Part A. Toxic / Hazardous Substances and Environmental Engineering, 42, 1241-1250.

LJUNG, K., SELINUS, O., OTABBONG, E., BERGLUND, M. 2006. Metal and arsenic distribution in soil particle sizes relevant to soil ingestion by children. Applied Geochemistry, 21, 1613-1624.

LJUNG, K.S., KIPPLER, M.J., GOESSLER, W., GRANDER, G.M., NERMELL, B.M. VAHTER, M.E. 2009. Maternal and Early Life Exposure to Manganese in Rural Bangladesh. Environmental Science & Technology, 7, 2595-2601.

LLOBETT JM, FALCO G, CASAS C, TEIXIDO A, DOMINGO JL. 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. Journal of Agricultural and Food Chemistry, 51, 838-842.

LONDESBOROUGH, S. MATTUSCH, J. WENNRICH. R. 1999. Separation of organic and inorganic arsenic species by HPLC-ICP-MS. Fresenius Journal Analytical Chemistry, 363, 577-581.

LONGNECKER MP, TAYLOR PR, LEVANDER OA, HOWE SM, VEILLON C, MCADAM PA. 1991. Selenium in diet, blood, and toenails in relation to human health in a seleniferous area. American Journal of Clinical Nutrition, 53, 1288–94.

LUCCHINI, R.G., MARTIN, C.J. AND DONEY, B.C. 2009. From Manganism to Manganese-Induced Parkinsonism: A Conceptual Model Based on the Evolution of Exposure. Neuromolecular Medicine, 11, 311-321.

LUOBA, A.I., GEISSLER, P.W., ESTAMBALE, B., OUMA, J.H., MAGNUSSEN, P., ALUSALA, D., AYAH, R., MWANIKI, D., FRIIS, H. 2004. Geophagy among pregnant and lactating women in Bondo District, western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 98, 734-741.

MACPHERSON, A., BARCLAY, M.N., SCOTT, R., YATES, R.W. 1997. Loss of Canadian wheat imports lowers selenium intake and status of the Scottish population. In: Fischer PW, L'Abbe MR, Cockell KA, Gibson RS, editors. Trace elements in man and animals. Ottawa: NRC Research Press;. p. 203–205.

MAEDA, S. 1994. Biotransformation of arsenic in the freshwater environment, in: Nriagu, J. O., (Ed.). Arsenic in the Environment, Part I, Cycling and Characterization, Wiley, New York, pp. 155-187.

MAHALINGAM, T.R., VIJAYALAKSHMI, S., KRISHNA PRABHU, R., THIRUVENGADASAMI, A., WILBER, A., MATHEWS, C.K., RADHA SHANMUGASUNDARAM, K. 1997. Studies on some trace and minor elements in blood: A survey of the Kalpakkam (India) population - Part III: Studies on dietary intake and its correlation to blood levels. Biological Trace Element Research, 57, 223-238.

MAIHARA, V.A., GONZAGA, I.B., SILVA, V.L., FÁVARO, D.I.T., VASCONCELLOS, M.B.A., COZZOLINO, S.M.F. 2004. Daily dietary selenium intake of selected Brazilian population groups. Journal of Radioanalytical and Nuclear Chemistry, 259, 465-468

MANDAL, B.K., AND SUZUKI, K.T. 2002. Arsenic round the world: a review, Talanta, 58, 201-235.

MARCUS, W.L. AND RISPIN, A.S. 1988. Threshold carcinogenicity using arsenic as an example. In C.R.Cothern, M.A. Mehlman and W.L. Marcus (Eds), Risk Assessment and Risk Magament of Industial and Environmental Chemicals. Princeton Scientific Publ. Co., Princeton, NJ, Vol. 15, pp 133-159

MARIN A.R.; MASSCHELEYN P.H.; PATRICK W.H. 1993. Soil redox-ph stability of arsenic species and its influence on arsenic uptake by rice. Plant and Soil, 152,245-253.

MARTI-CID, R., PERELLO, G., DOMINGO, J.L. 2009. Dietary Exposure to Metals by Individuals Living Near a Hazardous Waste Incinerator in Catalonia, Spain: Temporal Trend. Biological Trace Element Research, 131, 245-254.

MATOS-REYES, N. M., CERVERA, M. L., CAMPOS, R. C.,GUARDIA, M. 2010. Total content of As, Sb, Se, Te and Bi in Spanish vegetables, cereals and pulses and estimation of the contribution of these foods to the Mediterranean daily intake of trace elements. Food Chemistry. 122, 188–194.

MAYER, A. B., LATHAM, M. C., DUXBURY, J. M., HASSAN, N., FRONGILLO, E. A. 2011. A food system approach to increase dietary zinc intake in Bangladesh based on an analysis of diet, rice production and processing. Combating micronutrient deficiencies food-based approaches. Edited by Thompson, B. and Amoroso, L.

MAYER, A. B., LATHAM, M. C., DUXBURY, J. M., HASSAN, N., FRONGILLO, E. A., BISWAS, T.2010. The zinc content of rice in Bangladesh: relationship to soil, production methods, diets and the zinc status of children. Proceedings of the Nutrition Society, 69 (OCE4), E334.

McCARTY, K.M., HOUSEMAN, E.A., QUAMRUZZAMAN, Q., RAHMAN, M., MAHIUDDIN, G., SMITH, T., RYAN, L., CHRISTIANI, D.C. 2006. The impact of diet and betel nut use on skin lesions associated with drinking-water arsenic in Pabna, Bangladesh. Environment Health Perspective, 114, 334-340.

MEHARG, A. A., WILLIAMS, P. N., ADOMAKO, E., LAWGALI, Y. Y., DEACON, C., VILLADA, A., CAMBELL, R. C. J., SUN, G., ZHU, Y. G., FELDMANN, J., RAAB, A., ZHAO, F. J., ISLAM, R., HOSSAIN, S., YANAI, J. 2009. Geographical Variation in Total and Inorganic Arsenic Content of Polished (White) Rice. Environmental Science & Technology, 43, 1612-1617.

MEHARG, A.A., LOMBI, E., WILLIAMS, P.N., SCHECKEL, K. G., FELDMANN, J., RAAB, A. ZHU, Y., ISLAM, R. 2009. Speciation and Localization of Arsenic in White and Brown Rice Grains. Environmental Science and Technolology, 42, 1051-1057.

MEHARG, A.A., MAZIBUR, M.D. 2003. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. Environmental Science and Technolology, 37, 229-234.

MELTZER, H.M., NORHEIM, G., LOKEN, E.B., HOLM, H. 1999. Supplementation with wheat selenium induces a dose-dependent response in serum and urine of a Se-replete population. British Journal of Nutrition, 67, 287–94.

MIDDLETON, J.D. 1989. Sikor - an Unquantified Hazard. British Medical Journal, 298, 407-408.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (MAFF), UK, (1999). 1997 Total diet study: metals and other elements, Food Surveillance Information, sheet No.191.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD. 2000. Duplicate Diet Study of Vegetarians - Dietary Exposures to 12 Metals and Other Elements ((Shett 193). Food Surveillance Information Sheet.

MISHRA, K.P. 2009. Lead exposure and its impact on immune system: A review. Toxicology in Vitro, 23, 69-972.

MIYAZAKI, Y., KOYAMA, H., HONGO, T., SASADA, Y., NOJIRI, M., SUZUKI, S.2001. Nutritional consideration for changes in dietary habit and health promotion practices in community health care; from the view point of selenium. [Nippon koshu eisei zasshi] Japanese journal of public health, 48, 243-257.

MIYAZAKI, Y., KOYAMA, H., SASADA, Y., SATOH, H., NOJIRI, M., SUZUKI, S. 2004. Dietary Habits and Selenium Intake of Residents in Mountain and Coastal Communities in Japan. Nutritional Science and VitaminoIogy, 50, 309-319.

MONTASER, A., 1998. Inductively Coupled Plasma Mass Spectrometry, Wiley-VCH Inc., New York. USA. pp.1– 563.

MONTES-BAYON, M., DENICOLA, K., CARUSO, J. A., 2003. Liquid chromatography-inductively coupled plasma mass spectrometry. Journal of Chromatogrphy A, 1000, 457 – 476.

MOR, F. AND CEYLAN, S. 2008. Cadmium and lead contamination in vegetables collected from industrial, traffic and rural areas in Bursa Province, Turkey. Food Additives and Contaminants 25, 611-615.

MOSER, P.B., REYNOLDS, R.D., ACHARYA, S., HOWARD, M.P., ANDON, M.B., LEWIS, S.A. 1988. Copper, iron, zinc, and selenium dietary intake and status of Nepalese lactating women and their breast-fed infants. American Journal of Clinical Nutrition, 47, 729-734

MUCHUWETI, A., BIRKETT, J. W., CHINYANGA, E., ZVAUYA, R., SCRIMSHAW, M. D. & LESTER, J. N. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: Implications for human health. Agriculture Ecosystems & Environment, 112, 41-48.

MUNOZ, O., VELEZ, D. AND MONTORO, R. 1999.Optimization of the solubilization, extraction and determination of inorganic arsenic [As(III)+As(V)] in seafood products by acid digestion, solvent extraction and hydride generation atomic absorption spectrometry. Analyst, 124, 601-607.

MURPHY, J., HANNON, E.M., KIELY, M., FLYNN, A., CASHMAN, K.D. 2002. Selenium intakes in 18 - 64-y-old Irish adults. European Journal of Clinical Nutrition, 56, 402-408.

NAGVI, S.M., VAISHNAVI, C., SINGH, H. 1994. Toxicity and metabolism of arsenic in vegetables, in: J.O. Nriagu, (Ed.). Arsenic in the Environment. Part II: Human Health and Ecosystem Effects, Wiley, New York, pp. 55-91.

NAIDU, R., SMITH, E., OWENS, G., BHATTACHARYA, P., NADETAUM, P. 2006. Managing arsenic in the environment. From soil to human health. Csiro publishing.

NANDAKUMAR, A., THIMMASETTY, K. T., SREERAMAREDDY, N. M., VENUGOPAL, RAJANNA, T. C., VINUTHA, SRINIVAS, A. T., BHARGAVA, M. K. 1990. Briyish Journal of Cancer, 62, 847-851.

NASER, H., SHIL, N., MAHMUD, M., RASHID, M., :HOSSAIN, K. 2009. Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. Bangladesh Journal of Agricultural Research 34, 545-554.

NASREDDINE L., NASHALIAN O., NAJA F., ITANI L., PARENT-MASSIN D., NABHANI-ZEIDAN M., HWALLA N. 2010. Dietary exposure to essential and toxic trace elements from a Total diet study in an adult Lebanese urban population. Food and Chemical Toxicology, 48, 1262-1269.

NAVAS-ACIEN, A., SILBERGELD, E.K., STREETER, R.A., CLARK, J.M., BURKE, T.A. GUALLAR, E. 2006. Arsenic exposure and type 2 diabetes: A systematicview of the experimental and epidemiologic evidence. Environmental Health Perspectives, 114, 641-648.

NERMELL, B., LINDBERG, A.L., RAHMAN, M., BERGLUND, M., PERSSON, L.A., ARIFEEN, S.E. AND VAHTER, M. 2008. Urinary arsenic concentration adjustment factors and malnutrition. Environmental Research. 106, 212-218.

NHS. 2006. http://www.ic.nhs.uk/pubs/hse04ethnic

NOEL, L., LEBLANC, J.C., GUERIN, T. 2003. Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. Food Additives and Contaminants, 20, 44-56.

NORTON, G.J., DUAN, G., DASGUPTA, T., ISLAM, M.R., LEI, M., ZHU, Y., DEACON, C.M., MORAN, A.C., ISLAM, S., ZHAO, F.J., STROUD, J.L., MCGRATH, S.P., FELDMANN, J., PRICE, A.H., MEHARG, A.A. 2009. Environmental and genetic control of arsenic accumulation and speciation in rice grain: comparing a range of common cultivars grown in contaminated sites across Bangladesh, China, and India, Environmental Science and Technology, 43, 8381-8386.

NORTON, G. J., DEACON, C. M., XIONG, L., HUANG, S., MEHARG, A. A., PRICE, A. M. 2010. Genetic mapping of the rice ionome in leaves and grain: identification of QTLs for 17 elements including arsenic, cadmium, iron and selenium. Plant Soil, 329, 139–153.

NORTON, S. A. 1998. Betel: Consumption and consequences. Journal of the American Academy of Dermatology, 38, 81-88.

O'NEILL, P. 1990. Chapter 5 (Arsenic) in: Heavy Metals in soils. B.J. Alloway, (editor). Blackie and Son Ltd., London.

OHASHI, F., FUKUI, Y., TAKADA, S., MORIGUCHI, J., EZAKI, T., IKEDA, M. 2006. Reference values for cobalt, copper, manganese, and nickel in urine among women of the general population in Japan. International Archives of Occupational and Environmental Health, 80, 117-126.

OHNO K., YANASE T., MATSUO Y., KIMURA T., RAHMAN M.H., MAGARA, Y. 2007. Arsenic intake via water and food by a population living in an arsenic-affected area of Bangladesh, Science of the Total Environment, 381, 68-76.

ONIANWA, P. C., LAWAL, J. A., OGUNKEYE, A. A., OREJIMI, B. M. 2000. Cadmium and nickel composition of Nigerian foods. Journal of Food Composition And Analysis, 13, 61 - 969.

PAPPA, E.C., PAPPAS, A.C., SURAI, P.F. 2006. Selenium content in selected foods from the Greek marked and estimation of the daily intake. Science of The Total Environment, 372, 100 - 8.

PENDIAS, K. AND PENDIAS. 1984. Trace elements in soils and plants. CRC Press Inc. Boca Raton, pp. 171.

PETERS, S. C., BLUM, J. D., KLAUE, B. AND KARAGAS, M. R. 1999. Arsenic occurrence in New Hampshire drinking water. Environmental Science and Technology, 33, 1328-1333.

PETERS, U., POOLE, C., ARAB, L. 2001. Does tea affect cardiovascular disease? A meta-analysis. American Journal of Epidemiology, 154, 495-503.

PHE-Wastesite protocol text, Jule 1995.

PIERCE, B. L., ARGOS, M., CHEN, Y., MELKONIAN, S., PARVEZ, F., ISLAM, T., AHMED, A., HASAN, R., RATHOUZ, P. J., AHSAN, H. 2011. Arsenic Exposure, Dietary Patterns, and Skin Lesion Risk in Bangladesh: A Prospective Study. American Journal of Epidemiology, 173, 345-354.

PILSNER, J.R., HALL, M.N., LIU, X.H. AHSAN, H., ILIEVSKI, V., SLAVKOVICH, V., LEVY, D., FACTOR-LITVAK, P., GRAZIANO, J.H., GAMBLE, M.V. 2011. Associations of Plasma Selenium with Arsenic and Genomic Methylation of Leukocyte DNA in Bangladesh. Environmental Health Perspectives. 119, 113-118.

PILSNER, J.R., LIU, X.H., AHSAN, H., ILIEVSKI, V., SLAVKOVICH, V., LEVY, D., FACTOR-LITVAK, P., GRAZIANO, J.H., GAMBLE, MV. 2009. Folate Deficiency, Hyperhomocysteinemia, Low Urinary Creatinine, and Hypomethylation of Leukocyte DNA Are Risk Factors for Arsenic-induced Skin Lesions. Environmental Health Perspectives, 117, 254-260.

PRASAD, A. S. 1998. Zinc dietary trace element. Journal of Trace Elements in Experimental Medicine, 11, 61. 8. [Accessed 25 September 2011 from www.ZestRSA.co.za]

QIAN, YZ; CHEN, C; ZHANG, Q, LI, Y., CHEN, Z., LI, M. 2010. Concentrations of cadmium, lead, mercury and arsenic in Chinese market milled rice and associated population health risk. Food Control, 21, 1757-1763.

QUEIROLO, F., STEGEN, S., RESTOVIC, M., PAZ, M., OSTAPCZUK, P., SCHWUGER, M. J., MUNOZ, L. 2000. Total arsenic, lead, and cadmium levels in vegetables cultivated at the Andean villages of northern Chile. Science of the Total Environment, 255, 75-84.

RAGHUNATH R, TRIPATHI RM, SUSEELA B, BHALKE S, SHUKLA VK, PURANIK VD, 2006. Dietary intake of metals by Mumbai adult population, Science of The Total Environment, 356, 62-68.

RAGHUNATH, R., TRIPATHI, R.M., SUSEELA, B., BHALKE, S., SHUKLA, V.K., PURANIK, V.D. 2006. Dietary intake of metals by Mumbai adult population, Science of The Total Environment, 356, 62-68.

RDA, 2011. Recommended Daily Allowances. [Accessed 25 September 2011]. http://www.healthsupplementsnutritionalguide.com/recommended-daily allowances.html

ROBBERECHT, H.J, HENDRIX, P., VAN CAUWENBERGH, R., DEELSTRA, H.A. 1994. Actual dietary intake of selenium in Belgium, using duplicate portion sampling. Z Lebensm Unters Frosch, 199, 251–4.

ROBERTS, J., HUG, S., VOEGELIN, A., DITTMAR, J., KRETZSC, A., HMAR, R., WEHRLI, B., SAHA, G., BADRUZZAMAN, A., ALI, A. 2011. Arsenic Dynamics in Porewater of an Intermittently Irrigated Paddy Field in Bangladesh. Environmental Science and Technology, 45, 971–976.

ROSSIPAL, E., KRACHLER, M., LI, F., MICETIC-TURK, D. 2000. Investigation of the transport of trace elements across barriers in humans: studies of placental and mammary transfer. Acta Paediatrica, 89, 1190-1195.

ROYCHOWDHURY, T. UCHINO, T., TOKUNAGA, H., ANDO, M. 2002. Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. Food and Chemical Toxicology, 40, 1611-1621.

ROYCHOWDHURY, T., TOKUNAGA, H., ANDO, M. 2003.Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic-affected area of West Bengal, India. Science of The Total Environment, 308, 15-35.

RUDGE, C.V., ROLLIN, H.B., NOGUEIRA, C.M., THOMASSEN, Y., RUDGE, M.C., ODLAND, J.O. 2009. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. Journal of Environmental Monitoring, 11, 1322-1330.

SALVADOR, G. A. 2010. Iron in neuronal function and dysfunction. Biofactors, 36, 103-110.

SAMANTA, G., ROYCHOWDHURY, T., MANDAL, B.K., BISWAS, B.K., CHOWDURY, U.K., BASU, G.K., CHANDA, C.R. LODH, D., CHAKRABORTI,

D. 1999. Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India, and Bangladesh. Micro Chemical Journal 62, 174-191.

SANKARANARAYANAN, R., BLACK, R. J., SWAMINATHAN, R., PARKIN, D. M. 1998. An overview of cancer survival in developing countries. IARC Scientific Publications, 145, 135-173.

SANTAMARIA, A. B. 2008. Manganese exposure, essentility & Toxicity. Indian Journal of Medical Research, 128, 484 – 500.

SAPUNAR-POSTRUINIK, J., BAIULIC, D., KUBALA, H. 1996. Estimation of dietary intake of arsenic in the general population of the Republic of Croatia. Science of the Total Environment, 191, 119-123.

SARKER, M.A.H. 2002. Indigenous Fine Aromatic Rice Production: Bangladesh Perspective. 12-15 November 2002. Development of Basic Standard for Organic Rice Cultivation. 1st RDA/ARNOA International Conference. RDA and Dankook Univ., Korea.

SCANLON, K. A., MACINTOSH, D. L., HAMMERSTROM, K. A., RYAN, P. B. 1999. A longitudinal investigation of soild-food based dietary exposure to selected elements. J. Exposure anal. And Environ. Epidemology, 9, 485- 493.

SCHAFER, U., ANKE, M., SEIFERT, M., FISCHER, A.B. 2004. Influences on the manganese intake, excretion and balance of adults, and on the manganese concentration of the consumed food determined by means of the duplicate portion technique. Trace Elements and Electrolytes, 21, 68-77.

SCHETTLER, T. 2001. Toxic threats to neurologic development of children. Environmental Health Perspectives, 109, 813-816.

SCHOOF RA, YOST LJ, EICKHOFF J, CRECELIUS EA, CRAGIN DW, MEACHERDM, et al. 1999. A market basket survey of inorganic arsenic food. Food Chemical Toxicology, 37, 839–46.

SCHRAUZER, G.N. 2000. Selenomethionine: A review of its nutritional significance, metabolism and toxicity. Journal of Nutrition. 130, 1653-1656.

SENGUPTA, S., MUKHERJEE, P.K., PAL, T., SHOME, S. 2004. Nature and origin of arsenic carriers in shallow aquifer sediments of Bengal Delta, India. Environmental Geology, 45, 071-1081.

SERAJ, S. 2007. Puffed-rice Production, Adulterating Puffed-rice, and Public (Internet Edition). Awareness and Current Issue. (September, 2007). http://www.shykhseraj.com/press_room/writings_of_shykh_seraj.php [Accessed 26 September, 2011].

SHAKEEL, A., HUSSAIN, A., ALI, H., AHMAD, A. 2005. Transplanted fine rice (Oryza sativa L.) productivity as affected by plant density and irrigation regimes. International Journal of Agriculture and Biology, 7, 445–447.

SHARMA, R. K., AGRAWAL, M., MARSHALL, F. M. 2008. Atmospheric deposition of heavy metals (Cu, Zn, Cd and Pb) in Varanasi City, India. Environmental Monitoring and Assessment, 142, 269-278.

SHETTY, K. V. AND JOHNSON, N. W. 1999. Knowledge, attitudes and beliefs of adult South Asians living in London regarding risk factors and signsfor oral cancer. Community Dental Health, 16, 227-231.

SHIVOGA, W. A. AND MOTURI, W. N. 2009. Geophagia as a risk factor for diarrhoea. Journal of Infection in Developing Countries, 3, 94-98.

SIGEL, H. 1983. Metal ions in biological systems: Zinc and its role in biology and nutrition, Volume 15.

SIGNES-PASTOR, A.J., MITRA, K., SARKHEL, S., HOBBES, M., BURLO, F., DE GROOT, W. T., CARBONELL- BARRACHINA, A.A. 2008a. Arsenic speciation in food and estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. Journal of Agricultural and Food Chemistry. 56, 9469-9474.

SIGNES-PASTOR, A.J., MITRA, K., BURLO, F., CARBONELL-BARRACHINA, A.A. 2008b. Effect of two different rice dehusking procedures on total arsenic concentration in rice. European Food Research and Technology. 226, 561-567.

SIROT, V., SAMIERI, C., VOLATIER, J.L., VOLATIER J.L., LEBLANC J.C. 2008. Cadmium dietary intake and biomarker data in French high seafood consumers. Journal of Exposure Science and Environmental Epidemiology,18,2008.

SMITH, A.H., ARROYO, A. P., GUHA MAZUMDER, AND KOSNETT, M. J. 2000. Arsenic-induced skin lesions among Atacameno people in Northern Chile despite good nutrition and centuries of exposure. Environment Health Perspectives. 108, 617-620.

SMITH, L.K., BOTHA, J.L., BENGHIAT, A., STEWARD, W.P. 2003. Latest trends in cancer incidence among UK south Asians in Leicester. British Journal of Cancer, 89, 70-73.

SORKUN, H.C., BIR, F., AKBULUT, M., DIVRIKLI, U., ERKEN, G., DEMIRHAN, H., DUZCAN, E., ELCI, L., CELIK, I., YOZGATLI, U. 2007. The effects of air pollution and smoking on placental cadmium, zinc concentration and metallothionein expression. Toxicology, 238, 15-22.

SPALLHOLZ, J. E., BOYLAN, L. M., ROBERTSON, J. D., SMITH, L., RAHMAN, M. M., HOOK, J., RIGDON, R. 2008. Selenium and arsenic content of agricultural soils from Bangladesh and Nepal. Toxicological and Environmental Chemistry, 90, 203-210.

SPALLHOLZ, J. E., BOYLANA, L. M., RHAMAN, M. M. 2004. Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India. Science of The Total Environment, 323, 21–32

STREPPLER, M. 1991. Cadmium, In:Merian, E. (Ed.). Metals and their compounds in the environment, VCH, Weinneim, p 803.

SUN, G. F.LIU, S., LI, X., SUN, X., GUO, X. Y., QIAN, C., PI, J.B. 2001. Environmental Sciences, 8, 425.

SUN, Y., ZHOU, Q., LIU, W., AN, J., XU, Z., WANG, L. 2008. Joint effects of arsenic and cadmium on plant growth and metal bioaccumulation: A potential Cdhyperaccumulator and As-excluder Bidens pilosa L. Journal of Hazardous Materials, 165, 1023-1028.

SUNDE, R.A. AND HOEKSTRA, W.G. 1980. Structure, synthesis and function of glutathione-peroxidase, Nutrition Reviews, 38, 265-273.

SZPUNAR, J. 2000. Trace element speciation analysis of biomaterials by high-performance liquid chromatography with inductively coupled plasma mass spectrometric detection. TrAC Trends in Analytical Chemistry, 19, 127-137.

SZPUNAR, J. 2005. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. Analyst, 130, 442 – 465.

TAMM, C., SABRI, F., CECCATELLI, S. 2008. Mitochondrial-mediated apoptosis in neural stem cells exposed to manganese. Toxicological Sciences. 101, 310-320.

TANABE, K., YOKOTA, H., HIRONAKA, H., TSUSHIMA, S., KUBOTA, Y. 2001. Arsenic pollution of groundwater in Bangladesh, Applied Organometallic Chemistry, 15. 241-251.

TEMPLETON, D.M. ARIESE, F. CORNELIS, R. DANIELSSON, L.G. MUNTAU, H. VAN LEEUWEN, H.P. LOBINSKI. R. 2000. Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches (IUPAC Recommendations 2000). Pure and Applied Chemistry, 72, 1453-1470

TETENS I, HELS O, KHAN NI, THILSTED SH, HASSAN N. 2003. Rice-based diets inrural Bangladesh: how do different age and sex groups adapt to seasonal changes in energy intake? American Journal of Clinical Nutrition, 78, 406–13.

THE KUSHTIA TIMES. 2011. http://www.thekushtiatimes.com/25/07/2011/essentials-go-costlier-amidpanic-buying-hoarding/ [Accessed 11 October, 2011].

THE INDEPENDENT. 2011. http://theindependentbd.com/paper-edition/frontpage/129-frontpage/67969-toxic-poultry-fish-feed-swamps-market.html [Accessed 25 October, 2011].

THOMSON, J. 1997. Anaemia in pregnant women in eastern Caprivi, Namibia. South African Medical Journal, 87, 544-1547.

THORNTON, I. 1992. Source and pathways of cadmium in the environment. In: Nordberg, G. F., Herber, R. F. M., and Alessio. L. (Eds). Cadmium in the human environment. Toxicity and Carcinogenicity. International Agency for Research on Cancer, Lyon. IARC, Scientific Publications No. 118, p.149.

THORNTON, I. AND FAGAGO, M. 1997. The geochemistry of arsenic, in: C.O. Abernathy, R.L. Calderon, W.R. Cappell (Eds), Arsenic: Exposure and Health Effects, Chapman and Hall, Kluwer Academic Publishers, London, pp. 1-16.

TOKUNAGA, H. 2007. Risk assessment of arsenic for people who live in the area of arsenic-contaminated undergroundwater in Bangladesh. Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku, 1-16.

TRIPATHI, R. M., RAGHUNATH, R., KRISHNAMOORTHY, T. M. 1997. Dietary intake of heavy metals in Bombay city, India. Science of The Total Environment, 208, 149-159.

TRIPATHI, R.M., MAHAPATRA, S., RAGHUNATH, R., SASTRY, V.N., KRISHNAMOORTHY, T.M. 2000. Daily intake of manganese by the adult population of Mumbai. Science of The Total Environment, 250, 43-50.

TSAI, S. M., WANG, T. N., KO, Y. C. 1998. Cancer mortality trends in a Blackfoot disease endemic community of Taiwan following water source replacement. Journal of Toxicology and Environmental Health, A55, 389-404.

TSUDA, T., INOUE, T., KOJIMA, M., AKOI, S. 1995. Market basket and duplicate portion estimation of dietary intake of cadmium, mercury, arsenic, copper, manganese and zinc by Japanese adults. Journal of AOAC International, 78, 1363-1368.

TUPWONGSE, V., PARKPIAN, P., WATCHARASIT, P., SATAYAVIVAD1, P. 2007. Determination of levels of Mn, As, and other metals in water, sediment, and biota from Phayao Lake, Northern Thailand, and assessment of dietary exposure .Journal of Environmental Science and Health Part A, 42, 1029–1041.

TURKDOGAN, M. K., KILICEL, F., KARA, K., TUNCER, I. & UYGAN, I. 2003. Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey. Environmental Toxicology and Pharmacology, 13, 175-179.

UNB. 2011. 2 crore people suffering from kidney diseases in Bangladesh: Experts. http://www.unbconnect.com/component/news/task-show/id-43116 [Accessed 26 September, 2011].

US NATIONAL RESEARCH COUNCIL. 1999. Arsenic in drinking water, National Academy Press. Washington DC, USA, pp.41-46.

VAHTER, M. MARAFANTE, E. AND DENCKER, L. 1984. Tissue distribution and retention of arsenic 74-dimethylarsenic acid in mice and rats. Archives of Environmental Contamination and Toxicology, 13, 259-264

VANNOORT RW, HANNAH ML, PICKSTON L. New Zealand Total Diet Survey. Part 2: Contaminant Elements. (ESR Client Report FA 95y7, Wellington: ESR: Health) 1995.

VANWYK, C.W., STANDER, I., PADAYACHEE, A., GROBLERRABIE, A.F. 1993. The Areca Nut Chewing Habit and Oral Squamous-Cell Carcinoma in South-African Indians - a Retrospective Study. South African Medical Journal, 83, 425-429.

VEGA, L., STYBLO, M., PATTERSON, R., CULLEN, W., WANG, C. D. 2001. Germolec. Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes. Toxicology and Appled Pharmacology, 172, 225-232.

VERBRUGGEN, N.; HERMANS, C.; SCHAT, H. 2009. Mechanisms to cope with arsenic or cadmium excess in plants. Current Opinion in Plant Biology, 12, 364-372.

VERMEER, D.E. 1971. Geophagy among Ewe of Ghana. Ethnology, 10, 56.

VERSTRAETEN, S., AIMO, L., OTEIZA, P. 2008. Aluminium and lead: molecular mechanisms of brain toxicity. Archives of Toxicology, 82, 789-802.

VINAS, P., LOPEZ-GARCIA, I., MERINO-MERONO, B., CAMPILLO, N., HERNANDEZ-CORDOBA. M. 2003. Speciation of arsenic in baby foods and the raw fish ingredients using liquid chromatography – hydride generation-atomic absorption spectrometry, Chromatographia, 57, 611-616.

VOUTSA, D. AND SAMARA, C. 1998. Dietary intake of trace elements and polycyclic aromatic hydrocarbons via vegetables grown in an industrial Greek area. Science of The Total Environment, 218, 203-216.

WAALKES, M.P. 2000. Cadmium carcinogenesis in review. Journal of Inorganic Biochemistry, 79, 240-244.

WAKAO, N. KOYATSU, H., KOMAI, Y., SHIMOKAWARA, H., SAKURAI, Y., SHIOTA. H. 1988. Microbial oxidation of arsenite and occurrence of arsenite – oxidizing bacteria in acid –mine water from a sulfur-pyrite mine. Geomicrobiology Journal, 6, 11-24.

WALSH, A. 1995. The application of atomic absorption spectra to chemical analysis. Spectrochimica Acta. 7, 108-117.

WANG, L., XU, Z. R., JIA, X. Y., JIANG, J. F. HAN, X. Y. 2006. Effects of Arsenic (AsIII) on Lipid Peroxidation, Glutathione Content and Antioxidant Enzymes in Growing Pigs. Asian - Australasian Journal of Animal Sciences, 19, 727-733.

WANG, X. L., SATO, T., XING, B. S., TAO, S. 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Science of The Total Environment, 350, 28-37.

WASSERMAN, G.A., LIU, X.H., PARVEZ, F., AHSAN, H., FACTOR-LITVAK, P., VAN GEEN, A., SLAVKOVICH, V., LOLACONO, N.J., CHENG, Z.Q., HUSSAIN, L. 2004. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environment Health Perspectives, 112, 1329-1333.

WATTS, M.J, BUTTON, M, BREWER, T.S., JENKIN, GRT., HARRINGTON, C.F. 2008. Quantitative arsenic speciation in two species of earthworms from a former mine site. Journal of Environmental Monitoring, 10, 753-759.

WHITE, M.A. AND SABBIONI, E. 1998. Trace element reference values in tissues from inhabitants of the European Union. X. A study of 13 elements in blood and urine of a United Kingdom population. Science of The Total Environment. 216, 253-270.

WHO, 1982. Food Additives Series 18. Arsenic.

WHO, 1992a. Cadmium. Environmental Health Criteria 134. World Health Organisation, International Programme on Chemical Safety (IPCS), Geneva, Switzerland.

WHO. 1992b. Cadmium, WHO Geneva. International programme on chemical safety (IPCS). Environmental Health Criteria 135.

WHO, 2001a. Arsenic compounds, Environmental Health Criteria 224, 2nd. World health organization, Geneva.

WHO, 2001b. Zinc, Environmental Health Criteria 221. World health organization, Geneva.

WHO, 2004. Cadmium. Safety evaluation of certain food additives and contaminants.61stmeeting of the Joint FAO/WHO Expert Committee on FoodAdditives (JECFA). IPCS—International Programme on Chemical Safety. WHOFood Additives Series: 52, World Health Organization, Geneva, Switzerland.

WHO, 2008. An interview with Mahmuder Rahman Bangladesh's arsenic agony. Bulletin of the world Health Organization, 86 (1), 11-12. www.who.int/bulletin/volumes/86/1/08-040108.pdf (Accessed 20 October, 2011).

WHO. 2010a. Exposure to cadmium: A major public health concern, WHO Geneva. International programme on chemical safety (IPCS). Environmental Health Criteria.

WHO. 2010b. Exposure to lead: A major public health concern, WHO Geneva. International programme on chemical safety (IPCS). Environmental Health Criteria.

WILD, S., ROGLIC, G., GREEN, A., SICREE, R., KING, H. 2004. Global Prevalence of Diabetes, Estimates for the year 2000 and projections for 2030. Diabetes Care, 27, 5.

WILLIAMS, P.N., PRICE, A.H., RAAB, A., HOSSAIN, S.A., FELDMANN, J., MEHARG, A.A. 2005. Variation In Arsenic Speciation And Concentration In Paddy Rice Related To Dietary Exposure. Environmental Science & Technology, 39, 5531-5540.

WILLIAMS, P. N., ISLAM, M. R., ADOMAKO, E. E., RAAB, A., HOSSAIN, S. A., ZHU, Y. G., FELDMANN, J., MEHARG, A. A. 2006. Increase in rice grain arsenic for regions of Bangladesh irrigating paddies with elevated arsenic in groundwaters. Environmental Science & Technology. 40, 4903-4908.

WILLIAMS, P.N., RAAB, A., FELDMANN, J., MEHARG, A. A. 2007. Market basket survey shows elevated levels of as in South Central US processed rice compared to California: Consequences for human dietary exposure. Environmental Science & Technology, 41, 2178-2183.

WILLIAMS, P. N., ISLAM, S., ISLAM, R., JAHIRUDDIN, M., ADOMAKO, E., SOLIAMAN, A. R. M., RAHMAN, G. K. M. M., LU, Y., DEACON, C.,ZHU, Y.,MEHARG, A. A. 2009. Arsenic limits trace mineral nutrition (selenium, zinc, and nickel) in Bangladesh rice grain. Environmental Science & Technology, 43, 8430-8436.

WILPLINGER, M., ZOCHLING, S., PFANNHAUSER, W. 1999. An analysis of the manganese supply in Austria on the basis of a selected diet. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Research and Technology, 208, 251-253.

WOIDICH H AND PFANNHAUSER W. 1977. Heavy-Metals in Foods - Analytical Control in Austria, Nahrung-Food, 21, 685-695.

WOYWODT, A. AND KISS, A. 2002. Geophagia: the history of earth-eating. Journal of the Royal Society of Medicine, 95, 143-146.

YAMAUCHI, H. AND FOWLER, B. A. 1994. Toxicity and metabolism of inorganic and methylated arsenicals, in: J.O. Nriagu, (Ed.). Arsenic in the Environment. Part II: Human Health and Ecosystem Effects, Wiley, New York, pp. 35-53.

YAMAZAKI, C., ISHIGA, H., AHMED, F., ITOH, K., SUYAMA, K., YAMAMOTO, H. 2003. Vertical distribution of arsenic in Ganges delta sediments in Deuli Village, Bangladesh. Soil Science and Plant Nutrition, 49, 567-574.

YAN, S., LING, Q., BAO, Z., CHEN, Z., DONG, Z., ZHANG, B., DENG, B. 2009. Cadmium accumulation in pak choi (*Brassica chinensis* L.) and estimated dietary intake in the suburb of Hangzhou city, China. Food Additives & Contaminants Part B-Surveillance, 2, 74-78.

YANAI, J., NOGUCHI, J., YAMADA, H., SUGIHARA, S., KILASARA, M., KOSAKI, T, 2009. Function of geophagy as supplementation of micronutrients in Tanzania. Soil Science and Plant Nutrition, 55, 215-223.

YOUNG, S.L., KHALFAN, S.S., FARAG, T.H., KAVLE,, J.A., ALI, S.M., HAJJI, H., RASMUSSEN, K.M., PELTO, G.H., TIELSCH, J.M., STOLTZFUS, R.J. 2010. Association of Pica with Anemia and Gastrointestinal Distress among Pregnant Women in Zanzibar, Tanzania. American Journal of Tropical Medicine and Hygiene, 83, 144-151.

YOUNG, S.L., WILSON, M.J., MILLER, D., HILLIER, S. 2008. Toward a Comprehensive Approach to the Collection and Analysis of Pica Substances, with Emphasis on Geophagic Materials. Plos One, 3, 1-13.

YSART, G., MILLER, P., CREWS, H., ROBB, P., BAXTER, M., DE L'ARGY, C., LOFTHOUSE, S., SARGENT, C., HARRISON, N. 1999. Dietary exposure estimates of 30 elements from the UK Total Diet Study. Food Additives and Contaminants, 16, 391-403.

YUNUS, M. 2001. Craving for Nicotine: A Study on Tobacco Prevalence in Bangladesh. Bangladesh Institute of Development Studies, Report, December.

ZABLOTSKA, L.B., CHEN, Y., GRAZIANO, J.H., PARVEZ, F., VAN GEEN, A., HOWE, G.R., AHSAN H. 2008. Protective effects of B vitamins and antioxidants on the risk of arsenic-related skin lesions in Bangladesh. Environment Health Perspectives, 116, 1056-1062.

ZHENG, N., WANG, Q. C., ZHANG, X. W., ZHENG, D. M., ZHANG, Z. S., ZHANG, S. Q. 2007. Population health risk due to dietary intake of heavy metals in the industrial area of Huludao city, China. Science of The Total Environment, 387, 96-104.

ZLOTKIN, S.H., ATKINSON, S., LOCKITCH, G. 1995. Trace elements in nutrition for premature infants. Clinics in Perinatology, 22, 223-40.

10 Appendices

10.1 Appendix (1): Raw data using ICP-MS instrument

Table 10-1: Concentrations in the digested solution of some food (including rice, vegetables and fish) samples were measured by ICP-MS

		As	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
		(µg/kg)	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**
S69	Rice BD Broin	0.75	0.607	262	1.498	1.021	171.4
S70	Rice BD parboiled (Sylhet)	1.728	0.256	158.8	0.781	0.808	108.6
S71	Rice BD atap (unknown)	1.261	1.095	160.5	1.812	0.959	158.3
S74	Rice BD Boro	1.365	0.112	352.2	0.562	1.301	344.1
S75	Rice BD parboiled (Shail)	1.225	0.253	470.3	0.413	0.563	217.6
S76	Rice BD Binni	0.604	0.795	524.2	0.441	0.463	180.2
S77	Rice BD Aman	1.73	0.206	329.4	0.267	0.155	251.1
S78	Rice BD Zaria	1.076	0.697	563.8	0.477	0.56	244.8
S79	Rice BD Irri	1.451	0.856	430	0.627	0.329	221.7
S80	Rice BD Ala shail	1.135	0.33	367.6	0.29	0.397	185.6
S81	Rice BD Katari	2.056	0.042	250.7	0.301	0.279	245.9
S82	Data stem (vegetable)	0.44	3.368	793.2	2.868	10.59	340.3
S83	Nali shak	0.724	6.591	601.4	2.502	1.589	280.9
S84	Lal shak 03 fresh	1.747	32.65	999.8	33.77	1.293	1093
S85	Lal shak frozen IBCO	0.462	40.34	421.2	4.587	1.17	807.2
S86	Lal shak 02 fresh	1.565	36.13	1192	29.93	1.217	1112
S87	Lal shak 01 fresh	1.725	29.42	893.9	29.67	1.076	850.9
S88	Lal shak	0.625	38.63	400.7	5.111	0.663	3173
S89	Lal shak	0.459	31.42	333.4	3.192	1.256	2012
S90	Pui shak	0.547	5.808	1466	2.362	1.596	519.2
S126	pan Kashia leaves	0.284	0.14	1801	2.468	0.189	153.9
S127	pan Bangla leaves	4.131	0.19	352.2	5.906	0.252	112.4
S128	Beans 1	0.059	0.717	734.3	0.327	0.227	288.5
S129	Beans 2	0.1	0.41	447.7	0.956	0.259	346.4
S130	Beans 3	0.09	0.65	768.8	1.066	0.281	316.8

Table 9-1 continued

		As	**Cd**	**Mn**	**Pb**	**Se**	**Zn**
		(µg/kg)	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**	**(µg/kg)**
S141	Hilsha egg (Manmar)	64.1	0.099	44.84	0.125	19.72	412.1
S142	Hilsha flesh (Manmar)	99.72	0.182	11.33	0.749	7.22	69.32
S143	Hilsha flesh (Manmar)	85.56	0.16	4.097	0.292	8.196	63.92
S144	Hilsha flesh (Manmar)	113.8	0.276	9.796	0.967	8.533	105.3
S145	Hilsha flesh (BD)	54.52	0.232	12.36	0.869	22.59	155.1
S146	Hilsha flesh (BD)	83.93	0.178	6.559	0.953	15.59	89.86
S147	Hilsha egg (BD)	61.07	0.246	73.17	0.523	35.81	602
S148	Hilsha egg frozen (BD)	58.75	0.342	94.66	0.567	47.22	625.2
S149	Rice flour SRM	1.112	3.324	388.5	17.13	0.285	213.5
S150	Tomato leaves SRM	1.707	16.96	3153	7.796	1.559	279.3

Table 10-2: Raw data of some food samples (same samples in Table 9-1) determined using ICP-MS instrument. (Data: 7/12/2009)

Run	**75As**	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Blank ppb							
1	-0.001	-0.005	0	-0.204	-0.044	99.22%	98.42%	99.54%
2	-0.009	-0.007	0.002	-0.233	-0.019	99.94%	100.41%	100.03%
3	-0.009	-0.015	0	-0.211	0.014	100.84%	101.18%	100.43%
x	-0.007	-0.009	0.001	-0.216	-0.016	100.00%	100.00%	100.00%
s	0.005	0.005	0.001	0.015	0.029	0.81%	1.42%	0.45%
%RSD	70.9	60.24	102.3	6.945	180.3	0.813	1.423	0.448
	Std 20ppb							
1	18.19	19.71	19.59	18.39	21.18	99.32%	100.50%	101.21%
2	19.07	19.84	19.36	18.59	21.1	102.22%	100.65%	102.30%
3	18.66	19.33	19.44	18.79	20.9	100.76%	101.25%	102.57%
x	18.64	19.63	19.46	18.59	21.06	100.77%	100.80%	102.02%
s	0.437	0.266	0.114	0.2	0.147	1.45%	0.40%	0.72%
%RSD	2.347	1.354	0.586	1.074	0.697	1.443	0.393	0.704
	Std 40 ppb							
1	38.5	39.69	39.51	38.57	43.09	98.86%	97.86%	100.73%
2	38.07	40.46	40.45	38.3	43.14	98.16%	98.06%	101.37%
3	38.47	39.85	39.44	37.43	42.54	99.45%	99.18%	101.66%
x	38.35	40	39.8	38.1	42.92	98.82%	98.37%	101.26%
s	0.243	0.405	0.567	0.596	0.335	0.65%	0.71%	0.48%
%RSD	0.635	1.013	1.425	1.565	0.779	0.653	0.722	0.469
	Std 100ppb							
1	96.62	100	100.4	95.95	104.9	95.91%	95.92%	98.85%
2	96.57	99.93	100.4	94.72	105.8	95.78%	96.13%	99.52%
3	97.33	101.6	101.8	96.31	107.2	94.88%	95.49%	98.82%
x	96.84	100.5	100.9	95.66	106	95.52%	95.84%	99.06%
s	0.428	0.94	0.817	0.834	1.182	0.56%	0.33%	0.39%
%RSD	0.442	0.935	0.81	0.872	1.116	0.584	0.342	0.396
	Washout							
1	-0.002	0.025	0.03	-0.177	0.918	110.57%	104.07%	102.24%
2	-0.002	0.014	0.025	-0.158	0.896	110.62%	105.90%	103.51%
3	-0.009	0.021	0.026	-0.162	0.806	109.69%	106.83%	104.21%
x	-0.004	0.02	0.027	-0.166	0.873	110.29%	105.60%	103.32%
s	0.004	0.005	0.003	0.01	0.059	0.52%	1.40%	1.00%
%RSD	98.97	27	9.387	6.253	6.783	0.473	1.326	0.965
	Sample_69							
1	0.78	266.8	1.545	1.031	174.3	180.96%	158.30%	131.94%
2	0.714	257.9	1.492	1.006	170.6	184.96%	163.23%	136.20%
3	0.757	261.3	1.456	1.027	169.2	185.22%	164.11%	138.28%
x	0.75	262	1.498	1.021	171.4	183.71%	161.88%	135.47%
s	0.034	4.467	0.045	0.014	2.634	2.39%	3.13%	3.23%
%RSD	4.518	1.705	2.974	1.329	1.537	1.301	1.932	2.384

Table 9-2 continued

	75As	55Mn	208Pb	78Se	66Zn	45Sc	103Rh	193Ir
	Sample_70							
1	1.591	160.2	0.795	0.814	109.8	176.89%	157.42%	132.01%
2	1.832	159.2	0.774	0.762	109.3	178.76%	159.54%	134.31%
3	1.762	156.9	0.772	0.848	106.7	182.39%	161.36%	135.01%
x	1.728	158.8	0.781	0.808	108.6	179.35%	159.44%	133.78%
s	0.124	1.708	0.013	0.043	1.661	2.80%	1.97%	1.57%
%RSD	7.178	1.076	1.637	5.351	1.53	1.56	1.238	1.172
	Sample_71							
1	1.278	162.5	1.821	0.928	158.7	174.30%	153.34%	133.81%
2	1.249	160.1	1.805	0.959	157.9	174.97%	154.78%	136.97%
3	1.256	159	1.808	0.991	158.1	176.87%	155.07%	136.50%
x	1.261	160.5	1.812	0.959	158.3	175.38%	154.40%	135.76%
s	0.015	1.791	0.008	0.031	0.429	1.33%	0.92%	1.70%
%RSD	1.164	1.116	0.457	3.247	0.271	0.761	0.599	1.255
	Sample_74							
1	1.32	350.7	0.559	1.267	339.1	167.59%	148.36%	131.84%
2	1.409	351.3	0.569	1.355	347.4	167.91%	148.56%	132.51%
3	1.367	354.5	0.558	1.281	345.7	168.26%	149.43%	134.51%
x	1.365	352.2	0.562	1.301	344.1	167.92%	148.78%	132.95%
s	0.045	2.014	0.006	0.047	4.39	0.33%	0.57%	1.39%
%RSD	3.264	0.572	1.153	3.622	1.276	0.197	0.385	1.042
	Sample_75							
1	1.253	471.4	0.418	0.601	218.6	160.99%	145.67%	131.27%
2	1.13	466.9	0.41	0.449	216.7	163.85%	146.63%	131.86%
3	1.291	472.6	0.411	0.639	217.4	163.92%	146.38%	133.40%
x	1.225	470.3	0.413	0.563	217.6	162.92%	146.23%	132.18%
s	0.084	3.034	0.004	0.101	0.936	1.67%	0.50%	1.10%
%RSD	6.863	0.645	1.021	17.86	0.43	1.026	0.341	0.83
	Sample_76							
1	0.656	552.5	0.457	0.496	187.6	149.77%	136.32%	125.63%
2	0.51	509.9	0.424	0.413	176.3	157.52%	141.63%	131.82%
3	0.646	510.3	0.44	0.48	176.5	157.91%	142.41%	130.49%
x	0.604	524.2	0.441	0.463	180.2	155.07%	140.12%	129.31%
s	0.082	24.46	0.017	0.044	6.465	4.59%	3.32%	3.26%
%RSD	13.53	4.666	3.762	9.515	3.588	2.961	2.366	2.521
	Sample_77							
1	1.672	332.4	0.271	0.118	252	150.40%	135.99%	126.43%
2	1.736	325.9	0.267	0.158	250	153.48%	138.16%	128.65%
3	1.783	329.7	0.264	0.188	251.3	153.43%	138.57%	130.73%
x	1.73	329.4	0.267	0.155	251.1	152.44%	137.57%	128.60%
s	0.055	3.277	0.003	0.035	1.015	1.77%	1.39%	2.15%
%RSD	3.2	0.995	1.266	22.88	0.404	1.158	1.008	1.669

Table 9-2 continued

	75As	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Sample_78							
1	1.102	573.3	0.477	0.531	247	148.73%	136.14%	127.87%
2	1.156	554.8	0.476	0.537	241.5	151.59%	137.97%	127.37%
3	0.971	563.4	0.477	0.612	246	150.80%	137.93%	129.62%
x	1.076	563.8	0.477	0.56	244.8	150.37%	137.35%	128.29%
s	0.095	9.291	0.001	0.045	2.899	1.48%	1.05%	1.18%
%RSD	8.849	1.648	0.177	8.055	1.184	0.982	0.761	0.921
	Sample_79							
1	1.499	432.8	0.622	0.283	222.9	148.13%	134.09%	126.48%
2	1.53	432.4	0.631	0.313	224.1	147.10%	137.12%	129.37%
3	1.323	424.8	0.628	0.392	218	150.42%	136.50%	129.51%
x	1.451	430	0.627	0.329	221.7	148.55%	135.90%	128.45%
s	0.112	4.502	0.005	0.056	3.219	1.70%	1.60%	1.71%
%RSD	7.693	1.047	0.764	17.04	1.452	1.143	1.177	1.334
	Sample_80							
1	1.168	374.4	0.293	0.396	188.4	142.85%	131.38%	125.35%
2	1.194	361.7	0.29	0.472	182.8	146.82%	134.86%	127.98%
3	1.042	366.7	0.286	0.324	185.6	146.75%	133.13%	127.45%
x	1.135	367.6	0.29	0.397	185.6	145.47%	133.13%	126.93%
s	0.081	6.402	0.004	0.074	2.774	2.27%	1.74%	1.39%
%RSD	7.138	1.741	1.313	18.62	1.495	1.563	1.307	1.099
	Sample_81							
1	2.073	250.8	0.299	0.256	246.4	141.01%	129.92%	125.70%
2	2.191	252	0.3	0.303	247	143.21%	132.08%	126.13%
3	1.904	249.3	0.305	0.279	244.3	144.82%	132.64%	126.98%
x	2.056	250.7	0.301	0.279	245.9	143.01%	131.55%	126.27%
s	0.144	1.334	0.003	0.024	1.417	1.92%	1.44%	0.66%
%RSD	7.021	0.532	1.133	8.51	0.576	1.34	1.094	0.519
	Sample_82							
1	0.426	815.6	2.909	10.19	346.2	122.09%	97.30%	97.79%
2	0.422	793.4	2.852	10.83	343.3	128.63%	100.91%	101.53%
3	0.471	770.7	2.844	10.75	331.3	134.63%	105.24%	103.83%
x	0.44	793.2	2.868	10.59	340.3	128.45%	101.15%	101.05%
s	0.027	22.41	0.036	0.348	7.9	6.27%	3.98%	3.05%
%RSD	6.215	2.826	1.242	3.287	2.322	4.881	3.929	3.015
	Sample_83							
1	0.784	616.5	2.52	1.729	286.8	151.11%	126.83%	123.73%
2	0.756	592.6	2.491	1.598	278.1	156.92%	131.49%	126.59%
3	0.633	595	2.494	1.44	277.8	157.40%	133.21%	127.77%
x	0.724	601.4	2.502	1.589	280.9	155.14%	130.51%	126.03%
s	0.08	13.16	0.016	0.144	5.139	3.51%	3.30%	2.08%
%RSD	11.11	2.189	0.643	9.095	1.83	2.259	2.529	1.647

Table 9-2 continued

	75As	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Sample_84							
1	1.945	1003	34.34	1.353	1103	143.55%	116.19%	114.08%
2	1.567	1001	33.48	1.349	1093	145.04%	119.12%	117.60%
3	1.728	995.1	33.49	1.178	1083	149.13%	121.54%	118.44%
x	1.747	999.8	33.77	1.293	1093	145.91%	118.95%	116.71%
s	0.19	4.2	0.497	0.1	9.8	2.89%	2.68%	2.31%
%RSD	10.87	0.42	1.471	7.755	0.897	1.979	2.256	1.983
	Sample_85							
1	0.459	430	4.64	1.234	822	139.86%	115.01%	111.81%
2	0.496	418.3	4.538	1.119	803.7	143.40%	118.25%	115.03%
3	0.432	415.3	4.583	1.157	795.9	146.19%	117.93%	114.79%
x	0.462	421.2	4.587	1.17	807.2	143.15%	117.06%	113.88%
s	0.032	7.755	0.051	0.059	13.37	3.17%	1.78%	1.79%
%RSD	7.016	1.841	1.115	5.016	1.657	2.216	1.524	1.574
	Sample_86							
1	1.571	1172	29.06	1.205	1085	145.69%	121.49%	119.59%
2	1.606	1205	30.69	1.273	1140	143.74%	118.86%	116.63%
3	1.517	1198	30.05	1.172	1112	146.21%	121.95%	119.36%
x	1.565	1192	29.93	1.217	1112	145.21%	120.77%	118.53%
s	0.045	17.63	0.826	0.051	27.36	1.30%	1.67%	1.65%
%RSD	2.864	1.479	2.758	4.209	2.459	0.894	1.379	1.389
	Sample_87							
1	1.798	941.2	31.27	1.059	899.4	139.22%	114.35%	112.70%
2	1.754	870.8	28.97	1.088	830.5	147.75%	122.80%	119.64%
3	1.621	869.7	28.76	1.083	822.7	147.79%	123.04%	120.70%
x	1.725	893.9	29.67	1.076	850.9	144.92%	120.06%	117.68%
s	0.092	40.96	1.393	0.015	42.21	4.94%	4.95%	4.34%
%RSD	5.349	4.583	4.694	1.414	4.96	3.406	4.121	3.692
	Sample_88							
1	0.7	407.3	5.137	0.653	3229	137.10%	113.08%	112.69%
2	0.547	400	5.126	0.697	3154	139.91%	115.18%	114.79%
3	0.628	394.8	5.071	0.64	3137	139.95%	115.60%	115.58%
x	0.625	400.7	5.111	0.663	3173	138.99%	114.62%	114.36%
s	0.076	6.279	0.035	0.03	49.41	1.63%	1.35%	1.49%
%RSD	12.22	1.567	0.692	4.49	1.557	1.173	1.178	1.304
	Sample_89							
1	0.434	336.5	3.245	1.112	2046	133.97%	110.12%	109.71%
2	0.467	334	3.159	1.263	2011	137.93%	113.05%	113.26%
3	0.477	329.9	3.171	1.394	1980	139.21%	115.84%	115.19%
x	0.459	333.4	3.192	1.256	2012	137.04%	113.00%	112.72%
s	0.023	3.363	0.047	0.141	33.01	2.73%	2.86%	2.78%
%RSD	4.949	1.008	1.459	11.23	1.641	1.993	2.532	2.465

Table 9-2 continued

	75As	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Sample_90							
1	0.589	1482	2.393	1.552	525.3	137.33%	113.37%	112.40%
2	0.521	1453	2.347	1.571	516.3	141.03%	117.19%	114.76%
3	0.531	1463	2.345	1.664	515.9	142.80%	117.03%	115.60%
x	0.547	1466	2.362	1.596	519.2	140.39%	115.86%	114.26%
s	0.037	14.81	0.027	0.06	5.342	2.79%	2.16%	1.66%
%RSD	6.687	1.01	1.152	3.762	1.029	1.987	1.864	1.453
	Sample_126							
1	0.251	1797	2.443	0.116	151.8	127.29%	108.04%	113.47%
2	0.363	1802	2.472	0.185	155.1	129.27%	108.78%	114.25%
3	0.239	1804	2.49	0.265	154.9	130.10%	110.06%	115.38%
x	0.284	1801	2.468	0.189	153.9	128.89%	108.96%	114.37%
s	0.068	3.301	0.024	0.075	1.823	1.45%	1.02%	0.96%
%RSD	24.11	0.183	0.963	39.72	1.185	1.122	0.939	0.842
	Sample_127							
1	4.321	376.7	6.391	0.336	120.3	125.72%	103.48%	106.15%
2	4.082	340	5.674	0.214	108.4	135.95%	112.72%	115.72%
3	3.99	340	5.652	0.207	108.4	137.27%	113.21%	116.72%
x	4.131	352.2	5.906	0.252	112.4	132.98%	109.80%	112.86%
s	0.171	21.17	0.421	0.072	6.896	6.32%	5.49%	5.83%
%RSD	4.127	6.01	7.122	28.73	6.136	4.754	4.995	5.168
	Sample_128							
1	0.035	725.2	0.32	0.222	285	135.34%	116.67%	119.56%
2	0.06	733	0.338	0.216	289	136.56%	116.96%	119.57%
3	0.08	744.7	0.324	0.243	291.5	134.48%	116.26%	119.88%
x	0.059	734.3	0.327	0.227	288.5	135.46%	116.63%	119.67%
s	0.023	9.837	0.009	0.014	3.271	1.05%	0.35%	0.19%
%RSD	38.42	1.34	2.805	6.259	1.134	0.773	0.303	0.155
	Sample_129							
1	0.102	459.8	0.972	0.291	353.2	131.49%	113.21%	116.18%
2	0.131	439.4	0.934	0.214	343.3	136.06%	116.74%	118.80%
3	0.067	443.8	0.961	0.273	342.8	137.31%	116.98%	118.03%
x	0.1	447.7	0.956	0.259	346.4	134.95%	115.65%	117.67%
s	0.032	10.7	0.02	0.04	5.849	3.06%	2.11%	1.34%
%RSD	32.1	2.39	2.049	15.6	1.689	2.269	1.828	1.143
	Sample_130							
1	0.074	781.5	1.08	0.253	323.1	132.60%	116.59%	118.07%
2	0.116	761.7	1.056	0.228	314.6	137.31%	118.83%	120.32%
3	0.078	763	1.063	0.364	312.9	137.53%	119.08%	119.45%
x	0.09	768.8	1.066	0.281	316.8	135.81%	118.17%	119.28%
s	0.023	11.1	0.013	0.072	5.463	2.79%	1.37%	1.13%
%RSD	25.7	1.444	1.182	25.66	1.724	2.053	1.162	0.951

Table 9-2 continued

	75As	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Sample_141							
1	65.86	46.22	0.129	20.51	422	150.03%	133.13%	126.23%
2	63.14	44.49	0.124	19.3	411.7	153.60%	135.77%	130.19%
3	63.28	43.81	0.121	19.34	402.6	153.50%	137.32%	132.23%
x	64.1	44.84	0.125	19.72	412.1	152.38%	135.41%	129.55%
s	1.531	1.242	0.004	0.692	9.714	2.04%	2.12%	3.05%
%RSD	2.389	2.771	3.037	3.509	2.357	1.336	1.566	2.356
	Sample_142							
1	99.62	11.54	0.754	7.314	70.24	151.18%	130.55%	126.26%
2	99.72	11.44	0.746	7.253	69.42	152.25%	132.53%	127.85%
3	99.83	11.02	0.747	7.093	68.29	154.04%	132.63%	128.39%
x	99.72	11.33	0.749	7.22	69.32	152.49%	131.90%	127.50%
s	0.105	0.276	0.004	0.115	0.98	1.45%	1.18%	1.11%
%RSD	0.105	2.432	0.586	1.587	1.413	0.95	0.891	0.871
	Sample_143							
1	85.94	4.129	0.292	8.164	64.17	147.75%	127.11%	123.23%
2	84.64	4.069	0.293	8.235	64.03	149.49%	130.76%	126.19%
3	86.11	4.093	0.291	8.19	63.55	151.20%	130.60%	127.01%
x	85.56	4.097	0.292	8.196	63.92	149.48%	129.49%	125.48%
s	0.806	0.03	0.001	0.036	0.325	1.72%	2.07%	1.99%
%RSD	0.942	0.736	0.345	0.437	0.509	1.153	1.595	1.584
	Sample_144							
1	113.1	9.674	0.967	8.409	106	148.98%	129.34%	126.18%
2	113.5	9.866	0.974	8.475	105.5	150.54%	132.20%	127.32%
3	114.8	9.849	0.959	8.717	104.5	152.30%	132.42%	129.37%
x	113.8	9.796	0.967	8.533	105.3	150.61%	131.32%	127.62%
s	0.885	0.106	0.008	0.162	0.734	1.66%	1.72%	1.62%
%RSD	0.778	1.085	0.802	1.897	0.697	1.103	1.309	1.27
	Sample_145							
1	54.47	12.39	0.873	22.61	156.3	145.02%	127.06%	123.88%
2	54.77	12.58	0.866	22.77	155.7	145.38%	128.36%	125.67%
3	54.33	12.12	0.867	22.38	153.4	149.21%	128.82%	125.48%
x	54.52	12.36	0.869	22.59	155.1	146.54%	128.08%	125.01%
s	0.223	0.229	0.004	0.198	1.553	2.32%	0.92%	0.98%
%RSD	0.409	1.852	0.415	0.874	1.001	1.584	0.715	0.786
	Sample_146							
1	86.94	6.843	0.965	16.13	91.74	150.23%	129.36%	125.68%
2	81.54	6.393	0.945	15.13	88.45	155.21%	133.05%	129.77%
3	83.3	6.442	0.948	15.51	89.41	155.57%	134.00%	129.20%
x	83.93	6.559	0.953	15.59	89.86	153.67%	132.14%	128.22%
s	2.755	0.247	0.011	0.506	1.694	2.98%	2.45%	2.21%
%RSD	3.282	3.761	1.143	3.247	1.885	1.94	1.852	1.727

Table 9-2 continued

	75As	**55Mn**	**208Pb**	**78Se**	**66Zn**	**45Sc**	**103Rh**	**193Ir**
	Sample_147							
1	59.42	71.33	0.512	34.69	584.5	150.40%	133.51%	128.52%
2	61.85	74.25	0.526	36.74	608	147.73%	132.67%	127.91%
3	61.92	73.92	0.532	35.99	613.5	147.34%	133.66%	127.39%
x	61.07	73.17	0.523	35.81	602	148.49%	133.28%	127.94%
s	1.423	1.599	0.01	1.036	15.43	1.67%	0.53%	0.57%
%RSD	2.33	2.186	1.956	2.893	2.562	1.122	0.4	0.441
	Sample_148							
1	58.93	94.5	0.565	47.44	625.5	142.35%	127.26%	125.06%
2	58.94	95.28	0.566	47.57	628.9	143.37%	129.67%	126.25%
3	58.39	94.19	0.57	46.64	621.3	147.23%	131.89%	127.53%
x	58.75	94.66	0.567	47.22	625.2	144.32%	129.61%	126.28%
s	0.312	0.563	0.003	0.502	3.818	2.57%	2.32%	1.24%
%RSD	0.531	0.595	0.473	1.063	0.611	1.784	1.787	0.981
	Sample_149							
1	1.027	392	17.15	0.326	213.8	140.70%	127.91%	123.45%
2	1.082	387.7	17.19	0.253	213.3	142.77%	129.92%	124.89%
3	1.227	385.9	17.05	0.276	213.5	143.06%	129.95%	126.34%
x	1.112	388.5	17.13	0.285	213.5	142.18%	129.26%	124.89%
s	0.103	3.109	0.073	0.038	0.272	1.29%	1.17%	1.44%
%RSD	9.301	0.8	0.428	13.17	0.127	0.907	0.902	1.156
	Sample_150							
1	1.827	3213	7.906	1.658	284.1	123.12%	98.94%	104.32%
2	1.491	3086	7.691	1.477	274.4	128.91%	103.98%	108.05%
3	1.803	3161	7.792	1.543	279.3	128.17%	101.69%	107.88%
x	1.707	3153	7.796	1.559	279.3	126.74%	101.54%	106.75%
s	0.188	63.6	0.108	0.091	4.837	3.15%	2.53%	2.11%
%RSD	10.99	2.017	1.382	5.866	1.732	2.489	2.487	1.973

Table 10-3: Raw data of Fish and Rice of As speciation using HPLC-ICP-MS.

		AsChol	**AsBet**	**As^{III}**	**DMA**	**MMA**	**As^{V}**	
Ret Time (min)		1.99	3.035	5.039	7.305	8.38	10.016	
1ppb As mix-1			18467		14472	13099	9139	
10ppb As mix-1			190394		145423	127799	115070	
50ppb As mix-1			967338		723202	629613	604935	
1ppb $As^{3+/}$1				7998				
10ppb $As^{3+/}$1				104246				
50ppb $As^{3+/}$1				503310				
25ppb AsChol/1		2279261						
	AsC	**AsBet**	**As^{III}**	**DMA**	**AsSug.**	**unkn2**	**MMA**	**As^{V}**
Ret Time (min)	1.99	3.035	5.039	7.305	7.501	7.696	8.38	10.016
Fish flesh_1		56441		163891	51463			
Fish flesh_2		83473		216039	70642			
Fish egg_3		55898		132445	29355			
Fish flesh_4		5968		214063	51161			
Fish flesh_5		8347		245904	64274			
Fish egg_6		14194		57045	4581	6006		
Fish flesh_7		10074		220835	52216			
Fish flesh_8		22072		138430	31355			
Fish egg_9		19007		29401	1079	2293		
Fish egg_10		24480		12633	558.74	2324		
Fish flesh_25		43180		79661	15228			
Fish flesh_26		75098		131808	35787			
Fish flesh_27		45267		85968	13997			
Fish flesh_1R_45		58061		187464	46245			
Fish egg_3R_46		48539		125451	21993			
BCR -627_11	9588	828913		30159	2444	4010	1249	
BCR-627_35	8495	824722		31418	2393	3548		
BCR-627_47	10524	957325		35666	3373	3425		
		AsChol	**AsBet**	**As^{III}**	**AsSug.**	**DMA**	**MMA**	**As^{V}**
Ret Time (min)		1.99	3.035	5.039	7.128	7.305	8.38	10.016
1ppb As mix-1			20284			15510	14071	14195
10ppb As mix-1			206028			155013	141991	139777
50ppb As mix-1			1025107			761909	697431	704681
1ppb As^{3+}/1				10582				
10ppb As^{3+}/1				96117				
50ppb As^{3+}/1				526081				

AsSug.: Arsenosugar (phosphate)

Table 9-3 continued

		AsChol	**AsBet**	**As^{III}**	**AsSug.**	**DMA**	**MMA**	**As^{V}**
Ret Time (min)		1.99	3.035	5.039	7.128	7.305	8.38	10.016
Rice1_13				18020		2204		4410
Rice2_14				30246		1882		4172
Rice3_15				27554		4491		7908
Rice4_16				10758		2515	488.92	16902
Rice23_17				11632		3309		19712
Rice23R_43				11678		3324		23074
Rice66_18				27469		2947		2000
Rice68_19				41111		2926		3927
Rice69_20				41649		5149		3367
Rice75_21				22263		2643		2281
Rice83_22				29585		5473		5647
Rice89_28				9569		2645		2675
Rice93_29				20601		1147		1571
Rice93R_44				18050		4027		1714
Rice94_30				6476		1240		939.8
Rice96_31				10033		6849		2060
Rice98_32				10163		1745		1900
Rice100_33				12337		3841		10571
Rice103_34				8822		1103		1629
Rice105_37				12263		6816		18994
Rice106_38				27976		4463		10991
Rice107_39				22888		5413	953.27	23717
Rice110_40				23017		6501		29778
Rice112_41				24835	1180	4900	682.12	18290
Rice CRM1_23				30155		1454	623.86	6241
RiceCRM2_42				35171		1507		7565
1ppb As mix-1			21545			16562	15237	15385
10ppb As mix-1			213490			158207	150538	162659
50ppb As mix-1			1050312			785711	755663	797891
1ppb As3+/1				12436				
10ppb As3+/1				128172				
50ppb As3+/1				655458				
AsCol (25ppb)		2580583						

10.2 Appendix (2): Correlations between different elements in Bangladeshi rice.

Table 10-4: Paired arsenic versus other elements Correlations.

		N	**r value**	***P* value**
Pair 1	As-BORO & Mn-BORO	19	-.236	.236
Pair 2	As-BORO & Zn-BORO	19	.016	.937
Pair 3	As-BORO & Se-BORO	19	.154	.442
Pair 4	As-BORO & Cd-BORO	19	-.455	.017
Pair 5	As-BORO & Pb-BORO	19	.044	.828
Pair 6	As-AMAN & Mn-AMAN	11	-.106	.657
Pair 7	As-AMAN & Zn-AMAN	11	.003	.990
Pair 8	As-AMAN & Se-AMAN	11	-.044	.852
Pair 9	As-AMAN & Cd-AMAN	11	-.332	.050
Pair 10	As-AMAN & Pb-AMAN	11	.655	.002
Pair 11	As-Aromatic & Mn-Aromatic	34	-.233	.192
Pair 12	As- Aromatic & Zn- Aromatic	34	.071	.696
Pair 13	As- Aromatic & Se- Aromatic	34	-.349	.046
Pair 14	As- Aromatic & Cd- Aromatic	34	-.466	.006
Pair 15	As- Aromatic & Pb- Aromatic	34	-.044	.807
Pair 16	As-Parboiled & Mn-Parboiled	13	-.576	.105
Pair 17	As- Parboiled & Zn- Parboiled	13	-.696	.037
Pair 18	As- Parboiled & Se- Parboiled	13	.567	.112
Pair 19	As- Parboiled & Cd- Parboiled	13	.165	.671
Pair 20	As- Parboiled & Pb- Parboiled	13	.437	.240

Table 10-5: Paired selenium versus other elements Correlations (except arsenic).

		N	**r value**	***P* value**
Pair 1	Se-BORO & Mn-BORO	19	-.115	.567
Pair 2	Se-BORO & Zn-BORO	19	.207	.301
Pair 3	Se-BORO & Cd-BORO	19	-.189	.344
Pair 4	Se-BORO & Pb-BORO	19	.348	.075
Pair 5	Se-AMAN & Mn-AMAN	11	.187	.431
Pair 6	Se-AMAN & Zn-AMAN	11	.442	.051
Pair 7	Se-AMAN & Cd-AMAN	11	.204	.387
Pair 8	Se-AMAN & Pb-AMAN	11	.075	.754
Pair 9	Se- Aromatic & Mn- Aromatic	34	.491	.004
Pair 10	Se- Aromatic & Zn- Aromatic	34	.552	.001
Pair 11	Se- Aromatic & Cd- Aromatic	34	.551	.001
Pair 12	Se- Aromatic & Pb- Aromatic	34	.236	.185
Pair 13	Se-Parboiled & Mn-Parboiled	13	-.347	.360
Pair 14	Se-Parboiled & Zn-parboiled	13	-.072	.854
Pair 15	Se-Parboiled & Cd-Parboiled	13	.783	.013
Pair 16	Se-Parboiled & Pb-Parboiled	13	.948	.000

r: correlation coefficient, *P*: probability value.

Table 10-6: Paired Mn versus Zn, Cd and Pb Correlations.

		N	r value	*P* value
Pair 1	Mn-BORO & Zn-BORO	19	.599	.001
Pair 2	Mn-BORO & Cd-BORO	19	-.229	.250
Pair 3	Mn-BORO & Pb-BORO	19	.041	.837
Pair 4	Mn-AMAN & Zn-AMAN	11	.261	.267
Pair 5	Mn-AMAN & Cd-AMAN	11	.654	.002
Pair 6	Mn-AMAN & Pb-AMAN	11	.123	.605
Pair 7	Mn- Aromatic & Zn- Aromatic	34	.268	.132
Pair 8	Mn- Aromatic & Cd- Aromatic	34	.269	.131
Pair 9	Mn- Aromatic & Pb- Aromatic	34	.280	.114
Pair 10	Mn-Parboiled & Zn-Parboiled	13	.704	.034
Pair 11	Mn-Parboiled & Cd-Parboiled	13	-.411	.272
Pair 12	Mn-Parboiled & Pb-Parboiled	13	-.234	.544

r: correlation coefficient, *P*: probability value.

Table 10-7: Paired Zn versus Cd and Pb, and Cd versus Pb Correlations.

		N	**r value**	***P* value**
Pair 1	Zn-BORO & Cd-BORO	19	-.416	.031
Pair 2	Zn-BORO & Pb-BORO	19	.111	.581
Pair 3	Zn-AMAN & Cd-AMAN	11	.170	.475
Pair 4	Zn-AMAN & Pb-AMAN	11	.264	.260
Pair 5	Zn- Aromatic & Cd- Aromatic	34	.374	.032
Pair 6	Zn- Aromatic & Pb- Aromatic	34	-.049	.785
Pair 7	Zn-Parboiled & Cd-Parboiled	13	.166	.670
Pair 8	Zn-Parbolied & Pb-Parboiled	13	-.007	.987
Pair 9	Cd-BORO & Pb-BORO	19	.120	.553
Pair 10	Cd-AMAN & Pb-AMAN	11	-.011	.963
Pair 11	Cd- Aromatic & Pb- Aromatic	34	.037	.839
Pair 12	Cd-Parboiled & Pb-Parboiled	13	.760	.017

r: correlation coefficient, *P*: probability value.