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Ground Water Arsenic Contamination and Its Accumulation into Different Varieties of Rice

Mondal, A.H.M. Firoz Kabir

University of Rajshahi

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Ground Water Arsenic Contamination and Its Accumulation into Different Varieties of Rice



**A Thesis
Submitted to the Institute of Environmental Science (IES)
University of Rajshahi for the Degree of
DOCTOR OF PHILOSOPHY
IN
ENVIRONMENTAL SCIENCE**

**By
A.H.M. Firoz Kabir Mondal**

**Institute of Environmental Science
University of Rajshahi
Rajshahi-6205
Bangladesh**

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June 2016

Dedicated
to
My Family

DECLARATION

I do, hereby, declare that the thesis entitled **“Ground Water Arsenic Contamination and Its Accumulation into Different Varieties of Rice”** submitted for the degree of Doctor of Philosophy in the Institute of Environmental Science, University of Rajshahi, Bangladesh, is the record of my original research work. I further declare that this work has not been submitted earlier for the award of any other degree or diploma at the University of Rajshahi or any other universities or institutions.

Date: June 2016

(A.H.M. Firoz Kabir Mondal)

CERTIFICATE

This is to certify that **A.H.M. Firoz Kabir Mondal** has carried out Ph.D. dissertation entitled “**Ground Water Arsenic Contamination and Its Accumulation into Different Varieties of Rice**” in the Institute of Environmental Science, University of Rajshahi, Bangladesh. This dissertation or part thereof has not been submitted for the award of any degree or diploma elsewhere.

The data presented in this thesis produced by A.H.M. Firoz Kabir Mondal are new, genuine and original. Mr. Firoz has fulfilled all the requirements for the submission of the dissertation for Ph.D. degree in the field of environmental science under the University of Rajshahi.

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A.H.M. Firoz Kabir Mondal

ABSTRACT

HYV rice BR-11 and BRRI dhan-50 were grown in an open-field Gangetic soil conditions with arsenic amended irrigation water and normal tap-water in experimental plot at Institute of Environmental Science of University of Rajshahi during August to December 2011 and February to June 2012, respectively. Whereas HYV rice BRRI dhan-36, local Boro variety Somsa and local Aman varieties (Mowka and Shorna) were also cultivated in an actual paddy field conditions with arsenic contaminated STW irrigation water in experimental plots at Mandal para village of Shahbajpur union under Shibganj upazila of Chapai Nawabganj district of Bangladesh during February to June 2012 and July to November 2012, respectively. The main objectives of this study were to observe the effect of arsenic (As) on rice (*Oryza sativa* L.) plants and the effect of arsenic contaminated pump distance and paddy field elevation on arsenic accumulation into HYV Boro and Aman rice.

The tillers number, panicle length and grain yield of BR-11 rice were found to decrease significantly ($p \leq 0.05$) with increase of arsenic (As) concentration in irrigation water. Arsenic in irrigation water showed a strong positive correlations with arsenic accumulation into soil, straw and grain, and the trend of accumulation was found as water > soil > straw > grain.

Chlorophyll contents of BRRI dhan-50 rice leaves were significantly decreased with increasing of soil residual arsenic. Grain and straw yield were not significantly affected by soil residual arsenic. A significant ($p \leq 0.01$) increasing trend of arsenic accumulation into straw and grain was observed with increasing of soil residual arsenic.

Arsenic accumulation in drainage sediments, paddy field soil, rice straw and grain were decreased significantly ($p < 0.01$) with increasing of arsenic contaminated pump distance during dry season for Boro cultivation period. Arsenic accumulation in paddy soil, rice straw and grain had significant ($p < 0.01$) negative correlation with paddy field elevation from mean sea level during Aman period (rainy season). Dry season hybrid rice varieties (BRRI dhan 50, BRRI dhan 36) accumulated more arsenic than rainy season local non-hybrid rice varieties. Highest arsenic accumulation in rice grain was 0.97 ± 0.01 mg/kg, but not exceeded the WHO recommended permissible limit 1 mg/kg.

Local non-hybrid rice varieties should be selected for arsenic contaminated area. Rice cultivation in upland and irrigation from distanced pump is suggested for less arsenic contamination.

LIST OF ABBREVIATIONS

As	- Arsenic
BRRI	- Bangladesh Rice Research Institute
BGS	- British Geological Survey
BBS	- Bangladesh Bureau of Statistics
BCSIR	- Bangladesh Council of Scientific and Industrial Research
BMDA	- Barendra Multipurpose Development Authority
BADC	- Bangladesh Agriculture Development Co-operation
cm	- centimeter
DPHE	- Department of Public Health and Engineering
DTW	- Deep Tube Well
DMRT	- Duncan Multiple Range Test
ft	- Foot
g	- Gram
FAO	- Food and Agriculture Organization
ha	- Hectare
HTW	- Hand Tube Well
HYV	- High Yielding Variety
kg	- Kilogram
km	- Kilometer
L	- Liter
mg	- milligram
NCDs	- Non-communicable Diseases
ppm	- Parts per million
ppb	- Parts per billion
STW	- Shallow Tube Well
SPSS	- Statistical Package for Social Science
SRDI	- Soil Resources Development Institute
µg	- microgram
WHO	- World Health Organization

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Chapter- 1

Introduction

1.1 Arsenic

The name arsenic derived from the Greek word arsenikon, meaning potent. It was first documented by Albertus Magnus in 1250 (Emsley, 2001). Arsenic is a chemical element with symbol As and atomic number 33. The three most common arsenic allotropes are *metallic gray*, *yellow* and *black arsenic*, with gray being the most common (Norman, 1998). Arsenic is a toxic, ubiquitous element with metalloid properties and almost often present in environmental samples (i.e. soil, sediment, water, aerosol, rain, aquatics, vegetation, milk and in living matters etc.) (Craig, 1986; Abernathy *et al.*, 1997; WHO, 1999; Tamaki and Frankenberger, 1992). It occurs in many minerals, usually in conjunction with sulfur and metals, and also as a pure elemental crystal. Arsenic chemistry is complex, and exists in the form of both organic and inorganic compounds in four oxidation states: -3, 0, +3 and +5. Arsenic occurs naturally in two main forms, arsenite As(III) and arsenate As(V), where As(III) is considerably more toxic than As(V) (Smith *et al.*, 1998). The chemical species of arsenic, which can exist in the natural environment heavily influence its mobility, adsorptivity and toxicity (Ascar *et al.*, 2008). The most common inorganic trivalent arsenic compounds are arsenic trioxide, sodium arsenite, and arsenic trichloride. Pentavalent inorganic compounds are arsenic pentoxide, arsenic acid, and arsenate (e.g. lead arsenate and calcium arsenate). Common organic arsenic compounds are arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid), and arsenobetaine (NRC, 1999). Arsenic trioxide is only slightly soluble in water; in sodium hydroxide it forms arsenite, and with concentrated hydrochloric acid it forms arsenic trichloride. Sodium arsenite and sodium arsenate are highly soluble in water. Interchanges in valence state may occur in water solutions, depending on the pH of the solution, as well as the presence of other substances that can be reduced or oxidized (Reay and Asher, 1977). Arsenic is mobilized through a combination of natural processes such as weathering reactions, biological activity and volcanic emissions as well as through a range of anthropogenic activities. Most environmental arsenic problems are the result of mobilization under natural conditions (Welch and Stollenwerk, 2002).

1.2 Forms of Arsenic in Soils

Arsenic is widely found in earth's crust, often as arsenopyrite (FeAsS) in iron pyrite rocks, the form which is present beneath West Bengal and Bangladesh. Many partial research on arsenic have been carried out, relevant some works are stated below.

Mridha (1998) found that arsenic in the earth's crust is 1.8 ppm. It is roughly as abundant as molybdenum or tin. Arsenic is a crystalline metalloid with three allotropic forms that are yellow, black and gray. Chemically, it is present as compounds with oxygen, chlorine, sulphur, carbon and hydrogen on one hand and with lead, gold and iron on the other hand. Arsenic is present in the earth's crust on an average of 2-5 mg/kg. The most common arsenic bearing minerals are arsenopyrite (FeAsS), enargite (Cu_3AsS_4), orpiment (As_2S_3) and As_4S_4 .

Samanta *et al.* (1999) stated that inorganic arsenic is more toxic than organic arsenic and again arsenite (As^{III}) is more toxic than arsenate (As^{V}). Arsenite and arsenate are present in ground water at an approximate ratio of 1:1.

Takamatsu *et al.* (1982) found that the common arsenic species in soils are arsenite, arsenate, MMAA and DMAA. Inorganic arsenic is the predominant form of arsenic in soil and soil solution (Onken and Hossner, 1996).

WHO (2001) reported at least six groups of arsenic compounds are present in the environment i) Inorganic water-soluble compounds: As (III) oxide and As (V) oxide, soluble As (III) (arsenite) and As (V) (arsenate) salts ii) Inorganic compounds of low water solubility: some arsenate and arsenite salts, arsenides, arsenic selenide and arsenic sulphide iii) Organic arsenic compounds occurring naturally or as pesticides, e.g. DMA (dimethylarsenic acid or cacodylic acid) iv) Organic arsenic compounds occurring naturally in marine organisms, e.g. arsenobetaine, arsenocholine v) Organic arsenic compounds used as feed additives, e.g. arsanilic acid vi) Gaseous inorganic and organic arsenic compounds, e.g. arsine.

1.3 Sources of Arsenic in Soil

Sources of arsenic in soil are very significant for the production of crops. There are many sources of arsenic in soil. Rocks and minerals are the predominant sources of arsenic. Excess use of fertilizers, pesticides and the arsenic contaminated irrigation water are also the source of arsenic in soils. So, the sources of arsenic are important not only our crop production but also our environment. A few of research works have been done in this ground, some relevant works are discussed below in short.

Bhattacharya *et al.* (1998) confirmed that major anthropogenic sources of arsenic contamination in the environment are from coal combustion, copper, smelting, pesticide use, agricultural chemicals, agricultural burning, food additives, cotton desiccants, herbicide and arsenic chemical used for wood treatment (Grant and Dobbs, 1997).

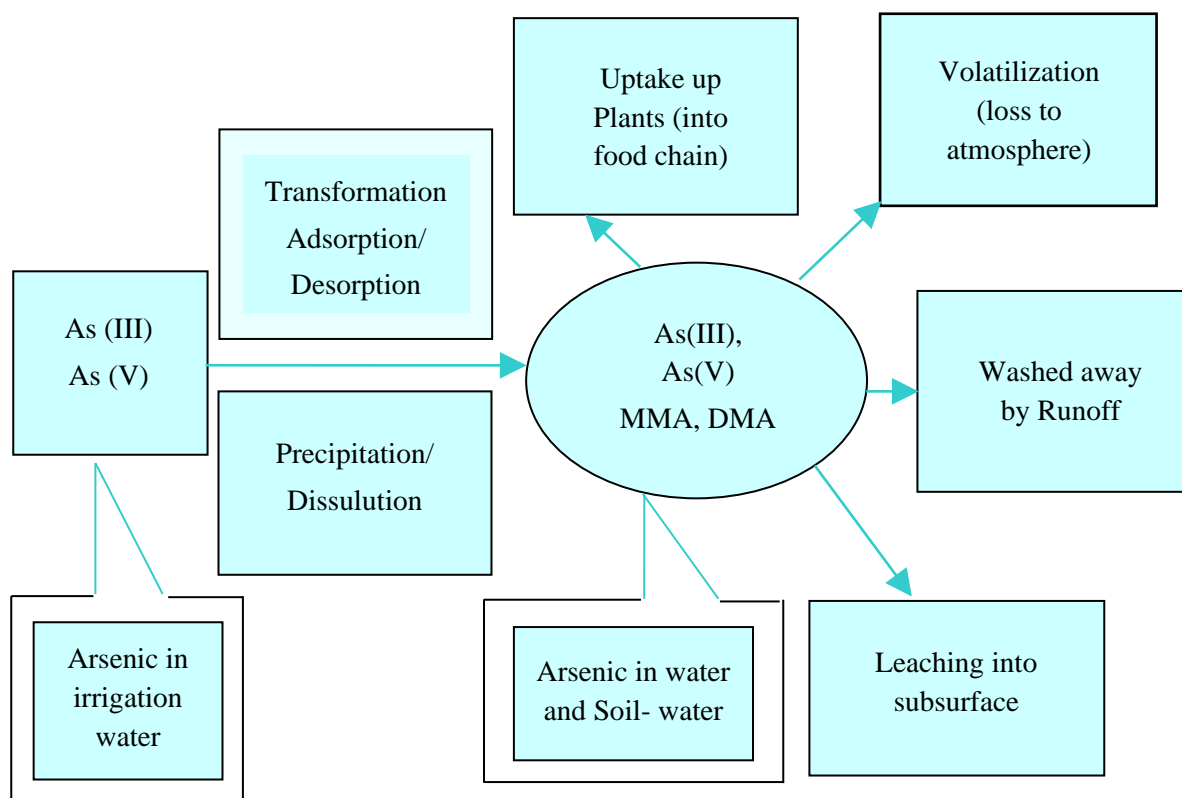
Ferguson and Gavis (1972) revealed that microorganisms increase the rate of arsenic release by catalyzing the oxidation of sulphide to sulphate and ferrous to ferric iron. Weathering of arsenic containing minerals is considered the major natural source of arsenic. Further, release of arsenic to the environment has been accruing from the use of arsenic compounds by the humans for several thousand years.

Oremland and Stolz (2003) stated that arsenic is a metalloid whose name conjures up images of murder. Nonetheless, certain prokaryotes use arsenic oxyanions for energy generation, either by oxidizing arsenite or by respiring arsenate. These microbes are phylogenetically diverse and occur in a wide range of habitats. Arsenic cycling may take place in the absence of oxygen and can contribute to organic matter oxidation. In aquifers, these microbial reactions may mobilize arsenic from the solid to the aqueous phase, resulting in contaminated drinking water. Arsenic may accumulate in soils through the use of arsenical pesticides, application of fertilizers, irrigation, oxidation of volatile arsines in air, dust from the burning fossil fuels and disposal of industrial, municipal, and animal wastes. Promotion of fertilizer application and irrigation farming practices and the use of land for waste disposal would thus enhance the arsenic inputs to soil environment.

O'Neill (1992) reported that microorganisms increase the rate of arsenic release by catalyzing the oxidation of sulphide to sulphate and ferrous to ferric. Further, release of arsenic to the environment has been accruing from the use of arsenic compounds by humans for several thousand years.

Smith *et al.* (1998) stated that arsenic enters the environment as a result of both natural and anthropogenic activities. The natural process of pedogenesis leads to mineral breakdown and translocation of the products as well as accessions from dust storms, volcanic eruptions and forest fires. Often the concentrations of arsenic released into the soil system by pedogenic processes are largely related to the origin and nature of the parent material.

Yan-Chu (1994) reported that arsenic is a naturally occurring terrestrial occurring element. The main source of arsenic in soils is the parent materials from which the soil is derived.



Source: Ali, *et al.*, 2003

Figure 1.1 Arsenic in the soil-water-plant environment

1.4 Production of Arsenic

Arsenic is widely distributed in the Earth's crust, which contains about 3.4 ppm arsenic (Wedepohl, 1991). It usually exists in nature in sulfide ores. Although there are more than 150 arsenic-bearing minerals (Budavari *et al.*, 2001; Carapella, 1992), arsenopyrite is by far the most common. Arsenic trioxide (white arsenic) is principally obtained as a by-product of smelting copper, lead, or gold ores. When these ores are smelted, the arsenic becomes gaseous and is trapped by electrostatic precipitators as a crude dust that is then roasted, whereby arsenic trioxide is driven off. (Pinto and McGill, 1953). Most commercially available arsenic compounds are produced from arsenic trioxide. The average annual world production of arsenic on the basis of limited data (1975-1977) was approximately 60,000 tons, and this level of production seems to be stable (WHO, 1981). In 2003, China was the world leader in the production of commercial-grade arsenic, followed by Chile and Peru (Brooks, 2003).

1.5 Uses of Arsenic

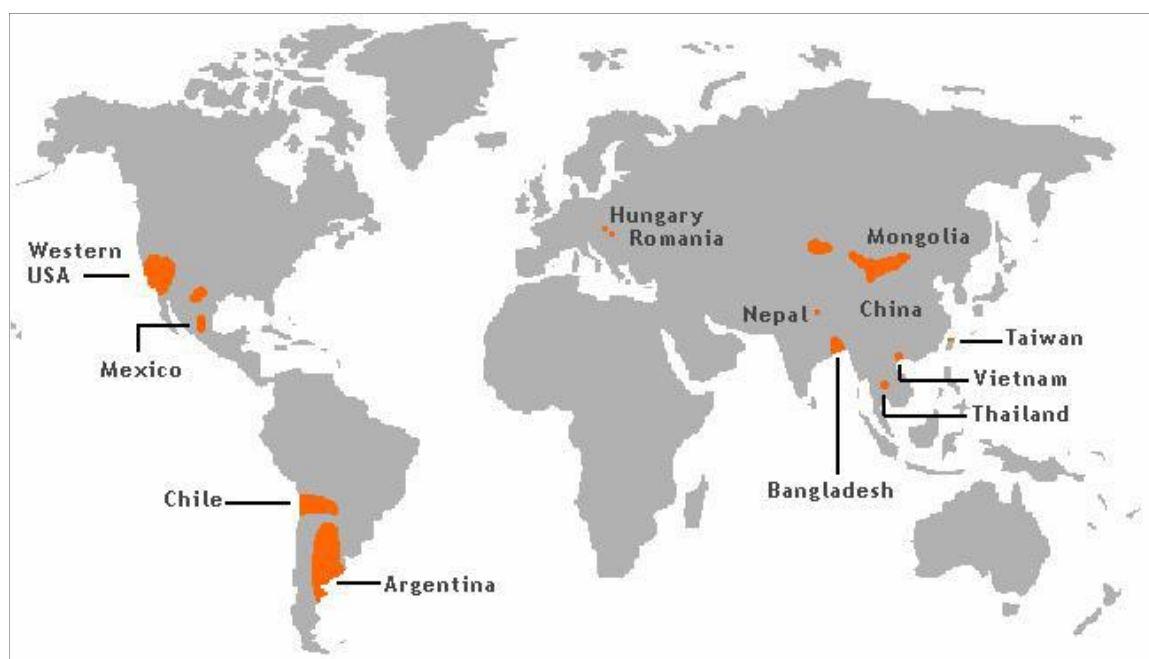
Arsenic was known as a therapeutic agent as early as 400 BC and has been widely used as such since then. From the nineteenth century onward, an inorganic arsenic compound known as Fowler's solution (liquor arsenicalis B.P. 1963; a potassium arsenite solution containing 7.6 g As/L) has been used for the treatment of leukemia, psoriasis, and chronic bronchial asthma, and organic arsenic compounds have been extensively used in the treatment of spirochetal and protozoal disease (Martindale, 1977; NRC, 1999). However, many countries have now banned the use of Fowler's solution. Recently, arsenic trioxide has reportedly been used in the treatment of acute promyelocytic leukemia (Gallagher, 1998; Wang, 2001). Recent studies have reported that As^{3+} produces anticancer effects in leukemic cells (Goussetis *et al.*, 2010; Zheng *et al.*, 2005), and induces autophagy and apoptosis in human fibrosarcoma (Chiu *et al.*, 2010). Altered gene regulation patterns are associated with decreased cell growth in As^{3+} -exposed human liver cancer (Yoo *et al.*, 2009), and breast cancer (Zhang *et al.*, 2011) cells.

The major current uses of arsenic are in pesticides (e.g. lead arsenate, calcium arsenate, and sodium arsenite), herbicides [e.g. monosodium arsenate and cacodylic acid (i.e. DMA)], cotton desiccants (e.g. arsenic acid), and wood preservatives (e.g. copper chromium arsenate). Arsenic is also used as a bronzing or decolorizing agent in the manufacture of glass and in the fabrication of opal glass and enamels. It has also been used in the manufacture of dyestuffs and chemical warfare gases, and is still used in the purification of industrial gases (sulfur removal). Elemental arsenic is used as an additive in the production of several alloys to increase hardness and heat resistance. In the livestock industry, organic arsenical is sometimes added to swine and poultry feed as an antimicrobial medicine. In 1999-2000, approximately 70% of the broiler industry added roxarsone to broiler poultry feed (Garbarino *et al.*, 2003). Recently, artificial gallium arsenide and indium arsenide crystals have become important materials in semiconductors, solar cells, and materials used for space research (Brooks, 2005; IARC, 2006)

1.6 Global Groundwater Arsenic Pollution

Contamination of groundwater, either from anthropogenic or natural sources with several social impacts, has now turned to be a major environmental concern in different parts of the world. Millions of people in several countries are exposed to high levels of As via

intake of As-rich groundwater. Elevated level of As in groundwater has been well documented in Chile, Mexico, China, Argentina, USA, and Hungary (Smedley and Kinniburgh, 2002) as well as in the Indian State of West Bengal, Bangladesh, and Vietnam (Bhattacharya *et al.*, 2002; Bhattacharya *et al.*, 1997; Bhattacharya *et al.*, 2007; Bundschuh *et al.*, 2009; Bhattacharya *et al.*, 2011). About 150 million people around the world are estimated to be affected globally with an increasing prospect as new affected areas are continuously discovered (Ravenscroft and Richards, 2009; Stroud *et al.*, 2011). Applying the WHO provisional guideline for drinking water of 10–50 ppb of As, a population of more than 100 million people worldwide is at risk, and of these more than 45 million people mainly in developing countries from Asia are at risk of being exposed to more than 50 ppb of As, which is the maximum concentration limit in drinking water in most of the countries in Asia (Ravenscroft and Richards, 2009). The major arsenicosis affected areas have been reported in large deltas and/or along major river basins across the world (Fendorf *et al.*, 2010) such as in Paraiba do Sul delta, Brazil (Mirlean *et al.*, 2014), Bengal delta (Mukherjee *et al.*, 2006a; Chakraborti *et al.*, 2010; Shukla *et al.*, 2010), Mekong delta, Cambodia (Sthiannopkao *et al.*, 2008), Danube river basin, Hungary (Nriagu *et al.*, 2007), Hetao river basin, Mongolia (Khan and Ho, 2011), Duero Cenozoic Basin, Spain (Gómez *et al.*, 2006), Zenne river basin, Belgium (Nriagu *et al.*, 2007), and Tulare Lake, USA (Cutler *et al.*, 2013). Groundwater concentration of As has been documented in the literature which reveals a very large range from less than 0.5 to 5000 ppb covering natural As contamination found in more than 70 countries (Ravenscroft *et al.*, 2009). Some of the best-documented and most severe cases of arsenic contaminated groundwater have been found in aquifers in Asia are as follows: (a) *Bangladesh*: most districts including Chandpur, Munshiganj, Noakhali, Satkhira, Chapi Nawabgonj; (b) *India*: West Bengal, Bihar; (c) *China*: Inner Mongolia Xinjiang and Shanxi Province; (d) *Nepal*: Terai region and (e) South America (*Argentina*: Salta province, *Mexico*: Legunea region) (Kibria *et al.*, 2010; Alaerts and Khouri, 2002; **Figure 1.2, Table 1.1**). In contaminated countries it shows a very large range from 1 to 5000 µg/L (**Table 1.1**)



Source: Wikipedia

Figure 1.2 Groundwater arsenic contamination areas in the world

Table 1.1 Worldwide Occurrences of Arsenic in Groundwater

Country	Source	Range in $\mu\text{g/L}$ or ppb	Estimated population exposed
Argentina	Natural	100-2000	200,000
Bangladesh	Natural – deriving from geological strata	<1-4700	57 millions exposed to $\text{As} > 10 \mu\text{g/L}$ and 35 millions exposed to $\text{As} > 50 \mu\text{g/L}$
Chile	Natural – associated with quaternary volcanism	900-1040	437,000
China	Natural, in reducing environment	NA	5.6 million
Ghana	Mining activities	NA	100,000
Hungary and Romania	Natural	2-176	400,000
India (West Bengal)	Natural – deriving from geological strata	<10-3700	Over 5 millions exposed to $\text{As} > 50 \mu\text{g/L}$; 300,000 suffering from arsenicosis
Mexico	Natural	1-5000	400,000
Nepal	Natural	<10-34	550,000 exposed to $\text{As} > 50 \mu\text{g/L}$ and 3.19 million exposed to $\text{As} > 10 \mu\text{g/L}$
Taiwan	Natural	10-1820	10,000
Thailand	Mining activities	1-5000	15,000
USA	Natural, geothermal and mining related sources	Varied	13 million exposed to $\text{As} = 10 \mu\text{g/L}$
Vietnam	Natural	1-3100	>1 million

Source: Kibria *et al.*, 2010; Garelick *et al.*, 2008

1.7 Groundwater Arsenic Pollution in the South-East Asia Excluding Bangladesh

Cambodia

During 2000, the government of Cambodia, with support from the WHO, conducted a survey of drinking water quality of water resources throughout the country (Berg *et al.*, 2007). Of 88 samples analyzed, 9% of the samples contained As above 10 µg/L. Various NGOs have tested about 5,000 tube-wells in rural areas of Cambodia by field testing kit and 20% samples had As levels above 50 µg/L and half were above 10 µg/L (Berg *et al.*, 2006). Buschmann *et al.* (2007) reported As contamination of groundwater in Cambodia with 350 people per km² are potentially exposed to chronic As poisoning. The concentration of As in drinking water ranged from 1 to 1,340 µg/L with an average of 163 µg/L (Buschmann *et al.*, 2007). Arsenic release from Holocene sediments between the Bassac and Mekong Rivers is most likely caused by reductive dissolution of metal oxides (Buschmann *et al.*, 2007). Berg *et al.* (2007) also reported that concentration of As in groundwater ranged from 1 to 1,610 µg/L with an average of 217 µg/L.

China

The presence of arsenicosis from drinking water has been reported in China since the 1980s (World Bank Policy Report 2005). Arsenic contamination in drinking water has been detected in Xinxiang, Shanxi, and parts of Inner Mongolia provinces. Groundwater conditions in the As-affected areas appear to be strongly reducing (World Bank Policy Report 2005).

India

Arsenic contamination has been reported in groundwater from several states of India. Arsenic contamination in the groundwater of West Bengal was first reported in the late 1980s (Bhattacharya *et al.*, 1997; Chakraborti *et al.*, 2004). It was estimated that around 6.5 million people had been ingesting water with As concentration exceeding

50 µg/L (Chakraborti *et al.*, 2004). It was reported that about 48% of 140,000 hand tube-well water samples analyzed from West Bengal exceeded 10 µg As/L and about 24% had As exceeding 50 µg/L (Mukherjee *et al.*, 2006a). Nine out of 19 districts (3,500 villages from 90 blocks) are affected by As concentrations above 50 µg/L (Mukherjee *et al.*,

2006a). The source of As in West Bengal is geogenic. Arsenic is present in the alluvial sediments of the Gangetic Delta. The mechanism and cause of As release and mobilization in groundwater has been explained by a series of plausible hypothesis such as oxidation of pyrite (Das *et al.*, 1996; Roy Chowdhury *et al.*, 1999; Chakraborti *et al.*, 2001), reduction of Fe-oxyhydroxides (Bhattacharya *et al.*, 1997; Nickson *et al.*, 1998, 2000), carbon reduction (Harvey *et al.*, 2002), and microbial reduction (Islam *et al.*, 2004; Akai *et al.*, 2004). Arsenic contamination in groundwater was first reported from the state of Bihar in 2002 (Chakraborti *et al.*, 2003), indicating that about 39% of 9,500 samples analyzed contained As above 10 µg/L and that 23% contained greater than 50 µg/L (Mukherjee *et al.*, 2006a). Of 4,513 persons screened, 525 (5.5%) were found with various types of As-related skin manifestations (Mukherjee *et al.*, 2006a). Arsenic contamination of groundwater in Uttar Pradesh was first discovered in late 2003 (Chakraborti *et al.*, 2004; Ahamed *et al.*, 2006; Mukherjee *et al.*, 2006a). 3,901 hand tubewell water samples had been analyzed from Uttar Pradesh. Of these, 46.6% contained As greater than 10 µg/L and 30.5% contained As exceeding 50 µg/L. These surveys demonstrated that 153 (15.5%) of 989 people screened had arsenical skin lesions (Mukherjee *et al.*, 2006a). Arsenic contamination of groundwater in Jharkhand state was reported in December 2003 (Bhattacharjee *et al.*, 2005; Mukherjee *et al.*, 2006a). Water samples taken from 1,024 tube-wells from 17 villages of Jharkhand state showed that 30% had levels of As exceeding 10 µg/L and 19.4% exceeded 50 µg/L (Mukherjee *et al.*, 2006a). This study also reported that 87 people were registered with arsenical skin lesions out of 320 screened (Mukherjee *et al.*, 2006a). Arsenic contamination in groundwater of Rajnandangaon district of Chhattisgarh was first identified in 1999 (Chakraborti *et al.*, 1999). The range of As in groundwater was <10–880 µg/L. The number of people at risk from As toxicity in this region was 10,000 (Pandey *et al.*, 1999) Chakraborti *et al.* (2008) reported that, out of 628 water samples analyzed from Manipur state, 63% samples contained As exceeded 10 µg/L and 40% exceeded 50 µg/L. In Assam, the highest levels of As were detected in Jorhat, Dhemaji, Golaghat, and Lakhimpur districts (Mukherjee *et al.*, 2006a). In Tripura, As was found in parts of West Tripura, north Tripura, and Dhalai districts in the range of 65–444 µg/L. In Arunachal Pradesh, As was detected in the Dibang district. In Nagaland, As was also found in seven locations in Mokokchung and five locations in Mon district. Although As contents beyond the WHO guideline value were found in a large number of groundwater samples, no arsenicosis patients had been

reported up to 2006 (Mukherjee *et al.*, 2006b). The sediment in the northern area contains a high amount of clay and organic matters, which may retain and release As in groundwater aquifers (Mukherjee *et al.*, 2006b).

Indonesia (Sumatra)

Groundwater investigations (Winkel *et al.*, 2008) in the lowlands of the south of the Indonesian island of Sumatra had indicated that an area of nearly 100,000 km² is vulnerable to As contamination, under reducing aquifer conditions. Based on analysis of groundwater samples from 102 randomly selected wells placed in the Holocene swamp deposits showed As concentrations were found to be above the WHO drinking water guideline (>10 µg/L) with a maximum of 65 µg/L.

Lao PDR

UNICEF has tested groundwater from wells in various regions (Attapeu, Savannakhet, Champassak, Saravan, Sekong, Khammuane, and Bolikamxay) of the Lao People's Democratic Republic (World Bank Policy Report 2005). Analysis of 200 water samples indicated that some samples had As concentrations above 10 µg/L (Fengthong *et al.*, 2002). Only one sample had As concentration of 112 µg/L, which was more than two times the Bangladesh threshold concentration of 50 µg/L.

Myanmar

The United Nations Development Programme (UNDP) and the United Nations Centre for Human Settlements (UNCHS) carried out preliminary testing of As in groundwater of Myanmar. Four percent of the samples out of 125 analyzed from Nyaungshwe in Shan state contained As above 50 µg/L (UNDPUNCHS 2001). Analysis of 1,912 shallow tubewells in four townships in the Ayeyarwaddy Division showed that 22% of samples contained As above 50 µg/L (World Bank Policy Report 2005). A test by the Water Resources Utilization Department (WRUD) showed that 15% of groundwater samples exceeded 50 µg/L out of 8,937 tube-wells analyzed. Merck field test kit was used in most of the surveys; the accuracy of the results is uncertain but likely to be limited (World Bank Policy Report 2005).

Nepal

The presence of As in groundwater is now recognized as a public health threat in Nepal (Tandukar *et al.*, 2001, 2006; Shrestha *et al.*, 2003). By 2003, analysis of 25,000 water samples indicated that 31% of the samples contained As above 10 µg/L and 8% contained above 50 µg/L. The highest concentration (2,620 µg/L) of As was detected in a well from Rupandehi district (Tandukar *et al.*, 2001). Based on their findings Tandukar *et al.* (2001) conclude that approximately 500,000 villagers from Terai region were potentially at risk of As poisoning. In the Terai region, deep tube-wells also contained low concentrations of As (World Bank Policy Report 2005). Low concentrations of As has been detected from 522 irrigation wells in the depth range >40–50 m (World Bank Policy Report 2005).

Pakistan

A total of 364 samples were analyzed from different district including Jhelum, Chakwal, Sargodha, and Gujarat. About 90% of the samples had As concentration less than 10 µg/L, and 2% exceeded 50 µg/L (Iqbal 2001). It was reported that 58% of the samples contained As above 10 µg/L, with the highest concentration detected being 906 µg/L, based on 49 groundwater samples analyzed from the Muzaffargarh district of Pakistan (Nickson *et al.*, 2005).

Taiwan

Arsenic contamination of groundwater in Taiwan was discovered in the early 1960s (Yeh, 1963). In the area of southwestern Taiwan, levels of As in wells range from 240 to 960 µg/L (Blackwell *et al.*, 1961). Chen *et al.* (1962) reported that the range of As concentrations in 34 artesian wells was 350–1,100 µg/L. Yeh (1963) reported that the range of As concentration was 340–900 µg/L, based on analysis of 11 wells. Lo (1975) reported that 18.7% wells had As above 50 µg/L, based on a nationwide survey of As in drinking water from 83,656 wells in 314 precincts and townships. Analysis of 3,901 tube-wells in 18 villages of four townships in Taiwan showed that the range of As in water was detected as <0.15– 3,590 µg/L (Chiou *et al.*, 2001). Tsai *et al.* (1998) reported 7,418 cases of hyperpigmentation, 2,868 of keratosis, and 360 of blackfoot diseases, based on their epidemiological study from As endemic areas of Taiwan. Diseases such as cancer (Chen *et al.*, 1988; Tsai *et al.*, 1998; Bates *et al.*, 1992), diabetes mellitus (Chen *et al.*, 2007), cardiovascular disease (Wang *et al.*, 2007), hypertension (Chen *et al.*, 2007), and obstetric problems (Yang *et al.*, 2003) have been reported from As-endemic areas of Taiwan.

Vietnam

High levels of As have been detected in the groundwater of Vietnam (Berg *et al.*, 2001, 2007; Agusa *et al.*, 2006; Shinkai *et al.*, 2007). Berg *et al.* (2001) reported concentrations of As in the groundwater of the Red River Delta that exceeded the WHO recommended value for drinking water (10 µg/L). The average As concentration was reported as 159 µg/L (n = 196) with a range of 1–3,050 µg/L (Berg *et al.*, 2001). It was reported that approximately 10 million people in the Red River Delta and 0.5–1.0 million in the Mekong River Delta were potentially at risk from As toxicity (Berg *et al.*, 2007). Agusa *et al.* (2006) recently reported the concentration of As in groundwater ranged from <0.1 to 330 µg/L, with approximately 40% of these above the WHO guideline value. In a study, it was reported that the concentration of As in groundwater of the Mekong River Delta of Vietnam varied from 0.9 to 321 µg/L and that 27% samples contained As above 10 µg/L (Shinkai *et al.*, 2007).

1.8 Arsenic Pollution in Bangladesh

Arsenic contamination of groundwater in Bangladesh was first reported in 1993, followed by official reporting of arsenicosis patients in 1994 (Khan *et al.*, 1997). It is estimated that more than 95% of the people in Bangladesh drink tube-well water and more than 50 million people are estimated to be ingesting arsenic-contaminated water (Khan *et al.*, 1997; BGS, 199; BGS, 2001; DGHS, 2012). Approximately 27% of shallow (<150 meter deep) wells in Bangladesh contain more than 50 µg/L arsenic (BGS, 2001). In Bangladesh, concentrations of arsenic in groundwater as high as 4.0 mg/L have been reported from Chatkhil of Noakhali district (BGS, 2001; BGS, 1999). The worst-affected area is in the southeast of Bangladesh, whereas in some districts more than 90% of tube-wells are affected (BGS, 2001; NAMIC, 2004; GOB, 2002). Arsenic contamination of ground water tapped through tube-wells had been reported from 62 out of 64 districts of Bangladesh (DGHS, 2012). In surveys covering 57,482 villages of 2934 unions in 270 upazillas, 29.12% of the 4,946,933 tube-wells were found to be arsenic contaminated. About 15% (8540) of the villages had more than 80% of their tube-wells contaminated and these villages are identified as “hot spots.” Among 66,034,962 individuals residing in 12,001,665 households in these upazillas, a total of 38,430 individuals had been reported as arsenicosis patients (NAMIC, 2004). Many of the health problems known to be related to arsenic toxicity have been reported in Bangladesh (Khan *et al.*, 1997; Ahmed *et al.*,

1997; Ahmed *et al.*, 2003; Ahmed *et al.*, 2001; Ahmed *et al.*, 1998; Sikder *et al.*, 1999; Tondel *et al.*, 1999; Rahman *et al.*, 1998; Milton *et al.*, 2001).

In the early 1970s, when people in Bangladesh mainly relied on surface water (ponds and rivers) and subsurface (dug-well) water sources, diarrheal diseases and cholera were widely prevalent. This had prompted the search for a microbially safe water source. This search had led to mass use of tube-wells. With the help of a hand-pump and pipes with strainers sunk a few meters into the ground, tube-wells yielded water that was reasonably safe from the point of microbial contamination that causes diarrheal diseases. Moreover, this means of obtaining water became relatively cheap and the water was easy to collect, and in subsequent years hundreds of thousands of tube-wells were installed on the basis of personal, governmental, and NGO initiatives. The tube-well installation initiative to supply safe water in Bangladesh was almost a total success, and increased the percentage of people with access to safe water from 77.6% in 1991 to 91.3% in 1994. Though the tube-well installation initiative provided safe water, a new problem regarding groundwater contamination surfaced. Tube-wells were found to yield water containing arsenic at levels not acceptable for consumption even by the Bangladesh water quality standard. The maximum allowable concentration of arsenic in drinking water in Bangladesh is 0.05 mg/L, which is five times higher than the World Health Organization provisional guideline value (0.01 mg/L) for arsenic in drinking water (Khan *et al.*, 1997; BGS, 2001; GOB, 2002; Ahmed *et al.*, 2003; WHO, 1996).

In 1994 the Department of Occupational and Environmental Health, National Institute of Preventive Medicine (NIPSOM), identified eight arsenicosis patients in Bangladesh (Khan *et al.*, 1997; DGHS, 2001). The contaminated tube-wells and patients were detected in the western part of Bangladesh in the village of Chamagram of Nawabganj District. Surveys for detection of arsenic in tube-wells throughout the country showed a widespread distribution of contaminated tube-wells. Regarding identification of arsenicosis patients in Bangladesh, up to 1997, 1625 arsenicosis patients had been identified and in 2008 the number of identified arsenicosis patients increased to 24,389. In 2012, 56,758 arsenicosis patients were identified by the Director General of Health Services (DGHS), Bangladesh (Khan *et al.*, 1997; DGHS, 2012). The WHO has predicted that 200,000–270,000 people will die of cancer from drinking As contaminated water in Bangladesh alone (WHO, 2001).

1.8.1 Geological Aspect of Groundwater Arsenic Contamination in Bangladesh

Groundwater is available at very shallow depths all over Bangladesh where the major aquifers are the Holocene alluviums and fan deposits and Pliocene fluvio-deltaic (Dupi Tila) sediments. Mio-Pliocene Tipam sands form minor aquifers in the hilly areas. The aquifers are highly transmissive and generally multilayered. The aquifer conditions vary from unconfined to leaky confined in the shallow alluvial deposits (Holocene alluviums) and are confined in the Dupi Tila and in deeper alluvial deposits. In the Ganges delta area the thickness of the recent sediment is higher and the Dupi Tila sandstone lies at greater depths. In the southern part (often called coastal plain) the thickness of the alluvial deposit is highest compared to other parts of the country. The aquifer systems in Bangladesh are geologically controlled and depend on the sedimentary characteristics, depositional environments, and other related parameters. Arsenic contamination in groundwater is reported to be greater in the Holocene alluviums and fan deposit areas of Bangladesh (BGS, 2001; Ahmed *et al.*, 2003; Umitsu M, 1993).

The Bengal Basin is one of the largest sedimentary basins in the world. Bangladesh occupies most of the present-day delta in the Bengal Basin. The Bengal Basin is bounded on the west and northwest by the Rajmahal Hills (Trap). The northeast is bounded by the Garo, Khasi, and Jaintia hills (west to east), which stretch for about 97 km from north to south and 240 km from east to west. In the far northeast, Shillong or Assam Plateau acts as a boundary. Generally, the Bengal Delta is often referred to as the “Ganges-Brahmaputra-Meghna Delta” (GBMD), which is still active. It is reported that sediments from the Himalayas, adjoining India and Burma, had contributed to the development of the Bengal Basin, and the Ganges-Brahmaputra-Meghna river system in addition to tectonic activity, climatic changes, and accompanying sea level changes had played a significant role. The Ganga-Brahmaputra river system had mainly contributed to the buildup of the Bengal Fan. This river system had carried enormous volumes of sediments from the Himalayan belt. The sediments are derived from the upland Himalayan catchments, the Indo-Burman ranges, and from basement complexes of the northern and western parts of West Bengal (Rajmahal Hills, Choto Nagpur Plateau, Shillong Plateau). Along with sediments from those areas many weathered minerals including arsenic had entered the basin and had been deposited in the delta over thousands of years. The high arsenic content areas are found in the catchments of the Ganges, Brahmaputra, and

Meghna rivers (Goodberg *et al.*, 2003; Goodberg *et al.*, 2000; Acharyya, 2005; Mukherjee *et al.*, 2009; Alam *et al.*, 2003; Acharyya, 2007; Ahmed *et al.*, 2004; Garzanti *et al.*, 2004; Uddin and Lundberg 1998).

A variety of anthropogenic sources have also been proposed as the cause of particular occurrences of elevated arsenic concentration in groundwater in the Bengal Basin, including industrial pollution and the use of agrochemicals and wood preservatives; only the mineralogical source within the sediments of the Bengal Basin is consistent with the full regional extent and magnitude of arsenic occurrence as observed. It is now widely accepted that arsenic in ground water of the region has a source within the sediments of the Bengal Basin originating possibly from the Himalayan region; it is of a natural origin (geogenic in nature) mainly from the GBM river system, especially in the Holocene period (Ahmed *et al.*, 2004; Garzanti *et al.*, 2004; Uddin and Lundberg, 1998; Acharyya *et al.*, 1999; Nickson *et al.*, 2000; Lowry, 2005; Clift, 2005).

1.8.2 Causes of Groundwater Arsenic Contamination in Bangladesh

The reasons behind leaching arsenic in the ground water in Bangladesh are not clear. Three mechanisms have been proposed to explain arsenic pollution of ground water in the “Ganges-Brahmaputra-Meghna Delta” (GBMD) (BGS, 2001; Acharyya *et al.*, 1999; Nickson *et al.*, 2000; Mallick and Rajagopal, 1996; Chowdhury *et al.*, 1998; Das *et al.*, 1996; Nickson *et al.*, 1998; McArthur *et al.*, 2001; Mukherjee and Bhattacharya, 2001):

1. Pyrite oxidation: It has been proposed that arsenic is present as arsenical pyrite in the alluvial sediments. Due to heavy withdrawal of ground water through shallow and deep tube-wells for irrigation and domestic purposes, a vacuum is created that leads to entry of atmospheric oxygen into the aquifer subsequent to aquifer drawdown, which in turn leads to oxidation of arsenical pyrite and as a result arsenic is released.

2. Anion (competitive) exchange of sorbed arsenic with phosphate from fertilizer: According to this hypothesis arsenic anions sorbed to aquifer minerals are displaced into solution by competitive exchange of phosphate anions derived from over application of fertilizer to surface soils. Phosphate derived from excessive use of phosphate fertilizer, from latrines, and from the fermentation/decay of buried peat deposits and other natural organic materials may leach into the aquifer and cause

displacement of arsenic from sorption sites on aquifer minerals as a result of competitive (anion) exchange, resulting in arsenic pollution in the aquifer. However, it has been suggested that competitive exchange with phosphate generated *in situ* may contribute to arsenic pollution but this contribution is believed to be small.

3. Reductive dissolution of FeOOH and release of sorbed arsenic to ground water:

The most widely accepted hypothesis is that, under anoxic conditions, reduction of iron oxyhydroxides (FeOOH) takes place, which results in release of sorbed arsenic to solution. Reduction of FeOOH is driven by microbial metabolism of organic matter.

1.8.3 Arsenic Distribution in Groundwater of Bangladesh

A number of surveys have been carried out over the last few years on As in groundwater of Bangladesh. The British Geological Survey (BGS) and the Department of Public Health and Engineering (DPHE), Bangladesh conducted a survey of 3,534 tube-well water samples covering the entire area of Bangladesh (BGS & DPHE 2001). The major screening program of tube-wells was conducted by the Bangladesh As Mitigation Water Supply Project (BAMWSP) and a number of nongovernmental organizations (NGOs) and international agencies including the Japan International Cooperation Agency, the Asia As Network, UNICEF, World Vision International, and the Watson Partnership (World Bank Policy Report 2005).

Kinniburgh & Smedley (2001) reported that naturally occurring high levels of As in the groundwater of Bangladesh where at least 30 million people are exposed to As-contaminated water of 50 µg/L. Arsenic has been detected in 60 of the 64 districts in the country above both the World Health Organization (WHO) threshold value (10 µg/L) and Bangladesh's standard of 50 µg/L (Smedley & Kinniburgh 2002; Bhattacharya *et al.*, 2002; Ahmed *et al.*, 2004).

Chakraborti *et al.* (2004) reported that about 43% of more than 50,000 hand tube-well water samples analyzed from all 64 districts of Bangladesh have As concentration above 10 µg/L, and 27% have As concentration above 50 µg/L.

A nationwide sample survey of tube-wells, mostly from the Department of Public Health Engineering (DPHE)-installed tube-wells located in 433 upazillas (eight samples per upazilla) from 61 districts (excluding the three hill districts), found that the arsenic

concentrations ranged from less than 0.00025 to 1.670 mg/L. The median and mean arsenic concentrations in the samples were 0.004 mg/L and 0.055 mg/L respectively. Among the surveyed “shallow” tube-wells (wells less than 150 m deep), 27% of them exceeded the Bangladesh standard for arsenic in drinking water (0.05 mg/L) and 1% “deep” wells (more than 150 m deep) exceeded the Bangladesh standard. About 9% of the tube-wells exceeded 0.20 mg/L, 1.8% exceeded 0.50 mg/L, and 0.1% exceeded 1.0 mg/L (Khan *et al.*, 1997; BGS, 2001; Karim, 2000).

Deep wells tapping depths of more than 150–200 m have, almost invariably, low arsenic concentrations, less than 5 µg/L and usually less than 0.5 µg/L. Wells from the older Plio-Pleistocene sediments of the Barind and Madhupur Tracts have low arsenic concentrations. The worst-affected areas of Bangladesh are south and east of Dhaka where in some villages more than 90% of the wells have arsenic concentrations above 50 µg/L. The ground waters are predominantly reducing as evidenced by geo-chemical analysis. Arsenic speciation studies have revealed a large range in the relative proportions of dissolved arsenate and arsenite. The modal proportion of arsenite appears to be between 50 and 60% of the total arsenic. Reducing arsenic-rich ground waters from Bangladesh have $\text{As}^{\text{III}}/\text{AsT}$ ratios varying between 0.1 and 0.9 but are typically around 0.5–0.6 (BGS, 2001; BGS, 1999; DPHE, 1999; Acharyya, 1997).

According to British Geological Survey (1999), 27% of the shallow hand tube-wells have been arsenic concentration exceeding 0.05 mg /L; in acute arsenic problem areas more than 75% of shallow tube-wells are contaminated of arsenic. Arsenic problem alone has reduced the national safe water supply coverage by about 15-25%. The most arsenic contaminated part of the country lies in the southern regions covering then districts Chandpur, Laksmipur, Comilla and Noakhali of Chittagonj division; Faridpur, Gopalganj, Munshiganj, Madaripur, Narayanganj, Shariatpur and Sherpur of Dhaka division; Jessor, Kushtia, Meherpur, Satkhira of Khulna division are the most arsenic contaminated areas of Bangladesh. Contaminated has also been found in south-west, north part of the west, north-east and north-central region. In the north-west region and south-west regions contaminated and uncontaminated wells are located close to each other whereas, in the south-east, particularly in Chandpur almost all the shallow wells are contaminated (**Figure 1.3**).

Testing of all wells using field kits conducted by BRAC, UNICEF and BAMWSP shows variations in the percentage of contamination wells in different upazila e.g., almost 100% contaminated in Hajiganj in Chandpur Districts compared to almost non contaminated in Porsha in Naogaon district (Ahmed, 2003). There is a large variability in the concentration ranges of arsenic within areas. There are variations in concentration ranges even in village scale where areas is low and high arsenic regions can occur as reported from Samta village of Sarsa thana of Jessor district (AAN, 1999).

Very high concentration can occur in a restricted area as found in Nawabganj sadar of Chapai Nawabganj district, Charuppur of Iswardi of Pabna district, Gopalganj sadar of Gopalganj district, Kachua, Hajiganj and Faridganj of Chandpur district, Ramganj of Laksmipur district, Begumganj of Noakhali districts, Kushtia sadar of Kushtia district, Sonargaon of Narayanganj district, Shreenagar of Munshiganj district and Samta of Sharsha of Jessor district etc (DPHE/BGS, 2000). These types of occurrence can be term as hot spots. Sonargaon is severely contaminated upazila under Narayanganj district where all the wells have been tested by BRAC under a UNICEF/DPHE programme. The union wise distribution of arsenic contaminated wells show that in wells are contaminated (up to 89%) where as in two unions only a small number in wells (>10%) are contaminated (BRAC, 2000). The Dupi Tila sediment underlie these union and most of of the wells are developed there. The study conducted by British Geological Survey (BGS), Department of public health and engineering (DPHE) and Mott McDonald Limited (MML) in two phases examined 3534 distributed water sample from 61 districts except three hilly districts (BGS, DPHE and MML,1999) with an average 58 samples per district and 8 samples per upazila. The study showed that 42% of all tube-wells sample exceeded 10 µg/L and 25% exceeded 50 µg/L arsenic concentration. When only shallow tube-wells were considered 46 and 27% exceeded 10 and 50µg/L, respectively. In case of deep tube-well sample arsenic contents of only 5% exceeded 10µg/L and 15% exceeded 50 µg/L.

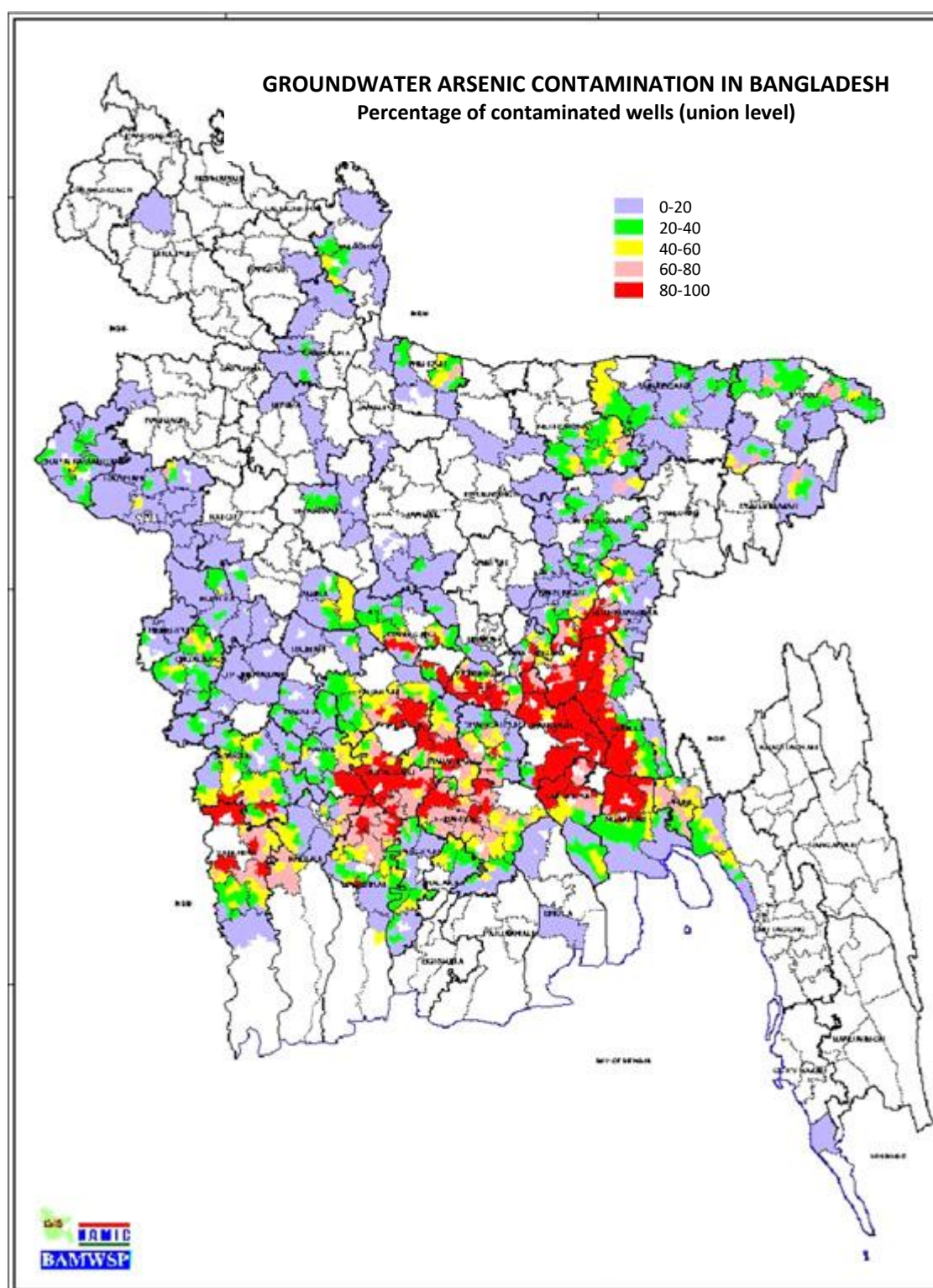


Figure 1.3 Distribution of arsenic contaminated tube-wells in Bangladesh

[Reproduced from National Arsenic Mitigation Information Centre (NAMIC) (2004). National screening program data, Bangladesh arsenic mitigation water supply plan. Department of Public Health Engineering, Bangladesh. (Source: Ahmed and Ahmed, 2014)]

1.9 Arsenic in Irrigation Water of Bangladesh

Widespread arsenic (As) pollution of shallow tube well (STW) irrigation water and increasing accumulation of As in soils have become a major threat to the production of rice, the staple food crop in Bangladesh (Hossain *et al.*, 2008; Panaullah *et al.*, 2009). The STWs, being the almost exclusive source of irrigation water, play a key role in the production of dry season winter rice (locally called Boro rice), which accounts for 50% of the total annual rice output in this country. Thus, Boro rice is an important determinant of food security or insecurity in Bangladesh, where due to a mounting population pressure, the balance between food demand and supply remains very delicate. Much of the groundwater aquifer of the major rice producing regions of the country is contaminated with As (Smedley *et al.*, 2002). Arsenic in irrigation water from contaminated shallow tube-wells is being transferred to soils, where it is potentially available for uptake by plants (Panaullah *et al.*, 2009; Islam *et al.*, 2007).

Boro rice accounts for about 55% of the total rice production in Bangladesh, and the irrigation water needed for its cultivation is mainly extracted from shallow tube wells (STWs) (MoA 2005). Many STWs deliver As concentrations above 50 µg/ L. About 86% of the total groundwater withdrawn is used for irrigating dry season crops, mainly Boro rice. Irrigation-water borne As from contaminated STWs accumulates in the soil (Panaullah *et al.*, 2003; Islam *et al.*, 2007).

Huq *et al.* (2003, 2006) stated that agriculture depends mainly on groundwater for irrigation in many areas of the Bengal basin, and in 40% of the net cultivable area in Bangladesh As contaminated groundwater represents the main water source for irrigation.

Ali *et al.* (2003a) reported that a huge amount of As is transferred every year from the contaminated aquifer to the surface water–soil–plant system. The As reaching the soil by irrigation could accumulate in the soil solid phase, could be released to the deep or surface water bodies, could be metabolized and possibly volatilized by microorganisms, and could be taken up by crops from the soil–water system.

Hossain *et al.* (2003) stated that dry-season irrigation with groundwater has enabled the expansion of rice production, greatly improving food security and economic opportunity for farm households in Bangladesh. But, extensive use of arsenic-contaminated groundwater for irrigation during the dry season threatens these benefits.

Following years of irrigation with groundwater, soil arsenic concentrations have risen, and arsenic is now transferring into rice at concentrations sufficient to decrease yields and create dangerous levels of arsenic in rice grains (Abedin *et al.*, 2002a; Brammer and Ravenscroft, 2009; Meharg and Rahman, 2003; Meharg *et al.*, 2009; Stroud *et al.*, 2011a; Williams *et al.*, 2006). Due to the large volumes of irrigation water required, as well as the cost of highly technical treatment options, there are currently no practical methods for large-scale removal of arsenic from these systems (Brammer, 2009; Brammer and Ravenscroft, 2009).

BBS (1996) reported that about 33% of total arable land of the country is under irrigation facilities. It is estimated that 83% of the total irrigated areas of Bangladesh are used for rice cultivation (Dey *et al.*, 1996). Irrigation is principally performed by a large number of shallow tube-wells (STWs) and deep tube-wells (DTWs). The water of STWs contained very high level of arsenic (Nickson *et al.*, 2000; McLellan 2002; van Geen *et al.*, 2003; Alam *et al.*, 2002). The use of arsenic contaminated underground water in irrigation for a prolong period of time may increase the concentrations of arsenic in agricultural soil and crops (Ullah 1998; Imamul Huq *et al.*, 2003; Rahman *et al.*, 2007b).

Meharg and Rahman (2003) showed that arsenic concentrations were higher in agricultural soils of those areas where shallow tube wells (STWs) have been in operation for longer period of time and arsenic contaminated underground water from those STWs have been irrigated to the crop fields in Bangladesh.

Panaullah *et al.* (2009) stated that 70% of arable land is irrigated with shallow tube wells which pump out many millions of cubic meters of As-contaminated groundwater every year for irrigating winter rice. It is estimated that over 1000 tonnes of As are transferred to paddy fields every year in Bangladesh through irrigation (Ali *et al.*, 2003), which has been linked to elevated concentrations of As in rice (Lu *et al.*, 2009).

Recently, it has become apparent that arsenic-contaminated irrigation water is adding significant amount of arsenic in the topsoil and in rice, which pose serious threat to sustainable rice cultivation in Bangladesh (Brammer and Ravenscroft, 2009; Dittmar *et al.*, 2010; Khan *et al.*, 2009; Khan *et al.*, 2010a; Khan *et al.*, 2010b; Meharg and Rahman, 2003). Since the agroecological and hydrogeological conditions of the S and SE Asian

countries are broadly similar, irrigation of arsenic-contaminated groundwater is supposed to produce similar effects on paddy rice of this region. In addition, paddy rice is considered to be one of the major and potential exposure sources of arsenic for humans (Meharg and Rahman, 2003; Mondal and Polya, 2008; Pillai *et al.*, 2010; Rahman *et al.*, 2008; Singh *et al.*, 2010; Tuli *et al.*, 2010; Williams *et al.*, 2006; Zavala and Duxbury, 2008) because of its increasing deposition in the topsoil from irrigation water and its subsequent uptake in rice grain (Dittmar *et al.*, 2010).

Irrigation with arsenic-contaminated groundwater may particularly affect rice cultivation in terms of production and contamination. There may be two main reasons for this — i) a large amount of underground water containing high level of arsenic has been irrigated for rice cultivation in most parts of S and SE Asia during dry season and ii) rice is the crop that is most susceptible to arsenic toxicity (Brammer and Ravenscroft, 2009). Due to the decrease of rainfall in this region, even in monsoon season, the dependency on groundwater for rice cultivation is expected to be increased in the coming years in order to increase crop production to meet the demands of the increasing population. This practice will increase additional arsenic deposition in topsoil.

Roberts *et al.* (2007) reported that arsenic contents in topsoil in Bangladesh have increased significantly over the last 15 years because of irrigation with arsenic rich groundwater. Other studies showed that arsenic concentrations remain unchanged at the start of two successive irrigation seasons suggesting that arsenic added during the first irrigation season had been leached by floodwater during the following monsoon season (Dittmar *et al.*, 2007). Thus, the rate of arsenic deposition from contaminated irrigation water would be higher in flat terrain soil than that in floodland soil. Another important concern regarding arsenic deposition in paddy soil is whether all arsenic delivered by the tube-wells is reached and deposited throughout the fields equally. In addition, how arsenic in irrigation water and soil contributes to its uptake in rice plant and grain is also important concern.

Several research groups postulated that irrigation pumping may flush arsenic from aquifers (e.g. Harvey *et al.*, 2003; McArthur *et al.*, 2004). Harvey *et al.* (2003) support this contention by comparing concentrations sampled from irrigation wells to concentrations from drinking water wells to show that irrigation wells, which flush much

greater quantities of water, have significantly lower arsenic concentrations. At a national scale, Ali (2003) estimated that each year groundwater irrigation removes from aquifers, and then applies to fields, about one million kilograms of arsenic. On the other hand, some evidence suggests that arsenic concentrations may rise after pumping commences.

Kinniburgh *et al.* (2003), van Geen *et al.* (2003) and McArthur *et al.* (2004) all provided strong statistical evidence that arsenic concentrations in domestic well water correlate to the age of the well, suggesting that arsenic concentrations may rise after a well is installed, perhaps because irrigation wells, which have much greater effects on the local groundwater system, are installed in the region at the same time as the domestic wells where arsenic is measured.

Alam and Sattar (2000), Das *et al.* (2008), Norra *et al.* (2005b), and Sarkar *et al.* (2012) all found a direct correlation between irrigating with arsenic contaminated water and elevated soil arsenic levels.

The extensive use of groundwater in irrigation of rice has resulted in elevated As in soils and crops. A study was undertaken to determine As concentrations in groundwater, soils, and crops in 16 districts of southwestern Bangladesh. Groundwater samples were collected from shallow-tube and hand-tube wells (STW and HTW) used for irrigation and drinking water. Soil and rice plants were sampled from the command area of the tube wells. Arsenic concentrations in 59% of STW samples exceeded 50 µg As /L. A positive relationship was observed between groundwater and soil As, implying that soil As level increases as a result of irrigation with contaminated water (Rahman *et al.*, 2010).

BBS (2004) reported the development of irrigated winter (Boro) season rice since the 1970s which accounts for 55% of the total rice production and has enabled the country to shift from chronic food shortages to self sufficiency by the mid-1990's (Baffes and Gautam 1996). About 70% of the total arable land area is irrigated from shallow tubewells (STW). Build up of As in soil associated with the use of As contaminated irrigation water has been shown to lead to elevated levels of As in paddy soil and soil solution (Meharg and Rahman 2003; Van Geen *et al.*, 2006; Dittmar *et al.*, 2007).

Hossain *et al.* (2003) observed that groundwater is widely used for irrigation during dry season (December to April), particularly for growing of the dry-season rice called boro which requires about 1 m of irrigation water each year.

BADC (2005) reported that a total of 925,152 shallow and 24,718 deep tube-wells were used for irrigation during the 2004 dry season and groundwater irrigation covered about 75% of the total irrigated area. Boro cultivation and irrigation have together increased since 1970, and from 1980 until present, the area under groundwater irrigation has increased by almost an order of magnitude (Harvey *et al.*, 2005).

MoA (2004) reported that during 2003 dry season, about 87% of the total irrigated area of about 4 million hectare (about 28% of the total area of the country) was under boro cultivation and boro accounted for about 49% of the total rice production. Thus, groundwater irrigation has greatly increased agricultural production in Bangladesh and the country's food security is heavily dependent on groundwater irrigation during the dry season.

Based on available information on the distribution of As concentration in groundwater (BGS and DPHE, 2001) and the area under shallow tube-well irrigation (BADC, 2005), Saha (2006) estimated that close to 1000 metric tons of As is cycled with irrigation water during the dry season of each year. The accumulation of As in rice field soil and its introduction into the food chain through uptake by the rice plant is of major concern. Rice production is reported to decrease by 10% at a concentration of 25 mg/kg As in soil (Xiong *et al.*, 1987).

The self-sufficiency, in rice production, the high yielding varieties (HYV) of rice have been cultivated widely in the country through the year. The rice cultivation is solely depend on underground water particularly in dry season in Bangladesh, since the sources of surface water like river, dam, pond etc. of this reason becomes dry throughout the season. Natural release of arsenic from aquifer rocks has been reported to contaminate this underground water in Bangladesh (Hopenhayn, 2006). It is clear that soil concentrations are increasingly over time because of irrigation with arsenic contaminated water. Long-term use of arsenic contaminated underground water in irrigation may result in the increase of its concentration in rice plants.

1.10 Arsenic in Soil of Bangladesh

Arsenic is the 20th most abundant element in the earth's crusts as a component of more than 245 minerals. These are mostly found in ores containing sulphide along with copper, nickel, lead, cobalt and other metals. Smelting of such ores produces arsenic trioxide as a by-product and a raw material in the preparation of multiple industrial arsenic chemicals.

The main sources of arsenic in soils are the parent materials from which they are derived. Several works on arsenic status in soils of Bangladesh have been carried out, some are stated below.

Ahmed *et al.* (2011) assessed the nature and extent of the risk of As contamination from irrigation water into soils of Bangladesh. They analyzed 263 paired groundwater and paddy soil samples covering highland (HL) and medium highland-1 (MHL-1) land types for geostatistical mapping of soil As and delineation of As contaminated areas in Tala Upazilla, Satkhira district. They also collected 74 non-rice soil samples to assess the baseline concentration of soil As for that area. The mean soil As concentrations (mg/kg) for different land types under rice and non-rice crops were: rice- MHL-1 (21.2)>rice-HL (14.1)>non-rice-MHL-1 (11.9)>non-rice-HL (7.2). Multiple regression analyses showed that irrigation water As, Fe, land elevation and years of tube-well operation are the important factors affecting the concentrations of As in HL paddy soils. Only years of tube-well operation affected As concentration in the MHL-1 paddy soils. Quantitatively similar increases in soil As above the estimated baseline-As concentration were observed for rice soils on HL and MHL-1 after 6–8 years of groundwater irrigation, implying strong retention of As added in irrigation water in both land types. Application of single geostatistical methods with secondary variables such as regression kriging (RK) and ordinary co-kriging (OCK) gave little improvement in prediction of soil As over ordinary kriging (OK). Comparing single prediction methods, kriging within strata (KWS), the combination of RK for HL and OCK for MHL-1, gave more accurate soil As predictions and showed the lowest misclassification of declaring a location “contaminated” with respect to 14.8mg As/kg, the highest value obtained for the baseline soil As concentration. Prediction of soil As buildup over time indicated that 75% or the soils cropped to rice would contain at least 30 mg/L As by the year 2020.

Biswas *et al.* (2003) assessed the levels of arsenic in soils by different extraction methods and found that the amount of arsenic extracted by each of the three assays decreased in the following order among thanas: Faridpur> Tala> Brahmanbaria> Paba> Senbag. There was a wide variation of arsenic content in soils both between and within thanas. The low arsenic levels in Senbag soil samples might be influenced by higher sand and lower clay contents. Conversely, the higher levels of arsenic in Faridpur sadar, Tala and Brahmanbaria soils might be due to the higher phyllosilicate and Fe oxide contents.

Geen *et al.* (2006) obtained soil and soil–water As profiles from 4 rice paddies in Bangladesh during the wet growing season (May– November), when surface water with little arsenic is used for irrigation, or during the dry season (January–May), when groundwater elevated in arsenic is used instead. In the upper 5 cm of paddy soil, accumulation of 13 ± 12 mg/kg acid-leachable As (n=11) was observed in soil from 3 sites irrigated with groundwater containing 80–180 $\mu\text{g/L}$ As, whereas only 3 ± 2 mg/kg acid leachable As (n=8) was measured at a control site. Dissolved As concentrations averaged 370 ± 340 $\mu\text{g/L}$ (n=7) in the upper 5 cm of the soil at the 3 sites irrigated with groundwater containing 80–180 $\mu\text{g/L}$ As, contrasting with soil water As concentrations of only 18 ± 7 $\mu\text{g/L}$ (n=4) over the same depth interval at the control site.

Hossain *et al.* (2008) studied spatial distribution of arsenic (As) concentrations of irrigation water, soil and plant (rice) in a shallow tube-well (STW) command area (8 ha), and their relationship with Fe, Mn and P. Arsenic concentrations of water in the 110 m long irrigation channel clearly decreased with distance from the STW point, the range being 68–136 mg/L. Such decreasing trend was also noticed with Fe and P concentrations, but the trend for Mn concentrations was not remarkable. Concerning soil As, the concentration showed a decreasing tendency with distance from the pump. The NH_4 -oxalate extractable As contributed 36% of total As and this amount of As was associated with poorly crystalline Fe-oxides. Furthermore only 22% of total As was phosphate extractable so that most of the As was tightly retained by soil constituents and was not readily exchangeable by phosphate. Soil As (both total and extractable As) was significantly and positively correlated with rice grain As (0.296 ± 0.063 mg/ g, n =56).

Hussain *et al.* (2001a) studied to determine the status of some selected soils in ten thanas of four arsenic affected district of Bangladesh. Thirty soil samples taking three from each of 10 thanas representing 4 districts viz., Gopalganj, Faridpur, Rajbari, and Madaripur of Bangladesh were collected. The soils were collected from 3 depths viz. 0-15, 15-30, and 30-45 cm from each location. The arsenic content in soils of Gopalganj, Faridpur, rajbari and Madaripur districts ranged from 3.96-25.09, 0-38.67, 1.32-36.99 and 1.32-38.19 ppm, respectively. Out of 30 samples, arsenic content was noticed for 21 samples at 21 samples at 0-15 cm depth (1.98-46.09 ppm), 26 samples at 15-30 cm depth (1.98-51.73 ppm) and 27 samples at 30-45cm depth (1.98-54.05 ppm). Nine samples at 0-15 cm, 4 samples at 15-30 cm and 3 samples at 30-45 cm were free from arsenic contamination. On the other

hand, 7 samples at 0-15 cm depth, 6 samples at 15-30 cm depth and 4 samples at 30-45 cm depth were found to be slightly arsenic contaminated.

Huq (2001) reported that arsenic in uncontaminated soil was about 3.9 mg/kg in the top soil and 3.85 mg/kg in the lower soil while, soils collected from agricultural field irrigated with arsenic contaminated water showed arsenic concentration was high as 34 mg/ kg in the surface and 28 mg/kg in the lower soils.

Hossain *et al.* (2001b) studied to determine the arsenic status of some selected soils in six thanas of three arsenic affected districts of Bangladesh. Eighteen soil samples taking three from each of 6 thanas representing 3 districts viz. Moulavibazar, Habiganj and Sylhet of Bangladesh were collected for the study. The soils were collected from 3 depths viz. 0-15, 15-30 and 30-45 cm from each location. The arsenic content in soils of Moulavibazar, Habiganj and Sylhet districts ranged from 1.32-31.89, 0-16.87 and 0.66-17.57 ppm, respectively. Out of 18 samples, arsenic content was noticed for 11 samples at 0-15 cm depth (1.98-25.76 ppm), 15 samples at 15-30 cm depth (3.96-30.68 ppm) and 14 samples at 30-45 cm depth (3.96-38.23 ppm). The highest arsenic content of 38.23 ppm was found at 30-45 cm depth in Sylhet district. Seven samples at 0-15 cm, 3 samples at 15-30 cm and 4 samples at 30-45 cm were free from arsenic contamination. Correlation study of arsenic contents of soils was done with some soil properties viz. sand, silt and clay contents, soil pH, EC and organic matter status. Results showed that arsenic content correlated significantly with different soil properties.

Islam *et al.* (2004) assessed the levels of arsenic in soils, and rice plants (grain and straw) in five districts viz. Pabna, Chapai Nawabganj, Rajbari, Faridpur and Gopalganj of the Gangetic Floodplains of Bangladesh. The arsenic concentrations for all soils varied widely between locations. Generally the arsenic levels in soils were higher in Rajbari and Faridpur compared to the other three districts. There was a good correlation between water-arsenic and soil-arsenic over the locations. None of the soils had arsenic level more than 20 µg/g. About 16 % grain samples had no detectable arsenic and on the other hand 14 % grains had arsenic level more than 1 ppm. Comparing varieties effects, the grain arsenic concentration in IR 8 and BRRI dhan 29 rice were higher in comparison with BRRI dhan 28 and pajira.

Islam *et al.* (2003) conducted a study with four soil samples (0-15 cm) collected from different levels of arsenic contaminated areas where arsenic contaminated irrigation water from shallow tube-well was used for rice cultivation to determine the maximum adsorption capacity, energy of adsorption and buffering capacity of arsenic. The test soils showed a large capacity of arsenic adsorption. The application of arsenic progressively increased the equilibrium solution concentration of arsenic. At the lower levels of equilibrium concentration, the adsorption of arsenic linearly increased, but at greater levels of equilibrium solution concentration the rate of arsenic adsorption decreased. Highest and lowest adsorptions were observed 2000 mg/kg and 1111 mg/kg in soil respectively. The highest and lowest arsenic buffering capacity were observed 405 and 185 in soil respectively. The arsenic adsorption parameters were highly correlated with clay content, FeO and MnO content of the soils and not with the total arsenic of the soils.

Jahiruddin *et al.* (2004) observed on pot experiments that there was a significant yield reduction at and above 10 ppm soil added arsenic. The arsenic concentration in rice grain increased with arsenic application either through irrigation water or added directly to the soil, still it did not exceed 1 ppm (maximum permissible limit). Again, the straw-arsenic concentrations in all cases were well above 1 ppm. It was further observed that application of P enhanced the arsenic accumulation in grain and straw while the P content in grain and straw remained unaffected by arsenic addition to soil. It might be possible that application of high levels of P may increase the arsenic toxicity in soils.

Jahiruddin *et al.* (2003) conducted two sets of pot experiments; one set with soil added arsenic and the other with irrigation water added arsenic and found that addition of arsenic in irrigation water up to 0.25 mg/L increased the crop growth as well as grain yield, thereafter the yield decreased with higher arsenic doses in irrigation water. For soil added arsenic, there was significant yield reduction at 10 mg/L or above arsenic doses. The arsenic concentration in rice grain increased with arsenic applications either added through irrigation water or added directly to the soil; still it did not exceed 1 ppm.

Jahiruddin *et al.* (2000) examined that soils of Gangetic alluvium contained more arsenic than that of Brahmaputra alluvium, some of the former soils had arsenic greater than 20 µg/g arsenic, the maximum acceptable limit for agricultural soils whereas the later soils all had arsenic level below 20 µg/g.

Khan *et al.* (2010) studied the consequences of arsenic from irrigation water and direct surface (0–15 cm) soil application under field conditions with wetland rice culture over 2 years. Twenty PVC cylinders (30-cm length and 30-cm diameter) were installed in field plots to evaluate the mobility and vertical distribution of soil As, As mass balance, and the resulting influences on rice yield and plant-As concentration in Boro (dry season) and transplanted (T.) Aman (wet season) rice over the 2-year growth cycle. Treatments included irrigation-water As concentrations of 0, 1 and 2 mg/L (Boro season only) and soil-As concentrations of 10 and 20 mg/kg. Following the 2-year cropping sequence the major portion (39.3–47.6%) of the applied arsenic was retained within the rooting zone at 0–15 cm depth, with 14.7–19.5% of the total applied As at the 5–10 cm and 10–15 cm soil depths compared to 1.3– 3.6% at the 35–40 cm soil depth. These results indicate the relatively low mobility of applied As and the likely continued detrimental accumulation of As within the rooting zone. Arsenic addition in either irrigation water or as soil-applied As resulted in yield reductions from 21 to 74 % in Boro rice and 8 to 80 % in T. Aman rice, the latter indicating the strong residual effect of As on subsequent crops. The As concentrations in rice grain (0.22 to 0.81 µg/g), straw (2.64 to 12.52 µg/g) and husk (1.20 to 2.48 µg/g) increased with increasing addition of As. These results indicate the detrimental impacts of continued long-term irrigation with As-contaminated water on agricultural sustainability, food security and food quality in Bangladesh.

Khan *et al.* (2009) conducted an experiment involving four soils and three irrigation-water As concentrations in undisturbed soil columns during 2004-2006, with Boro (winter dry-season, flood-irrigated rice, using As-contaminated water for irrigation), T. Aman (summer monsoon, rain-fed flooded rice), Boro, and T. Aman rice grown in sequence, to examine the fate of added As from irrigation water during flooded rice culture. The As content in the column leachate represented only 1.17–5.08% of the total applied As, indicating the substantial build-up of soil As. Most of the irrigation-applied As remained in the zone of maximum root activity at the top 0–15 cm of soil. Increased As concentrations in soil from the application of As-contaminated water resulted in substantially reduced rice-grain yields and increased As concentrations in rice grain and straw, indicating the potential hazards of continued irrigation with As contaminated water to sustainable rice production and food security in South Asia.

Martin *et al.* (2013) assessed how soil and environmental properties interact in affecting the fixation/mobilization of As in the soils of the Ganges and Meghna agricultural zones. For this purpose, soil samples from different districts in the Ganges and Meghna floodplains have been characterized, different pools of As, Fe, Mn and P have been quantified and the results have been related to the main soil and environmental characteristics of the two areas. The As content in groundwater and the baseline As concentration in the parent material in the two zones would point to a higher As accumulation in the Meghna floodplain soils, however the Ganges floodplain soils had higher contents of As in all fractions, proving the key role of the factors controlling the release/fixation of As. The soils of the two floodplains, in fact, differed for most physicochemical properties. The ones from the Ganges floodplain were calcareous, with finer texture and generally richer in Fe but oxalate extractable Fe was higher in the Meghna floodplain soils, suggesting a higher degree of waterlogging. This is in agreement with the averagely longer duration and higher depth of submersion of the soils of this area, which enhanced Fe dynamics and favored the release of the less tightly bonded As forms. The competing effect of P was probably similar in the two areas, since P concentrations did not differ significantly among the two soil series. However, more P was Olsen extractable in the Meghna floodplain soils, in contrast with As, that was more easily extracted from the Ganges floodplain soils. The concentration and potential mobility of the retained As were hence greater in the soils of the Ganges floodplain.

Mridha (1998) reported that arsenic occurs widely in the alluvial aquifers of Bangladesh, beneath the floodplains of the Ganges, Brahmaputra and Meghna rivers. Native arsenic in virgin soils ranges from 0.2 to 40 µg/g, with an average content of about 5 µg/g. He also reports that arsenic in soils is highly mobile and any retention of arsenic in soils would occur by adsorption, especially if the soils contained iron or aluminium oxides.

Nuruzzaman (1995) had examined the arsenic status of industrial polluted soil around Dhaka City and reported a higher concentration of arsenic (>20 µg/g) in soils particularly near Tannery industry.

Polizzotto *et al.* (2013) conducted experiments to analyze processes impacting arsenic transport in irrigation water flowing over bare rice-field soils in Bangladesh. Dissolved concentrations of As, Fe, P, and Si varied over space and time, according to whether

irrigation water was flowing or static. Initially, under flowing conditions, arsenic concentrations in irrigation water were below well-water levels and showed little spatial variability across fields. As flowing-water levels rose, arsenic concentrations were elevated at field inlets and decreased with distance across fields, but under subsequent static conditions, concentrations dropped and were less variable. Laboratory experiments revealed that over half of the initial well-water arsenic was removed from solution by oxidative interaction with other water-column components.

Introduction of small quantities of soil further decreased arsenic concentrations in solution. At higher soil-solution ratios, however, soil contributed arsenic to solution via abiotic and biotic desorption. Collectively, these results suggest careful design is required for land-based arsenic-removal schemes

Panaulla *et al.* (2003) assessed the levels of arsenic in soils, water and rice plants (grain and straw) in five thanas viz. Brahmanbaria sadar, Senbag, Faridpur sadar, Paba and Tala of Bangladesh. Arsenic concentration in the STW waters varied widely, the maximum arsenic concentration being 0.6 mg/L and the minimum <0.01 mg/L. Total soil arsenic exceeded 10 mg/kg and it went up to 60 mg/kg. The correlation between water arsenic and soil arsenic was poor for all thanas. The straw arsenic content was about 10 times than the grain arsenic content. The plant arsenic concentrations correlated poorly with water or soil arsenic content and also indicate that arsenic content appeared to be more prominent in case of soil than water or rice grain. However, it was not clear from their study that whether the spatial variability of arsenic in soil was dependent on irrigation water or background arsenic.

Rahman *et al.* (2010) undertaken a study to determine As concentrations in groundwater, soils, and crops in 16 districts of southwestern Bangladesh. Groundwater samples were collected from shallow-tube and hand-tube wells (STW and HTW) used for irrigation and drinking water. Soil and rice plants were sampled from the command area of the tube wells. Arsenic concentrations were determined using an atomic absorption spectrometer equipped with flow injection hydride generator. Groundwater samples contained <10 to 552 µgAs/L. Arsenic concentrations in 59% of STW samples exceeded 50 µg As/L, the national standard for As in drinking water. Unlike groundwater, most of the surface water samples contained <10 µg As/L. Concentrations of As in the soils from the command area

of the tube wells ranged from 4.5 to 68 mg/kg. More than 85% of the soils contained <20 mg As/kg. The mean As concentration in the rice grain samples was 0.23 mg/kg, which is much less than the maximum food hygiene standard. A positive relationship was observed between groundwater and soil As, implying that soil As level increases as a result of irrigation with contaminated water. However, irrigation water As did not show any relation with rice grain As. Their findings suggest that surface water bodies are a safe source of irrigation water in the As-contaminated areas.

Rashid *et al.* (2004a) determined the levels of arsenic in water in different areas from 17 districts of Bangladesh and results showed that in Charghat area, the highest arsenic content in water was 0.5 ppm and that of Chapai Nawabganj area was 0.3 ppm. It was observed that arsenic concentration started to increase with the beginning of dry season and continued up to May/June and to decrease with the beginning of the monsoon and reached at the minimum level after the monsoon. Arsenic content was higher in shallow aquifer than that in the deeper aquifer.

Rashid *et al.* (2004b) stated that arsenic concentration was found to be the highest with HTWs (27%), followed by STWs (21%) and the least with DTWs (7%). The middle layers (i.e. those between 40-160 feet) reflected the highest levels of arsenic contamination in groundwater. The shallower layers up to 35 feet and the deeper layers below 160 feet below the surface showed uniformly low (safe) levels of arsenic. Arsenic for age, the three types of tube-wells tested were found to have no relationship with arsenic contamination. In case of lateral zoning with a 4 Km assigned distance, most of the unsafe wells were within the 1st zone and gradually decreased with the increase of distance from the rivers.

Stroud *et al.* (2011) investigated the dynamics of As concentration and speciation in paddy fields during dry season (boro) rice cultivation at 4 sites in Bangladesh and West Bengal, India. Three sites which were irrigated with high As groundwater had elevated As concentrations in the soils, showing a significant gradient from the irrigation inlet across the field. Arsenic concentration and speciation in soil pore water varied temporally and spatially; higher As concentrations were associated with an increasing percentage of arsenite, indicating a reductive mobilization. Concentrations of As in rice grain varied by 2-7 fold within individual fields and were poorly related with the soil As concentration. A field site

employing alternating flooded-dry irrigation produced the lowest range of grain As concentration, suggesting a lower soil As availability caused by periodic aerobic conditions.

Saha and Ali (2007) monitored arsenic (As) concentrations in the soil layers of 12 rice fields located in four As affected areas and two unaffected areas in Bangladesh during 2003. In the unaffected areas, where irrigation water contained little As ($<1 \mu\text{g/L}$), As concentrations of rice field soils ranged from 1.5 to 3.0 mg/kg and did not vary significantly with either depth or sampling time throughout the irrigation period. In the As affected areas where the irrigation water contained elevated As (79 to 436 $\mu\text{g/L}$), As concentrations of rice field soils were much higher compared to those in the unaffected areas and varied significantly with both depth and sampling time. For the top 0 to 150 mm of the soil, the As concentration increased significantly at the end of the irrigation season (May–June 2003). About 71% of the As that is applied to the rice field with irrigation water accumulates in the top 0 to 75mm soil layer by the end of the irrigation season. After the wet season during which the rice fields were inundated with flood/rain water, the As concentrations in the soil layer decreased significantly and were reduced to levels comparable to those found in soil samples collected at the beginning of the irrigation period. The long-term As accumulation in agricultural soil appears to be counteracted by biogeochemical pathways leading to As removal from soil Ullah (1998) reported that arsenic concentration in Bangladesh soils ranged from 4-8 $\mu\text{g/g}$. However, in areas where irrigation is performed with arsenic contaminated ground water, soil arsenic level can reach up to 83 $\mu\text{g/g}$.

1.11 Arsenic into Rice

Long-term use of arsenic contaminated ground water to irrigate crops, especially paddy rice (*Oryza sativa* L) has resulted in elevated soil arsenic levels in Bangladesh. There is, therefore concern regarding accumulation of arsenic in rice grown on these soils. In this fields some works have been done, relevant works are discussed bellow in brief.

Azad *et al.* (2009) conducted a pot-culture experiment in open-field conditions with highly cultivated locally transplanted (T) aman rice (*Oryza sativa* L.) named BR-22 in arsenic (As)-amended soil (0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg/kg As) of Bangladesh to see the effect of As on the growth, yield and metal uptake of rice. Arsenic

was applied to soil in the form of sodium arsenate (Na_2HAsO_4). Arsenic affected the plant height, tiller and panicle numbers, grain and straw yield of T-aman rice significantly ($P \leq 0.05$). The grain As uptake of T-aman rice was found to increase with increase of As in soil and a high grain As uptake was observed in the treatments of 30–50 mg/kg As-containing soil. These levels exceed the food hygiene concentration limit of 1.0 mg/kg As. However, the straw As uptake varied significantly ($P \leq 0.05$) from a low concentration of As in soil (5 mg/kg) and the highest uptake was noticed in 20 mg/kg As treatment.

Adomako *et al.* (2009) conducted a field survey in arsenic impacted and non-impacted paddies of Bangladesh to assess how arsenic levels in rice (*Oryza sativa* L.) grain are related to soil and shoot concentrations. Ten field sites from an arsenic contaminated tube-well irrigation region (Faridpur) were compared to 10 field sites from a non affected region (Gazipur). Analysis of the overall data set found that both grain and shoot total arsenic concentrations were highly correlated ($p < 0.001$) with soil arsenic. Median arsenic concentrations varied by 14, 10 and 3 fold for soil, shoot and grain respectively comparing the two regions. The reason for the sharp decline in the magnitude of difference between Gazipur and Faridpur for grain arsenic was due to an exponential decline in the grain/shoot arsenic concentration ratio with increasing shoot arsenic concentration. When the Bangladesh data were compared to EU and US soil–shoot–grain transfers, the same generic pattern could be found with the exception that arsenic was more efficiently transferred to grain from soil/shoot in the Bangladesh grown plants. This may reflect climatic or cultivar differences.

Abedin and Meharg (2002a) conducted a germination study on rice seeds and a short-term toxicity experiment with different concentrations of arsenite and arsenate on rice seedlings. Percent germination over control decreased significantly with increasing concentrations of arsenite and arsenate. Arsenite was found to be more toxic than arsenate for rice seed germination. There were varietal differences among the test varieties in response to arsenite and arsenate exposure. The performance of the dry season variety Purbachi was the best among the varieties. Germination of Purbachi was not inhibited at all up to 4 mg/L arsenite and 8 mg/L arsenate treatment. Root tolerance index (RTI) and relative shoot height (RSH) for rice seedlings decreased with increasing concentrations of arsenite and arsenate. Reduction of RTI caused by arsenate was higher than that of arsenite. In general, dry season varieties have more tolerance to arsenite or arsenate than the wet season varieties.

Abedin *et al.* (2002b) conducted a greenhouse experiment where rice (*Oryza sativa* L) was irrigated with arsenate-contaminated water containing arsenite, arsenate, dimethylarsinic acid, and monomethylarsinic acid. The short-term uptake kinetics for these four arsenic species were determined in 7 day old excised rice roots. High affinity uptake (0-0.0532mM) for arsenite and arsenate with eight rice varieties, covering two growing seasons, Boro rice (dry season) and T. Aman rice (wet season), showed that uptake of both arsenite and arsenate by boro varieties was less than that of Aman varieties. Arsenite uptake was active, and taken up at approximately the same rate as arsenate. Greater uptake of arsenite, compared with arsenate, was found at higher substrate concentration (low affinity uptake system). Competitive inhibition of uptake with phosphate showed that arsenite and arsenate were taken up by different uptake systems because arsenate uptake was strongly suppressed in the presence of phosphate, whereas arsenite transport was not affected by phosphate. At a slow rate, there was a hyperbolic uptake of monomethyl arsenic acid, and limited uptake of dimethyl arsenic acid.

Abedin *et al.* (2002c) was conducted a green house study to examine the effects arsenic contaminated irrigation water on the growth of rice and uptake and speciation of arsenic. Treatments of the greenhouse experiment consisted of two phosphate doses and seven different arsenate concentrations ranging from 0 to 8 mg of As/L applied. Increasing the concentration of arsenic in irrigation water significantly decreased plant height, grain yield, the number of field grains, grain weight and root biomass, while the arsenic concentration in root, straw and rice husk increased significantly. The concentrations of arsenic in rice straw (up to 91.8 mg/kg for the highest As treatment) were of the same order of magnitude as root arsenic concentrations (up to 107.5 mg/kg), suggesting that arsenic can be readily translocated to the shoot. The high arsenic concentration may have the potential for adverse health effects on the cattle and an increase of arsenic exposure in human via the plant-animal-human pathway.

Bhattacharya *et al.* (2013) conducted a greenhouse pot experiment on three high yielding, one hybrid and four local rice varieties to investigate the uptake, distribution and phytotoxicity of arsenic in rice plant in West Bengal India. 5, 10, 20, 30 and 40 mg/kg dry weights arsenic dosing was applied in pot soil and the results were compared with the control samples. All the studied high yielding and hybrid varieties (Ratna, IET 4094, IR 50 and Gangakaveri) were found to be higher accumulator of arsenic as compared to all but one local rice variety, Kerala Sundari. In these five rice varieties accumulation of

arsenic in grain exceeded the WHO permissible limit (1.0 mg/kg) at 20 mg/kg arsenic dosing. Irrespective of variety, arsenic accumulation in different parts of rice plant was found to increase with increasing arsenic doses, but not at the same rate. A consistent negative correlation was established between soil arsenic and chlorophyll contents while carbohydrate accumulation depicted consistent positive correlation with increasing arsenic toxicity in rice plant.

Bhattacharya *et al.* (2010) investigated to assess the level of severity of arsenic contamination, concentrations of arsenic in irrigation water, soil, rice, wheat, common vegetables, and pulses, intensively cultivated and consumed by the people of highly arsenic affected Nadia district, West Bengal, India in Ganga-Meghna-Brahmaputra basin, one of the major arsenic-contaminated hotspot in the world. Results revealed that the arsenic-contaminated irrigation water (0.318– 0.643 mg /L) and soil (5.70–9.71 mg /kg) considerably influenced in the accumulation of arsenic in rice, pulses, and vegetables in the study area. Arsenic concentrations of irrigation water samples were many folds higher than the WHO recommended permissible limit for drinking water (0.01 mg /L) and FAO permissible limit for irrigation water (0.10 mg/L). But, the levels of arsenic in soil were lower than the reported global average of 10.0 mg /kg and was much below the EU recommended maximum acceptable limit for agricultural soil (20.0 mg/kg). The total arsenic concentrations in the studied samples ranged from <0.0003 to 1.02 mg/ kg. The highest and lowest mean arsenic concentrations (milligrams per kilogram) were found in potato (0.654) and in turmeric (0.003), respectively. Higher mean arsenic concentrations (milligrams per kilogram) were observed in Boro rice grain (0.451), arum (0.407), amaranth (0.372), radish (0.344), Aman rice grain (0.334), lady's finger (0.301), cauliflower (0.293), and Brinjal (0.279). Apart from a few potato samples, arsenic concentrations in the studied crop samples, including rice grain samples were found not to exceed the food hygiene concentration limit (1.0 mg/ kg).

Bhattacharya *et al.* (2010) investigated the presence of arsenic in irrigation water and in paddy field soil to assess the accumulation of arsenic and its distribution in the various parts (root, straw, husk, and grain) of rice plant from an arsenic affected area of West Bengal. Results showed that the level of arsenic in irrigation water (0.05–0.70 mg/L) was much above the WHO recommended arsenic limit of 0.01 mg/L for drinking water. The paddy soil gets contaminated from the irrigation water and thus enhancing the

bioaccumulation of arsenic in rice plants. The total soil arsenic concentrations ranged from 1.34 to 14.09 mg/kg. Soil organic carbon showed positive correlation with arsenic accumulation in rice plant, while soil pH showed strong negative correlation. Higher accumulation of arsenic was noticed in the root (6.92 ± 0.241 – 28.63 ± 0.225 mg/kg) as compared to the straw (1.18 ± 0.002 – 2.13 ± 0.009 mg/kg), husk (0.40 ± 0.004 – 1.05 ± 0.006 mg/kg), and grain (0.16 ± 0.001 – 0.58 ± 0.003 mg/kg) parts of the rice plant. However, the accumulation of arsenic in the rice grain of all the studied samples was found to be between 0.16 ± 0.001 and 0.58 ± 0.003 mg/kg dry weights of arsenic, which did not exceed the permissible limit in rice (1.0 mg/kg according to WHO recommendation). Two rice plant varieties, one high yielding (Red Minikit) and another local (Megi) had been chosen for the study of arsenic translocation. Higher translocation of arsenic was seen in the high yielding variety (0.194–0.393) compared to that by the local rice variety (0.099–0.161). An appreciable high efficiency in translocation of arsenic from shoot to grain (0.099–0.393) was observed in both the rice varieties compared to the translocation from root to shoot (0.040–0.108).

Chaturvedi (2006) carried a greenhouse experiment by using two *Oryza sativa* genotypes (Mahsuri and CN 1035-60). Mahsuri showed greater sensitivity to arsenic toxicity than CN1035-60. Arsenic concentration in rice plants was found to be directly proportional to soil arsenic concentration. Arsenic concentration in the stem varied from 0.9 to 13.8 mg/kg, while that in the root varied from 1.7 to 36.5 mg/kg. Arsenic was accumulated in the roots in much higher amounts as compared to above ground biomass (stem). Genotype CN1035-60 had significantly less uptake of arsenic than genotype Mahsuri might be because of varietals difference in some physiological or morphological attributes of the root system.

Chino (1981) stated, there is no evidence that arsenic is essential for plant growth but it has phytotoxic effects on different crops. Arsenic is translocated to many parts of the plants, most is found in old leaves and roots. The yield limiting arsenic concentrations in plant tissue are 4-5 ppm in cotton and 1 ppm in soybeans. In rice, the critical level in tops ranges from 20 to 100 ppm arsenic; and in roots 1000 ppm. Tillering is also severely depressed with high concentration of arsenic Dilday *et al.* (2000) reported that arsenic influenced the straight head of rice (*Oryza sativa*) which is a physiological disorder that results in blank florets and distorted lemma and palea, and in extreme cases, can result in

almost a total loss of yield. Twelve cultivars including ten of the most popular cultivars were grown in the southern United States for their response to arsenic (i.e. monosodium methanearsenate, MSMA). On a scale of low to high susceptibility cv. Cocodrie, Kaybonnet, Bengal, and Mars were the most susceptible to straight head at the rate of 6 lb/acre level of MSMA. Cultivars that showed tolerance to arsenic also appeared to be tolerant to straight head.

Garnier *et al.* (2010) monitored As and Fe concentrations in soil water and in the roots of rice plants, primarily the Fe plaque surrounding the roots, during the 4-month growing season at two sites irrigated with groundwater containing ~130 µg/L As and two control sites irrigated with water containing <15 µg/L As in Arai hazari upazila, 30 km northeast of Dhaka in Bangladesh. At both sites irrigated with contaminated water, As concentrations in soil water increased from <10 µg/L to >1000 µg/L during the first five weeks of the growth season and then gradually declined to <10 µg/L during the last five weeks. At the two control sites, concentrations of As in soil water never exceeded 40 µg/L. At both contaminated sites, the As content of roots and Fe plaque rose to 1000–1500 mg/kg towards the middle of the growth season. It then declined to ~300 mg/kg towards the end, a level still well above As concentration of ~100 mg/kg in roots and plaque measured throughout the growing season at the two control sites. These time series, combined with simple mass balance considerations, demonstrate that the formation of Fe plaque on the roots of rice plants by micro-aeration significantly limits the uptake of As by rice plants grown in paddy fields. Large variations in the As and Fe content of plant stems at two of the sites irrigated with contaminated water and one of the control sites were also recorded. The origin of these variations, particularly during the last month of the growth season, needs to be better understood because they are likely to influence the uptake of As in rice grains.

Hua *et al.* (2013) conducted a field experiment on three rice cultivars grown in both monosodium methane arsonate (MSMA)-treated and -untreated soils under continuous or intermittent flood water management practices to assess the arsenic uptake by rice and determine rice cultivar response to soil MSMA level at the United State Department of Agriculture-Agriculture Research Service (USDA-ARS) Dale Bumpers National Rice Research Center near Stuttgart, Arkansas. Results indicated that the grain yield and the occurrence of straighthead disease were cultivar-dependent and influenced by soil As level and water management practices. Straighthead-resistant cultivars yielded more and

had lower grain As than the susceptible ones. Elevated soil As with continuous flood management significantly reduced the grain yield of susceptible cultivars by >89% due to substantially increased straighthead, which were induced by increased As content in grains. Yield reduction by MSMA treatment could be partially mitigated with intermittent flood water practice. The As accumulation was found to be associated with soil iron redox transformation influenced by the water management. This study demonstrates that the selection of less As-susceptible cultivars and intermittent flood water practice could be effective means to lower the As accumulation in grains and minimize the occurrence of the As-induced straighthead symptom and yield reduction.

Hu *et al.* (2007) studied to assess the effects of two sulfur (S) sources (SO_4^{2-} , S^0), and three rates of S application (0, 30, 120 mg S/kg) on the formation of iron plaque in the rhizosphere, and on the root surface of rice, and As (arsenic) uptake into rice (*Oryza sativa* L.) in a combined soil-sand culture experiment. Significant differences in As uptake into rice between +S and -S treatments were observed in relation to S sources, and rates of S application. Concentrations of As in rice shoots decreased with increasing rates of S application. The mechanism could be ascribed to sulfur, induced the formation of iron plaque, since concentrations of Fe in iron plaque on quartz sands in the rhizosphere, and on the root surface of rice increased with increasing rates of S application. The results suggest that sulfur fertilization may be important for the development approaches to reducing As accumulation in rice.

Immamul Huq *et al.* (2006) examined the response of two varieties of rice plant (BR28 and BR29) to arsenic accumulation added from two sources (AsIII and AsV) under two different water regimes (100 and 75% of field capacity). Treatments added to soil were 0, 10, 20 and 40 mg As/kg soil. The accumulation was greater in the arsenite treated soil than that in the arsenate treated one, indicating the higher phytoavailability of As III. Most of the arsenic taken up by plants was sequestered in the root, followed by straw and grain. Arsenic in straw and grain was lower for plants of both varieties at 75% of field capacity. However, there have been some varietals differences in the response to As III or As V.

Islam *et al.* (2004) conducted a pot experiment to see the effects of irrigation water on Boro rice and the residual effects on T-Aman rice. There were eight treatments consisting of control, 0.1, 0.25, 0.50, 0.75, 1.0, 1.50 and 2.0 ppm arsenic added through irrigation

water. The irrigation water added arsenic up to 0.25 ppm enhanced the plant height, panicle length, filled grain/panicles, 1000 grain weight and finally the grain yield of Boro rice and the further doses of depressed then plant growth, yield and yield components. The concentration of arsenic in rice grain in straw of Boro rice increased significantly with increasing arsenic concentration in the irrigation water. Application of arsenic added to first crop had significant residual effects on the second crop in respect of plant height, panicle length, grains/panicle, grain and straw yields.

Jiang *et al.* (2014) conducted a study to explore the effects of soil available phosphorus on As uptake by rice, and identify the effects of soil properties on arsenic transfer from soil to rice under actual field conditions in China. 56 pairs of topsoil and rice samples were collected. The relevant parameters in soil and the inorganic arsenic in rice grains were analyzed, and then all the results were treated by statistical methods. Results show that the main factors influencing the uptake by rice grain include soil pH and available phosphorus. The eventual impact of phosphorus is identified as the suppression of As uptake by rice grains. The competition for transporters from soil to roots between arsenic and phosphorus in rhizosphere soil has been a dominant feature.

Kang Li Jun *et al.* (1996) studied arsenic contents of rice in a pot experiment. The rice was grown on loam soil with available arsenic contents of 1.3, 6.0, 7.8 or 10.3 mg/kg and total arsenic contents of 1.3, 27.7, 36.6 and 56.0 mg/kg, respectively. Increasing levels of arsenic decreased plant height, number of effective tillers, dry weight of above ground parts and 1000 grain-weight. Yields decreased from 48.7 g/pot with the lowest rate of arsenic to 17.9g with the highest rate. Content of arsenic was higher in roots than in stems plus leaves or in grain, but in all parts the content increased as soil arsenic increased. The contents of arsenic in stems plus leaves were more closely related to soil total and available arsenic than those of roots or grain.

Kiss *et al.* (1992) conducted a pot experiment with solution culture with rice. Arsenic markedly reduced plant weight and the effects of arsenic were cultivar-dependent.

Liu *et al.* (2004) conducted a soil-glass bead culture system to investigate characteristic of iron plaque and arsenic accumulation and speciation in the mature rice plants. Data showed that arsenic in iron plaque was sequestered mainly with amorphous and

crystalline iron oxides and that arsenate was the predominant species. There was significant variation in iron plaque formation between genotypes and the distribution of arsenic in different components of mature rice plants followed the following order: iron plaque>root> straw> husk> grain for all genotypes. Arsenic accumulation in grain differed significantly among genotype.

Li Xiaoping *et al.* (1996) studied the phytotoxicity of arsenic to rice. Soils were treated with different concentration of arsenic compounds, incubated for different periods before being planted with rice. A significant negative correlation between the incubation periods prior to sowing increased the level of arsenic phytotoxicity whereas N and K applications helped alleviate the symptoms.

Meharg and Rahman (2002) investigated the arsenic level in rice grains from Bangladesh. Rice grain grown in the regions where arsenic is buildup in the soil had high arsenic concentrations, with three rice grain samples having levels above 1.7 $\mu\text{g/g}$. These typical grain arsenic levels contributed considerably to arsenic quantity of 0.1 mg/L. Arsenic levels in rice can be further elevated in rice growing on arsenic contaminated soils, potentially greatly increasing arsenic exposure of the Bangladesh population.

Montenegro and Mejia (2001) carried out field and greenhouse experiments in rice to evaluate the effect of the Cd and As content in irrigation waters on soils, and on the physiological parameters of rice growth, the amount of arsenic accumulated in different parts of rice plants, and the yield and other aspects of rice crop. The results showed that rice reached its maximum height when neither element was present in the irrigation waters; an increase in arsenic content of irrigation waters induced a 10% reduction of grains per panicle; when irrigation waters used contained the highest concentration of Cd and As yields were significantly reduced; the maximum yield was obtained when Cd and As were absent in irrigation water; concentration of Cd and As progressively increased in rice plants with the increase of both elements in the irrigation waters; and the presence of Cd and As in irrigation waters apparently did not affect the milling quality of the rice.

Marin *et al.* (1992) found that uptake most of the arsenite, arsenate and MMAA accumulated in the root while DMAA was readily translocated to the shoot. Marin *et al.* (1993) also found easy and quick translocation of DMAA when rice plants were grown in

DMAA solution at 0-1.6 mg As/L resulting in higher concentrations of arsenic in rice shoots than that of roots. Irrespective of arsenic chemical forms root arsenic concentration was 10.5 mg/kg in the 0.05 mg As/L treatment, which increased to 212.7 mg/kg in the 0.8 mg As/L treatment.

Norton *et al.* (2013) conducted a study to investigate the effect of increased organic matter in the soil on the release of arsenic into soil pore water and accumulation of arsenic species within rice grain. It was observed that high concentrations of soil arsenic and organic matter caused a reduction in plant growth and delayed flowering time. Total grain arsenic accumulation was higher in the plants grown in high soil arsenic in combination with high organic matter, with an increase in the percentage of organic arsenic species observed. The results indicate that the application of organic matter should be done with caution in paddy soils which have high soil arsenic, as this may lead to an increase in accumulation of arsenic within rice grains. Results also confirm that flooding conditions substantially increase grain arsenic.

Odanaka *et al.* (1987) reported higher accumulation of arsenic in roots than any other plant parts. The arsenic chemical form (species) was found to be more important than arsenic concentration in growing media in determining the phytotoxic effect of arsenic on rice. Among the four arsenicals used, arsenite and MMAA were phytotoxic to rice.

Odanaka *et al.* (1987) showed evidence of very little transformation of arsenic species in rice shoot and root from the chemical speciation study. However, similar study (Heitkemper *et al.*, 2001) on rice grain speciation showed that rice grain may contain a considerable proportion of organic arsenic species. However, the proportion of organic species (DMAA and MMAA) in rice grain may vary depending on the source of grain and methods of extraction.

Panaullah *et al.* (2009) studied to assess the impact of As contaminated irrigation water on soil-As content and rice productivity over two winter-season rice crops in the command area of a single tube-well in Faridpur district, Bangladesh. After 16–17 years of use of the tube-well, a spatially variable build up of As and other chemical constituents of the water (Fe, Mn and P) was observed over the command area, with soil-As levels ranging from about 10 to 70 mg/kg. A simple mass balance calculation using the current

water As level of 0.13 mg As/L suggested that 96% of the added arsenic was retained in the soil. When BRRI dhan 29 rice was grown in two successive years across this soil-As gradient, yield declined progressively from 7–9 to 2–3 t ha⁻¹ with increasing soil-As concentration. The average yield loss over the 8 ha command area was estimated to be 16%. Rice-straw As content increased with increasing soil-As concentration; however, the toxicity of As to rice resulted in reduced grain-As concentrations in one of the 2 years. The likelihood of As-induced yield reductions and As accumulation in straw and grain has implications to agricultural sustainability, food quality and food security in As-affected regions throughout South and Southeast Asia.

Rahman *et al.* (2007a) conducted a glass house study to investigate the accumulation of arsenic in tissues of five widely cultivated rice (*Oryza sativa* L.) varieties of Bangladesh namely BRRI dhan 28, BRRI dhan 29, BRRI dhan 35, BRRI dhan 36, BRRI hybrid dhan 1. Arsenic concentrations were measured in straw, husk and brown and polish rice grain to see the differential accumulation of arsenic among the rice varieties. The results showed that the concentrations of arsenic in different parts of all rice varieties increased significantly ($p < 0.05$) with the increase of its concentrations in soil. The rice varieties did not showed significant differences in arsenic accumulation in straw, husk, brown and polish grain when the concentrations of arsenic in soil was low. However, at higher concentrations of arsenic in soil, different rice varieties showed significant differences in the accumulations of arsenic in straw, husk and grain. Significantly higher concentrations of arsenic in straw and husk of rice were observed in BRRI hybrid dhan 1 compared to those of other varieties. The BRRI dhan 28 and 35 concentrated significantly higher amount of arsenic in brown and polish rice grain compared to those of other rice varieties. The results imply that arsenic translocation from root to shoot (straw) and husk was higher in hybrid variety compared to those of nonhybrid varieties. Arsenic concentrations in brown and polish rice grain of five rice varieties were found to follow the trend: BRRI dhan 28 > BRRI dhan 35 > BRRI dhan 36 > BRRI dhan 29 > BRRI hybrid dhan 1. The order of arsenic contents in tissues of rice was: straw > husk > brown rice grain > polish rice grain.

Rahman *et al.* (2007c) conducted a study to investigate the accumulation and distribution of arsenic in different fractions of rice grain (*Oryza sativa* L.) collected from arsenic affected area of Bangladesh. The agricultural soil of study area has become highly contaminated with arsenic due to the excessive use of arsenic-rich underground water

(0.070 ± 0.006 mg/L, $n = 6$) for irrigation. Arsenic content in tissues of rice plant and in fractions of rice grain of two widely cultivated rice varieties, namely BRRI dhan28 and BRRI hybrid dhan1, were determined. Regardless of rice varieties, arsenic content was about 28- and 75-folds higher in root than that of shoot and raw rice grain, respectively. In fractions of parboiled and non-parboiled rice grain of both varieties, the order of arsenic concentrations was; rice hull > bran polish > brown rice > raw rice > polish rice. Arsenic content was higher in non-parboiled rice grain than that of parboiled rice. Arsenic concentrations in parboiled and non-parboiled brown rice of BRRI dhan28 were 0.8 ± 0.1 and 0.5 ± 0.0 mg/kg dry weight, respectively while those of BRRI hybrid dhan1 were 0.8 ± 0.2 and 0.6 ± 0.2 mg/kg dry weight, respectively. However, parboiled and non parboiled polish rice grain of BRRI dhan28 contained 0.4 ± 0.0 and 0.3 ± 0.1 mg/kg dry weight of arsenic, respectively while those of BRRI hybrid dhan1 contained 0.43 ± 0.01 and 0.5 ± 0.0 mg/kg dry weight, respectively.

Rahman *et al.* (2008) conducted a glass house experiment to investigate the effects of inorganic arsenic on straight head disease in rice. BRRI dhan 29 was grown in soils spiked with arsenic at the rate of 10, 20, 30, 40, 50, 60, 70, 80 and 90 mg As/kg and on a control treatment was also run to compare the results. With the increase of soil arsenic concentrations, the severity of straight head increased significantly, up to the 50 mg of As/kg soil treatments. Straight head resulted in sterile florets with distorted lemma and palea, reduced plant height, tillering, panicle length and grain yield. Straight head caused approximately 17-100 % sterile florates formation and about 16-100 % less of grain yield. Straight head also cause the reduction of panicle formation and panicle length significant and panicle formation was found to be reduced 21-95 % by straight head.

Rahman *et al.* (2007b) conducted a glass house study to investigate the accumulation of arsenic in different parts of five widely cultivated rice varieties. The results showed that the concentrations of arsenic in different parts of all rice varieties increased significantly with increase of its concentrations in soil. However, at higher concentration of arsenic in soil, different rice varieties showed significant differences in the concentrations of arsenic in straw and husk of rice were observed in BRRI hybrid dhan 1 compared to those of other varieties. The results imply that arsenic translocation from root to shoot and husk was higher in hybrid variety compared to those of non-hybrid variety. The order of arsenic contents in tissue of rice was straw > husk > grain.

Rahman *et al.* (2006) conducted a study to investigate the accumulation and distribution of arsenic in different fractions of rice grain collected from arsenic affected area of Bangladesh. The agricultural soil of study area has become highly contaminated with arsenic due to the excessive use arsenic rich underground water (0.07 ± 0.006 mg/L, n=6) for irrigation. Arsenic content in tissues of rice plant and in fraction of rice grain of two widely cultivated rice varieties, namely BRRI dhan 28 and BRRI hybrid dhan 1, were determine. Regardless of rice varieties, arsenic content was about 28 and 75 folds higher in root than that of shoot and raw rice grain, respectively. The order of arsenic concentrations was: rice hull > bran polish > brown rice > raw rice > polish rice. Arsenic content was higher in non-parboiled rice grain than that of parboiled rice. Arsenic concentration in parboiled rice and non parboiled brown rice of BRRI dhan 28 were 0.8 ± 0.1 and 0.5 ± 0.0 mg/kg dry weight, while those of BRRI hybrid dhan 1 were 0.8 ± 0.2 and 0.6 ± 0.2 mg/kg dry weight respectively.

Smith *et al.* (2008) conducted a small scale rice paddy experiment to evaluate the uptake of As by rice in Australia. Arsenic concentrations in rice tissue increased in the order grain <<leaf<stem<<<root with the As concentration in the rice grain, in some cases, exceeding the maximum Australian permissible concentration of 1 mg/kg. Speciation of As in rice tissue was performed using a modified protein extraction procedure and trifluoroacetic acid extraction. Whilst higher As recoveries were obtained using trifluoroacetic acid extraction, both methods identified arsenite and arsenate as the major As species present in the root, stem and leaf, however, arsenite and dimethylarsinic acid (DMA) were the major As species identified in the grain. Notably, DMA comprised 85 to 94% of the total As concentration in the grain. The high proportion of organic to inorganic As in the grain has implications on human health risk assessment as inorganic As species are more bioavailable than methylated As species.

Schoof *et al.* (1999) in their market basket survey found 56 percent of organic species in rice grain while in another study, organic species (DMAA and MMAA) measured by the same group was 19 percent. Uptake, accumulation and phytotoxicity may also differ depending on the cultivar used. Xie and Huang (1998) also observed significant differences among the rice cultivars for uptake and accumulation of arsenic in different plant parts.

Slancheva *et al.* (1999) conducted a pot experiment where rice (*Oryza sativa*) cv. Krasnodarski 424 was grown on alluvial soil and treated with 25, 50 or 100 mg As/kg soil (as Na_3AsO_4) with or without seed inoculation with *Azospirillum brasilense*. Concentrations of arsenic higher than 50 mg/kg were toxic to the plants. Arsenic reduced chlorophyll a and b, and carotenoid concentrations in chloroplasts, panicle number, filled grains and grain yield.

Talukder *et al.* (2012) under taken pot experiments to investigate the effects of water management (WM), arsenic (As) contaminated soil-water and Phosphorus (P) rates on As uptake in rice plants. There were 18 treatments comprising of three each of As rates (0, 20 and 40 mg/kg soil) and P rates (0, 12.5 and 25 mg/kg soil) and two WM (aerobic and anaerobic) strategies on winter (boro var. BRRI dhan 29) and monsoon (aman var. BRRI dhan 32) rice at the Wheat Research Center (WRC), Nashipur, Dinajpur, Bangladesh. Arsenic concentrations in rice grain and straw increased significantly ($p < 0.01$) with the increasing As rates in the soil. Arsenic availability in soil pore-water solution was less (58%) under aerobic WM (redox potential $-E_h = +135$ to $+138$ mV; pH—6.50 at 24.3°C) as compared to anaerobic WM (flooded: $E_h = -41$ to -76 mV; pH-6.43 at 23°C). The highest total grain As content 2.2370.12 mg/kg and 0.62370.006 mg/kg was found in T₆ (P_{12.5} As₄₀-anaerobic) and T₉ (P₂₅ As₄₀-anaerobic) in BRRI dhan 29 and BRRI dhan 32, respectively, which was significantly higher (41–45 %) than in the same As and P treatments for pots under aerobic WM. The As content in rice straw (up to 24.770.49 ppm in BRRI dhan 29, 17.370.49 mg/kg in BRRI dhan 32 with the highest As level) suggested that As can more easily be translocated to the shoots under anaerobic conditions than aerobic condition. BRRI dhan 29 was more sensitive to As than BRRI dhan 32. Under aerobic WM, P soil amendments reduced As uptake by rice plants. The study demonstrated that aerobic water management along with optimum P amendment and selection of arsenic in efficient rice varieties are appropriate options that can be applied to minimize As accumulation in rice which can reduce effects on human and cattle health risk as well as soil contamination.

Talukder *et al.* (2011) conducted a field experiments to examine the effects of water management (WM) and Phosphorus (P) rates on As uptake, rice growth, yield and yield attributes of winter (boro) and monsoon (aman) rice in an As contaminated soil-water at Gobindagonj, Gaibandha, Bangladesh in 2004 and 2005. Significantly, the highest

average grain yields ($6.8870.07 \text{ t ha}^{-1}$ in boro $6.3870.06 \text{ t ha}^{-1}$ in aman) were recorded in permanent raised bed (PRB; aerobic WM: $E_h = + 360\text{mV}$) plus 100 % P amendment. There was a 12 % yield increase over conventional till on flat (CTF; anaerobic WM : $E_h = - 56 \text{ mV}$) at the same P level. In boro, the As content in grain and As content in straw were about 3 and 6 times higher in CTF compared to PRB, respectively. The highest total As content ($0.64670.01 \text{ ppm}$ in grain and $10.9370.19 \text{ ppm}$ in straw) was recorded under CTF, and the lowest total As content ($0.24770.01$ and $1.55470.09 \text{ ppm}$ in grain and straw, respectively) was recorded under PRB (aerobic WM). The results suggest that grain and straw As are closely associated in boro rice. The furrow irrigation approach of the PRB treatments consistently reduced irrigation input by 29–31 % for boro and 27–30 % for aman rice relative to CTF treatments in 2004 and 2005, respectively, thus reducing the amount of As added to the soil from the As-contaminated irrigation water. Yearly, 30 % less As was deposited to the soil compared to CTF system through irrigate on water during boro season. High As concentrations in grain and straw in rice grown using CTF in the farmers' field, and the fact that using PRB reduced grain As concentrations to value less than half of the proposed food hygiene standard.

Tsutsumi (1980) found elevated arsenic concentration in rice straw (up to 149 mg As/kg dry weight) when rice was grown in soil amended with sodium arsenate at different levels ($0\text{--}312.5 \text{ mg As/kg}$). Heitkemper *et al.* (2001) also found that rice grain generally has lower arsenic concentration and the concentration remains much below the maximum permissible limit of 1 mg As/kg .

Xu *et al.* (2008) investigated the dynamics of arsenic speciation in the soil solution under both flooded and aerobic condition and compared arsenic accumulation in rice shoot and grain in greenhouse experiment. Flooding of soil led to a rapid mobilization of arsenic, mainly as arsenite, in the soil solution. Arsenic concentration in the soil solution were 7–16 and 4–13 times higher under the flooded than under the aerobic conditions in the control without arsenic addition and in the As treatments. Arsenic accumulation in the shoots and grain was markedly increased under flooded conditions was the main reason for an enhanced arsenic accumulation by flooded rice and growing rice aerobically can dramatically decrease the arsenic transfer from soil to grain.

Xie and Huang (1998) found higher arsenic concentration in rice grain (husked rice) of some cultivars exceeding the maximum permissible limit (1 mg As/ kg) when grown in contaminated fields. Although there are reports of transformation of arsenic species in the plant system (Nissen and Benson, 1982) information on transformation in the rice plants is limited.

Xie and Huang (1994) stated that arsenic uptake and accumulation is greatly affected by arsenic concentration in soil or nutrient media and increased greatly with increasing levels. Arsenic concentration pattern in rice plant parts generally follow the pattern: root>straw>husk>whole grain>husked rice.

1.12 Arsenic in Food Chain

Many previous reports demonstrated that foodstuffs collected from arsenic epidemic areas contain significant concentrations of arsenic.

Roychowdhury *et al.* (2002) reported the arsenic concentrations in individual composites of cooked items, collected from an arsenic epidemic area of West Bengal, India, as rice (between 374.17 and 666.57 mg/kg), freshwater fish (between 830 and 900 mg/kg), potato curry (186 mg/kg), potato skin fried in oil (617 mg/kg), leaf of vegetables (578 mg/kg), mixed vegetable (277.33 mg/kg), pulses (143 mg/kg).

Das *et al.* (2004) reported arsenic concentrations exceeding the food safety limits in *Calocasia antiquorum* (between 0.09 and 3.99 mg/kg), potato (between 0.07 and 1.36 mg/kg), *Ipomoea reptans* (between 0.1 and 1.53 mg/kg) collected from an arsenic epidemic area of Bangladesh. Arsenic deposition in cattle body (Bruce *et al.*, 2003; Thornton *et al.*, 1979) and tissues and milk of sheep (Shariatpanahi *et al.*, 1984) has also been reported.

Liao and Ling (2003) conducted an experiment on arsenic bioaccumulation in tilapia fish (*Oreochromis mossambicus*) and found that the highest 95th percentile of potential health risk for inorganic arsenic ranged from 7.36×10^{-4} to 1.12×10^{-3} for the subsistence fishers of Blackfoot disease area of Taiwan.

Thus, it is evident that not only “soil–water–human” but also “plant–human” and “plant–animal–human” may be other potential food chain pathways of arsenic accumulation in human body, though arsenic contaminated drinking water is the major and direct source. To figure out the fact regarding arsenic poisoning in human body through these food chain pathways, a few investigations have been done on this field.

1.12.1 Arsenic Absorption by Plants and Crops

The accumulation of arsenic in plant parts is important for our human body. High arsenic in irrigation water and soil appears to result of arsenic in straw and grain of rice plants. Rice grain and straw is widely used as human food and cattle feed in Bangladesh and India. High arsenic in rice grain and straw may result in adverse impacts on human and animal health.

Arsenic concentration in crops depends on the plant species, variety and growth conditions. The use of arsenic contaminated water poses a risk to both food safety and crop yield. It is very unfortunate that farmer's knowledge is still limited in quantifying and managing risks. Arsenate has toxic effects on some plant species (Meharg and Hartley, 2002). Arsenate has a very similar chemistry to phosphate. Arsenite reacts with the sulphhydryl groups of enzymes inhibiting cellular functions. Seventy five percent of arsenic in the leaves was present as arsenite which is toxic to plants (Lombi *et. al*, 2002). In this fields some works have been done, relevant works are discussed bellow.

Abedin *et al.* (2002a) reported by based on greenhouse study that high arsenic in irrigation water and soil appears to result in higher concentration of arsenic in root, stem and leaf of rice plants. This result suggests that arsenic can be easily translocated paddy shoot. Since rice straw is widely used as cattle feed in Bangladesh and India, high arsenic in rice stem and leaf (i.e., in straw) may result in adverse health impacts on cattle and increase human arsenic exposure via the plant-animal-human pathway.

Anastasia and Kender (1973) reported that uptake of arsenic by plants occurs primarily through the root system, and the highest arsenic concentrations are reported in plant roots and tubers.

Bondara and Lina Qiying (2003) conducted an experiment to determine the effect of arsenic on pteridophyte, a fern, commonly known as Chinese Brake fern (*Pteris vittata* L.). The emergence of this arsenic loving ferns offers a great promise to phytoremediation, a plant driven environmentally being lean up process where in the roots take up colossal amount of a toxic metal from soils and rapidly sequester into their above ground portions. Plants capable of accomplishing such feature used termed as hyper accumulators. Chinese Brake fern qualifies as an arsenic hyper accumulator and thus has potential application in phytoremediation of arsenic contaminated sites.

Broeck-K-Vanden *et al.* (1997) reported that mungbean seedlings would be used as a bio-indicator of arsenic contamination. Endogenous arsenic concentrations in mungbean tissue were determined by inductively coupled plasma mass spectrometry. Experiments were carried out with arsenate and arsenite additions of 0.5-50 μM arsenic to the growth medium. The arsenate uptake increased with increasing arsenate concentrations in the growth medium, the highest accumulation occurring in the roots. Arsenite seemed to be far more toxic than arsenate (lethal dosage at $>10 \mu\text{M}$). Phosphate decreased the toxic effects of arsenate but had no effect on the arsenite uptake.

Carbonell-Barrachina *et al.* (1997a) reported that in a hydroponic culture, arsenic uptake and concentration in root of *Phaseolus Vulgaris* cv. Buenos Aires increased upon increased NaAsO_2 concentration in the nutrient solution. Upon uptake, arsenic was readily translocated to the aerial organs and approximately half of the absorbed arsenic was transported to the upper plant parts. The arsenic concentration in pods always remained below the recommended limit for arsenic content in fruit and edible vegetable products. Arsenite was phytotoxic.

Carbonell-Barrachina *et al.* (1997b) studied the effect of arsenic concentration of tomato cv. Marmande and bean (*Phaseolus vulgaris*) cv. BuenosAires grown in crushed volcanic rock. Tomato and bean plants were grown in nutrient solution containing arsenic at 0, 2, 5, or 10 mg/L. Arsenite was phytotoxic to both plant species; tomato plants, however, were more tolerant than bean plants. Bean plants exhibited symptoms of arsenic toxicity, and plants treated with 10 mg As/L were dead after 36+ days of treatment. Arsenic in tomato root tissue seems to be so effectively compartmentalized that's its impact on plant growth and metabolism was minimal. However, in bean plants upon uptake, arsenic was readily transported to shoots and accumulated to high concentrations in leaf tissue. The observed differences in absorption and translocation of arsenite or its metabolized species by tomato and bean plants were probably responsible for the difference in plant tolerance of arsenic pollution.

Carbonell-Barrachina *et al.* (1998 a) showed the effects caused by arsenite on the processes of uptake and accumulation of Ca, K, Mg, N and P in tomato plants (*Lycopersicum esculentum* cv. Marmende). Tomato plants were grown in a nutrient solution (hydroponically) containing arsenic (as sodium arsenite, NaAsO_2) at 2, 5 or 10

mg As/L. Vegetative growth and fruit yield were affected by arsenic concentration in the nutrient solution. Plant growth was significantly restricted by arsenic. Fresh fruit production decreased to 60.7%, 47.3% and 23.3% at 2, 5 and 10 mg As/L, respectively, compared to the control.

Das *et al.* (2004) determined the level of contamination in 100 samples of crop, vegetables and fresh water fish collected from three different regions in Bangladesh. Arsenic concentrations were determined by hydride generation atomic absorption spectrophotometry. All 11 samples of water and 18 samples of soil exceeded the expected limits of arsenic. No samples of rice grain (*Oryza sativa* L.) had arsenic concentrations more than the recommended limit of 1.0 mg/kg. However, rice plants, especially the roots had a significantly higher concentration of arsenic (2.4 mg/kg) compared to stem (0.73 mg/kg) and rice grains (0.14 mg/kg). Arsenic contents of vegetables varied; those exceeding the food safety limits included Kachu sak (*Colocasia antiquorum*) (0.09–3.99 mg/kg, n = 9), potatoes (*Solanum tuberosum*) (0.07–1.36 mg/kg, n = 5), and Kalmi sak (*Ipomoea reptans*) (0.1–1.53 mg/kg, n = 6). Lata fish (*Ophicephalus punctatus*) did not contain unacceptable levels of arsenic.

Gulz and Gupta (2000) carried out an experiment to investigate the ecotoxicological risks of arsenic. They assessed the arsenic risk for the plant-man, plant-animal-man pathway and transfer of arsenic from soil to plants. Therefore, pea, maize, ryegrass and rape were planted in arsenic containing nutrient solutions to test the uptake of arsenic by plants. The results showed that all plants in these experiments were able to accumulate higher amounts of arsenic than 1 mg As/kg (dry weight) which is the currently accepted health limit for human consumption.

Jacobs *et al.* (1970) expected that tuber crops (e.g. potatoes) could be to have higher arsenic concentrations than other crop types when grown in polluted soils. This appears not to be the case, since potatoes grown in a sandy soil that received arsenic additions ranging from 45 to 720 kg As/ha accumulated only 0.5 mg As/kg in the tuber. Jiang and Singh (1994) conducted two greenhouse experiments to investigate the effects of different sources of arsenic application on the yield of ryegrass (*Lolium perenne*) and barley (*Hordeum vulgare*) and the arsenic concentration in crop tissue grown in a sand and loam soil. In the first experiment (3 yr), arsenic was applied either as sodium arsenite or as

disodium hydrogen arsenate at the rate of 0, 2, 10, 50, and 250 mg As/kg soil in year 1. In years 2 and 3, only the residual effect of arsenic applied in year 1 was investigated. In the second experiment (5 Yr), a NPK (16-7-12) fertilizer containing 10, 100, and 3000 mg As/Kg as sodium arsenite was applied at rates of 750 and 600 mg/kg to ryegrass and barley, respectively with lime and without lime. Arsenite had a more inhibiting effect on crop yield than arsenate. The residual effect of arsenic resisted up to year 3, this was dependent on arsenic application rate, soil type and crop species. The yield reduction and the increase of arsenic concentration in crop were related to arsenic application rates. The arsenic rates of 50 and 250 mg As/kg resulted in significant yield reduction and marked increase of arsenic concentration in both crops and these rates could be considered as critical for both crops. In both experiments, the yield reduction and the increase in arsenic concentration in crop tissue were lower in the loam soil than in the sand and barley straw contained greater arsenic levels than barley grain, indicating that greater proportion of the arsenic taken up by this crop was retained in the vegetative organs.

Klose and Braun (1997) studied the arsenic content of soil and uptake by crops as included fodder plants, spring barley, potatoes, maize, winter rape, pasture grasses and clover. The soil arsenic content of all the soils tested was over 50 mg/kg soil. In maize, rape, barley and potatoes, arsenic content ranged from 0.04 to 1.31 mg As/kg dry matter when grown on soil containing 60-362 mg As/kg soil. In experiments with pasture grasses soil arsenic content ranged from 90 to 1050 mg As/kg soil and plant arsenic content ranged from 0.18 to 6.7 mg/kg dry matter.

Kiss *et al.* (1992) conducted a pot experiment using sandy chernozem soil and treated by arsenic through irrigation water as well as in soil on spring barley cv. Spartan. They observed that plant growth was reduced by arsenic and the leaves showed red or yellow discoloration. Irrigation with water containing arsenic increased plant arsenic content more than the soil arsenic treatment. In a similar trial with onions cv. Makoi, they also found that uptake of arsenic was greater by leaves than by roots in bulbs.

Laizu (2007) reported that the total 400 vegetables samples of 20 varieties of three categories were collected from a local market of Dhaka city. Speciation of arsenic (inorganic arsenic, MMA and DMA) and the amount of inorganic phosphate were

estimated. There was no significant variation in the concentration of inorganic phosphate levels among the three categories of vegetables. But in case of arsenic accumulation the fruiting vegetables, root and tube vegetables and leafy vegetables showed significant variation. Significant negative relations were observed between inorganic phosphate and inorganic arsenic in different types of fruiting vegetables. The fruiting vegetables contained low level of arsenic, in root and other vegetable, significant relation was present in arum. But in case arum loti significant positive relationship was observed between inorganic phosphate and DMA.

Martin *et al.* (2000) studied the distribution of arsenic in the stems of fruit trees grown in soils exposed to arsenical pesticides in Canada using neutron activation analysis. The results showed it to be confined mainly to heartwood near the pith and active xylem tissue in the most recent annual growth rings.

Otte *et al.* (1995) reported arsenic accumulation in the rhizosphere by wetland plants leads to precipitation of iron oxyhydrates. Arsenic and zinc have a high binding affinity for iron oxyhydroxides and accumulate in iron plaque around the roots of *Aster tripolium*. The soil under *Halimione portulacoides* contained more Fe, Zn and As per unit volume than under *Spartina anglica*. The concentration of Zn and As were higher in the rhizosphere as expected. It is suggested that differences in redox characteristics were most important in differing the concentration of Fe, Zn and As. Arsenic was enriched in the iron plaque of *Spartina anglica* but not in *Halimione portulacoides*.

Tlustos *et al.* (1998) conducted a pot experiment with three soils (fluvisol, chernozem and luvisol) differing in physical and chemical properties and in the total arsenic soil content, 5 kg of each soil was mixed with N, P, and K fertilizers, and As was applied as an aqueous solution. Sodium arsenate, sodium arsenite and dimethyl arsinic acid were applied at rates of 10 and 100 mg As/pot. Radishes cv. Duo was sown twice at each pot, with the first growing period immediately after application of the arsenic solutions and the second one a year later. The amount of available soil arsenic was determined by extraction of 0.01 mol. CaCl_2 solution per litre after each harvest. The higher arsenic rate caused significant yield reduction in all treatments. The most toxic effect was found after application of dimethylarsinic acid. In fluvisols and chernozems, plants died in the first growing period, but in the second period plants died only in fluvisols. Plants growing at the

lower arsenic rate accumulated more arsenic in leaves than in roots. Arsenic accumulation differed in plants treated with the higher rate of arsenic, probably due to protection of the assimilatory organs. The arsenic contents were higher in roots than in leaves.

Vaughan (1993) studied that the accumulation of arsenic in the edible parts of most plants is generally low. Plants seldom accumulate arsenic at concentrations hazardous to human and animal health because phytotoxicity usually occurs before such concentrations are reached.

Walsh and Keeney (1975) also reported that the major hazard for animal and human systems is ingesting arsenic from plants grown in contaminated soils or directly consuming arsenic contaminated water.

Wachaupe (1983) showed that plant uptake of arsenic from arsenic contaminated soils is a four-step process with the ultimate step being the one of a toxic reaction in the plant (1) arsenic is adsorbed to the root surface (2) arsenic moves from the exterior to the interior of roots (3) arsenic is translocated to some site of action and (4) a toxic biochemical reaction occurs in the plant. After arsenic has entered the plant, it is translocated throughout the plant freely; this is especially the case for arsenate. Translocation within the plant occurs both symplastically and appoplastically, with a common pathway being root → xylem → leaves.

Yu GuoYing *et al.* (1995) conducted a pot experiment to study the combined impact of Cd, Pb, Cu, Zn and As pollution on soybean cv. Liaofeng 241 growth. Under the conditions of combined pollution, the accumulation of heavy metals in soybean roots was in the order of As > Pb > Cu > Cd > Zn and that in stem and leaf was in the order of Cd > Cu > Zn > As > Pb. Interactions between nutrients depended on their concentrations and relative proportions. Arsenic and Cu were the main elements toxic to soybean growth. The relative ionic intensity was an effective index for indicating and controlling the integrated impact of combined pollution

1.12.2 Arsenic in Fish and Seafood

The highest mean total As concentrations in the European study by EFSA (2009) were found in fish and other seafood with a mean value of 2.38 mg/kg dw (typically 2– 60 mg As/kg dm). The inorganic As values in the data collected by EFSA were on average 2% of the total As for “Fish and fish products” and 1.2% for “Seafood and seafood

products'', with inorganic As concentrations below 0.2 mg As/kg dm. The low concentration of inorganic As in fish, was recently substantiated in a large study on inorganic As in 900 individual fish from Norwegian waters, where concentrations below 0.015 mg/kg were reported for all samples (Julshamn *et al.*, 2012).

However, there are some exceptions, where high proportions of inorganic As have been demonstrated in seafood, including certain seaweeds of the *Saragassum fusiforme* family, which may contain up to >70% of inorganic As (Holdt and Kraan, 2011). Other examples include blue mussels (*Mytilus edulis*) from certain areas in Norway, which have shown inorganic As concentrations up to 5.8 mg/kg (Sloth and Julshamn, 2008) and in some freshwater fish from Thailand (up to 2.6 mg/ kg dry mass inorganic As) (Jankong *et al.*, 2007).

Elevated As concentrations in the soil and groundwater may as well have an effect on the inland fisheries. According to Chowdhury and Maharajan (2001), 77% of the total fish production in Bangladesh was from inland fisheries of which 63% were from open water flood plains, river capture fisheries and the rest, 27% were from closed water ponds and tank culture fisheries. There is an indication in the literature data that higher As concentration in water leads to elevated As concentrations in fish and that very high concentrations can have toxic effects on fish (Erickson *et al.*, 2011).

1.12.3 Arsenic in Food of Animal Origin

The mean As concentration in food of animal origin (e.g. meat, meat products, offal, egg, milk and dairy based products) was in the range of 0.004–0.015 mg/kg wet weight, with maximum reported total As concentrations of 1.05, 0.182 and 0.66 mg/kg for meat, milk products and eggs indicating incidences of elevated As concentrations (EFSA, 2009).

Roychowdhury *et al.* (2002) reported that the villagers in the West Bengal area occasionally eat egg (once per week) and meat (once per month) indicating that their exposure through these food items was very limited.

Sigrist *et al.* (2010) studied the effect of As concentration in drinking water on total As levels in cow's raw milk. The As drinking water concentrations were up to 300 µg/L, whereas the As concentration in milk were below 2.2 µg/L indicating the relatively low transfer of ingested As to the milk in cow metabolism.

The high As concentrations reported in the study by Roychowdhury *et al.* (2002) could be due to contamination with As contaminated water, since it is customary in many countries to add water to milk.

1.12.4 Arsenic Transfer through Food Chain

The pattern of arsenic accumulation and its transfer from one trophic level to another is important.

Mason *et al.* (2000) reported a decrease of arsenic levels with the increase of higher trophic level. He also suggested that the subsequent transfer of arsenic to higher trophic levels is related to both the ability of the organisms to depurate and the mode of accumulation, either directly from water or from foodstuffs. Total arsenic concentrations in organisms after accumulation from foodstuffs decreased one order of magnitude per elevation of the trophic level.

Klose and Braun (1997) studied the arsenic content in soil and uptake by crops including fodder plants, spring barley, potatoes, maize, winter rape, pasture grass and clover. In maize, rape, barley and potatoes, arsenic content ranged from 0.04 to 1.31mg/kg dry matter when grown on 60–362 mg of As/kg soil. In experiment with pasture grasses, plant arsenic content ranged from 0.18 to 6.7 mg/kg dry matter when the soil arsenic content ranged from 90 to 1050 mg/kg soil. Limited reports are available on bioaccumulation of arsenic in different consumers of trophic levels such as animals, insects, birds and also men.

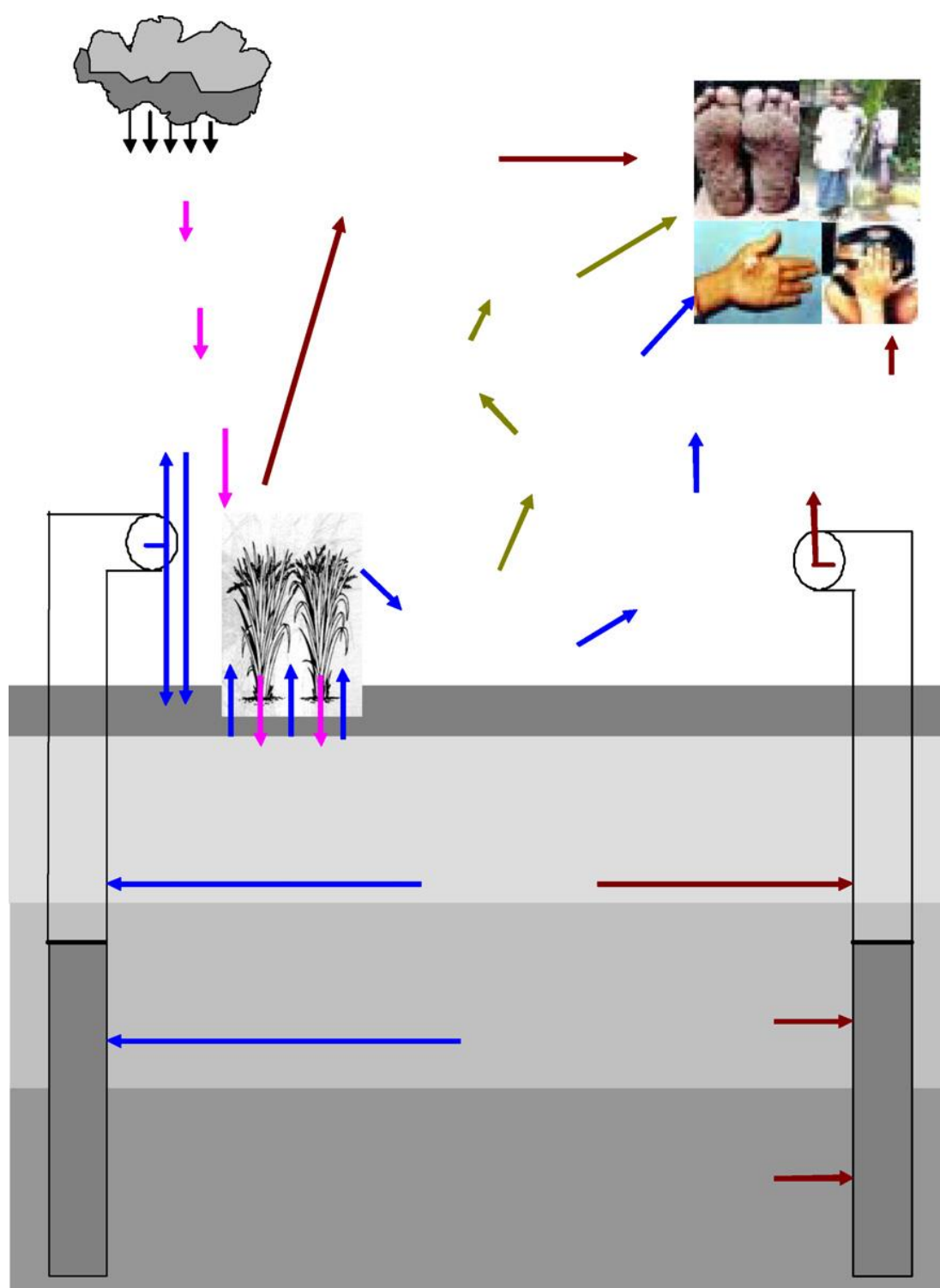


Figure 1.4 Arsenic Transfer through Food Chain

[Populations of Bangladesh, one of the severely arsenic-affected areas of the world, have been exposed to arsenic poisoning from drinking water directly. There are some other possible food chain pathways of terrestrial ecosystem through which humans may be exposed to arsenic poisoning from many sources as they are one of the topmost consumer of the ecosystem. (Rahman *et al.*, 2008)]

Because of low concentrations in terrestrial plants, arsenic accumulation in animals from this source is also low. Direct ingestion of arsenic from soil could be a major source of dietary arsenic for grazing livestock (Thornton *et al.*, 1979). Bruce *et al.* (2003) also reported direct ingestion of arsenic from soil. Rahaman *et al.* (2008b) estimated that about 1% of the arsenic in the soil was actually absorbed by the cattle, while the remaining is excreted directly. There have been different possible food chain pathways of natural ecosystem through which human beings (when considered as the topmost consumer of terrestrial ecosystem) may be exposed to arsenic toxicity (**Figure 1.4**).

1.12.5 Human Exposure to Arsenic through Food Chain

Batista *et al.* (2011) evaluated the concentration of total arsenic and five main chemical species of arsenic (As^{3+} , As^{5+} , DMA, MMA and AsB) in 44 different rice samples (white, parboiled white, brown, parboiled brown, parboiled organic and organic white) from different Brazilian regions using high-performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC–ICP-MS). The mean level of total arsenic was 222.8 $\mu\text{g/g}$ and the daily intake of inorganic arsenic (the most toxic form) from rice consumption was estimated as 10% of the Provisional Tolerable Daily Intake (PTDI) with a daily ingestion of 88 g of rice. Inorganic arsenic (As^{3+} , As^{5+}) and dimethylarsinic acid (DMA) are the predominant forms in all samples. The percentages of species were 38.7; 39.7; 3.7 and 17.8 % for DMA, As^{3+} , MMA and As^{5+} , respectively.

Li *et al.* (2011) Quantified inorganic arsenic (iAs) exposure from food for different population groups in China. By analyzing the data from the China National Nutrition and Health Survey (CNNHS) and collecting reported values of iAs in major food groups, they developed a framework of calculating average iAs daily intake for different regions of China. Based on this framework, cancer risks from iAs in food was deterministically and probabilistically quantified. They estimated for health risk due to the ingestion of food products contaminated with arsenic. Both per individual and for total population estimates were obtained. For the total population, daily iAs intake is around 42 $\mu\text{g/day}$, and rice is the largest contributor of total iAs intake accounting for about 60%. Incremental lifetime cancer risk from food iAs intake is 106 per 100,000 for adult individuals and the median population cancer risk is 177 per 100,000 varying between regions. Sensitive analysis indicated that cancer slope factor, ingestion rates of rice, aquatic products and inorganic

arsenic (iAs) concentration in rice were the most relevant variables in the model, as indicated by their higher contribution to variance of the incremental lifetime cancer risk. They concluded that rice might be the largest contributor of iAs through food route for the Chinese people.

Rahman *et al.* (2013) assessed the daily consumption by adults of arsenic (As) and other elements in drinking water and home-grown vegetables in a severely As- contaminated area of Bangladesh. Most of the examined elements in drinking water were below the World Health Organization (WHO) guideline values except As. The median concentrations of As, cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), Mn, nickel (Ni), and zinc (Zn) in vegetables were 90 µg/kg, 111 µg/kg, 0.80 mg/kg, 168 µg/kg, 13 mg/kg, 2.1 mg/kg, 65 mg/kg, 1.7 mg/kg, and 50 mg/kg, respectively. Daily intakes of As, Cd, Cr, Co, Cu, Pb, manganese (Mn), Ni, and Zn from vegetables and drinking water for adults were 839 µg, 2.9 µg, 20.8 µg, 5.5 µg, 0.35 mg, 56.4 µg, 2.0 mg, 49.1 µg, and 1.3 mg, respectively. The health risks from consuming vegetables were estimated by comparing these figures with the WHO/FAO provisional tolerable weekly or daily intake (PTWI or PTDI). Vegetables alone contribute 0.05 µg of As and 0.008 mg of Cu per kg of body weight (bw) daily; 0.42 µg of Cd, 8.77 mg of Pb, and 0.03 mg of Zn per kg bw weekly.

Rahman *et al.* (2009) measured As concentrations in rice grain collected from households in As-affected villages of Bangladesh where groundwater was used for agricultural irrigation and estimated the daily intake of As consumed by the villagers from rice. The median and mean total As contents in 214 rice grain samples were 131 and 143 µg/kg, respectively, with a range of 2–557 µg/kg (dry weight, dw). Arsenic concentrations in control rice samples imported from Pakistan and India and on sale in Australian supermarkets were significantly lower ($p < 0.001$) than in rice from contaminated areas. Daily dietary intake of As from rice was 56.4 µg for adults (males and females) while the total daily intake of As from rice and from drinking water was 888.4 and 706.4 µg for adult males and adult females, respectively. From their study, it appears that the villagers are consuming a significant amount of As from rice and drinking water.

Rahman *et al.* (2008) conducted a study to find out the possible food chain pathways through which arsenic may enter into human body in Bangladesh. Arsenic concentration in rice grain was 0.5 ± 0.02 mg/kg with the highest concentrations being in grains grown

on soil treated with 40 mg As/kg soil. With the average rice consumption between 400 and 650 g/day by typical adults in the arsenic-affected areas of Bangladesh, the intake of arsenic through rice stood at 0.20–0.35 mg/day. With a daily consumption of 4 L drinking water, arsenic intake through drinking water stands at 0.2 mg/day. Moreover, when the rice plant was grown in 60 mg of As/kg soil, arsenic concentrations in rice straw were 20.6 ± 52 at panicle initiation stage and 23.7 ± 0.44 at maturity stage, whereas it was 1.6 ± 0.20 mg/kg in husk. Cattle drink a considerable amount of water. So alike human beings, arsenic gets deposited into cattle body through rice straw and husk as well as from drinking water which in turn finds a route into the human body. Arsenic intake in human body from rice and cattle could be potentially important and it exists in addition to that from drinking water.

Rahman *et al.* (2006) conducted a field level experiment in Bangladesh to investigate the influence of cooking methods on arsenic retention in cooked rice. Rice samples were collected directly from a severely arsenic affected area and also from an unaffected area, to compare the results. Rice was cooked according to the traditional methods employed by the population of subjected areas. Arsenic concentrations were 0.40 ± 0.03 and 0.58 ± 0.12 mg/kg in parboiled rice of arsenic affected area, cooked with excess water and 1.35 ± 0.04 and 1.59 ± 0.07 mg/kg in gruel for BRRI dhan28 and BRRI hybrid dhan1, respectively. In non-parboiled rice, arsenic concentrations were 0.39 ± 0.04 and 0.44 ± 0.03 mg/kg in rice cooked with excess water and 1.62 ± 0.07 and 1.74 ± 0.05 mg/kg in gruel for BRRI dhan28 and BRRI hybrid dhan1, respectively. Total arsenic content in rice, cooked with limited water (therefore gruel was absorbed completely by rice) were 0.89 ± 0.07 and 1.08 ± 0.06 mg/kg (parboiled) and 0.75 ± 0.04 and 1.09 ± 0.06 mg/kg (non-parboiled) for BRRI dhan28 and BRRI hybrid dhan1, respectively. Water used for cooking rice contained 0.13 and 0.01 mg of As/L for contaminated and non-contaminated areas, respectively. Arsenic concentrations in cooked parboiled and non-parboiled rice and gruel of non-contaminated area were significantly lower ($p < 0.01$) than that of contaminated area. The results imply that cooking of arsenic contaminated rice with arsenic contaminated water increases its concentration in cooked rice.

Wong *et al.* (2013) conducted a total diet study, the dietary exposure of the Hong Kong people, including various age-gender subgroups, and inorganic arsenic was estimated for assessing the associated health risk. Food samples, which represented the Hong Kong

people's diet, were collected and prepared "as consumed" for analysis. Concentrations of inorganic arsenic, as sum of arsenite (As(III)) and arsenate (As(V)) were determined in 600 composite samples by using inductively coupled plasma mass spectrometry. The dietary exposures were estimated by combining the analytical results with the local food consumption data of the adult population. The mean and 95th percentile of inorganic arsenic exposures of the Hong Kong people were 0.22 and 0.38 $\mu\text{g/kg}$ body weight (bw)/day, respectively. Among the 12 age-gender subgroups, the respective exposures ranged from 0.19 to 0.26 $\mu\text{g/kg}$ bw/day and from 0.33 to 0.46 $\mu\text{g/kg}$ bw/day. The main food category that contributed inorganic arsenic was "cereals and their products" (53.5% of the total exposure), particularly rice.

It is clear from previous reports that arsenic deposits in tissues of crop plants grown in arsenic-rich soil irrigated with arsenic contaminated water. Arsenic accumulation has been reported in maize (Sadiq, 1986), barley and ryegrass (Jiang and Singh, 1994), rice (Duxbury *et al.*, 2003; Abedin *et al.*, 2002a; Marin *et al.*, 1992; Bae *et al.*, 2002; Onken and Hossner, 1995; Rahman *et al.*, 2004; D'llio *et al.*, 2002), *Spartina alterniflora* (Carbonell *et al.*, 1998 b) too. The accumulation of arsenic in plants occurs primarily through the root system and the highest arsenic concentrations have been reported in plant roots and tubers (Anastasia and Kender, 1973; Marin *et al.*, 2003). Therefore, tuber crops are expected to have higher arsenic contents than that of other crops when those are grown in arsenic contaminated soil. The concentration of arsenic in edible parts of most plants is generally low (Vaughan, 1993; O'Neil, 1995). Plants seldom accumulate arsenic at concentrations hazardous to human and animal health because, phytotoxicity usually occurs before such concentrations are reached (Walsh and Keeney, 1975). Although human may be exposed to arsenic from a variety of environmental sources, food constitutes the largest source of arsenic intake with smaller contribution from air and drinking water (Chen and Lin, 1994). In a tropical country like Bangladesh, water consumption is normally very high. Most of the arsenic affected areas are villages where people are involved in agrarian manual labor. Daily water consumption by an adult ranged between 4 and 6L (Farmer and Johnson, 1990) and when the arsenic concentration in drinking water is 0.05 mg/L, the acceptable limit for drinking water in Bangladesh (though in many areas, arsenic concentrations in drinking water has been found to be more than this), an adult is expected to intake 0.2–0.3 mg of As/day from drinking water.

The average daily rice consumption by an adult of this area is between 400 and 650 g raw rice grain (Duxbury *et al.*, 2003). Bae *et al.* (2002) reported that the concentration of arsenic in cooked rice was higher than that of raw rice. Rahman *et al.* (2006) also reported elevated concentrations of arsenic in cooked rice when the rice was cooked with arsenic-contaminated water and the gruel was not discarded after cooking. This was because the arsenic in water was absorbed by cooked rice. Ackerman *et al.* (2005) found 89–105% absorption of arsenic by rice from total volume of water [1:1–4:1 (water: rice)] used in cooking for two different contaminated drinking water. Moreover, most of the arsenic in drinking water is dissolved as toxic inorganic forms, while the species of arsenic in raw and cooked rice are poorly characterized (Duxbury *et al.*, 2003). Schoof *et al.* (1999) reported that between 30% and 85% of arsenic in rice is inorganic. These reports suggest that intake of arsenic from rice and its potential to human exposure should not be ignored.

Tsutsumi *et al.* (1980) reported 149 mg of As/kg dry weight in rice straw when soil arsenic concentration was 313 mg/kg. Abedin *et al.* (2002a) found 25 mg of As/kg dry weight in rice straw when the plant was irrigated by 2mg of As/L water. Cattle are one of the primary consumers of terrestrial ecosystem. They feed on rice straw and husk and drink water as well. Though there is no direct report of arsenic accumulation in cattle body from rice straw or husk, the consequence of exposure to this toxic element in organs such as the liver and kidneys of this animal is well reported (WHO, 2001). Bruce *et al.* (2003) reported arsenic accumulation in liver and other tissues of tailing paddock animals though the accumulation was insignificant to cause chronic toxicity or any immediate perceivable contamination. Because Bruce *et al.* (2003) conducted their experiment for a short time (240 days); they expected more accumulation of arsenic in cattle if the experiment were for longer time. Straw given to cattle in UK contained less than 0.20 mg As/kg (Nicholson *et al.*, 1999), though arsenic metabolized by the cattle is dependent on the arsenic species in the straw and on the metabolism of cattle (Abedin *et al.*, 2002b). In another experiment, Shariatpanahi *et al.* (1984) reported that sheep fed on methylarsonate showed a significant increase of arsenic accumulation in their tissues and milk. Although there have not been found adequate data on the presence of arsenic in milk and meat of the cattle of Bangladesh and those imported from West Bengal, India (another arsenic epidemic area, where arsenic contamination in ground water is alarming), there is an ample scope of arsenic deposition in cattle body, especially from high arsenic-containing rice straw and husk.

Thus, the possible deposition of arsenic in human body not only be from drinking water but also from beef and mutton through “Plant–Animal–Man” and some other food chain pathways (**Figure. 1.4**). All studies suggest that the possible health risk of human beings from arsenic toxicity through “Plant–Animal–Man” food chain pathway should not be ignored.

1.13 Effects of Arsenic Toxicity on Human Health in Bangladesh

Chronic exposure to arsenic has been linked to adverse health effects in human populations. Arsenic is a known carcinogen and has potentialities of producing cancers at multiple sites, notably in the skin, bladder, kidneys, prostate, and lungs (WHO, 2001; ATSDR, 2005; WHO, 1981). Arsenic is known to have both cancer and non-cancer health effects (WHO, 2001; ATSDR, 2005; WHO, 1981). Many of the health problems related to arsenic exposure, ranging from the classical dermatological signs such as melanosis (**Figure 1.5**), keratosis (**Figures 1.6 and 1.7**), and leukomelanosis (**Figure 1.8**) to respiratory problems, anemia, weakness, conjunctival congestion, diabetes mellitus, hypertension, hepatopathy, peripheral neuropathy, non-pitting edema of lower limbs (**Figure 1.9**), adverse reproductive outcomes, gangrene (Figure 1.10), Bowen’s disease (**Figure 1.11**), cardiovascular and cerebrovascular diseases, peripheral vascular disease, and skin cancers (**Figures 1.12 and 1.13**), are already evident among the arsenic-exposed population in Bangladesh (Ahmed, 2003; Ahmad, 2001; Ahmad, 1998;Dhar,1997; Ahmad, 1999a; Sikder, 1999; Tondel,1999;Rahman,1998;Rahman,1998;Milton,2001)

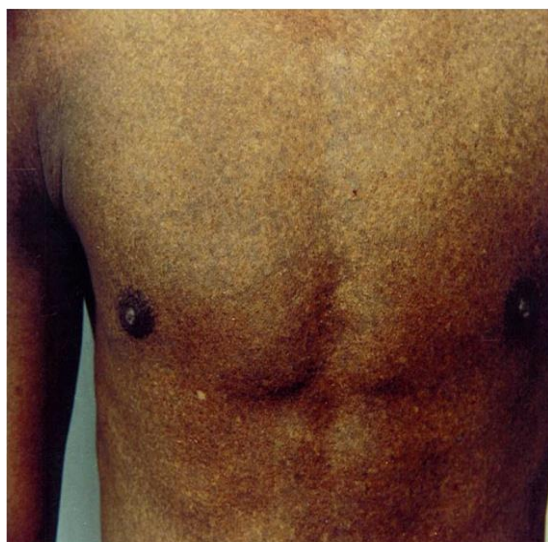


Figure 1.5 Melanosis



Figure 1.6 Keratosis of Palm



Figure 1.7 **Keratosis of Sole**



Figure 1.8 **Leukomelanosis**

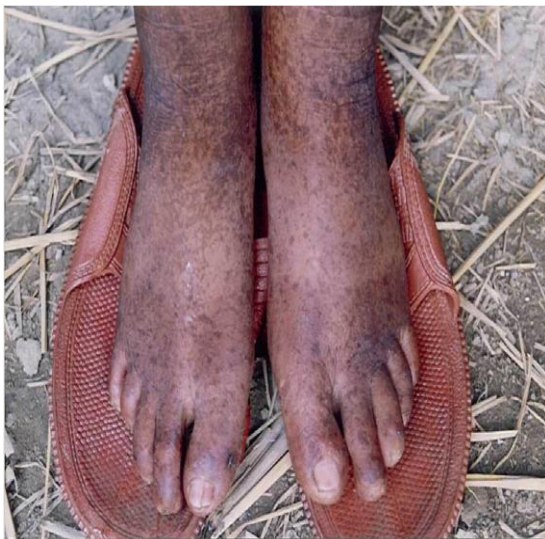


Figure 1.9 **Bilateral Non-pitting Edema**



Figure 1.10 **Gangrene of Foot**



Figure 1.11 Bowen's Disease



Figure 1.12 Squamous Cell Carcinoma of Palm



Figure 1.13 Squamous Cell Carcinoma of Scalp

In Bangladesh, the disease due to chronic arsenic toxicity is known as arsenicosis. For identification and diagnosis of arsenicosis cases the WHO case diagnosis and management protocol is usually followed. The disease is usually first diagnosed on the

basis of skin manifestations. According to WHO protocol (WHO, 2005), arsenicosis has been defined as a chronic health condition arising from prolonged ingestion of arsenic above the safe dose for at least 6 months, usually manifested by characteristic skin lesions of melanosis and keratosis, occurring alone or in combination, with or without the involvement of internal organs.

In different studies (**Table 1.2**) it was reported that almost 100% of arsenicosis patients had melanosis. Keratosis was found in about 58.8 to 80% of the reported cases.

Table 1.2 Common Manifestations of Chronic Arsenic Toxicity Patients

Arsenicosis Manifestations	<i>n</i> = 110	<i>n</i> = 96	<i>n</i> = 363	<i>n</i> = 250	<i>n</i> = 116	Total
Melanosis	100.0	98.9	99.5	100.0	100.0	99.7
Keratosis	58.8	92.7	68.9	80.0	79.3	75.5
Conjunctival congestion		9.4	15.7	12.0	25.0	15.3
Chronic cough	33.8	25.8	23.7	20.0	26.7	21.8
Weakness	88.2			100.0	93.0	93.7
Pedal edema	4.4		2.8		6.9	4.7
Skin cancer	2.9	1.04	0.8	1.0		1.4

Source : (Ahmad, 1997; Ahmad, 1999a; Sikder, 1999; Hoque, 1996; Sikder, 2000)

Other common manifestations were chronic cough (20.0 to 33.8%), conjunctival congestion (9.4 to 25.0%), and pedal edema (2.5 to 6.9%). Pedal edema is a complication of arsenicosis, usually bilateral, and is mainly reported from Bangladesh and West Bengal, India. However, one of the major complications of the arsenicosis patient is weakness and in Bangladesh weakness was found among 88.2 to 100% of arsenicosis patients (Khan, 1997; Ahmad, 1999a; Sikder, 1999).

Table 1.3 Prevalence of Some Non-communicable Diseases (NCDs) Among the Arsenic-Exposed Population

NCDs	Exposed to Arsenic			Unexposed			Statistical Test
	Yes	No	Total	Yes	No	Total	
Diabetes	21	142	163	25	829	854	CPR 4.4
	12.9%	87.1%	100.0	2.9%	97.1%	100.0	(95%, CI 2.5–7.7)
Hypertension	198	1283	1481	9	105	114	CPR 1.7
	13.4%	86.6%	100.0	7.9%	92.1%	100.0	(95%, CI 0.8–3.3)
Respiratory effect	29	65	94	13	111	124	CPR 2.9
	30.8%	69.2%	100.0	10.5%	89.5%	100.0	(95%, CI 1.6–5.0)

Source: (Ahmad, 1997; Ahmed, 2003; Ahmad, 2001; Ahmad, 1998; Dhar, 1997; Ahmad, 1999a; Sikder, 1999; Tondel, 1999; Rahman, 1998; Rahman, 1998; Milton, 2001)

Occurrence of non-communicable diseases such as hypertension and diabetes mellitus is higher among the arsenic-exposed population compared to the non-exposed population. Studies conducted on a Bangladesh population had demonstrated increased occurrence of hypertension, diabetes mellitus, and chronic respiratory disorders in terms of chronic cough and asthma among arsenicosis patients compared to those having no exposure to arsenic (Rahman, 1998; Rahman, 1998; Milton, 2001; Islam, 2005; Nabi, 2005; Parvez, 2010; Chen, 2011.). Studies revealed (**Table 1.3**) that the crude prevalence ratio for diabetes mellitus among arsenicosis patients was 4.4 (95%, CI 2.5–7.7). The crude prevalence ratio for hypertension was 1.7 (95%, CI 0.8–3.3) and that for respiratory disorders was 2.9 (95%, CI 1.6–5.0).

A study conducted in Bangladesh compared ECG findings among the arsenicosis, non-arsenicosis, and non-exposed groups of respondents, and revealed that overall, abnormal ECG findings were high (58%) among arsenicosis cases and highly significant ($p < 0.001$) (Ahmad, 2006).

Another study revealed that mortality due to cardiovascular diseases among the arsenic-exposed population was high in Bangladesh: it was found that 43% of total arsenic-related deaths was due to such disease (Chen, 2011).

An extreme manifestation of lower extremity arterial disease (LEAD) is gangrene, which has been reported in a number of studies focusing on the health effects of arsenic in Bangladesh (Khan, 1997; Ahmad, 1999a).

Khan *et al.* (2010) found that the prevalence and risk of LEAD was higher among the population whose drinking water source(s) contained arsenic in excess of 0.05 mg/L than among those whose drinking water source(s) did not contain excess arsenic (<0.05 mg/L). A normal ankle brachial systolic pressure index (ABSPI) was found more frequently in the arsenic-exposed (13.3%) than in the non-exposed (2.5%) group. Further, they found that the prevalence and risk of LEAD was higher if patients had developed signs of arsenicosis (melanosis \pm keratosis).

Table 1.4 Adverse Pregnancy Outcomes Among Exposed and Non-exposed Population

Adverse Pregnancy Outcomes	Arsenic		<i>P</i> -value
	Exposed	Non-exposed	
Abortion	68.8	23.7	$z = 2.65, p = 0.008$
Still birth	53.1	23.7	$z = 2.0, p = 0.046$
Pre-term birth	68.8	27.1	$z = 2.45, p = 0.014$

Source: (Ahmad, 2001)

For arsenic-exposed mothers, adverse pregnancy outcome has been reported from various parts of the world. In Bangladesh, adverse pregnancy outcome has also been evident in studies (Ahmad, 2001; Milton, 2005; Kwok, 2006).

A study (**Table 1.4**) that compared pregnancy outcomes in terms of live birth, stillbirth, spontaneous abortion, and pre-term birth among arsenic-exposed and non-exposed women revealed that spontaneous abortion, stillbirth, and pre-term birth rates were significantly higher among those women who had been exposed to arsenic-contaminated water than among those who had not been exposed ($p = 0.008$, $p = 0.046$, and $p = 0.018$, respectively). The participants of the study had been matched for age, socio-economic status, education, and age at marriage (Ahmad, 2001).

In another study, the odds ratios for spontaneous abortion, stillbirth and neonatal deaths were found to be 2.5 (95% CI 1.5-4.3), 2.5 (1.3-4.9) and 1.8 (0.9-3.6) respectively in mothers whose drinking water arsenic content was greater than 50 µg/L compared to that in mothers whose drinking water arsenic level was 50 µg/L or less (Kwok, 2006).

It is known that arsenic is neurotoxic; studies conducted in Bangladesh showed reduced mental development among children aged 1–5 years. In that study 18% of the children who were exposed to arsenic-contaminated water (arsenic levels >50 µg/L) showed abnormal mental development and reduced intellectual function compared to the children who did not drink arsenic-contaminated water (Akhtar, 2007). Another study conducted among children aged 8–11 years revealed an inverse association of arsenic exposure through drinking water with lower developmental scores of the children (Parvez, 2011).

Arsenic is a known carcinogen and has the potential to produce cancers at multiple sites, notably in the skin, bladder, kidneys, prostate, and lungs (WHO, 2001; ATSDR, 2005;

WHO, 1981.) In Bangladesh, skin cancer, lung cancer, and renal cancer have been reported in different studies (Ahmad, 1998; Chen, 2004; Mostafa, 2013). However, skin cancers were more common and the types of skin cancers are squamous cell cancer and basal cell cancer. Bowen's disease is a precancerous skin lesion and is evident among the arsenicosis patients of Bangladesh (Ahmad, 1997; Ahmad, 1998; Alam, 2010). It is estimated that there will be a doubling of the potential lifetime mortality risk from cancer in Bangladesh due to arsenic in drinking water and it is indicated that the overall lifetime mortality risk due to cancer of the lung, liver, and bladder resulting from such exposure will be 229.6 per 100,000 population (Chen, 2004).

1.14 Epidemiology of Arsenicosis in Bangladesh

Arsenicosis is prevalent in the rural areas of Bangladesh, and is associated with age, sex, socio-economic conditions, and nutritional status of the host. In Bangladesh, most arsenicosis patients are between 20 and 40 years of age. People are being exposed to arsenic mainly through consuming arsenic-contaminated tube-well water. Arsenicosis has been found to be more common among males (53.7%) in comparison to females (46.3%). Female arsenicosis patients are found to be associated with low concentrations of arsenic in tube-well water. A significantly low dose of arsenic intake was found among females (1.321 mg) compared to males (1.734 mg). These findings indicate that females are more susceptible to the toxic effects of chronic arsenic exposure. Arsenicosis patients are mostly from low socio-economic backgrounds and suffering from malnutrition. The majority of these patients are in a mild or moderate stage, and severe cases or cases with complications are few (Ahmad, 1997; Ahmad, 1998; Ahmad, 1999a; Sultana, 2012; Ahmad, 1999a).

Das *et al.* (2009) analyzed 39,000 biological samples for urine As speciation from both patients and non-patients in Bangladesh. 83% of the samples had As above normal level. Arsenical skin lesions were identified in 13,118 people, screening 114,841 from West Bengal and Bangladesh. About 6.1% of 5,000 children in Bangladesh and 1.7% of 14,000 in West Bengal were found to have arsenical skin lesions.

Ahmad *et al.* (1999b) carried out a study in a village in Jessore district, Bangladesh, to identify the epidemiological characteristics of arsenicosis. Eighty-seven per cent of the tube-wells had arsenic concentration more than the WHO maximum permissible limit of 0.05

mg/L. The mean arsenic concentration was 0.240 mg/L and the maximum concentration was 1.371 mg/L. Of the total 3606 villagers, 10% (363) were found to be suffering from arsenicosis. Most of the arsenicosis patients were between 10 to 39 years of age. There were more male patients (52.6%). There were no patients among villagers who consumed tube-well water having arsenic levels less than 0.082 mg/L. The majority (93.4%) of the patients were in the first and second stage of arsenicosis. With increasing exposure to arsenic, a simultaneous increase in the severity of clinical manifestations of arsenicosis was observed ($F = 43.699$; $p = 0.000$). The time-weighted arsenic exposure varied from 0.248 to 5.482 mg day⁻¹ and the mean was 1.918 mg day⁻¹. Melanosis was present in almost all the patient (99.5%) and keratosis was present in 68.9%. Cancer (basal cell epithelioma) was present in three (0.8%) patients. The duration of clinical manifestations of arsenicosis varied from 1 to 12 years and the majority were suffering for 4-6 years.

Ahmad *et al.* (1997) reported that of the 1273 people exposed to arsenic contaminated water supply 7.5 % showed clinical manifestations of arsenicosis in Rajarampur village in the Nawabgonj district of north-western Bangladesh. The majority of these (59.4%) were female. There were no cases below 7 years of age. The most frequently seen clinical manifestations were melanosis (98.9%), keratosis (92.7%), hyperkeratosis (45.8%), depigmentation (29.2%), anorexia (26.0%) and cough (25.0%). Hepatomegaly was detected in 3.2% of the population and there was one case of squamous-cell carcinoma.

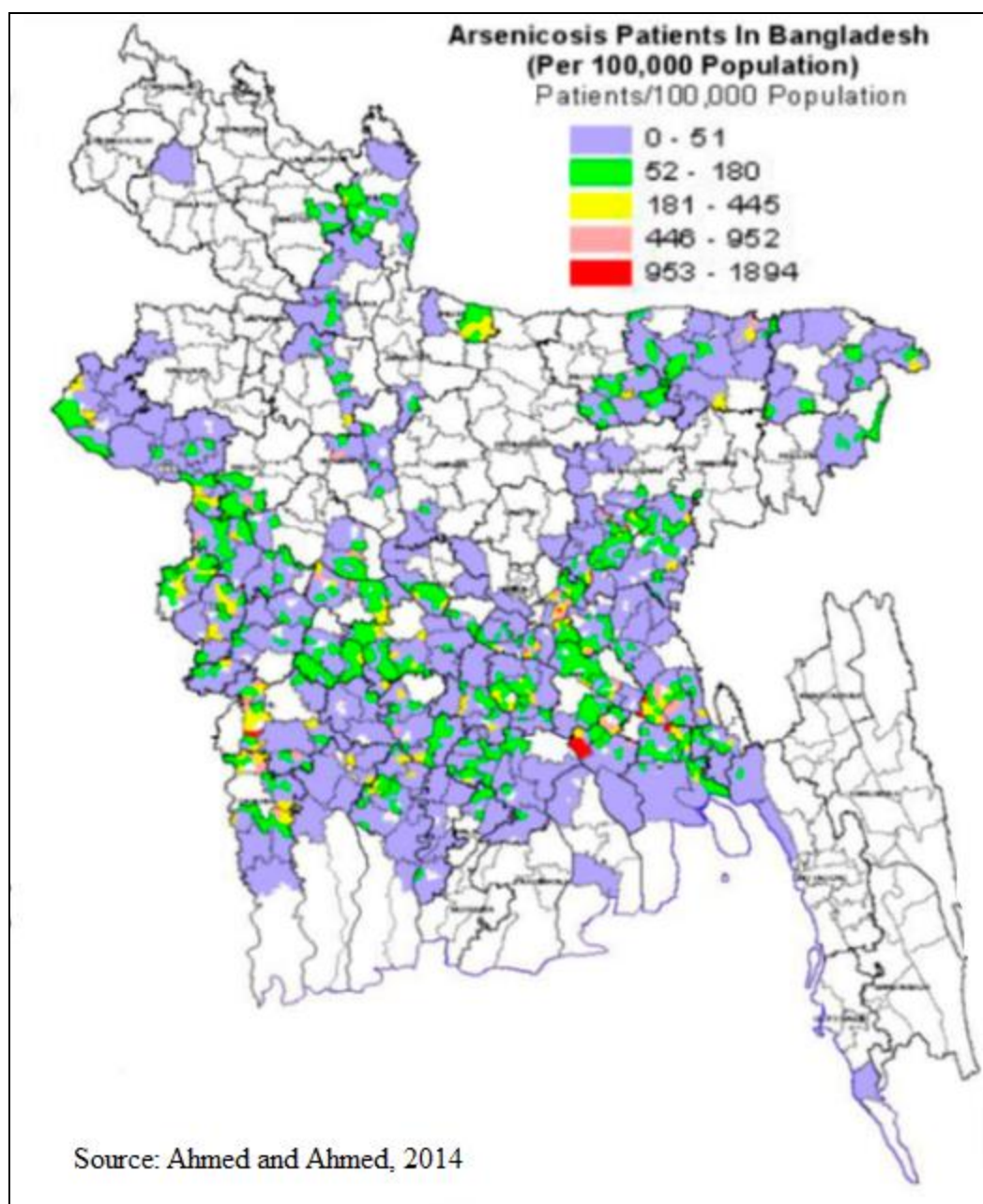


Figure 1.14 Arsenicosis Patients in Bangladesh

1.15 Socio-Economic Impacts of Arsenicosis in Bangladesh

Arsenic contamination in ground water and thereby the occurrence of arsenicosis is not only a major public health problem but leads to many social problems and economic setbacks in rural Bangladesh. Studies found that poor people are more affected than others by arsenicosis. Skin lesions are higher among men compared to women, and women are more socially damaged than men. The majority of rural people believe that arsenicosis is a contagious disease and could spread from person to person through

physical contact, or through contact with objects touched or used by individuals with arsenicosis (Sultana, 2012; Ahmad, 2006; APSU, 2006).

In Bangladesh, women suffering from arsenicosis are ignored in society and may be divorced or abandoned, and unmarried women find difficulty getting married. Female arsenicosis patients are less likely to receive treatment and face further problems getting treatment from a healthcare facility. Social and cultural values make it difficult for them to attend to their own health care and travel to service providers. Other concerns for female patients needing treatment are long waiting times, discrimination in service delivery, and inadequate separate facilities for female patients (Ahmad, 2006; APSU, 2006).

Besides social problems, there are some economic consequences of arsenicosis to consider. Many arsenic victims who are ill are found to be too weak to work, lose their jobs, or are refused work because of fears of contagion. In a study, the majority (58.6%) of the respondents said they faced various economic setbacks (Ahmad, 2006; APSU, 2006).

Regarding access to a healthcare facility for arsenicosis treatment, about half (50.7%) of arsenicosis patients face difficulties getting treatment. On the other hand, the length of time needed to recover from symptoms of arsenicosis lead to a loss of faith in the efficacy of treatment, which extends to laxity in seeking health care. Study findings reveal that a significant proportion (79.9%) of arsenicosis patients are found to access alternative health care, which includes homeopathy, village doctors, and *kabiraj* (Ahmad, 2006).

1.16 Rationale of the Study

Considering the nature of the problem, arsenic contamination in Bangladesh has been recognized as a major public health issue. Arsenic contaminated tube-wells are widely distributed throughout Bangladesh, except in three hilly districts. About 50 million people are at risk of arsenic toxicity. Besides common skin manifestations, increased risks of non-communicable diseases (NCDs), adverse pregnancy outcomes, and skin cancers among arsenicosis patients are evident and could become a big public health problem. The most important concern is increased incidence of cancers due to long-term exposure to low dose arsenic which could overburden the economy and health system of Bangladesh. The environmental health crisis stemming from arsenic contamination of

ground water is currently one of the world's greatest environmental health crises. Millions of people continue to be exposed to arsenic through water and food. The population in Bangladesh will be confronting the consequences of arsenic contamination in water supply, agriculture, and health sectors for generations to come.

Although arsenic associated diseases are not contagious, people suffering from such diseases are facing serious social and economic problems. They can't mix with people who hate them and have matrimonial problems. They are not allowed to sit in a tea stall and they are waiting for losing jobs.

The economy of Bangladesh is primarily based on agriculture. More than 70 to 80 percent of the labour forces are involved in agriculture. Most of the people in Bangladesh rely on rice for consumption. Paddy rice is generally used for local consumption, selling to urban communities and for export. Rice production has increased substantially during the last 30 years following the installation of tube-wells. The extraction of groundwater for irrigation has been increased a large from the last few years. Arsenic in groundwater may pose an even more insidious threat. From a major review of studies conducted in Bangladesh, concludes that people may be exposed to arsenic not only through drinking water, but indirectly through food crops irrigated by arsenic contaminated groundwater. This is why millions of people are exposed to high levels of arsenic from drinking water and thousands are suffering from arsenic poisoning but little is known about the fate and transport of arsenic in the food chain. Health risks assessments of arsenic in foods and food consumption patterns, which are not yet robust enough for Bangladesh. This situation is creating a lot of problem for human and animal being as well as our environment. The adverse impact of arsenic ingestion to human and animal health has led to significant economic loss through human loss, health and medical costs and decline in crop productivity.

Rice is an efficient accumulator of arsenic and thus irrigation with arsenic contaminated groundwater and soil may induce human health hazard via water-soil-plant-human pathway. Although human exposure to arsenic is thought to be caused mainly through arsenic contaminated underground drinking water, the use of this water for irrigation enhances the possibility of arsenic uptake into crop plants especially rice. It is the staple food for the people of arsenic prevalent South (S) and South-East (SE) Asian countries including Bangladesh. In this region, arsenic contaminated groundwater has been used

not only for drinking and cooking purposes but also for rice cultivation during dry season. Irrigation of arsenic-contaminated groundwater for rice cultivation has resulted high deposition of arsenic in topsoil and uptake in rice grain. Rice is the major food exposure route to inorganic As followed by other grain and vegetables. Inorganic arsenic is the main species of S and SE Asian rice (80 to 91% of the total arsenic). Excessive and long-term human intake of toxic inorganic As with food and water is causing arsenicosis, which is disfiguring, disabling, and leading to potentially fatal diseases. The people of Bangladesh eat an average of 450 g rice a day. In addition to drinking water, dietary intake of arsenic from rice is supposed to be another potential source of exposure, and to be a new disaster. The continued presence of arsenic in soil will have long-term effect on crop productivity and quality. This will in turn impact on the local and international economy.

Agriculture sector is playing a vital role regarding employment generation, foreign currency earning and poverty alleviation in Bangladesh. Its importance is increasing day by day. Government of Bangladesh has given an especial attention in this sector. So, it is very much important to minimize the concentration of arsenic in irrigation water for the production of crop to maintain its proper environment and to explore the sustainable management system. Irrigation with arsenic-rich groundwater poses threats to sustainable agriculture in Bangladesh. The extent and urgency of those threats urgently need to be assessed. There is a rich body of literature on arsenic (As) contamination of groundwater and its consequences for human health via drinking water. Less is known however, on the impacts that flow from the use of arsenic rich groundwater for irrigation or the effectiveness of arsenic remediation in agricultural systems. There is a burning need to search for safe water for irrigation.

This study will find out remedial measures that will reduce arsenic toxicity in crops. This study will also be helpful for the policy makers for reviewing the policy in water management in agricultural sector. This study may also be a guideline to the future researchers.

1.17 Objectives of the Study

Bangladesh has no standard of permissible level for arsenic in irrigation water yet. Most of the research works on arsenic accumulation into rice plants conducted with pot experiments. Limited researches have been done in open field soil conditions. Detailed information is needed to produce arsenic free rice in open field soil conditions. Therefore,

open field experiments were conducted to find out the safe arsenic level in irrigation water and secure water management system for rice cultivation to produce arsenic free rice considering the following specific objectives:

- i. To reveal the effects of arsenic amended irrigation water on BR-11 rice (*Oryza sativa* L) grown in open field.
- ii. To determine the effects of residual arsenic in soil on BRRI dhan-50 (*Oryza sativa* L) grown in open field.
- iii. To verify arsenic accumulation into rice (*Oryza sativa* L) cultivated with arsenic contaminated STW groundwater in open field condition.

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Chapter- 2

**Effect of Arsenic Amended Irrigation Water
on BR-11 Rice (*Oryza sativa L.*) Grown in
Open Field Gangetic Soil Condition**

Abstract

Rice was grown in an open-field Gangetic soil condition with arsenic amended irrigation water in experimental plot at Institute of Environmental Science, University of Rajshahi University to see the effect of arsenic (As) on growth and yield of rice (*Oryza sativa L.*) and to observe the trend of arsenic (As) accumulation into rice and soil. A popular HYV rice variety named BR-11 was cultivated with arsenic amended irrigation water (0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/L As containing water) in a green house made of transparent polyethylene paper. Sodium arsenate (Na_2HAsO_4) was added to irrigation water for arsenic source. Arsenic accumulation of rice straw, grain and soil were investigated. The tillers number, panicle length and grain yield of BR-11 rice were found to decrease significantly ($p \leq 0.05$) with increase of arsenic (As) concentration in irrigation water. The highest plant height and straw yield was observed in 0.5 mg/L treatment, whereas highest tillers number, panicles number, panicle length and grain yield were found in control treatment. The lowest values of these parameters were observed in the treatment of 4.0 mg/L As containing irrigation water. A significant ($p \leq 0.01$) increasing trend of arsenic accumulation in straw, grain and soil was found with increase of arsenic in irrigation water. The highest level of arsenic in straw, grain and soil was observed in the treatment of 4.0 mg/L As containing irrigation water and lowest level in control treatment. Arsenic in irrigation water showed a strong positive correlations with arsenic accumulation into soil, straw and grain, and the trend of accumulation was found as water > soil > straw > grain.

2.1 Introduction

Widespread arsenic (As) pollution of shallow tube well (STW) irrigation water and increasing accumulation of As in soils have become a major threat to rice, the staple food crop of Bangladesh (Marin *et al.*, 1992; Yan *et al.*, 2005). Arsenic in rice could become an additional health hazard for 80 million people of 59 districts out of 64 districts (Brammer, 2009; Rahman *et al.*, 2008). About 70% of the total arable land area is currently irrigated from shallow tube wells (BGS and DPHE, 2001). Rice covers about 75% of the total cropped area and 83% of the irrigated area (Abedin and Meharg, 2002b). Arsenic contaminated irrigation water has been shown to lead to elevated levels of As in paddy soil and soil solution (Meharg and Rahman 2003; Van Geen *et al.*, 2006; Dittmar *et al.*, 2007; Hossain *et al.*, 2008; Khan *et al.*, 2009).

Human exposure to As is mainly through the intake of drinking water and foods, such as rice grains, that contain elevated amounts of As (Duxbury and Panaullah, 2007). The Food and Agriculture Organisation/World Health Organisation (FAO/WHO) recommended a provisional tolerable weekly intake of not more than 15 µg inorganic As/kg body weight. But, the average daily intake of As from rice for a Bangladeshi adult is approximately 100 µg As (400 g dry wt × 0.25 µg As/g), which is 5 times higher than the 20 µg As intake from consumption of 2 L of water at the WHO limit of 10 µg/L (Panaullah *et al.*, 2009).

Soil is a principal sink of As in the environment and as most of the arsenical residues have low solubility and low volatility, they generally accumulate in the top soil layers (Woolson *et al.*, 1973). Arsenic dynamics and uptake by rice is impacted by complex soil variables and environmental and management factors. Different soils can behave differently (Talukder *et al.*, 2012). Arsenic species, particularly arsenate and arsenite, are readily assimilated by rice plants from soil and soil solution (Abedin *et al.*, 2002a, b). The presence of high As concentrations in agricultural soils and the use of As contaminated irrigation water may affect the movement of As in soil and its accumulation into rice (Abedin *et al.*, 2002a). The paddy soil gets contaminated from the irrigation water and thus enhancing the bioaccumulation of arsenic into rice plants (Bhattacharya *et al.*, 2010a). Rice grown in soils of Bangladesh contaminated with arsenic of 14.5 ± 0.1 mg/kg could be considered safe for human consumption (Rahman *et al.*, 2007b). But, concentrations of As in soils could reach

~30 mg/kg (compared to a crustal content of ~2 mg/kg) in areas where groundwater is generally elevated in As and has been used for irrigation for over a decade (Meharg and Rahman, 2003). Dittmar *et al.* (2010) also found that annually there was an estimated $4.4 \pm 0.4 \text{ kg ha}^{-1} \text{ a}^{-1}$ As deposited through irrigation water and in the top 40 cm soil, the mean As accumulation over three years were recorded to $2.4 \pm 0.4 \text{ kg ha}^{-1} \text{ a}^{-1}$.

Under aerobic soil conditions, it is considered that most As remains bound to iron oxides and unavailable to absorb by the plants (Lauren and Duxbury, 2005). Growing rice in an aerobic situations where As is adsorbed on oxidized Fe surfaces and renders it largely unavailable to uptake by the rice plant (Lauren and Duxbury, 2005; Duxbury and Panaullah, 2007). Arsenic is more available in soil water due to flooding (Onken and Hossner, 1995). Rice growing in the anaerobic situation was found to absorb the highest amount of As among all grain crops (Marin *et al.*, 1993). The As content of lowland paddy-rice grain is generally much higher than that of upland rice or other cereal crops (Schoof *et al.*, 1999; Williams *et al.*, 2007) because of the high availability of soil As under anaerobic conditions (lowland and flooded) as compared to upland (aerobic) rice. Arsenic may also be present as arsenate where uptake is interrupted by phosphate (Abedin *et al.*, 2002c), whereas arsenite is found in flooded anaerobic soils and can be readily taken up by plant cells allowing its passage into the plant parts (Meharg and Jardine, 2003), which is not affected by phosphate (Abedin *et al.*, 2002b; Creger and Peryea, 1994). The anaerobic conditions in paddy soils lead to a mobilization of arsenite (Marin *et al.*, 1993; Takahashi *et al.*, 2004; Xu *et al.*, 2008; Li *et al.*, 2009), which is taken up efficiently via the silicic acid pathway in rice (Ma *et al.*, 2008). Anaerobic water management is the main reason for the high enhanced As uptake in rice (Talukder *et al.*, 2012).

Rice sulphate nutrition plays an important role in regulating arsenic translocation from roots to shoots (Zhang *et al.*, 2011). Lihong and Guilan (2009) discovered that Phosphorus (P) deficiency increased the sensitivity of rice to arsenate and increasing of external phosphate supply could alleviate As toxicity.

Rice is more efficient in As accumulation compared with other cereal crops, such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Williams *et al.*, 2007; Su *et al.*, 2010). Artificially elevated levels of As in irrigation water or soil can reduce growth and productivity of rice (Abedin *et al.*, 2002b; Delowar *et al.*, 2005; Islam *et al.*, 2004). Arsenic toxicity affects the photosynthesis which ultimately results in the reduction of

rice growth and yield (Rahman *et al.*, 2007a). Use of high As irrigation water decrease the rice yields (Jahiruddin *et al.*, 2004; Heikens *et al.*, 2007; Panaullah *et al.*, 2009). The global normal range of As concentration in rice is 0.08–0.20 mg/kg (Zavala and Duxbury, 2008). But, rice grains contained As as high as 1.835 mg of As/kg have been found in Bangladesh (Meharg and Rahman, 2003). Khan *et al.* (2009) stated that increasing arsenic concentrations of both soil and irrigation water resulted significantly increased As concentrations in both rice grain and straw. Lu *et al.* (2009) found elevated arsenic concentrations in rice grain from many parts of Bangladesh. Daum *et al.* (2001) found that rice grain accumulated relatively large amounts of arsenic from soils which not contaminated by arsenic. Ali *et al.* (2003) reported that although the As content of paddy soil and various parts of the rice plant other than rice grains was significantly higher in two areas of Bangladesh impacted by As compared to a control area, the average As content of rice grains (0.5 mg/kg) from the same three areas was similar. Hironaka and Ahmad (2003) also stated the average and range of As concentrations in the combined Bangladesh sample of rice grains (n=62) was not significantly different from the outcome of analyses of rice grown in Japan (n=30) on water that was presumably not elevated in As. Norra *et al.* (2005) observed no difference in As content for rice grains grown in fields irrigated with high and low-As irrigation water in West Bengal, India. Abedin *et al.* (2002a) conducted a greenhouse study using irrigation water with extremely high As concentrations (up to 8 mg/L) and observed that the As content of aman rice grains was to be much less sensitive to the As content of irrigation water compared to other parts of the rice plant. Abedin *et al.* (2002b) also found in a very similar study that the As content of rice grains instead showed a modest response, an increase from 0.3 to 0.5 mg As/kg, to irrigation water containing 1 mg As/L.

However, to the best of my knowledge a few research works have been done in open-field gangetic soil conditions to observe the effect of arsenic contaminated irrigation water on rice (*Oryza sativa* L.). Therefore, detailed information is needed to know and explain the problem perfectly. Thus the present experiment was conducted considering the following specific objectives:

- i. to observe the effect of irrigation water arsenic on growth and yield of BR-11 rice,
- ii. to detect the trend of arsenic accumulation into rice, and finally
- iii. to discover the safe level of arsenic in irrigation water for rice cultivation.

2.2 Materials and Methods

2.2.1 Experimental Site

This field experiment was conducted in a green house made of transparent poly-ethylene paper, situated within 24°22'10.2'' to 24°22'10.3'' N and 88°38'21.7'' to 88°38'21.8'' E at Institute of Environmental Science of Rajshahi University in north-western part of Bangladesh during August to December 2011. The study site has sub-tropical and humid climate with adequate sunshine during day time.



Plate 2.1 Experimental Plot of BR-11 Rice at Institute of Environmental Science, University of Rajshahi

2.2.2 Soil Condition

The experiment was conducted in gangetic soil condition. The properties of soil are given in Table 2.1.

Table 2.1 Soil Conditions in Experimental Site

Soil parameters	Values
Total Nitrogen	0.04±0.02%
Available P	25.3±0.04 ppm
Available K	0.21±0.03 mol/kg
Available S	15.7±0.05 ppm
Available p ^H	Z 0.68±0.04 ppm 7.7±0.03
Organic matter	0.80±0.05
Background total arsenic (As).	5.60±0.05 ppm

2.2.3 Rice Variety

Rice variety, BR-11 is very popular HYV variety in Bangladesh. This rice variety was cultivated during this experiment.

2.2.4 Seedling Transplantation

Thirty-five (35) days old seedlings were uprooted carefully from the seedbed in the morning from the Bangladesh Rice Research Institute, Rajshahi station and four seedlings for each hill with three replications were transplanted on the same day in experimental field on 7th August 2011. The seedlings which died within 6 days of transplantation were discarded and new seedlings were replaced.

2.2.5 Intercultural Application

2.2.5.1 Fertilizer Application

To support the plant growth, urea, triple super phosphate (TSP), murate of potash (MP) and gypsum fertilizer were applied for nitrogen, phosphorus, potassium, and sulfur, respectively. The first split (one third of the dose) of urea and full doses of all other fertilizers were incorporated into the soil by hand before two days of seedling transplantation. The second and third splits of urea were applied after 30 (maximum tillering stage) and 70 (panicle initiation stage) days of transplantation, respectively. One insecticide named fighter was applied into the soil to kill the insects and aphids those attacked the rice plants.

2.2.5.2 Arsenic Source

Sodium arsenate (Na_2HAsO_4) was applied as arsenic source in this experiment.

2.2.5.3 Irrigation and Treatment

Six arsenic treatments 0.0, 0.1, 0.5, 1.0, 2.0, and 4.0 mg/L As containing irrigation water were applied in this experiment. After transplantation of rice seedlings, 3-4 cm water above soil level was maintained in each treatment throughout the growth period. Irrigation was stopped before 10 days of harvest.

2.2.6 Chlorophyll Measurement

The chlorophyll from rice leaves during flowering stage was extracted in 80% acetone and chlorophyll contents were measured at 663 nm and 645 nm in a spectrophotometer. From the absorption coefficients, the amount of chlorophyll was calculated.

2.2.6.1 Procedure

- i) At first, the rice leaves were cut into small pieces leaving away the midribs, mixed thoroughly and 1 gm of the leaves were taken into a clean mortar to grind them.
- ii) The tissues were grinding to fine pulp with the addition of 20 ml of 80% acetone.
- iii) The supernatant were taken into 100 ml volumetric flask after centrifuging (5,000 rpm for 5 min) the samples.
- iv) Then the residues were grinding with 20 ml of 80% acetone and centrifuged again and transferred the supernatant to the same volumetric flask.
- v) The pestle and mortar thoroughly washed with 80% acetone and collected the clear washing in the volumetric flask.
- vi) Then the homogenate was filtered through filter paper (Whatman no.1) and made a volume of 100 ml with 80% cold acetone.
- vii) Finally, the optical density of each solution was measured at 663 and 645 nm against (80% acetone) the solvent blank

2.2.7 Measurement of Plant Growth and Yield

The growth and yield elements of rice were collected and recorded. Rice plant height and number of tiller were recorded for the measurement of plant growth. Plant height was measured from the ground level to the top of the panicle at the full growth of plant using meter scale. Plants of all hills were measured and averaged from each plot. The number of tiller in each plot was counted at the maximum tillering stage. Panicle length was measured from basal node of the rachis to the apex. The number of grains per panicle of all fertile tillers was counted. Thousand grains from each plot were counted and weighed. The grain and shoot biomass/plot (defined as the remaining above ground portion of the rice plant after the spikelets have been removed) were dried and weighed. The result was expressed gm/plot.

2.2.8 Samples Collection and Preservation

2.2.8.1 Soil Samples Collection and Preservation

Soil samples were collected from 0–15 cm depth in 15 cm² area by composite sampling from the fields irrigated with arsenic contaminated water and transferred to airtight polyethylene bags. The samples were immediately air dried at room temperature after collection. Finally, the samples were dried in the Hot Air Oven at 60°C for 72 h and were stored in airtight polyethylene bags at room temperature with proper labeling.

2.2.8.2 Rice Plant Samples Collection and Preservation

The rice plants were cut at 4 cm above the soil. Rice grain was harvested at their maturity stage (120 days after transplantation) on 7th December 2011. Then the collected samples (straw and rice grain) from each treatment were tagged properly and sun dried for 3 days and then keeping the samples on a table. Finally, the samples were dried in the Hot Air Oven at 60°C for 72 h and were stored in airtight polyethylene bags at room temperature with proper labeling. Proper care was taken at each step to minimize any sort of contamination.

2.2.9 Total Arsenic Measurement Methods

Soil, rice straw and grain samples were digested separately following the heating block digestion procedure (Rahman *et al.* 2007c). Rice straw and grain samples were digested by HNO₃-HClO₄ and soil samples by HNO₃-H₂SO₄-HClO₄ for measuring arsenic concentrations in hydride generation atomic absorption spectrophotometer.

2.2.9.1 Sample Digestion

- i) At first, the oven dried samples were ground and passed through 2.0 mm pore sized sieve to get homogenized representative powder sample.
- ii) Then about 0.5 g of the sample was taken into clean dry digestion tubes and 5 ml of concentrated nitric acid (HNO₃) was added to it. The mixture was allowed to stand over night under fume hood.
- iii) In the following day, the digestion tubes were placed on a heating block and heated at 60°C for 2 h.
- iv) The tubes were then allowed to cool at room temperature.
- v) Then about 2 ml of concentrated perchloric acid (HClO₄) was added to the plant samples.

- vi) For the soil samples 3 ml of concentrated sulfuric acid (H_2SO_4) was added in addition to 2 ml of concentrated perchloric acid (HClO_4).
- vii) Then the tubes were heated at 160°C for about 4–5 h.
- viii) The heating was stopped when the dense white fume of perchloric acid (HClO_4) was emitted.
- ix) The content was then cooled, diluted to 25 ml with de-ionized water.
- x) Filtered through Whatman No. 42 filter papers for soil samples and Whatman No. 41 for plant samples and finally stored in polyethylene bottles.

All glassware and plastic bottles were previously washed with 2% HNO_3 followed by rinsing with de-ionized water and drying.

2.2.9.2 Sample Arsenic Analysis

The total arsenic of the digested soil, rice straw and grain samples were analyzed by flow injection hydride generation atomic absorption spectrophotometer (FI-HG-AAS, Perkin Elmer Analyst 400) using external calibration through arsenate as standard (Welsch *et al.* 1990). For each sample three replicates were taken and the mean values were obtained on the basis of calculation of those three replicates.

2.2.10 Soil Sample Analysis Methods

Chemical properties of initial composite soil sample were analyzed. The chemical properties included soil total N, available P, exchangeable K, available S, available Zn contents, pH and organic matter.

2.2.10.1 Total Nitrogen (N)

The micro-Kjeldahl method was used for estimating total nitrogen of soil. The soil was digested with H_2O_2 and concentrated H_2SO_4 in presence of a catalyst mixture ($\text{K}_2\text{SO}_4:\text{CuSO}_4.5\text{H}_2\text{O}:\text{Se}$ in the ratio of 10:1:0.1) and the nitrogen in the digest was determined by distillation with 40% NaOH followed by titration of distillate trapped in H_3BO_3 with 0.01N H_2SO_4 (Page *et al.*, 1982).

2.2.10.2 Available Phosphorus (P)

Following the Olsen *et al.* (1954) method available phosphorus was extracted from the soil with 0.5 M NaHCO_3 solution, pH 8.5. Phosphorus in the extract was then determined by developing blue colour with reduction of phosphomolybdate complex and the colour intensity

was measured colorimetrically at 660 nm wavelength (Page *et al.*, 1982). The P concentration of extract was calculated by fitting the absorbance reading to the standard curve.

2.2.10.3 Exchangeable K

According to Page *et al.* (1982) method exchangeable K was determined on 1N NH₄OAc (pH 7.0) extract of the soil by using flame photometer.

2.2.10.4 Available Sulphur (S)

Available S content of soil was measured by extracting the soil with CaCl₂ (0.15%) solution as explained by Page *et al.* (1982). The extractable S was estimated by developing turbidity by adding acid seed solution (20 ppm S as K₂SO₄ in 6N HCl) and BaCl₂ crystals. The intensity of turbid was estimated by spectrophotometer at 420 nm wavelength.

2.2.10.5 Available Zn

DTPA extraction method (Hunter, 1984) was applied for measuring available Zn content in soil.

2.2.10.6 Soil pH

A glass electrode pH meter was used for measuring soil pH, the soil-water ratio being maintained at 1:2.5 (Jackson, 1962).

2.2.10.7 Organic Matter Content

Wet oxidation method of Walkey and Black (1934) was applied for determining organic carbon in soil volumetrically. The organic matter content was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor).

2.2.11 Statistical Analysis of Experimental Data

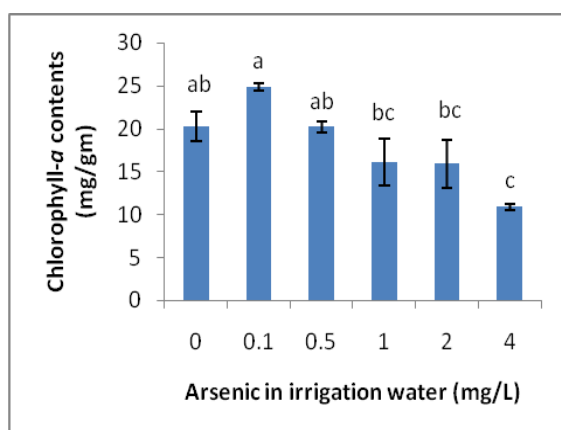
The analysis of variance (ANOVA) was done following the F-statistics. Duncan's multiple range test (DMRT) was used for mean comparisons of the treatment at 5 % level of probability by SPSS, version 15.0 for windows software. Pearson's correlation was also carried out to find out the correlation among arsenic concentration in irrigation water, irrigated field soil and in different parts (straw, grain) of the rice plant. Graphical statistical analyses were done with the help of Microsoft Excel software.

2.3 Results and Discussion

2.3.1 Effect of Arsenic Amended Irrigation Water on Chlorophyll Contents of BR-11 Rice Leaves

2.3.1.1 Chlorophyll-*a* Contents in Leaves of BR-11 Rice

Miteva and Merakchiyska (2002) conducted an experiment with two rice varieties and found that both chlorophyll-*a* and *b* contents in rice leaf decreased with the increase of soil arsenic concentrations. Increased arsenic concentrations caused an alternation of the chloroplast shape, manifested in its rounding. Other manifestations are concaving membrane, bending and partial destruction as well as changes in the accumulation and flow of assimilates which results in the decrease of chlorophyll content in rice leaf. In the present study the chlorophyll-*a* contents in BR-11 rice leaf were affected significantly ($p=0.002$) by irrigation water arsenic. The highest chlorophyll-*a* contents ($24.83 \pm 0.39a$) in rice leaf was found in 0.1 mg/L As containing treatment and the lowest ($10.91 \pm 0.38c$) in 4 mg/L As containing treatment (**Table 2.2** in Appendix-1). Chlorophyll-*a* contents were increased up to 0.1 ppm treatment and thereafter decreased significantly ($p \leq 0.05$) with increasing arsenic concentration in irrigation water (**Figure 2.1.1**). Moreover, a strong significant ($p \leq 0.01$) negative correlation was also found between chlorophyll-*a* contents and irrigation water arsenic (**Figure 2.1.2**)



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.1.1 Effect of irrigation water arsenic on chlorophyll-*a* contents of BR-11rice leaves

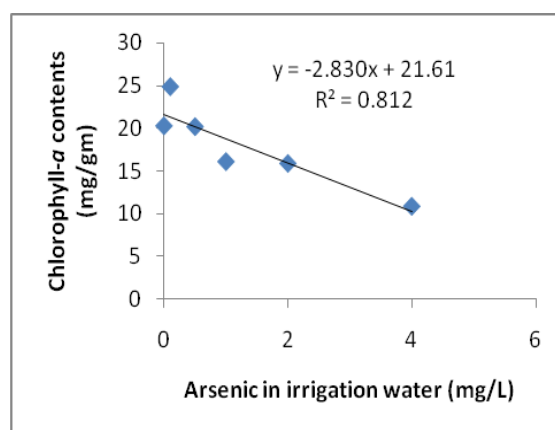
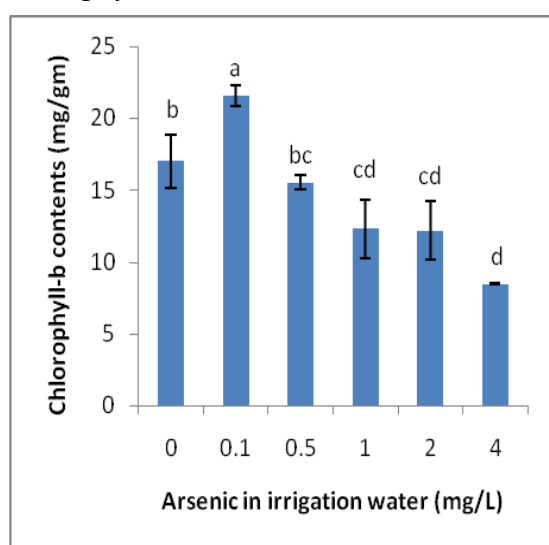


Figure 2.1.2 Correlation between irrigation water arsenic and chlorophyll-*a* contents of BR-11 rice leaves

2.3.1.2 Chlorophyll-*b* Contents in Leaves of BR-11 Rice

The chlorophyll-*b* contents in BR-11rice leaf were affected significantly ($p = 0.001$) by irrigation water arsenic. The highest chlorophyll-*b* contents (21.56 ± 0.73 mg/gm) in rice leaf was found in 0.1 mg/L As containing treatment and the lowest ($8.46 \pm 0.03d$) in 4

mg/L As containing treatment (**Table 2.2** in Appendix-1). Chlorophyll-*b* contents were increased up to 0.1 ppm treatment and thereafter decreased significantly ($p \leq 0.05$) with increasing arsenic concentration in irrigation water (**Figure 2.2.1**). Moreover, a strong significant ($p \leq 0.01$) negative correlation was also found between chlorophyll-*b* contents and irrigation water arsenic (**Figure 2.2.2**). Rahman *et al.* (2004) also observed that soil arsenic concentrations showed negative correlations with the chlorophyll-*a* and chlorophyll-*b* contents in rice leaf.



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.2.1 Effect of irrigation water arsenic on chlorophyll-*b* contents of BR-11 rice leaves

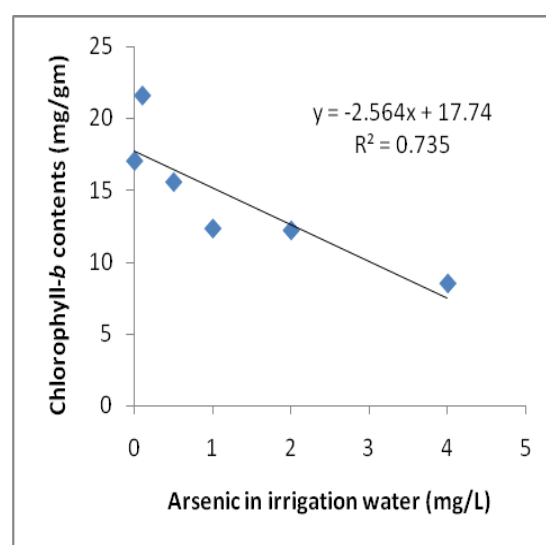
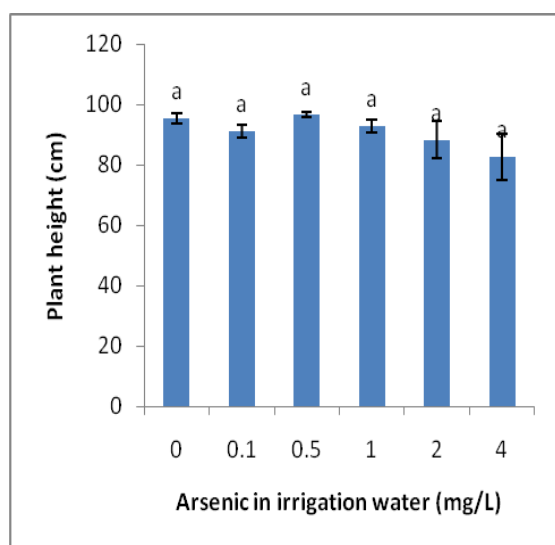


Figure 2.2.2 Correlation between irrigation water arsenic and chlorophyll-*b* contents of BR-11 rice leaves

2.3.2 Effect of Arsenic Amended Irrigation Water on Growth of BR-11 Rice

2.3.2.1 Plant Height

Azad *et al.* (2009) found that arsenic (As) had a significant ($p \leq 0.5$) effect on the reduction of plant height of T-Aman rice. Present experiment found that plant height was increased up to the application of 0.5 mg/L arsenic and thereafter at higher concentration of arsenic resulted a gradual decrease of plant height (**Figure 2.3.1**). Xie and Huang (1994) also reported that lower concentration of arsenic through irrigation water had stimulatory effect for rice. The tallest (96.64 ± 0.73 cm) and smallest (82.66 ± 7.6 cm) plant were found in 0.5 and 4.0 mg/L arsenic treatments, respectively (**Table 2.3** in Appendix-1). This study found a negative correlation between arsenic in irrigation water and plant height (**Figure 2.3.2**). Abedin *et al.* (2002b) also found that use of arsenic contaminated irrigation water significantly reduced plant height.



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.3.1 Effect of irrigation water arsenic on Plant height of BR-11rice

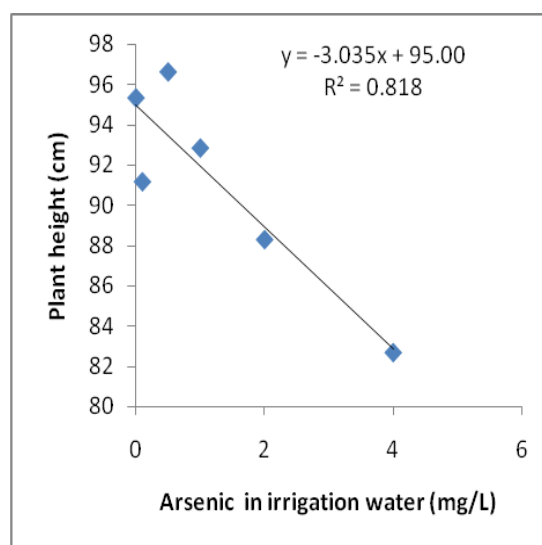
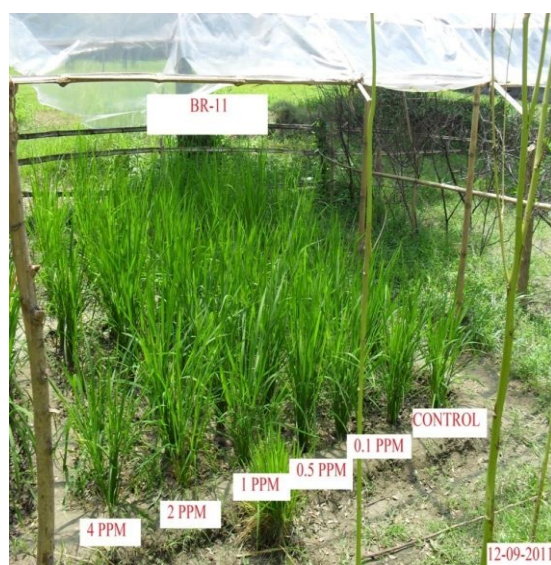


Figure 2.3.2 Correlation between irrigation water arsenic and Plant height of BR-11rice



Early Stage



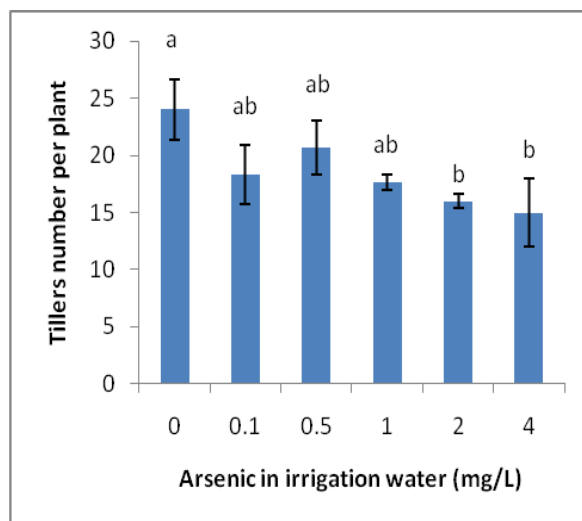
Final Stage

Plate 2.2 Effect of Arsenic Amended Irrigation Water on Growth of BR-11 Rice

2.3.2.2 Tillering

Chino (1981) reported that tillers of rice to be severely depressed with high concentration of As. In this study arsenic in irrigation water up to 1.0 mg/L did not affect the tillers number significantly. But a higher concentration of arsenic significantly decreased the total number of tillers per plant (**Figure 2.4.1**). Abedin *et al.* (2002b) also observed that tillers number was reduced significantly with increase of arsenic concentration in

irrigation water up to 8 mg/L. The highest number (24.00 ± 2.65) of tillers was observed in control treatment and lowest number (15.00 ± 3) was observed in 4.0 mg/L arsenic treated plot (**Table 2.3** in Appendix-1). A significant ($p=0.02$) negative correlation was found between arsenic in irrigation water and tillers number (**Figure 2.4.2**). Khan *et al.* (2010) also found that the addition of arsenic significantly reduced tillering.



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.4.1 Effect of irrigation water arsenic on Tillering of BR-11rice

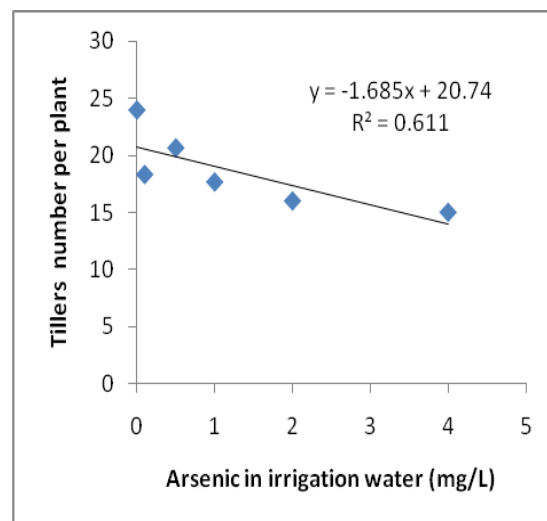
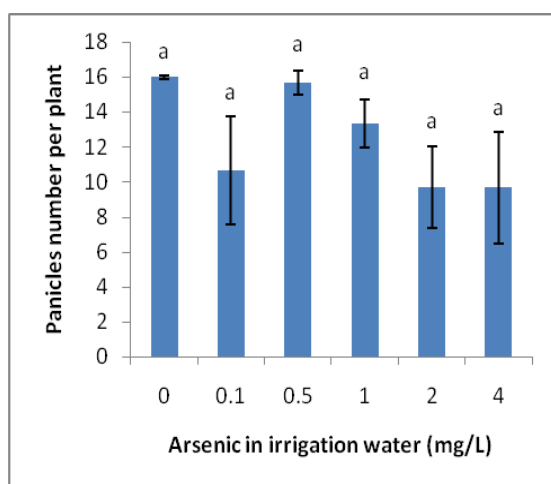


Figure 2.4.2 Correlation between irrigation water arsenic and Tillering of BR-11rice

2.3.2.3 Panicle Number

Azad *et al.* (2009) reported that the panicle numbers of T-aman rice were not affected at low doses of As in soil but significantly affected the panicles number at higher doses. This study found that panicle numbers were decreased with increase of arsenic concentration in irrigation water but the differences were not statistically significant (**Figure 2.5.1**). The highest panicle number (16.00 ± 02) was observed in control treatment and the lowest panicle number (9.67 ± 3.18) was found in 4.0 mg/L treatment (**Table 2.3** in Appendix-1). Arsenic in irrigation water and panicle number had a negative correlation (**Figure 2.5.2**).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.5.1 Effect of irrigation water arsenic on panicle number of BR-11rice

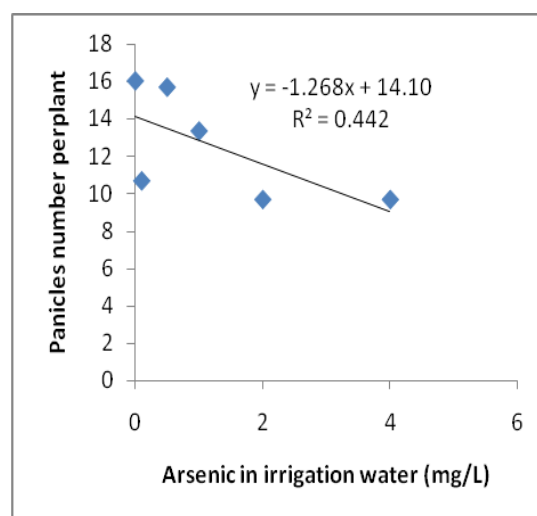
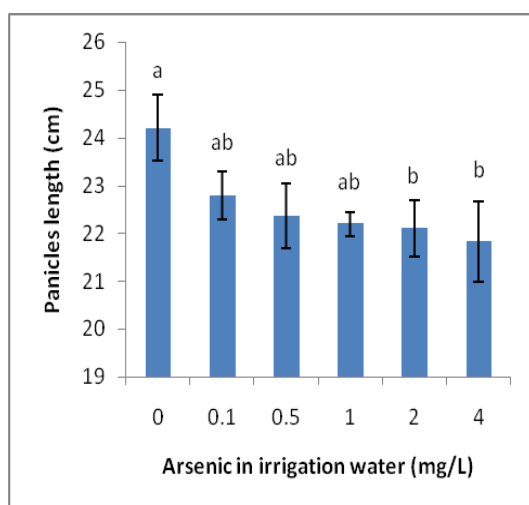


Figure 2.5.2 Correlation between irrigation water arsenic and panicle number of BR-11rice

2.3.2.4 Panicle Length

The panicle lengths were affected significantly by irrigation water arsenic. The highest panicle length (24.20 ± 0.69 cm) and the lowest panicle length (21.83 ± 0.84 cm) were observed in control and 4.0 mg/L arsenic treated plot, respectively (Table 2.3 in Appendix-1). The panicle lengths were decreased significantly ($p \leq 0.05$) with increase of arsenic in irrigation water (Figure 2.6.1). Azad *et al.* (2009) also found that the panicle lengths of T-aman rice were not affected at low doses of As in soil but affected significantly at higher doses of As. Arsenic in irrigation water and panicles length had a negative correlation (Figure 2.6.2).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.6.1 Effect of irrigation water arsenic on panicle length of BR-11rice

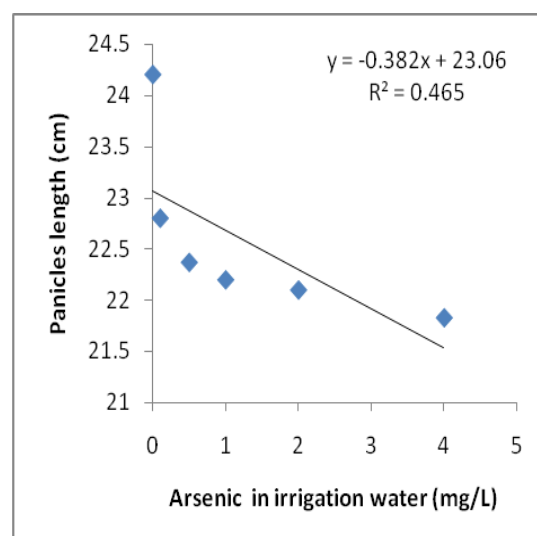
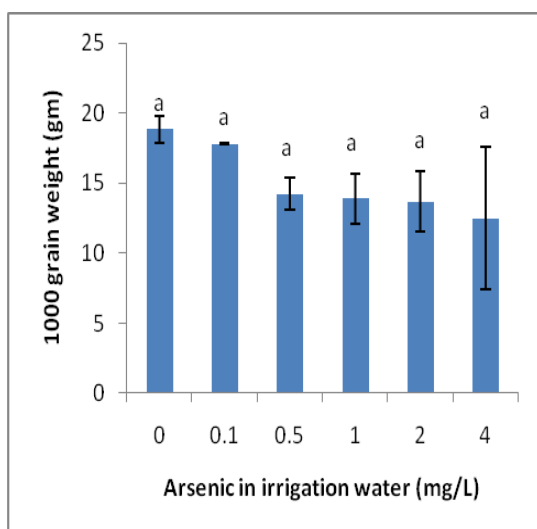


Figure 2.6.2 Correlation between irrigation water arsenic and panicle length of BR-11rice

2.3.2.5 1000 Grain Weight

Abedin *et al.* (2002b) found that presence of arsenic as arsenate at a higher concentration in irrigation water significantly reduced ($p < 0.001$) the 1000 grain weight. The thousand grain weight was decreased with increasing arsenic in irrigation water but the differences were not statistically significant (**Figure 2.7.1**). Tsutsumi (1980) also reported that As could reduced 1000 grain weight. The highest grain weight ($18.84 \pm 0.94\text{g}$) and lowest grain weight ($12.5 \pm 5.11\text{g}$) were recorded in control and 4.0 mg/L arsenic treatment plot, respectively (**Table 2.3** in Appendix-1). Arsenic in irrigation water and thousand grain weight were related antagonistically (**Figure 2.7.2**). Wang *et al.* (2006) also reported that 1000 grain weight was significantly reduced with increased As level in soil treated with two organoarsenic compound ($p < 0.01$).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.7.1 Effect of irrigation water arsenic on 1000 Grain weight of BR-11rice

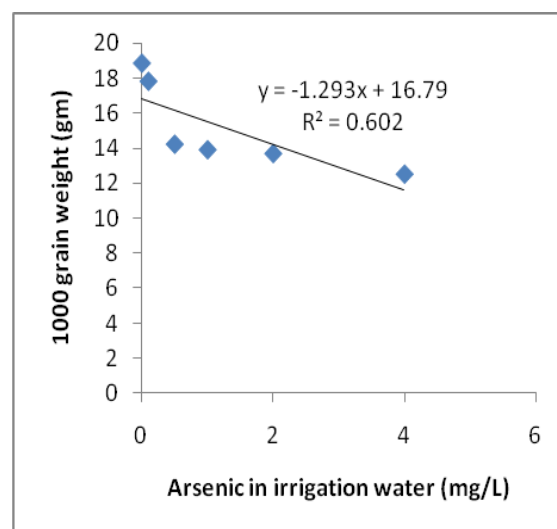


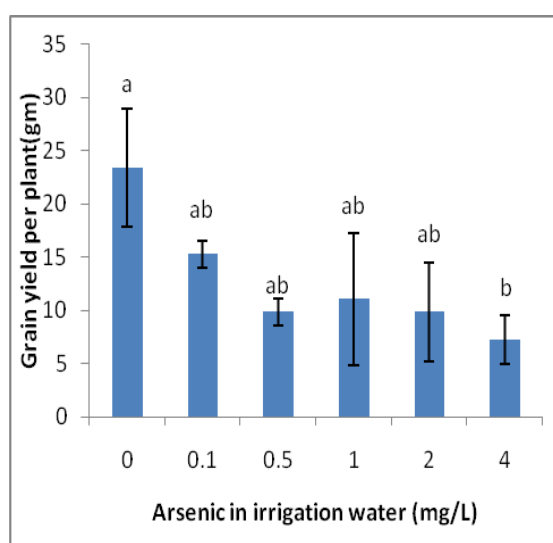
Figure 2.7.2 Correlation between irrigation water arsenic and 1000 Grain weight of BR-11rice

2.3.3 Effect of arsenic amended irrigation water on yield of BR-11 rice

2.3.3.1 Grain Yield

Abedin *et al.* (2002b) reported that grain yield was decreased significantly ($p < 0.001$) with increase of arsenic concentration in irrigation water. Grain yield of BR-11 rice was decreased significantly with increase of arsenic concentration in irrigation water (**Figure 2.8.1**). There were also some reports of rice grain yield reduction due to As application for rice (Farn *et al.*, 1988; Milan *et al.*, 1988; Gilmour and Wells, 1988; Liu and Gao, 1987; Tsutsumi, 1980). The highest grain yield ($23.38 \pm 5.55 \text{ g}$) and lowest grain yield

(7.24 ± 2.3 g) were found in control and 4.0 mg/L arsenic treated plot, respectively (**Table 2.4** in Appendix-1). The grain yield was found to decrease considerably by 58.04% and 69.03% compared to control in 2.0 and 4.0 mg/L arsenic treatments, respectively (**Table 2.4** in Appendix-1). Hossain *et al.* (2009) also reported that grain yield of rice was decreased as the level of arsenic addition was increased, and the yield was reduced drastically with the 30 mg As/kg addition. Moreover, grain yield and arsenic concentration in irrigation water had a negative correlation (**Figure 2.8.2**). Panaullah *et al.* (2009) also found rice grain was negatively correlated with soil-As concentration ($r^2=0.91$).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.8.1 Effect of irrigation water arsenic on grain yield of BR-11rice

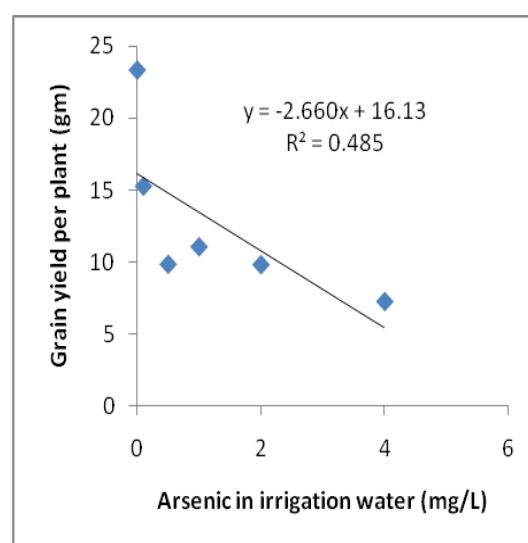
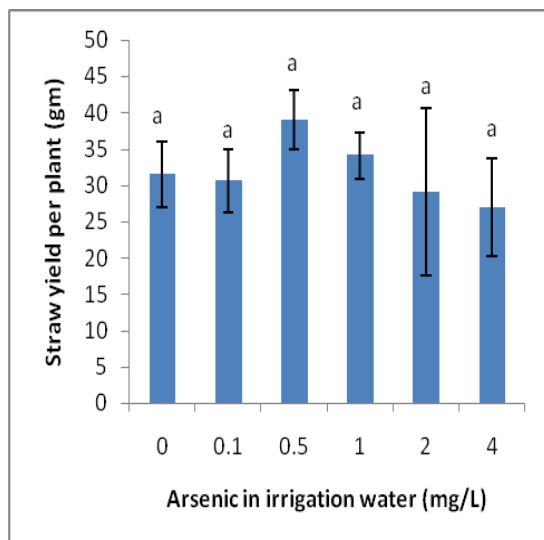


Figure 2.8.2 Correlation between irrigation water arsenic and grain yield of BR-11rice

2.3.3.2 Straw Yield

Khan *et al.* (2010) reported that straw yield was decreased significantly with As addition in irrespective of season, year, method and level of As application. The straw yield of BR-11 rice was increased at 0.5 mg/L arsenic treatment and thereafter straw yield were decreased with increase of arsenic concentration in irrigation water but the differences were not statistically significant (**Figure 2.9.1**). The highest straw yield (39.07 ± 4.08 g) and lowest straw yield (27.01 ± 6.74 g) were found in 0.5 mg/L and 4.0 mg/L arsenic treatments, respectively (**Table 2.4** in Appendix-1). Arsenic concentration in irrigation water and straw yield had a negative correlation (**Figure 2.9.2**). Hossain *et al.* (2009) also found a negative relationship between straw yield and arsenic dose. The straw yield decreased by 7.77% and 14.25% compared to control in 2.0 mg/L and 4.0 mg/L arsenic

treatments, respectively (**Table 2.4** in Appendix-1). Abedin *et al.*, (2002b) also found that straw yield were significantly ($p < 0.001$) reduced with increase of arsenate concentration in irrigation water.



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.9.1 Effect of irrigation water arsenic on straw yield of BR-11rice

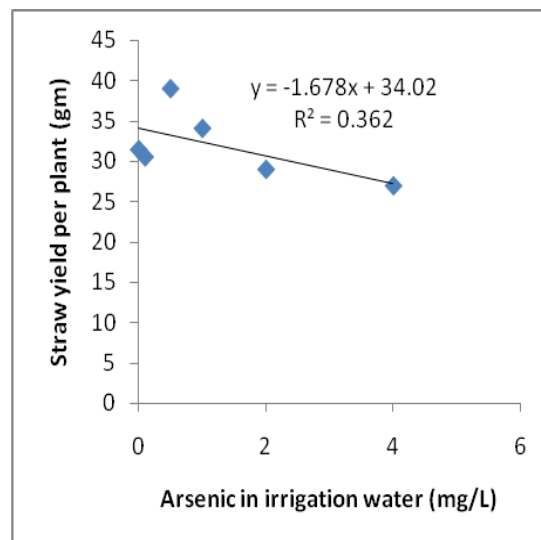
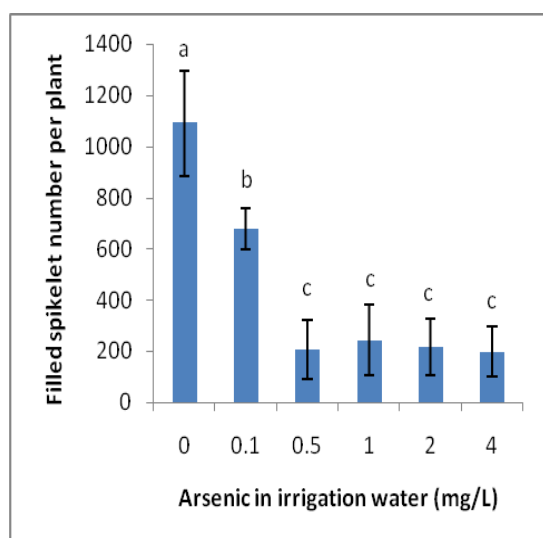


Figure 2.9.2 Correlation between irrigation water arsenic and straw yield of BR-11rice

2.3.3.3 Spikelet Number

2.3.3.3.1 Filled Spikelet Number

The filled spikelet number per plant of BR-11 rice was significantly ($p = 0.002$) affected by irrigation water arsenic. The highest (1091.67 ± 206.10) and lowest (197.33 ± 97.58) filled spikelet number were found in control and 4mg/L As containing treatments, respectively (**Table 2.5** in Appendix-1). Filled spikelet numbers per plant were decreased significantly with increasing arsenic concentration in irrigation water (**Figure 2.10.1**). Abedin *et al.* (2002b) also found that the number of grain pot^{-1} decreased significantly with the level of contamination of irrigation water by arsenate. Moreover, a negative correlation was found between filled spikelet number and irrigation water arsenic (**Figure 2.10.2**).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.10.1 Effect of irrigation water arsenic on filled spikelet number of BR-11rice

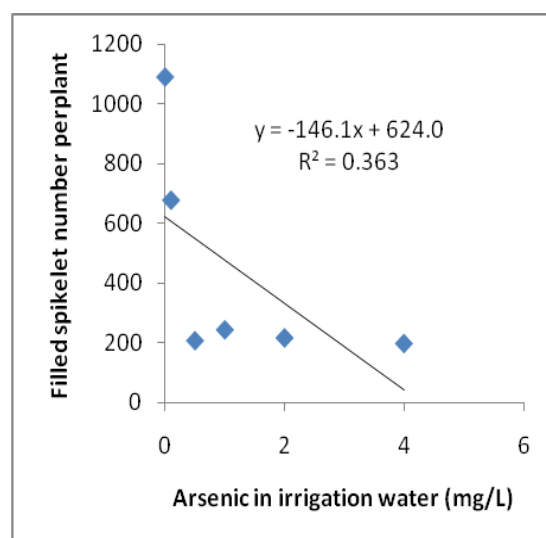
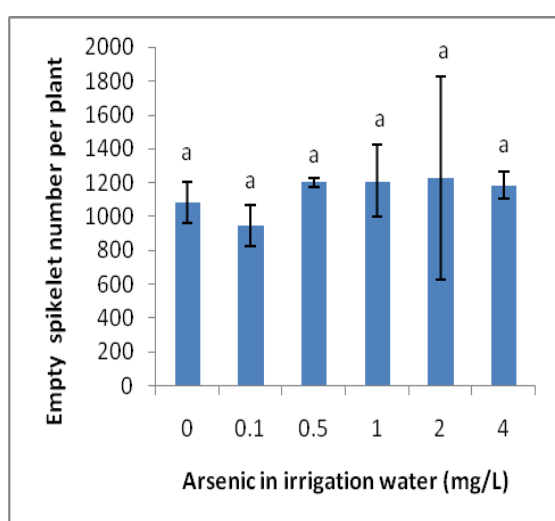


Figure 2.10.2 Correlation between irrigation water arsenic and filled spikelet number of BR-11rice

2.3.3.3.2 Empty Spikelet Number

The highest (1185.00 ± 80.47) empty spikelet number per plant of BR-11 rice was found in 4 mg/L As containing treatment and the lowest ($945.00 \pm 120.45a$) in 0.1 mg/L As containing treatment (**Table 2.5** in Appendix-1). Empty spikelet numbers per plant were increased with increasing arsenic concentration in irrigation water but the differences were not statistically significant (**Figure 2.11.1**). Moreover, a negative correlation was found between empty spikelet number and irrigation water arsenic (**Figure 2.11.2**).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.11.1 Effect of irrigation water arsenic on empty spikelet number of BR-11rice

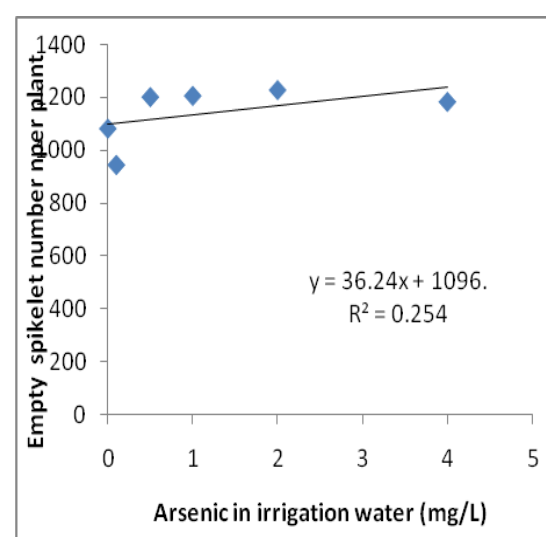


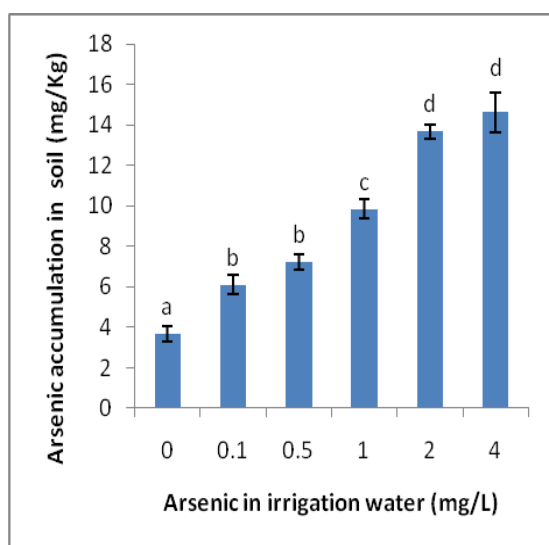
Figure 2.11.2 Correlation between irrigation water arsenic and empty spikelet number of BR-11rice

2.3.4 Effect of Arsenic Amended Irrigation Water on Arsenic Accumulation

2.3.4.1 Arsenic Accumulation in Soil

The background arsenic concentration in soil of BR-11 paddy field was 5.60 mg/kg. Arsenic accumulation in soil was significantly ($p \leq 0.01$) affected by irrigation water arsenic. Khan *et al.* (2010) observed that the As addition in irrigation water during crop growth or directly to soil before transplanting of rice significantly increased soil-As concentrations. Arsenic accumulation in paddy soil was increased significantly ($p \leq 0.01$) with increase of arsenic concentration in irrigation water (**Figure 2.12.1**). Meharg and Rahman (2003) estimated that if the field is irrigated with 0.1 mg As/L irrigation water, soil As would increase by 1 mg/kg per year. Up to 1 mg/L As in irrigation water, the soil arsenic not exceeded the world standard but thereafter arsenic concentration in paddy soil was found higher than the world standard for soil (**Table 2.6** in Appendix-1). Ahsan *et al.* (2009) also showed that the average arsenic concentration in Faridpur was more than three times higher than the world standard (10 mg/kg). A significant ($p \leq 0.01$) strong positive correlation between irrigation water arsenic and arsenic accumulation in paddy soil was also observed (**Figure 2.12.2**).

Other researchers have also reported high accumulation of arsenic in the soil surface layers from irrigation water arsenic. Khan *et al.* (2009) observed that the increases in soil As concentration at the 0–15 cm depth was 1.7–3.0 mg kg⁻¹ year⁻¹ when 1 mg As/L was added in irrigation water during the Boro season. Islam *et al.* (2005) found that the concentration of As in the rice field would increase by 0.50 mg kg⁻¹ year⁻¹ when 0.10 mg As/L is added in irrigation water of Boro rice. Saha and Ali (2007) observed an enrichment of As in the top soil of rice fields irrigated with As contaminated ground water.



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.12.1 Effect of irrigation water arsenic on arsenic accumulation in soil of BR-11 paddy field

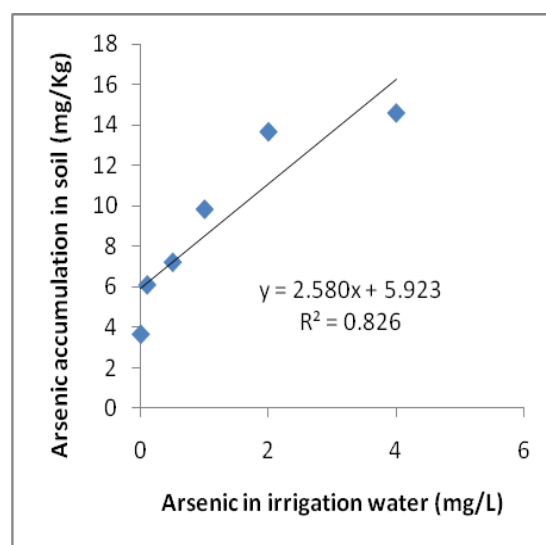
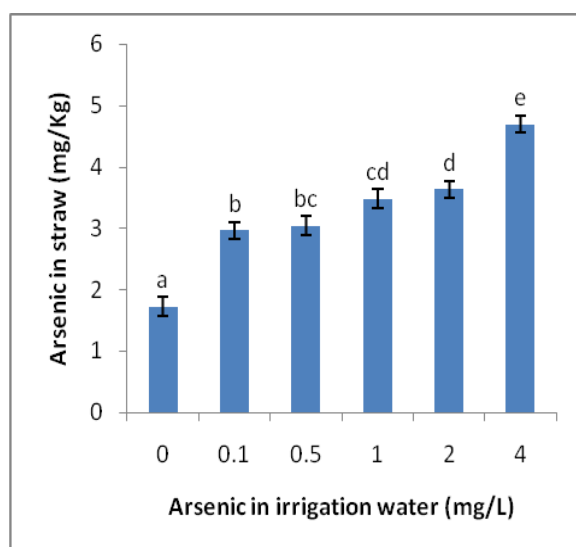


Figure 2.12.2 Correlation between irrigation water arsenic and arsenic accumulation in soil of BR-11 paddy field

2.3.4.2 Arsenic Accumulation in Straw

Islam *et al.* (2004) reported a significant increase of arsenic in rice straw with increase of arsenate concentrations in irrigation water. In this study arsenic accumulation in straw of BR-11 rice was found to increase significantly ($p \leq 0.01$) with increase of arsenic concentration in irrigation water (**Figure 2.13.1**). The highest arsenic accumulation in straw (4.69 ± 0.14 mg/kg) was found in 4 mg/L treatment and lowest (1.72 ± 0.16 mg/kg) in control (**Table 2.6** in Appendix-1). Tsutsumi (1980) detected an elevated arsenic concentration in rice straw (up to 149 mg/kg As by dry weight) when rice was grown in soil amended with sodium arsenate at different levels (0–312.5 mg/kg As). Panaullah *et al.* (2009) also stated that rice-straw arsenic content increased with increasing of soil-arsenic concentration. Azad *et al.* (2009) observed that straw accumulated twice as much arsenic than the grain. The present study also found that arsenic accumulation in straw was much higher than grain. As like as soil a significant ($p \leq 0.01$) strong positive correlation between irrigation water arsenic and arsenic accumulation in straw of BR-11 rice was observed (**Figure 2.13.2**)



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.13.1 Effect of irrigation water arsenic on arsenic accumulation in straw of BR-11rice

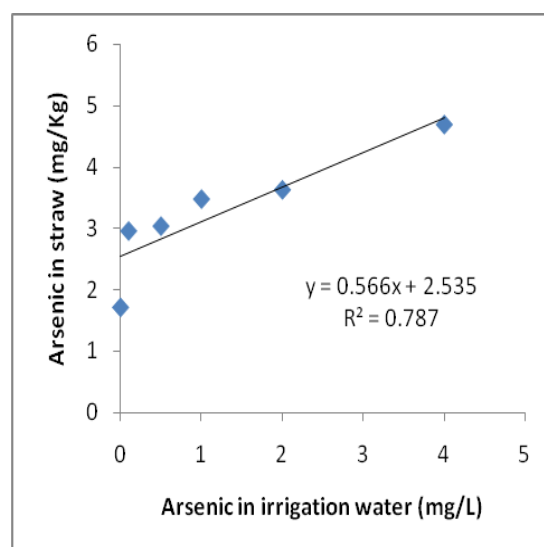
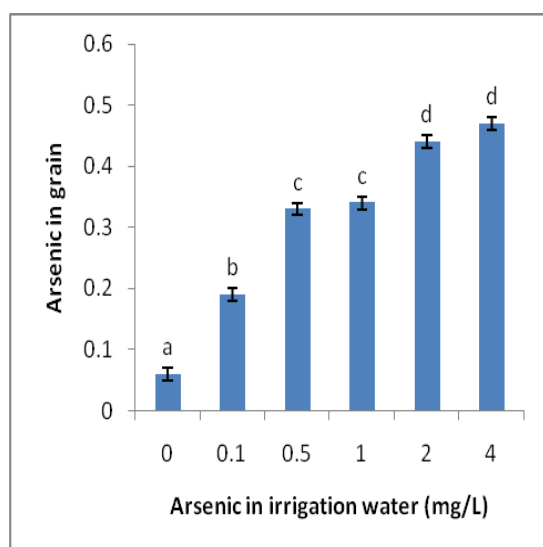


Figure 2.13.2 Correlation between irrigation water arsenic and arsenic accumulation in straw of BR-11rice

2.3.4.3 Arsenic Accumulation in Grain

From the arsenic uptake study of T-aman rice, Azad *et al.* (2009) found that the arsenic in rice grain increases with increase of arsenic in soil, but did not vary significantly up to 20 mg/kg As treatment. Meharg and Rahman (2003) observed that arsenic uptake and accumulation was greatly affected by arsenic contamination in soil and increased greatly with increasing levels of arsenic. Islam *et al.* (2004) reported arsenic in rice grain (0.30 mg/kg dry weight) when the rice was grown with 2.0 ppm arsenic contaminated irrigation water. This study also found that arsenic accumulation in grain of BR-11 rice was significantly ($p \leq 0.01$) affected by irrigation water arsenic. Arsenic accumulation in grain was increased significantly with increase of arsenic concentration in irrigation water (**Figure 2.14.1**). The highest level of arsenic accumulation in grain (0.47 ± 0.03 mg/kg) and the lowest level of arsenic accumulation in grain (0.06 ± 0.01 mg/kg) was observed in 4 mg/L and control treatments, respectively (**Table 2.6** in Appendix-1). Schoof *et al.* (1998) also stated that rice grain generally has lower arsenic concentration and the concentration remains much below the maximum permissible limit of 1 mg/kg As. Moreover, a significant ($p \leq 0.01$) strong positive correlation between arsenic in irrigation water and arsenic accumulation in grain of BR-11 rice was also found (**Figure 2.14.2**).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 2.14.1 Effect of irrigation water arsenic on arsenic accumulation in grain of BR-11rice

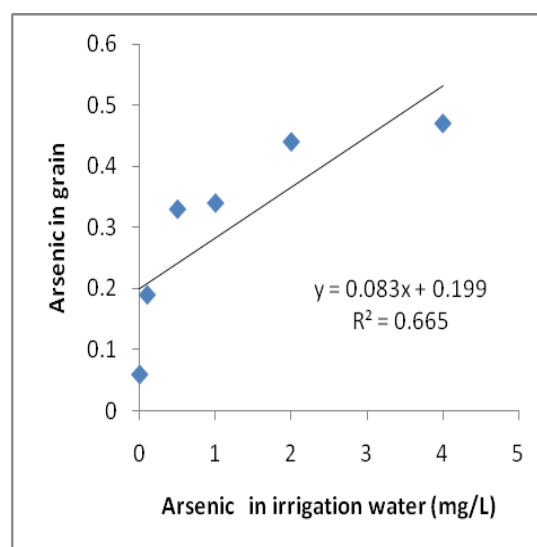


Figure 2.14.2 Correlation between irrigation water arsenic and arsenic accumulation in grain of BR-11rice

2.4 Conclusions

Rice growth and yield were significantly ($p \leq 0.05$) affected by arsenic amended irrigation water. Lower concentration of arsenic in irrigation water (up to 0.5 mg/L) stimulated the rice growth and yield but higher concentration of arsenic in irrigation water (above 0.5 mg/L) reduced the rice growth and yield markedly.

Arsenic accumulation in soil, rice straw and grain was also significantly ($p \leq 0.01$) affected by irrigation water arsenic. Arsenic accumulation in soil, rice straw and grain followed a strong positive correlation trend with irrigation water arsenic concentration. The trend of arsenic accumulation into rice in Gangetic soil condition is as follows: irrigation water > soil > straw > grain.

2.5 Recommendations

- Farmers of Bangladesh should minimize the use of irrigation water for rice cultivation.
- The level of arsenic in irrigation water for rice cultivation should be within 0.5 mg/L
- Rain water and surface water should be stored for rice cultivation

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Chapter- 3

**Effect of Residual Arsenic on BRRI
dhan-50 (*Oryza sativa* L) Grown in
Open Field Gangetic Soil Condition**

Abstract

Rice was grown in open field Gangetic soil condition with normal tap-water in experimental plot at Institute of Environmental Science, University of Rajshahi to see the effect of residual soil arsenic on growth and yield of rice (*Oryza sativa* L.) as well as to observe the trend of arsenic accumulation into rice plant and soil. A popular HYV Boro rice variety named BRRI dhan-50 was cultivated in experimental plots containing 3.67, 6.11, 7.23, 9.85, 13.68, 14.61 mg/kg residual arsenic in soil which accumulated from previous season irrigation water containing 0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mg/L arsenic, respectively. Arsenic accumulation in rice straw and grain were investigated. Chlorophyll contents of BRRI dhan-50 rice leaves were significantly decreased with increasing of soil residual arsenic. Grain and straw yield were not significantly affected by soil residual arsenic. A significant ($p \leq 0.01$) increasing trend of arsenic accumulation into straw and grain was observed with increasing of soil residual arsenic. The highest level of arsenic in straw (2.06 mg/kg) and grain (0.57 mg/kg) was found in plot containing 14.61 mg/kg soil residual arsenic in soil. Residual arsenic in soil showed a negative correlation with arsenic accumulation into straw and grain. The trend of arsenic accumulation was found as soil>straw>grain.

3.1 Introduction

Rice is the staple food for around 50% of the world's population contributing over 70% of the energy and 50% of protein provided their daily food intake (IRRI, 1993). It is the world's second largest cereal crop, the most important cereal grown in Bangladesh, per capita cereal consumption is 150.4 kg year⁻¹ of which rice is 91% (Alam *et al.*, 2002), provide 73% of the populations caloric intake in this country (Del & Dorosh, 2001).

Rice may accumulate considerable amounts of essential elements, but also toxic elements such as arsenic (Williams *et al.*, 2007; Meharg *et al.*, 2009). Among agricultural crops, rice is the greatest contributor to inorganic arsenic (As) uptake to humans through food (Kile *et al.*, 2007; Mondal and Polya, 2008; Meharg *et al.*, 2009). Health risks associated with the chronic low dose uptake of arsenic (As) have been of great concern in Southeast Asia, where rice is a staple crop (Yamaguchi *et al.*, 2011). Zavala and Duxbury (2008) reported a global normal range of 0.08-0.20 mg/kg for arsenic concentration in rice. Rice grains collected in arsenic contaminated districts of Bangladesh had concentrations that were 10-fold higher than the normal level of about 0.2 µg/g As (Meharg & Rahman, 2003; Islam *et al.*, 2004). The average daily intake of arsenic from rice for a Bangladeshi adult is approximately 100 µg As (400 g dry wt x 0.25 µg/g), which is 5 times of 20 µg As intake from consumption of 2L water at the WHO limit of 10 µg/L (Panullah, 2009).

Rice-rice is the main cropping sequence of Bangladesh. Higher grain yield compared to rainy season, motivate the farmers to cultivate rice under irrigated condition during the post winter season. Farmers apply 1200-1400 mm water to meet the higher evapotranspiration (3-5.5 mmday⁻¹) demand during the growing period of summer rice and more than 60% of this met through ground water (Sarkar, 2001; Huq and Naidu, 2005). Boro rice accounts for about 55% of the total rice production in Bangladesh, and the irrigation water needed for its cultivation is mainly extracted from shallow tube wells (STWs) (MOA, 2005). Many shallow tube wells (STWs) deliver arsenic concentrations above 50 µg/L. About 86% of the total ground water withdrawn is used for irrigation in dry season crops, mainly Boro rice (Khan, 2009). In a rice-rice cropping system irrigation water is applied during the Boro rice (dry season rice), and the following Aman rice (wet-season rice) is grown with natural rainfall. Irrigation with arsenic contaminated

groundwater directly affects the immediate Boro rice (Abedin, Cotter-Howells, and Meharg 2002b), and the residual arsenic in soil may affect the Aman rice (Duxbury *et al.*, 2003). The use of contaminated irrigation water for paddy fields has resulted in an elevated level of arsenic in rice grain (Meharg and Jardine, 2003; Meharg, 2004; Williams *et al.*, 2006). Abedin *et al.* (2002b) observed in a greenhouse study that increasing arsenate concentration from 0 to 8 mg/L in irrigation water severely reduced plant height, grain yield, the number of filled grains, grain weight, and root biomass.

Arsenic has been reported to interfere with metabolic processes and to inhibit plant growth, sometimes leading to death at higher concentrations (Baker *et al.*, 1976; Marin *et al.*, 1992; Reed and Sturgis, 1936; Schweizer, 1967). Arsenic uptake and translocation in rice plants are affected by a wide range of factors including soil arsenic speciation, soil physical and chemical properties, irrigation water, and fertilizer management (Brammer, 2009; Zhao, *et al.*, 2009; Lu *et al.*, 2009; Norton *et al.*, 2009). Rice grain can accumulate relatively large amounts of arsenic even from soils not contaminated by arsenic (Daum *et al.*, 2001). The statutory permissible for straw fed to cattle is 0.2 mg/kg (Nicholson *et al.*, 1999), but it could be up to 92 µg/g when rice plants were irrigated with arsenic contaminated ground water (Abedin *et al.*, 2002 b). The availability of arsenic to the rice plant might also be subjected to the geographic location, soil properties, redox condition and cropping season (Meharg and Rahman, 2003).

Accumulation of arsenic in paddy soil can cause phytotoxicity to rice plants and significant reduction in grain yield, thus threatening the long term sustainability of the rice cropping system in the affected areas (Khan *et al.*, 2009). Contamination of arsenic in paddy soils is a widespread problem due to irrigation of arsenic laden groundwater in south and southeast Asia (Brammer, 2009). The elevated arsenic concentrations in soil can be directly linked to crop cultivation in Bangladesh via the use of arsenic contaminated ground water for irrigation purpose (Williams *et al.*, 2007). Rice growing in the anaerobic situation was found to score the highest amount of arsenic among all grain crops (Marin *et al.*, 1993). Boro rice is exposed to arsenic caused by both soil and irrigation water, whereas the Aman rice is exposed to arsenic through the natural soil arsenic in addition to the buildup of arsenic over time due to use of contaminated irrigation water (Duxbury and Panaullah, 2007). Duxbury *et al.* (2003) reported that the

mean As concentration for Boro (winter season) rice (183 $\mu\text{g/kg}$) was 1.5 times higher than Aman (monsoon season) rice (117 $\mu\text{g/kg}$), based on 150 paddy rice samples from different districts of Bangladesh. Azad *et al.* (2009) observed an increase grain arsenic uptake of transplanted Aman rice with the increase of arsenic treatment in soil and found that 30-50 mg/kg arsenic containing soil produced rice grains with arsenic levels exceeding the WHO recommended permissible limit of 1.0 mg/kg. Use of arsenic contaminated groundwater has the potential to increase soil arsenic over time (Panaullah *et al.*, 2009; Dittmar *et al.*, 2010). Application of 1 m depth of water containing 0.1 mg As/ L to a single rice crop would increase soil arsenic in the top 15 cm of soil by about 0.5 mg/kg assuming uniform distribution of arsenic across soil in a tube well command area and retention of added as in soil (Ahmed *et al.*, 2011). Kabata-Pendias and Pendias (1992) recommended 20 mg of As/kg soil as the safe level of arsenic in agricultural soil for crops. Unfortunately, in several countries, the level of arsenic in contaminated agricultural soils is much higher than the acceptable level (Mohan & Pittman, 2007). Das *et al.* (2002) reported the worldwide naturally occurring average arsenic concentration in soil is 10 mg/kg. Background concentrations of arsenic in Bangladeshi soils are 4-8 mg As/kg. However, in areas where irrigation is carried out with arsenic contaminated groundwater, soil arsenic level can reach up to 83 mg As/kg (Ullah, 1998). Alam & Sattar reported elevated arsenic concentrations up to 57 mg/kg in soils collected from different locations of four districts of Bangladesh. Meharg and Rahman (2003) reported that the arsenic concentrations of Bangladeshi soils may reach ~ 30 mg/kg in the areas where groundwater having elevated concentration of arsenic has been used for over a decade in irrigating crops. Heikens *et al.* (2007) stated that soil concentrations of arsenic are increasing with time because of irrigation, but it is unclear under what conditions and in what time frame this takes place, which makes it difficult to quantify the risk. Duxbury and Panullah also assessed that arsenic accumulation in soils could be increased at the rate of 1 mg/kg crop⁻¹ through the use of 1.5 m of irrigation water containing 0.1 mg As/L, resulting in no net loss of arsenic from the soil environment, indicated strong residual effects on following crops (Khan *et al.*, 2010). Dittmar *et al.* (2010) also found that annually there was an estimated $4.4 \pm 0.4 \text{ kg ha}^{-1} \text{ a}^{-1}$ As deposited through irrigation water. In the top 40 cm soil, the mean arsenic accumulation over three years were recorded to $2.4 \pm 0.4 \text{ kg ha}^{-1} \text{ a}^{-1}$, implying that there was an average loss of arsenic was

2.0 kg h⁻¹ a⁻¹. Robert *et al.* (2007) and Ali *et al.* (2003) estimated that over 1000 tones of arsenic are transferred to paddy fields every year in Bangladesh through irrigation, which has been linked to elevated concentration of arsenic in rice (Lu *et al.*, 2009). Panaullah *et al.* (2009) calculated that 96% of the added arsenic at their Faridpur, Bangladesh, study site was retained in the topsoil at the end of the irrigation season. Despite seasonal losses, arsenic had accumulated in the irrigated top soils over the 15-17 years of irrigation reported of the Munshiganj and Faridpur sites in Bangladesh (Brammer, 2009). The yearly application of arsenic contaminated ground water as irrigation for Boro rice production has resulted in a gradual increase in soil-arsenic concentration (Heikens *et al.*, 2007). The anaerobic conditions of flooded paddy soil are responsible for the reductive dissolution of the Fe oxy-hydroxides, and the release of the adsorbed arsenate which causes arsenic (As) to be mobilized as arsenite in the soil pore water (Khan *et al.*, 2009, 2010), which is taken up efficiently via the silicic acid pathway in rice (Ma *et al.*, 2008). Anaerobic soil environment converts ferric iron (Fe³⁺) into ferrous iron (Fe²⁺) and thereby enhances the release of arsenic, which in turn make rice more efficient in arsenic uptake than of other cereal crops (Williams *et al.*, 2007; Zhao *et al.*, 2010). At higher soil arsenic concentration, rice plant shows different toxicity symptoms related to physiological and agronomical characteristics such as growth and yield reduction, reduced root and shoot length and biomass production (Rahman *et al.*, 2004; Abedin *et al.*, 2002b; Meharg and Rahman, 2003), reduced chlorophyll content, panicle length and panicle number (Rahman *et al.*, 2007 a, b).

Khan *et al.* (2010) found that addition arsenic either irrigation water or in soil decreased grain yields by 21-74% in Boro rice and 8 to 80% in Aman rice, the latter indicating the strong residual effects of arsenic on subsequent crops. Williams *et al.* (2006) analyzed the arsenic contents of rice from markets in 25 districts across the whole of Bangladesh and found good correlations between the arsenic contents of Aman (wet-season rice, usually not irrigated) and Boro (irrigated, dry season rice), with high average arsenic contents in Aman rice in districts with extensive irrigation of Boro paddy. Khan *et al.* (2009) reported that the application of arsenic in the first (Boro rice 2004) and third (Boro rice 2005) crops had residual effects on the concentrations of arsenic in rice grain and straw of subsequent T. Aman rice crops, and rice grain of both crops had higher arsenic concentration than those of the T. Aman crops (Khan *et al.*, 2009). Ahmed *et al.* (2011)

analyzed 38 rice varieties released by the Bangladesh Rice Research Institute (BRRI) and found that the mean grain arsenic concentration was significantly higher in the Boro (0.290 mg As/kg) than in the Aman (0.154 mg As/kg) season. Hossain *et al.* (2008) reported that arsenic concentration in grain and straw of Boro rice increased significantly with increasing concentration of arsenic in irrigation water, and residual arsenic from previous Boro rice showed a very similar pattern in the following Aman rice although arsenic concentration in Aman rice grain and straw over the treatment was almost half of the arsenic levels in Boro rice grain.

No clear guidelines for irrigated soils and irrigation water have yet been established in Bangladesh. Most of the recent studies focusing on inorganic arsenic and impacts on arsenic uptake by rice and grain yield (Hua *et al.*, 2013). However, limited research has been done on residual effects of arsenic contaminated irrigation water on rice, cultivated with natural rain water. In particular, the relation between soil residual arsenic and rice plant is needed to be investigated. Therefore, the aim of this experiment was to investigate the residual effects of arsenic-contaminated irrigation water on the following rice plant (BRRI dhan 50 rice variety), cultivated with normal tape water. The specific objectives were,

- i) to observe the effect of soil residual arsenic on rice growth and yield
- ii) to measure the effect of soil residual arsenic on chlorophyll contents of rice leaves, and
- iii) to analyze the effect of soil residual arsenic on arsenic accumulation into rice grain and straw.

3.2 Materials and Methods

3.2.1 Experimental Site

This experiment was conducted in a field, situated within 24°22'10.2" to 24°22'10.3" N latitude and 88°38'21.7" to 88°38'21.8" E longitude at Institute of Environmental Science of Rajshahi University in the north-western part of Bangladesh during February to May 2012. The study site has sub-tropical and humid climate with adequate sunshine during day time.



Plate 3.1 Experimental Plot of BRRI dhan-50 Rice at Institute of Environmental Science, University of Rajshahi

3.2.2 Soil Condition

The experiment was conducted in gangetic soil condition. The properties of soil are given in **Table 3.1**.

Table 3.1 Soil Conditions in Experimental Site

Previous Season irrigation water Arsenic (ppm)	Residual As in Soil (ppm)	Total N (%)	Available P (ppm)	Available K (mol/kg)	Available S (ppm)	Available Z (ppm)	pH	Organic matter (%)
0.0	3.67	0.06	22.7	0.14	8.9	0.86	8.4	1.25
0.1	6.11	0.03	24.5	0.23	9.8	1.63	8.3	0.60
0.5	7.23	0.07	21.9	0.17	9.1	1.76	8.3	1.34
1	9.85	0.04	21.1	0.19	10.8	1.90	8.3	0.80
2	13.68	0.07	31.7	0.21	10.4	2.03	8.2	1.44
4	14.62	0.05	30.2	0.18	12.9	1.93	8.3	1.05

3.2.3 Rice Variety

BRRI dhan 50 rice variety was cultivated during this experiment.

3.2.4 Seedling Transplantation

Thirty-five (35) days old seedlings were uprooted carefully from the seed bed in the morning from the Bangladesh Rice Research Institute, Rajshahi station and four seedlings for each hill with six replications were transplanted on the same day in experimental field

on 22nd February 2012. The seedlings which died within 6 days of transplantation were discarded and new seedlings were replaced.

3.2.5 Intercultural Application

3.2.5.1 Fertilizer Application

To support the plant growth, urea, triple super phosphate (TSP), murate of potash (MP) and gypsum fertilizer were applied for nitrogen, phosphorus, potassium, and sulfur, respectively. The first spilt (one third of the dose) of urea and full doses of all other fertilizers were incorporated into the soil by hand before two days of seedling transplantation. The second and third splits of urea were applied after 30 (maximum tillering stage) and 70 (panicle initiation stage) days of transplantation, respectively. One insecticide named fighter was applied into the soil to kill the insects and aphids those attacked the rice plants.

3.2.5.2 Arsenic Source

Sodium arsenate (Na_2HAsO_4) was applied as arsenic source during irrigation water in previous season.

3.2.5.3 Irrigation and Treatment

Six arsenic treatments 0.0, 0.1, 0.5, 1.0, 2.0, and 4.0 mg/L As containing irrigation water were applied in previous season rice cultivation. Normal tape water was applied for irrigation in this experiment. After transplantation of rice seedlings, 3-4 cm water above soil level was maintained in each plot throughout the growth period. Irrigation was stopped before 10 days of harvest.

3.2.6 Chlorophyll Measurement

The chlorophyll from rice leaves during flowering stage was extracted in 80% acetone and chlorophyll contents were measured at 663 nm and 645 nm in a spectrophotometer. From the absorption coefficients, the amount of chlorophyll was calculated.

3.2.6.1 Procedure

- i) At first, the rice leaves were cut into small pieces leaving away the midribs, mixed thoroughly and 1 gm of the leaves were taken into a clean mortar to grind them.

- ii) The tissues were grinding to fine pulp with the addition of 20 ml of 80% acetone.
- iii) The supernatant were taken into 100 ml volumetric flask after centrifuging (5,000 rpm for 5 min) the samples.
- iv) Then the residues were grinding with 20 ml of 80% acetone and centrifuged again and transferred the supernatant to the same volumetric flask.
- v) The pestle and mortar thoroughly washed with 80% acetone and collected the clear washing in the volumetric flask.
- vi) Then the homogenate was filtered through filter paper (Whatman no.1) and made a volume of 100 ml with 80% cold acetone.
- vii) Finally, the optical density of each solution was measured at 663 and 645 nm against (80% acetone) the solvent blank.

3.2.7 Measurement of Plant Growth and Yield

The growth and yield elements of rice were collected and recorded. Rice plant height and number of tiller were recorded for the measurement of plant growth. Plant height was measured from the ground level to the top of the panicle at the full growth of plant using meter scale. Plants of all hills were measured and averaged from each plot. The number of tiller in each plot was counted at the maximum tillering stage. Panicle length was measured from basal node of the rachis to the apex. The number of grains per panicle of all fertile tillers was counted. Thousand grains from each plot were counted and weighed. The grain and shoot biomass/plot (defined as the remaining above ground portion of the rice plant after the spikelets have been removed) were dried and weighed. The result was expressed gm/plot.

3.2.8 Samples Collection and Preservation

3.2.8.1 Soil Samples Collection and Preservation

Soil samples were collected from 0–15 cm depth in 15 cm² area by composite sampling from the fields irrigated with arsenic contaminated water and transferred to airtight polyethylene bags. The samples were immediately air dried at room temperature after collection. Finally, the samples were dried in the Hot Air Oven at 60°C for 72 h and were stored in airtight polyethylene bags at room temperature with proper labeling.

3.2.8.2 Rice Plant Samples Collection and Preservation

The rice plants were cut at 4 cm above the soil. Rice grain was harvested at their maturity stage on 30th May 2012. Then the collected samples (straw and rice grain) from each treatment were tagged properly and sun dried for 3 days and then keeping the samples on

a table. Finally, the samples were dried in the Hot Air Oven at 60°C for 72 h and were stored in airtight polyethylene bags at room temperature with proper labeling. Proper care was taken at each step to minimize any sort of contamination.

3.2.9 Total Arsenic Measurement Methods

Soil, rice straw and grain samples were digested separately following the heating block digestion procedure (Rahman *et al.* 2007c). Rice straw and grain samples were digested by HNO₃-HClO₄ and soil samples by HNO₃-H₂SO₄-HClO₄ for measuring arsenic concentrations in hydride generation atomic absorption spectrophotometer.

3.2.9.1 Sample Digestion

- i) At first, the oven dried samples were ground and passed through 2.0 mm pore sized sieve to get homogenized representative powder sample.
- ii) Then about 0.5 g of the sample was taken into clean dry digestion tubes and 5 ml of concentrated nitric acid (HNO₃) was added to it. The mixture was allowed to stand over night under fume hood.
- iii) In the following day, the digestion tubes were placed on a heating block and heated at 60°C for 2 h.
- iv) The tubes were then allowed to cool at room temperature.
- v) Then about 2 ml of concentrated perchloric acid (HClO₄) was added to the plant samples.
- vi) For the soil samples 3 ml of concentrated sulfuric acid (H₂SO₄) was added in addition to 2 ml of concentrated perchloric acid (HClO₄).
- vii) Then the tubes were heated at 160°C for about 4–5 h.
- viii) The heating was stopped when the dense white fume of perchloric acid (HClO₄) was emitted.
- ix) The content was then cooled, diluted to 25 ml with de-ionized water.
- x) Filtered through Whatman No. 42 filter papers for soil samples and Whatman No. 41 for plant samples and finally stored in polyethylene bottles.

All glassware and plastic bottles were previously washed with 2% HNO₃ followed by rinsing with de-ionized water and drying.

3.2.9.2 Sample Arsenic Analysis Methods

The total arsenic of the digested soil, rice straw and grain samples were analyzed by flow injection hydride generation atomic absorption spectrophotometer (FI-HG-AAS, Perkin Elmer A Analyst 400) using external calibration through arsenate as standard (Welsch *et*

al., 1990). For each sample three replicates were taken and the mean values were obtained on the basis of calculation of those three replicates.

3.2.10 Soil Sample Analysis

Chemical properties of initial composite soil sample were analyzed. The chemical properties included soil total N, available P, exchangeable K, available S, available Zn contents, pH and organic matter.

3.2.10.1 Total Nitrogen (N)

The micro-Kjeldahl method was used for estimating total nitrogen of soil. The soil was digested with H_2O_2 and concentrated H_2SO_4 in presence of a catalyst mixture ($\text{K}_2\text{SO}_4:\text{CuSO}_4.5\text{H}_2\text{O}:\text{Se}$ in the ratio of 10:1:0.1) and the nitrogen in the digest was determined by distillation with 40% NaOH followed by titration of distillate trapped in H_3BO_3 with 0.01N H_2SO_4 (Page *et al.*, 1982).

3.2.10.2 Available Phosphorus (P)

Following the Olsen *et al.* (1954) method available phosphorus was extracted from the soil with 0.5 M NaHCO_3 solution, pH 8.5. Phosphorus in the extract was then determined by developing blue colour with reduction of phosphomolybdate complex and the colour intensity was measured colorimetrically at 660 nm (Page *et al.*, 1982). The P concentration of extract was calculated by fitting the absorbance reading to the standard curve.

3.2.10.3 Exchangeable K

According to Page *et al.* (1982) method exchangeable K was determined on 1N NH_4OAc (pH 7.0) extract of the soil by using flame photometer.

3.2.10.4 Available Sulphur (S)

Available S content of soil was measured by extracting the soil with CaCl_2 (0.15%) solution as explained by Page *et al.* (1982). The extractable S was estimated by developing turbidity by adding acid seed solution (20 ppm S as K_2SO_4 in 6N HCl) and BaCl_2 crystals. The intensity of turbid was estimated by spectrophotometer at 420 nm wavelength.

3.2.10.5 Available Zn

DTPA extraction method (Hunter, 1984) was applied for measuring available Zn content in soil.

3.2.10.6 Soil pH

A glass electrode pH meter was used for measuring soil pH, the soil-water ratio being maintained at 1:2.5 (Jackson, 1962).

3.2.10.7 Organic Matter Content

Wet oxidation method of Walkey and Black (1934) was applied for determining organic carbon in soil volumetrically. The organic matter content was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor).

3.2.11 Statistical Analysis of Experimental Data

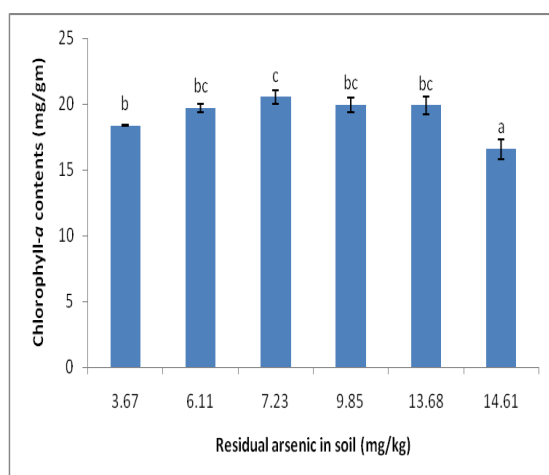
The data was subjected to one-way ANOVA using SPSS 15.0 software, where means and standard errors were calculated for the six replicates. The means were compared using the Duncan significance test at the 0.05 level and the correlation amongst the residual arsenic in soil, rice growth and yield parameters, As concentration in rice grain and straw were established using the Pearson's correlation. Graphical statistical analyses were done with the help of Microsoft Excel software.

3.3 Results

3.3.1 Effect of Soil Residual Arsenic on Chlorophyll Contents of BRRI dhan-50 Rice Leaves

3.3.1.1 Chlorophyll-a Contents in Leaves of BRRI dhan-50 Rice

The chlorophyll-a contents of BRRI dhan-50 rice were significantly ($p \leq 0.01$) affected by soil residual arsenic. The highest chlorophyll-a contents (20.53 ± 0.52) was found in 7.23 mg/kg As containing treatment and the lowest (16.58 ± 0.76) in 14.6 mg/kg As containing treatment. Up to the 7.23 mg/kg As containing treatment the Chlorophyll-a contents were increased, but thereafter decreased significantly with increasing soil residual arsenic concentration. The chlorophyll-a contents negatively correlated with soil residual arsenic (**Figure 3.1.1**), (**Figure 3.1.2**) and (**Table 3.2** in Appendix-2).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.1.1 Effect of soil residual arsenic on chlorophyll-a contents of BRRI dhan-50 rice leaves

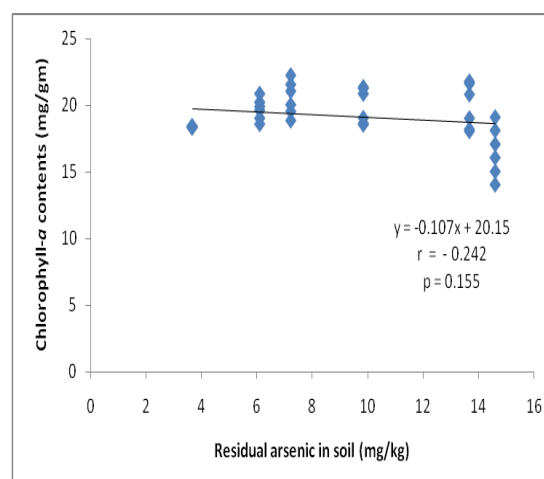
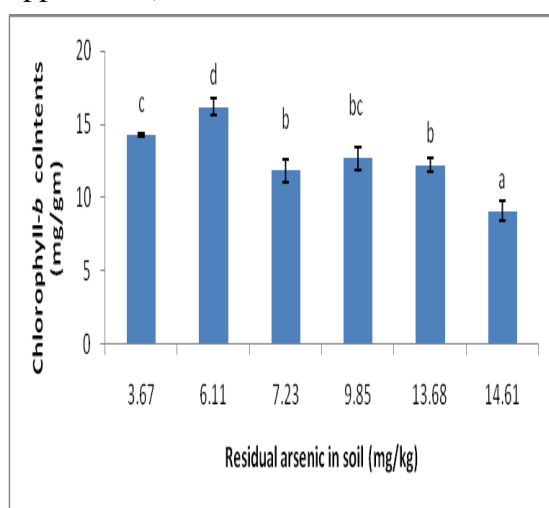


Figure 3.1.2 Correlation between soil residual arsenic and chlorophyll-a contents of BRRI dhan-50 rice leaves

3.3.1.2 Chlorophyll- b Contents in Laves of BRRI dhan-50 Rice

The chlorophyll-b contents of BRRI dhan-50 leaves were significantly ($p \leq 0.01$) affected by soil residual arsenic. Up to the 6.11 mg/kg As containing treatment chlorophyll-b contents were increased significantly. The chlorophyll-b contents were decreased significantly from 12.66 ± 0.74 mg/gm in 9.85 mg/kg As treatment to 9.08 ± 0.69 mg/gm in 14.61 mg/kg As containing treatment. Chlorophyll-b contents had significant negative correlation with soil residual arsenic (Figure 3.2.1), (Figure 3.2.2) and (Table 3.2 in Appendix-2).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.2.1 Effect of soil residual arsenic on chlorophyll-b contents of BRRI dhan-50 rice leaves

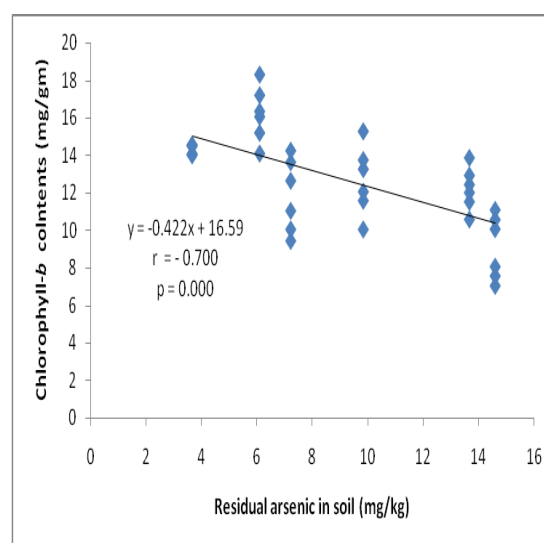
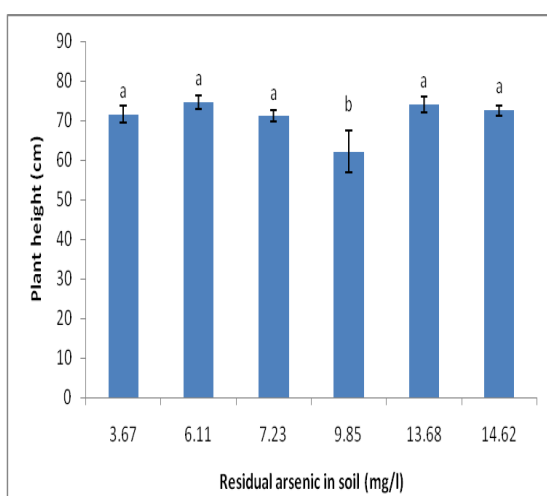


Figure 3.2.2 Correlation between soil residual arsenic and chlorophyll-b contents of BRRI-dhan-50 rice leaves

3.3.2 Effect of Soil Residual Arsenic on Growth of BRRI dhan-50 Rice

3.3.2.1 Plant Height

Plant heights of BRRI dhan-50 rice were significantly affected by soil residual arsenic. It increased up to 74.60 ± 1.63 cm at 6.11 mg/kg As containing treatment. But, after that plant heights were decreased significantly with increasing soil residual arsenic concentration. The smallest plant height was observed in 9.58 mg/kg As containing treatment. A negative insignificant correlation was found between plant height and soil residual arsenic (**Figure 3.3.1**), (**Figure 3.3.2**) and (**Table 3.2** in Appendix-2).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.3.1 Effect of soil residual arsenic on Plant height of BRRI-dhan -50 rice

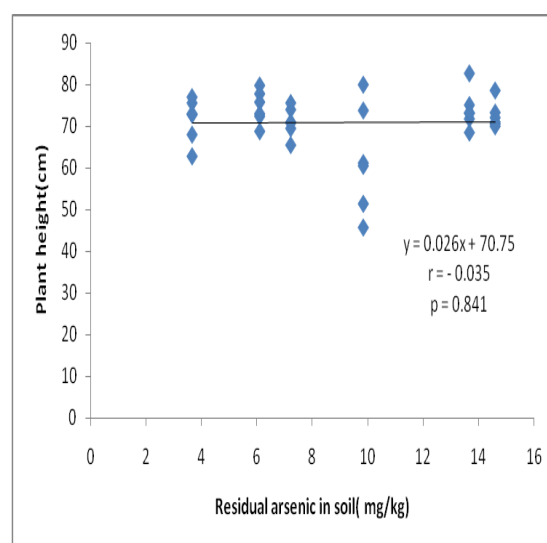


Figure 3.3.2 Correlation between soil residual arsenic and Plant height of BRRI dhan-50 rice



Early Stage

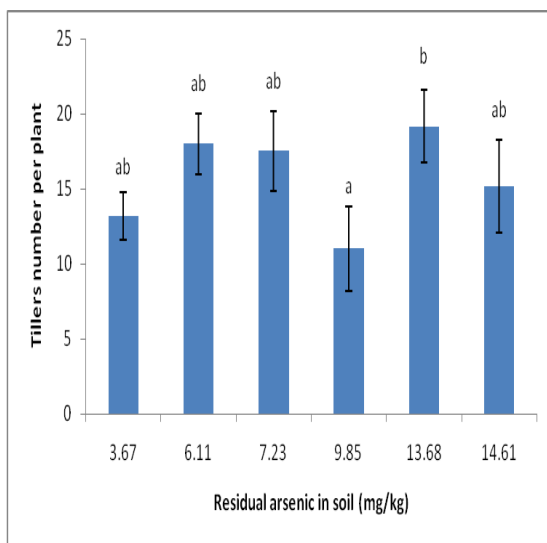


Final Stage

Plate 3.2 Effect of Soil Residual Arsenic on Growth of BRRI dhan-50 Rice

3.3.2.2 Tillering

Tillers number per plant of BRRI dhan-50 rice were significantly affected by soil residual arsenic. It decreased significantly from 18 ± 2.04 in 6.11 mg/kg As containing treatment to 11.00 ± 2.8 in 9.85 mg/kg As containing treatment. No significant correlation was observed between tillering and soil residual arsenic (**Figure 3.4.1**), (**Figure 3.4.2**) and (**Table-3.2** in Appendix-2).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.4.1 Effect of soil residual arsenic on Tillering of BRRI dhan-50 rice

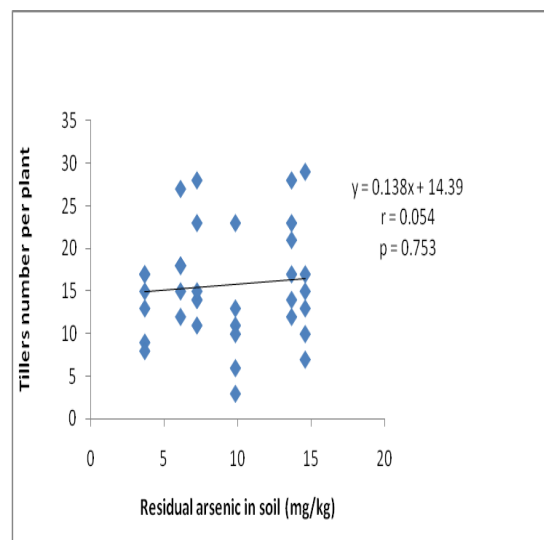
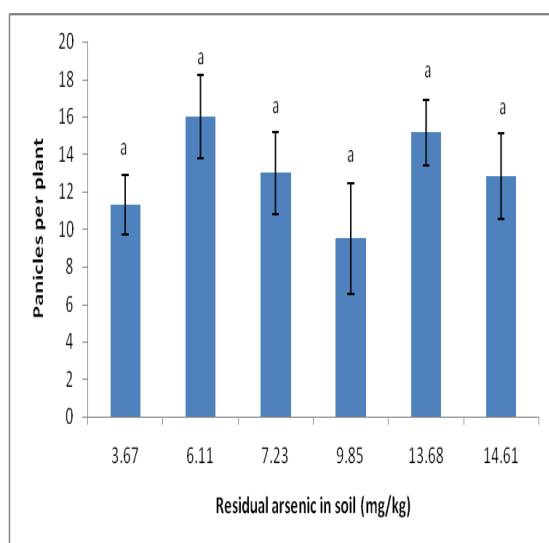


Figure 3.4.2 Correlation between soil residual arsenic and Tillering of BRRI dhan-50 rice

3.3.2.3 Panicles Number

Panicles number per plant of BRRI dhan-50 rice were not significantly affected by residual soil arsenic. The highest panicle number per plant (16 ± 2.2) was found in 6.11 mg/kg As containing treatment and the lowest (9.5 ± 2.97) in 9.85 mg/kg As containing treatment. Panicles number per plant gradually decreased with increasing soil residual arsenic from 7.23 mg/kg to 9.85 mg/kg. No significant correlation had observed between panicles number and soil residual arsenic (**Figure 3.5.1**), (**Figure 3.5.2**) and (**Table 3.2** in Appendix-2).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.5.1 Effect of soil residual arsenic on panicle number of BRRI dhan-50 rice

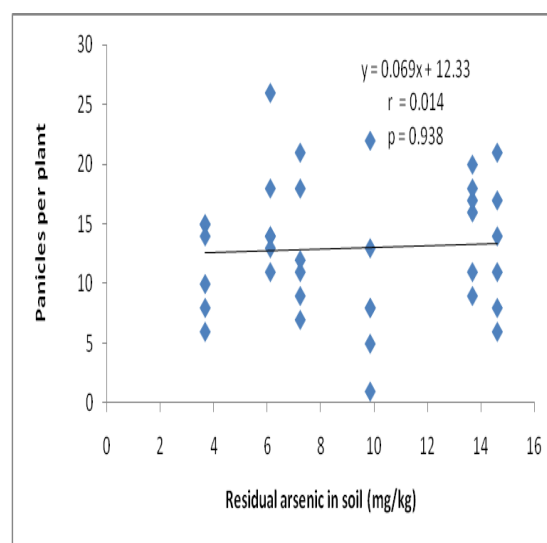
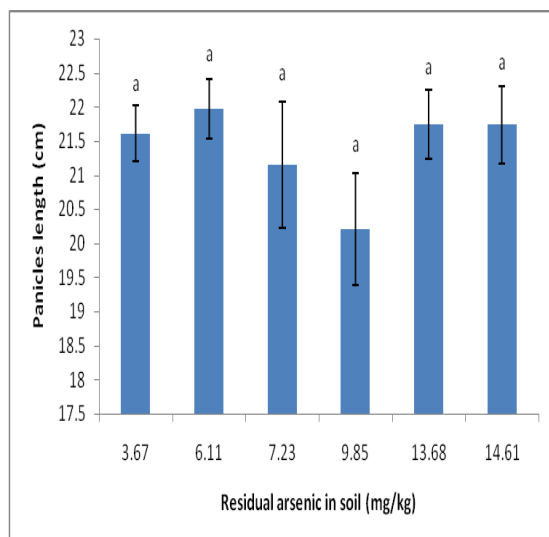


Figure 3.5.2 Correlation between soil residual arsenic and panicle number of BRRI dhan-50 rice

3.3.2.4 Panicles Length

Panicles length of BRRI dhan-50 rice were not significantly affected by soil residual arsenic. The tallest panicle (21.96 ± 0.44 cm) was detected in 6.11 mg/kg As containing treatment and the smallest panicle (20.21 ± 0.81 cm) in 9.85 mg/kg As containing treatment. A negative insignificant correlation was observed between panicle length and soil residual arsenic (Figure 3.6.1), (Figure 3.6.2) and (Table 3.2 in Appendix-2).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.6.1 Effect of soil residual arsenic on panicle length of BRRI dhan-50 rice

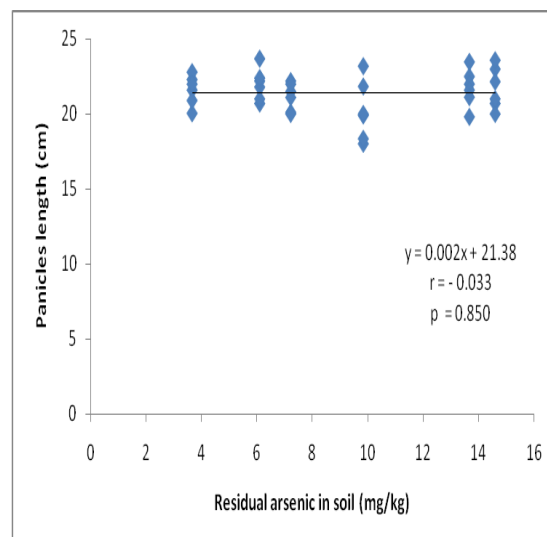
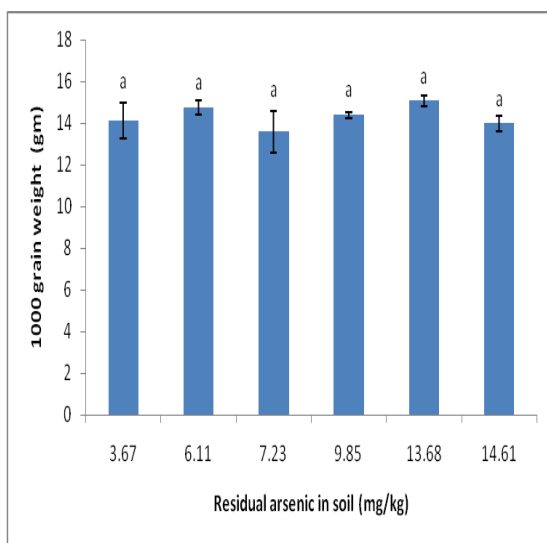


Figure 3.6.2 Correlation between soil residual arsenic and panicle length of BRRI dhan-50 rice

3.3.2.5 1000 Grain Weight

Thousand grain weights of BRRI dhan-50 rice were not significantly affected by soil residual arsenic. The highest weight (14.77 ± 0.34 gm) was found in 6.11 mg/kg As containing treatment and the lowest weight (13.58 ± 0.99 gm) in 7.23 mg/kg As containing treatment (**Table 3.2** in Appendix-2). The differences of 1000 grain weight were not significant with increasing soil residual arsenic concentration. No significant correlation was found between thousand grain weight and soil residual arsenic (**Figure 3.7.1**), (**Figure 3.7.2**).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.7.1 Effect of soil residual arsenic on 1000 Grain weight of BRRI dhan-50 rice

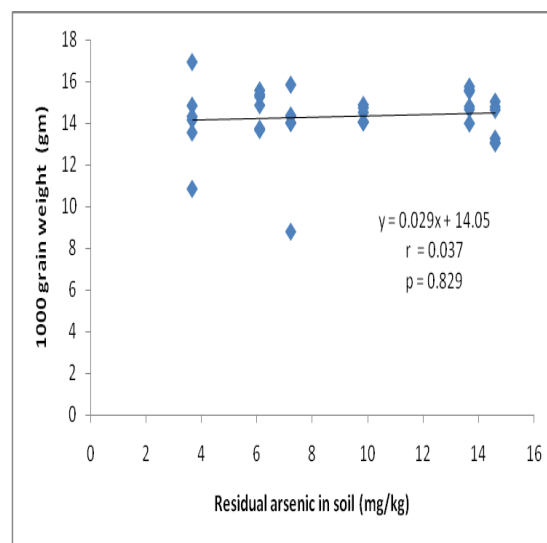
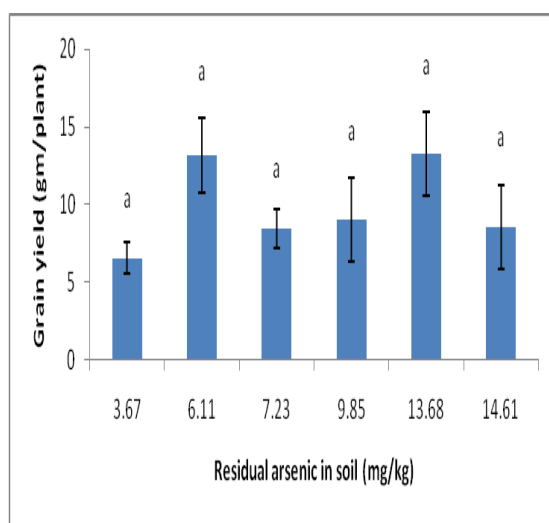


Figure 3.7.2 Correlation between soil residual arsenic and 1000 Grain weight of BRRI dhan-50 rice

3.3.3 Effect of Soil Residual Arsenic on Yield of BRRI dhan-50

3.3.3.1 Grain Yield

Grain yields per plant of BRRI dhan-50 rice were not significantly affected by soil residual arsenic. The differences of grain yield among each other soil residual arsenic treatment were not significant (**Figure 3.8.1**). The range of yield was between 6.55 ± 1.02 gm to 13.25 ± 2.66 gm (**Table 3.4** in Appendix-2). No significant correlation was found between grain yield per plant and residual arsenic (**Figure 3.8.2**).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.8.1 Effect of soil residual arsenic on grain yield of BRRI dhan-50 rice

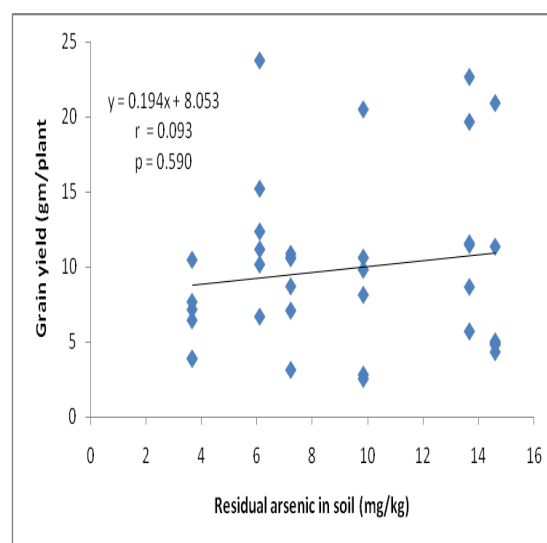
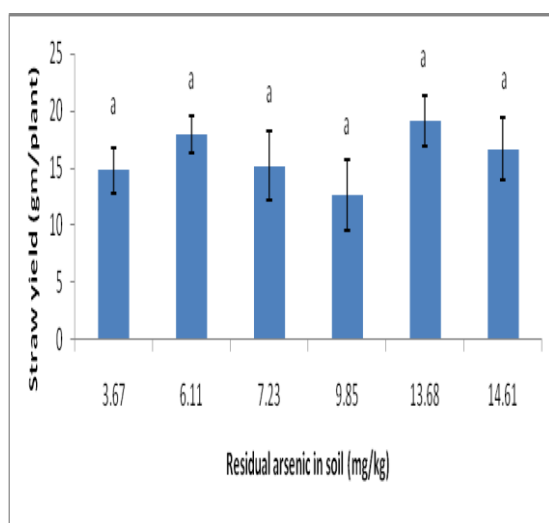


Figure 3.8.2 Correlation between soil residual arsenic and grain yield of BRRI dhan-50 rice

3.3.3.2 Straw Yield

Straw yields per plant of BRRI dhan-50 rice were not significantly affected by soil residual arsenic. Straw yield was decreased from 17.94 ± 1.58 gm in 6.11 mg/kg As containing treatment to 12.63 ± 3.15 gm in 9.85 mg/kg As containing treatment. But, the differences of straw yield were not significant among each other residual arsenic concentration. No significant correlation was detected between straw yield and soil residual arsenic (**Figure 3.9.1**), (**Figure 3.9.2**) and (**Table 3.4** in Appendix-2).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.9.1 Effect of soil residual arsenic on straw yield of BRRI dhan-50 rice

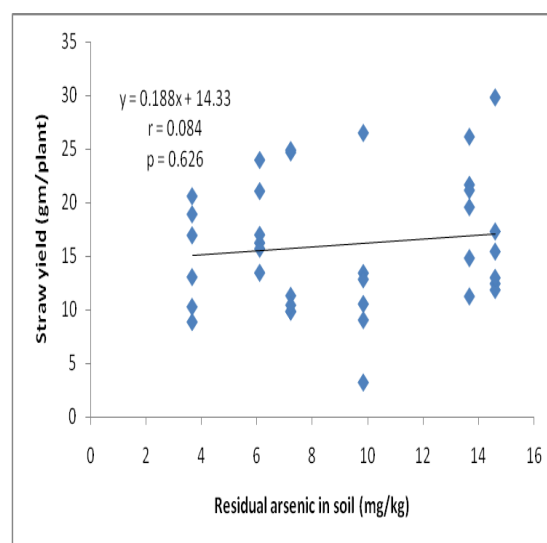
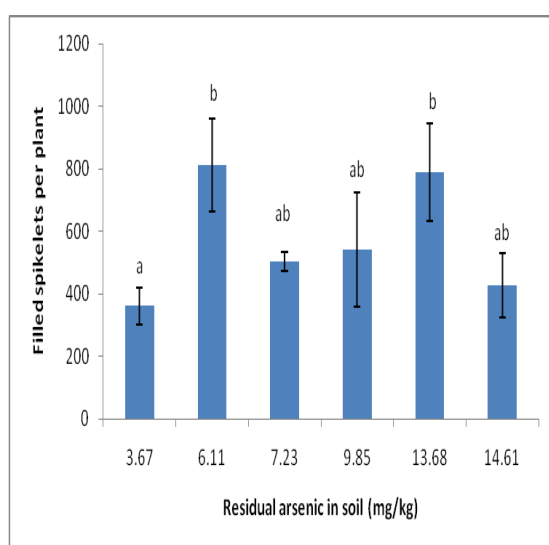


Figure 3.9.2 Correlation between soil residual arsenic and straw yield of BRRI dhan-50 rice

3.3.3.3 Spikelet Number

3.3.3.3.1 Filled Spikelet numbers

Filled spikelets of BRRI dhan-50 rice were significantly affected by soil residual arsenic. The lowest filled spikelet number (362.16 ± 58.77) was found in 3.67 mg/kg As containing treatment and highest (812 ± 149) in 6.11 mg/kg As containing treatment. The differences of filled spikelet number from each other treatments were significant (**Figure 3.10.1**). No significant correlation was found between filled spikelet number and soil residual arsenic (**Figure 3.10.2**).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.10.1 Effect of soil residual arsenic on filled spikelet number of BRRI dhan-50 rice

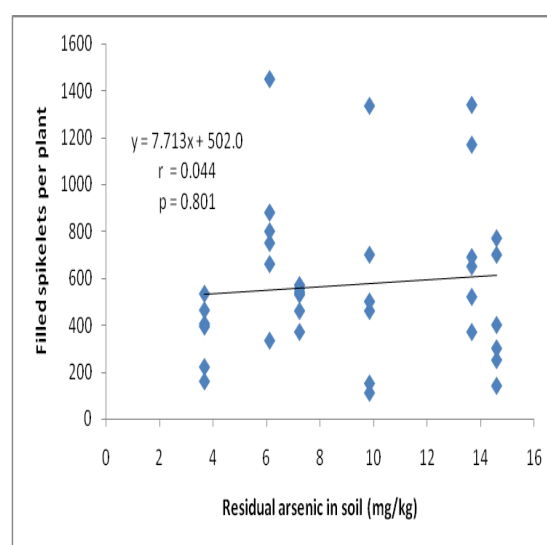
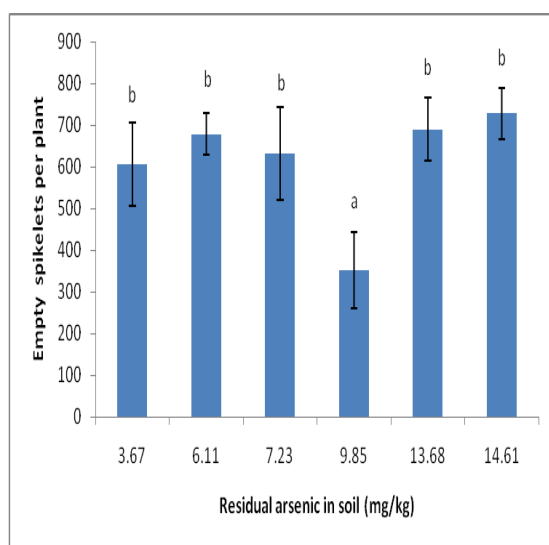


Figure 3.10.2 Correlation between soil residual arsenic and filled spikelet number of BRRI dhan-50 rice

3.3.3.3.2 Empty Spikelet Number

Empty spikelets number per plant of BRRI dhan-50 rice were significantly affected by soil residual arsenic (**Figure 3.11.1**). Up to 7.23 mg/kg As containing treatment the differences of empty spikelet number were not significant from each other. But, after that it increased significantly from 352.00 ± 91 in 9.85 mg/kg As contain treatment to 728.33 ± 61.39 in 14.61 mg/kg As containing treatment (**Table 3.5** in Appendix-2). No significant correlation was detected between empty spikelet number and soil residual arsenic (**Figure 3.11.2**).



Bars (mean ± SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.11.1 Effect of soil residual arsenic on empty spikelet number of BRRI dhan-50 rice

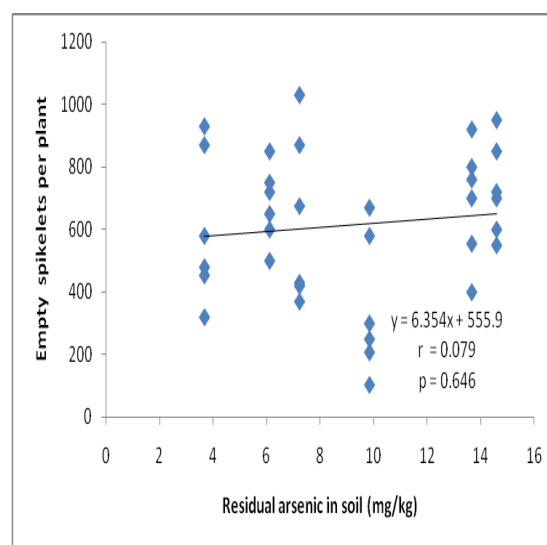
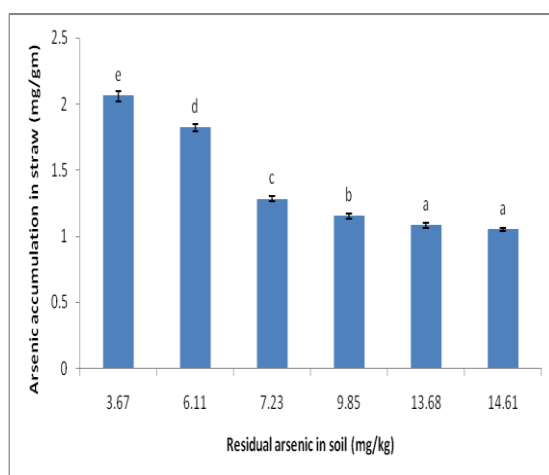


Figure 3.11.2 Correlation between soil residual arsenic and empty spikelet number of BRRI dhan-50 rice

3.3.4 Effect of Soil Residual Arsenic on Arsenic Accumulation in BRRI dhan-50 Rice Plant

3.3.4.1 Arsenic Accumulation in Straw

Arsenic accumulation in straw of BRRI dhan-50 was significantly affected by soil residual arsenic. The lowest arsenic accumulation (1.05 ± 0.01 mg/kg) in straw and the highest arsenic accumulation (2.06 ± 0.04 mg/kg) in straw were found in 3.67 mg/kg and 14.61 mg/kg As containing treatment, respectively (**Table 3.6** in Appendix-2). Arsenic accumulation in straw was increased significantly with increase of residual arsenic concentration (**Figure 3.12.1**). A significant negative correlation was observed between soil residual arsenic and arsenic accumulation in straw (**Figure 3.12.2**).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.12.1 Effect of soil residual arsenic on arsenic accumulation in straw of BRRI dhan-50 rice

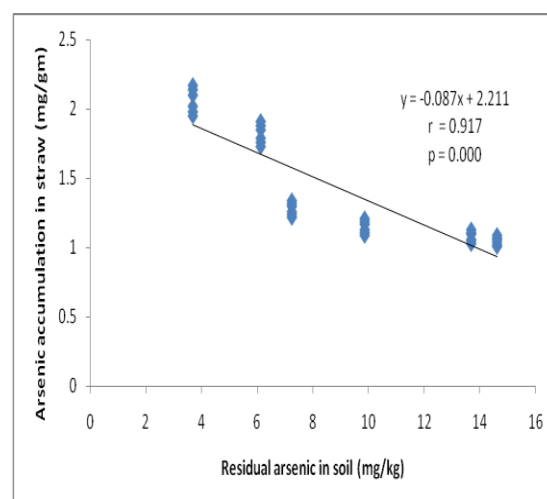
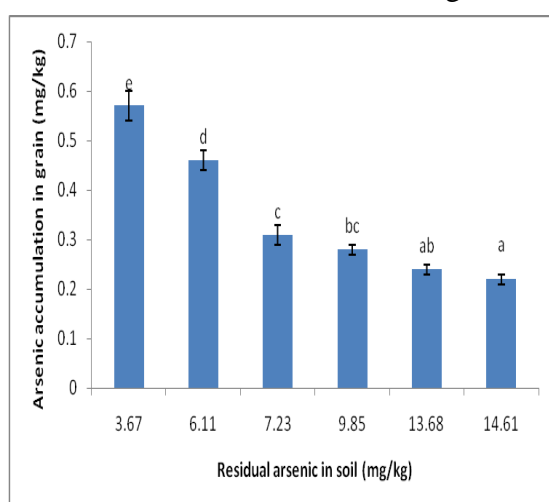


Figure 3.12.2 Correlation between soil residual arsenic and arsenic accumulation in straw of BRRI dhan-50 rice

3.3.4.2 Arsenic Accumulation in Grain

Arsenic accumulation in grain of BRRI dhan-50 rice was significantly affected by soil residual arsenic. The lowest arsenic accumulation (0.22 ± 0.01 mg/kg) in grain and the highest arsenic accumulation (0.57 ± 0.03 mg/kg) in grain were found in 3.67 mg/kg and 14.61 mg/kg A containing treatment, respectively (**Table 3.6** in Appendix-2). Arsenic accumulation in grain was increased significantly with increasing residual arsenic concentration (**Figure 3.13.1**). As like as straw a significant negative correlation was observed between soil residual arsenic and arsenic accumulation in grain of BRRI dhan-50 rice (**Figure 3.13.2**).



Bars (mean \pm SE) having the same letters do not differ significantly at $p \leq 0.05$ by DMRT.

Figure 3.13.1 Effect of soil residual arsenic on arsenic accumulation in grain of BRRI dhan-50 rice

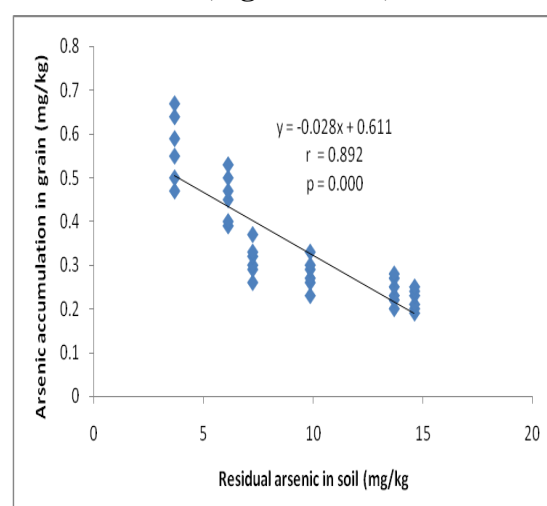


Figure 3.13.2 Correlation between soil residual arsenic and arsenic accumulation in grain of BRRI dhan-50 rice

3.4 Discussion

3.4.1 Effect of Soil Residual Arsenic on Chlorophyll Contents of Rice Plant

Photosynthesis is the most important biochemical event on earth. It serves as the World's largest solar battery. Photosynthesis converts massive amount of sunlight into electrical and then chemical energy (Hall and Rao, 1999).

In plants the most important pigment is chloroplast. It consists of two types of chlorophylls, chlorophyll 'a' and chlorophyll 'b'. They differ from each other chemically and absorb light of different wavelengths to perform photosynthesis (Rahman *et al.*, 2007b). Bhattacharya *et al.* (2013) reported both chlorophyll 'a' and 'b' contents in rice leaf of eight varieties were decreased with increasing soil arsenic concentration. Arsenic exposure had greater effect on chlorophyll content of rice leaf. IR 64 variety showed more decrease in chlorophyll content at higher concentration and duration (Ahmad *et al.*, 2012).

In the present study, both chlorophyll 'a' and 'b' contents of BRRI dhan-50 rice leaf were also found to decrease with increase of soil arsenic concentrations (**Figure 3.1.1 and 3.2.1**). This is in agreement with the observation by Rahman *et al.* (2007b). Arsenic toxicity may impair the electron transport process in thylakoids (Maiti and Biswas, 2006) and thus may damage chloroplast, which ultimately result in the decrease of chlorophyll contents in rice leaf (Choudhury *et al.*, 2011). Bhattacharya *et al.* (2002) observed a consistent negative correlation between soil arsenic treatments and both chlorophyll 'a' and 'b' content in rice plant. In the present study a significant negative correlation was also observed between soil residual arsenic and chlorophyll b contents of BRRI dhan 50 rice plants.

3.4.2 Effect of Soil Residual Arsenic on Growth and Yield of Rice

Arsenic toxicity affects the photosynthesis which ultimately results in the reduction of rice growth and yield (Rahman *et al.*, 2007b).

Plant height, tiller numbers, panicle numbers, panicle lengths and 1000- grain weight were recorded to determine the effect of soil residual arsenic on rice growth. Certainly, the reduction of rice plant growth, in terms of tillering, plant height and shoot biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Xie and Haung, 1998; Jahan *et al.*, 2003; Rahman *et al.*, 2004) though the phytotoxicity at lower soil arsenic concentration was not significant (Rahman *et al.*,

2007b). In the present study, the growth parameters of BRRI dhan-50 rice plants were not significantly affected by lower soil residual arsenic concentrations, accumulate from previous season irrigation water (**Table 3.2** in Appendix-2), which is in good agreement with previous studies.

At low soil arsenic concentration, displacement of soil phosphate by arsenate increased the availability of phosphate to the plant, which results in the increase of plant growth (Jacobs *et al.*, 1970; Duel and swoboda, 1972). Thus, Kabata-Pendias and Pendias (1992) recommended the safe level of arsenic in agricultural soil as 20 mg As/kg. In the present study, soil residual arsenic concentrations were within the safe level (**Table 3.2** in Appendix-2). The soil residual arsenic may affect the Aman rice (Duxbury *et al.*, 2003). The application of different concentrations of arsenic in irrigation water had significant effect on yield-contributing characteristics of Aman rice regarding plant height, filled grains panicle⁻¹, and unfilled grains panicle⁻¹ (Hossain *et al.*, 2007).

In this study, the application of different concentrations of arsenic in previous season irrigation water had significant residual effects on yield-contributing parameters of following BRRI dhan 50 rice regarding plant height and empty spikelet number per plot (**Table 3.2** in Appendix-2).

The number of fertile tillers pot⁻¹ and 1000-grain weight were not significantly affected by arsenic application in the previous crop (Hossain. *et al.*, 2007).

In the present study the number of fertile tillers pot⁻¹ and 1000-grain weight were also not significantly affected by arsenic application in the previous crop.

Application of arsenic in the previous Boro rice did not significantly reduced the straw yield of Aman rice inspite of a general trend of decrease in straw yield (Hossain *et al.*, 2007), similar to the present study (**Table 3.3** in Appendix-2). There was a significant residual effect of arsenic on the grain yield of Aman rice for application of arsenic to Boro rice (Hossain *et al.*, 2007), similar to Islam *et al.* (2004), but disagree with in the present study.

Rice yield was measured on the basis of grain yield, straw yield, filled spikelet and empty spikelet number per plant. There is no evidence that arsenic is essential for plant growth,

but it has phytotoxic effects on different crops. Arsenic is translocated to many parts of the plants; mostly, it is found in old leaves and roots. In rice, the critical level in tops ranges from 20 to 100 mg/kg As, and in roots 1,000 mg/kg As (Odanaka *et al.*, 1987). The tillers of rice are reported to be severely depressed with high concentrations of arsenic (Chino, 1981). Azad *et al.* (2009) reported that the number of tillers per pot of T-Aman rice was not significantly affected up to 20 mg/kg of As in soil. Just like the tiller numbers, the panicle numbers of T-Aman rice were also not affected at low doses of arsenic in soil. The panicles length also followed the same kind of trend.

Na-arsenate as arsenic in irrigation water was applied in previous season rice cultivation to observe the residual effect on next season rice plant. This arsenate is found to affect the root development and reduce the plant height of one variety of rice (Abedin *et al.*, 2002a). Some studies have reported that plant roots are unable to accumulate the essential nutrients from soil in the presence of excess arsenic because As (III) reacts with the sulphhydryl groups of proteins (Speer, 1973), causing disruption of root functions of plants (Orwick *et al.*, 1976).

Azad *et al.* (2009) reported that the grain yield of T-Aman rice markedly declined at 20 mg/kg As treatment and above. The reduced grain yield was reported in another study when rice was grown in arsenic enriched irrigation water for up to 170 days during the post- transplantation growing period (Abedin *et al.*, 2002 b) Arsenic toxicity induced chlorosis symptoms in the youngest leaf of rice by decreasing the chlorophyll content (Shaibur *et al.*, 2006).

Tsutsumi (1980) in his pot experiment, with different concentrations of arsenite in soil, observed no reduction of rice plant height up to 125 mg As/kg, but did observe 63% reduction of plant height at 312.5 mg As/kg. Marin *et al.* (1992), in their hydroponic experiment, observed shorter rice plants when grown with 0.8 mg/L arsenite and monomethylarsonic acid (MMAA), and a higher number of tillers in the dimethylarsenic acid (DMAA) treatment. However, they found no significant reduction in plant height when plants were exposed to arsenate.

No difference in root biomass (Marin *et al.*, 1992) and total dry-matter weight (Onken and Hossner, 1995) were observed when rice plants were treated with arsenics and

arsenate. The reduction of shoot height due to arsenic exposure can be an important consideration for rice cultivation as reduced shoot height will decrease rice leaf area, net photosynthesis (Marin *et al.*, 1993), and ultimately rice yield (Abedin *et al.*, 2002b). Khan *et al.* (2009) reported without arsenic addition, grain yields were reduced by up to 30% for BRRI dhan 29 (Boro) and up to 60% for BRRI dhan 33 (T. Aman) across the soil-arsenic gradient.

Studies of arsenic accumulation in soils (Heikens *et al.*, 2007) have suggested that the long term use of As contaminated water for irrigation would result in an increase soil-As concentration, which has long-term implications to crops productivity and food quality. Yield reduction in rice as a result of long term use of arsenic contaminated irrigation water has recently been demonstrated by Panaullah *et al.* (2009). Present study found that rice grain yields were not significantly correlated with the quantity of residual soil arsenic accumulated from previous only one season arsenic contaminated irrigation water, which is in agreement with the previous observations.

The high retention of applied arsenic in the upper soil layers is important, since most of the arsenic remained in the rice rooting zone, where it would have the greatest impact on plant growth (Khan *et al.*, 2010). Arsenic has been associated with metabolic processes and found to inhibit plant growth, sometimes leading to death (Marin *et al.*, 1993).

Arsenic concentrations in soil that are toxic to vital plant process have varied greatly in different studies due to interaction of different arsenic (As) chemical form with plant cultivars and many environmental factors (Wang *et al.*, 2006).

Onken and Hossner (1995) found that treatment with 5 mg Ag/kg (sodium arsenite or sodium arsenate), could increase dry weight. Rice growth and yield were usually depressed when grown in soils containing high levels of arsenic under both greenhouse and field conditions (Akter *et al.*, 2005; Williams *et al.*, 2005). Mei & Wong (2009) reported that the grain and straw biomass all of 25 rice cultivars were reduced when grown in soil with the addition of 100 mg Ag/kg compared to the control. Similar results have been reported by Williams *et al.* (2005) when rice was grown in soil with the addition of 100 mg Ag/kg. Akter *et al.* (2005) showed that arsenic in soil damaged the roots of rice resulting in inhibition of nutrient uptake.

The bioavailability of arsenic in paddy soil is very important for understanding the variation of arsenic accumulation in rice, which may also be related to the geographic location, soil properties, redox condition and cropping season (Meharg and Rahman, 2003). The key soil factor reported to govern arsenic bioavailability of plants include pH and organic matter (Bhattacharya *et al.*, 2010), redox status (Marin *et al.*, 1993), clay content (Sheppard, 1992), and the presence of poorly crystalline iron (Fe) oxide. (Takahashi *et al.*, 2004); Bogdan and Schenk, 2009).

Fu *et al.* (2011) reported that rice grain As concentrations correlated significantly to soil arsenic speciation, organic matter and soil P contents and could be best predicted by humic acid bound and Fe-Mn oxides bound arsenic fractions. Arsenic uptake and translocation in rice plants are affected by a wide range of factors including soil arsenic speciation, soil physical & chemical properties, irrigation water, and fertilizer management (Brammer, 2009; Zhao *et al.*, 2009; Lu *et al.*, 2009; Norton *et al.*, 2009). In general there is an increase in arsenic mobility as the soils become more acid, particularly at pH values below 5 when arsenic binding such as Fe and Al oxy-compound become more soluble (Carbonell-Barrachina *et al.*, 1999). Signes-Pastor *et al.* (2007) reported that low pH values (< 5.5) resulted in higher soluble concentrations of arsenic in soil solution. Fu *et al.* (2011) reported that organic matter (OM) present in the paddy soil might reduce arsenic bioavailability for the rice plant.

Arsenite and arsenate behave as analogs of silicic and phosphate, respectively, in terms of plant transport. Inorganic arsenic (As_i) enters the roots either as arsenite taken up by rice roots through the highly efficient silicon (Si) transport pathway, or as arsenate via arsenate/phosphate co-transporters (Norton *et al.*, 2010, and references therein). Therefore, silicic acid and phosphate may compete with arsenic for uptake and result in a decrease of arsenic concentration in the plant (Bogdan and Schenk, 2009; Norton *et al.*, 2010).

When paddy soil flooding, the reducing condition and the subsequently dissolve of Fe-Mn (hydr)oxides would lead to the leaching of Fe-Mn (hydr) oxides bound arsenic into the soil solution (Blodau *et al.*, 2008). It has been reported that arsenic can be released by the reductive dissolution of FeOOH or MnOOH linked to humic substances (Selim Reza *et al.*, 2010). The Fe hydroxides in soil and solution have a very strong binding affinity for arsenate (Meng *et al.*, 2002; Liu *et al.*, 2004 a, b), and a possible capacity to oxidize

arsenite to arsenate (Otter *et al.*, 1991). Iron plaque may be a barrier or a buffer to the uptake of arsenic (Liu *et al.*, 2004 a, b). Hu *et al.* (2007) reported that sulfur induced enhancement of iron plaque formation on the root surface of rice.

The arsenic (As) concentration in soil is related to the geological substratum from which the soil is formed (Hsu *et al.*, 2012). A rather wide range of As concentrations has been found in soils around the world, with an average of 5-10 mg/kg in uncontaminated soils (O' Nail, 1995). Anionic arsenic species are readily adsorbed on colloidal particles and poorly crystalline Fe-oxide phases because they have a net positive surface charge at approximately neutral pH. Fe oxyhydroxides are therefore headed as important arsenic sinks in soil and water (Dittmar, *et al.*, 2007; Brammer and Ravenscroft, 2009).

Arsenic accumulates in soil during dry season irrigation with arsenic- contaminated groundwater (Dittmer *et al.*, 2007; Saha and Ali 2007; Lu *et al.*, 2009), which can lead to spatial variation in arsenic concentration across paddy fields (Dittmer *et al.*, 2007; Hossain *et al.*, 2008) and during monsoon flooding season, some of the accumulated arsenic is lost from the paddy field in the floodwater (Dittmar *et al.*, 2010; Roberts *et al.*, 2010). The anaerobic conditions in paddy soil lead to mobilization of arsenite (Marin *et al.*, 1993; Takahashi *et al.*, 2004; Xu *et al.*, 2004; Li *et al.*, 2009). Arsenite oxidation in the standing water may be catalyzed by microbial, surface and photochemical pathways (Roberts *et al.*, 2007). Arsenic dynamics in the soil solution phase were influenced more by total soil As than by standing or irrigation water.

Arsenic mobility is largely governed by adsorption on mineral surfaces, especially Fe(hydro)oxides (Smith and Naidu, 2008). Several factors affecting these processes: pH (Onken and Adriano, 1997), dissolved arsenic carbon (Grafe *et al.*, 2001), competing anions such as phosphate, carbonate, sulfate and to a minor extent chloride and nitrate (Appelo *et al.*, 2002; Goh and Lim, 2005; Livesey and Huang, 1981), aging process (Fendorf *et al.*, 2004), and microbially mediated redox transformations (Langner and Inskeep, 2000; Zobrist *et al.*, 2000). Precipitation, co-precipitation and dissolution of As with other phases, can also play an important role (Alexandratos *et al.*, 2007; Roberts *et al.*, 2007). Smedley and Kinniburgh (2002) repoted that 5-10 mg As/kg baseline soil correlation. In Bangladesh rice fields irrigated with wells containing up to 130 µg As/L accumulated up to 60 m/kg (Hossain *et al.*, 2008).

Background levels of arsenic in rice paddy soils range from 4 to 8 $\mu\text{g/g}$ (Alam and Sattar, 2000; Williams *et al.*, 2006), which can reach up to 83 $\mu\text{g/g}$ in area where the crop land has been irrigated with arsenic-contaminated groundwater (Williams *et al.*, 2006). Robers *et al.* (2007) reported that arsenic contents in topsoil in Bangladesh have increased significantly over the last 15 years because of irrigation with arsenic rich groundwater. Other studies showed that arsenic concentrations remain unchanged at the start of two successive irrigation seasons suggesting that arsenic added during the first irrigation season had been leached by flood water during the following monsoon season (Dittmar *et al.*, 2007). The rate of arsenic deposition from contaminated irrigation water would be higher in flat terrain soil than in floodland soil.

In the present study, arsenic accumulation in soil increased significantly with increasing arsenic level in irrigation water which is in agreement with previous studies.

Considering 1.0 g/cm^3 bulk density, 0.4 porosity, 600 mm average maximum yearly irrigation, 7 cm infiltration depth and 1.5 $\mu\text{g/L}$ As input, the yearly arsenic load in topsoil will be 12.9 mg/kg/yr and during 30 years irrigation period 385.9 mg As/kg will be accumulated in topsoil (Casentini *et al.*, 2011). The global average level of arsenic in soil is 10.0 mg/kg (Das *et al.*, 2002) and EU recommended maximum acceptable limit for agricultural soil is 20.0 mg/kg (Rahman *et al.*, 2007b).

In the present study, up to 1 ppm and 4 ppm arsenic treatment the level of arsenic in paddy soil did not exceeded the global average level and the EU recommended maximum acceptable level of arsenic in agricultural soil, respectively (**Table 3.2** in Appendix-2). The arsenic contents of the soil was found to be significantly correlated with the arsenic content of irrigation water (Bahattacharya *et al.*, 2010), is in good agreement with present study.

Thus there is a high possibility of increase of arsenic concentration in the soil in near future, if the trend of using large amount of arsenic contaminated groundwater for irrigation. Similar prediction was earlier given by Das *et al.* (2004).

Bhattacharya *et al.* (2010) reported that the paddy soil gets contaminated from the irrigation water and the total soil arsenic concentration ranged from 1.34 to 14.09 mg kg^{-1} . The present study also found similar result and the total soil arsenic concentration ranged

from 3.67 to 14.61 mg/kg (**Table 3.2** in Appendix-2). Su *et al.* (2010) demonstrated that arsenic uptake and translocation by rice is more efficient than by wheat and barley. An important reason for the relatively high accumulation of arsenic by rice is the chemistry of the soil in paddy field (Hsu *et al.*, 2012).

In the present study, more arsenic accumulated in the straw (1.05 to 2.06 mg/kg) than grain (0.22 to 0.57 mg/kg) of BRRI dhan 50 rice plant. This observation agrees closely with the findings of Hsu *et al.* (2012), Bhattacharya *et al.* (2010), Azad *et al.* (2009), Smith *et al.*, (2007), Liu *et al.* (2006) and Abedin *et al.* (2002b).

Hsu *et al.* (2012) reported that most arsenic accumulated in the root (20.9-188 mg/kg), followed by the straw (0.7-5.79 mg/kg), husk (1.05-4.13 mg/kg) and grain (0.29-0.66 mg/kg). The actual mechanism by which more arsenic accumulates in the root than in the shoot of rice is still not well understood (Hsu *et al.*, 2012). Rice maintains relatively high redox potentials in the rhizosphere by maintaining a continuous flux of O₂ from shoots to the roots. The release of O₂ enables the accumulation of Fe oxyhydroxides in the rhizosphere, causing of Fe plaque to form around the root; it binds arsenic and thereby reduces the translocation of arsenic to the above ground tissues- straw, husk, and grain (Garnier *et al.*, 2010; Liu *et al.*, 2006). When the rice dies, the flux O₂ to the rhizosphere ceases, potentially causing the Fe plaque to dissolve, promoting the release of arsenic into the soil (Norra *et al.*, 2005). The root arsenic concentrations were not measured in the present study.

Talukder *et al.* (2012) reported that total straw arsenic content was markedly lower in aerobic vs. anaerobic in BRRI dhan 29 (from 6.42 to 12.46 mg/kg) and in BRRI dhan 32 (5.48 to 9.11 mg/kg), respectively. In the present study, a positive relationship was found between arsenic in straw and grain, implying that grain arsenic content progressively decreased with the decreased of straw arsenic which is in good agreement with Talukder *et al.* (2012) and Duxbury and Panullah (2007).

Talukder *et al.* (2012) reported that anaerobic water management is the main reason for the high enhanced arsenic uptake in rice and phosphorus has a positive impact on arsenic mitigation under aerobic water management.

Abedin *et al.* (2002b) observed accumulation of arsenic as high as 92 mg/kg in rice straw. Much higher arsenic accumulation ability in rice straw by hybrid rice varieties as compared to non-hybrid varieties had been formerly reported by Rahman *et al.* (2007b). The

accumulation of high levels of arsenic in the rice straw is a potential threat to cattle that consume the contaminated straw, and thus indirectly to human health, through presumably contaminated bovine meat and milk (Abedin *et al.*, 2002c; Rahman *et al.*, 2008). Bhattacharya *et al.* (2010) reported the accumulation of arsenic in straw during Boro rice season was in the range between 1.34-2.13 mg/kg. In the present study, arsenic in straw of BRRI dhan 50 rice was in the range between 1.05-2.06 mg/kg (**Table 3.6** in Appendix-2), is in which good agreement with the previous findings of Bhattacharya *et al.* (2010). Khan *et al.* (2010) reported the As concentrations in rice straw varied over season.

Azad *et al.* (2009) reported the reduction level of As uptake in straw above 20 mg/kg As treatment may be for dwarfism of plant height or due to enzyme saturation for the active transport of arsenic in tissue.

The same type of declining arsenic uptake pattern for straw was found for one variety of rice grown in a greenhouse (Rahman *et al.*, 2008), is in good agreement with the current study. Rahman *et al.* (2007b) reported at lower soil arsenic concentration (up to 20 mg/kg soil) the arsenic contents in straw of the five rice varieties were statistically identical, though they differed significantly at higher levels of soil arsenic (30 mg/kg soil). The statutory permissible for straw fed to cattle is 0.2 mg/kg (Nicholson *et al.*, 1999). Numerous studies confirmed that the formation of iron plaque around rice root would be an important barrier to inorganic arsenic uptake (Hossain *et al.*, 2009) and inorganic arsenic and Fe were strongly co-precipitated around the root zone (Seyfferth *et al.*, 2010).

Meharg and Rahman (2003) found 0.1-0.5 mg/kg As in grains of rice that were grown in North America and Taiwan in soils that were not contaminated with arsenic. Stroud *et al.* (2011) reported that grain arsenic range was between 0.22 mg/kg to 0.46 mg/kg, in rice paddies that were irrigated with groundwater that contained arsenic at four sites in Bangladesh and west Bengal. Hsu *et al.* (2012) reported that the accumulation of arsenic in rice grains ranged from 0.29 to 0.66 mg/kg, in paddy soils contained total arsenic ranged from 11.8 to 112 mg/kg that were irrigated long term with As-containing groundwater in south western Taiwan. In the present study, the accumulation of arsenic in rice grain ranged from 0.22 to 0.57 mg/kg, below the limit in rice (1.0 mg/kg) recommended by WHO (Abedin *et al.*, 2002b), which is in good agreement with the previous reports. The maximum tolerable daily intake level of inorganic arsenic for a 60 kg adult is 0.126 mg (WHO, 1997). A study indicated that rice grain can accumulate

relatively large amounts of arsenic even from soil not contaminated by arsenic (Daum *et al.*, 2001). The bioavailability of arsenic to the rice plant in different soil types is also an important factor. The pathway of As transfer from root and shoot to grain and the physiological process of stress were important influential factors on As absorption in rice which resulted in varietal variation (Ye and Yin 2012).

Rice cultivars with high porosities tended to possess higher rates of radial oxygen loss (ROL), and higher capacity for restricting the transfer of As to above ground tissues (Mei *et al.*, 2009). Geng *et al.* (2006) showed distinct varietal differences in the ability of rice to cope with physiological stress resulting from arsenic exposure. Grain arsenic must be derived from either direct xylem transport from root or remobilization of shoot As pools through the phloem during grain filling (Norton *et al.*, 2010). Ye and Yin (2012) reported that arsenic concentrations of polished rice grain were significantly affected by genotype and soil type in China. Bhattacharya *et al.* (2013) reported that the accumulation of arsenic in rice grain exceeded the WHO recommended permissible limit in rice (1.0 mg/kg) at the 20 mg/kg dry weight arsenic dosing in pot soil.

Arsenic phytotoxicity may inhibit arsenic accumulation in rice grain (Panaullah *et al.*, 2009). Because of the arsenic phytotoxicity soil pore water arsenic concentration was found to correlate with straw arsenic concentration, but not with grain arsenic concentration (Khan *et al.*, 2010). Brammer (2009) reported that soil analyses alone may not be sufficient to indicate the risk of high arsenic contents in rice grain. Stroud *et al.* (2011) reported the within field variation in rice grain arsenic concentration could not be satisfactorily explained by soil arsenic concentration. They found no relationship between grain arsenic concentrations and soil total arsenic in four paddy field site (Faridpur, Sonargaon, De Ganga and Nonaghata) in the Bengal delta.

Fu *et al.* (2011) reported rice grain arsenic rises steeply at soil arsenic concentrations lower than 3.6 mg/kg and gently at higher concentrations. Rahman & Hasegawa (2011) revealed that arsenic concentrations in rice grain from different countries, which provided the range of arsenic concentrations in raw rice worldwide from 0.11 to 2.05 mg/kg. They also reported arsenic concentrations in raw rice varied significantly with its origin, types and cultivars, and even with the growing seasons. Talukder *et al.* (2011) reported significant reduction of arsenic concentration in grain of Boro rice (BRRI dhan 29) in aerobic water management with P amended soil.

Panaullah *et al.* (2009) reported limited range of 0.3-0.6 mg/kg for the arsenic content of rice grown in paddies containing 10-70 mg/kg As in soil and level as high as 2500 µg/L As in soil water. In contrast, rice grains from the greenhouse study by Xu *et al.* (2008) collected from plants grown flooded pots, both unamended and amended with arsenic, contained much higher levels of arsenic in rice grains (1-2.5 mg/kg). Panaullah *et al.* (2009) found grain arsenic level up to 0.54 mg/kg at their lower soil arsenic level (11 mg/kg) which is in good agreement with current study. Adomako *et al.* (2009) at 10 tube-well sites in Gazipur district where groundwater has low arsenic concentrations, found grain arsenic levels up to 0.35 mg/kg at a minimum soil arsenic level of 2.4 mg/kg. In a field trial in Bangladesh with the rice variety BRRI dhan 29 grown under flooded soil conditions, Panaullah *et al.* (2009) found a negative correlation between grain arsenic and soil arsenic levels, which is in good agreement with the present experiment.

3.5 Conclusions

Residual arsenic in soil not exceeded the maximum acceptable limit (20 mg/kg) for agricultural soil. Rice growth and yield of BRRI dhan-50 were not significantly affected by soil residual arsenic which accumulated in previous season irrigation water arsenic. Chlorophyll contents of rice leaves were significantly affected by soil residual arsenic. Arsenic accumulation in straw and grain was also significantly affected by soil residual arsenic. The accumulation of arsenic in rice grain not exceeded the WHO recommended permissible limit in rice (1.0 mg/kg). Rice straw accumulated more arsenic than grain. Arsenic accumulation into straw and grain was followed a negative correlation with soil residual arsenic.

3.6 Recommendations

Rice cultivation in residual arsenic paddy field should be with rain water or surface water.

- Farmers of Bangladesh should be practiced rice cultivation in aerobic paddy field conditions.
- Flooded land should be selected for next dry season paddy field.

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Chapter- 4

Arsenic Accumulation into Different Varieties of Rice (*Oryza sativa* L) Cultivated with Arsenic Contaminated STW Water

Abstract

Rice was grown in an open-field Gangetic soil conditions with arsenic contaminated STW irrigation water in experimental plots at Mandal para village of Shahbajpur union under Shibganj upazila of Chapai Nawabganj district in Bangladesh to see the effect of arsenic (As) on rice (*Oryza sativa* L.) and to observe the effect of arsenic contaminated pump distance and paddy field elevation on arsenic accumulation into HYV Boro rice in dry period and local Aman rice in rainy season. A popular HYV Boro rice variety named BRRI dhan-36 and local rice variety named Somsa were cultivated with 0.5 mg/L arsenic contaminated STW ground water in an actual paddy field situation during Boro season. Distance of the experimental plots were 10, 50, 100, 200, 300 ft from the irrigation pump. Elevation of the experimental plots were 65.54 ± 0.09 , 65.38 ± 0.08 , 65.15 ± 0.13 , 64.95 ± 0.09 , 64.47 ± 0.14 ft from mean sea level. Local Aman rice variety named Mowka and Shorna were cultivated in the same experimental plots during Aman (rainy) season. Arsenic accumulation in drainage sediments, paddy field soil, rice straw and grain were decreased significantly ($p < 0.01$) with increasing of pump distance from paddy field during dry season for Boro cultivation period. Arsenic accumulation in paddy soil, rice straw and grain had significant ($p < 0.01$) negative correlation with paddy field elevation from mean sea level during Aman (rainy) season. Arsenic accumulation in rice straw during Boro and Aman season ranged from 2.58 ± 0.01 to 2.75 ± 0.02 mg/kg and 1.51 ± 0.04 to 1.76 ± 0.01 mg/kg, respectively. Arsenic accumulation in rice grain during Boro and Aman season ranged from 0.76 ± 0.02 to 0.97 ± 0.01 mg/kg and 0.10 ± 0.01 to 0.07 ± 0.01 mg/kg, respectively. Dry season rice varieties accumulated more arsenic than rainy season rice varieties. HYV rice varieties accumulated more arsenic than local rice. Highest arsenic accumulation in rice grain was 0.97 ± 0.01 mg/kg, but not exceeded the WHO recommended permissible limit 1 mg/kg.

4.1 Introduction

The problem of arsenic contamination in groundwater is not just restricted to Bangladesh. Other countries in S and SE Asia have also been reported to have high levels of arsenic in groundwater (Dahal *et al*, 2008; Nordstrom, 2002). It has been well recognized that consumption of arsenic contaminated foods leads to carcinogenesis (Mandal and Suzuki, 2002). Chronic effects of arsenic toxicity on humans have been reported from most of the countries in South (S) and South-East (SE) Asia through its widespread water and crop contamination (Kohnhorts, 2005; Mukherjee *et al*, 2006; Smedley, 2005). Over 50 million people living in the Ganga-Meghna-Bramhaputra plain are at risk through severe arsenic toxicity (Chakraborty *et al.*, 2004; Pal *et al.*, 2007).

Arsenic in groundwater and its fate and transport in the environment is a major concern in Bangladesh. It has been estimated that 35-77 million people are exposed to As-contaminated well water (Smith *et al.*, 2000; Rabbani *et al.*, 2002). Chakraborti *et al.* (2004) reported that about 43% of more than 50,000 hand tube-well water samples analyzed from all 64 districts of Bangladesh had As concentrations above 10 µg/L (the recommended minimum level of As in drinking water set by world health organization, WHO), and 27.5% have As concentration above 50 µg/L (the maximum permissible level of As in drinking water of Bangladesh). Based on these levels it has been estimated that between 200,000-270,000 people will die of cancer as a result of the ingestion of As-contaminated water in Bangladesh alone (WHO, 2001). Among the arsenic contaminated districts in Bangladesh, the severely affected Chapai Nawabganj district deserves special mention in terms of level of arsenic contamination and area coverage. In rural Bangladesh, farmers are generally not aware of the foods and Agricultural Organization (FAO) guideline value of arsenic in irrigation water (0.10 mg/L) (FAO,1985), and as the irrigation system in this areas is mostly dependant on groundwater, there is a high possibility of transfer of arsenic from contaminated irrigation water and soil to crops.

Arsenic contaminated groundwater is used not only for drinking purpose but also for crop irrigation, particularly for the paddy rice (*Oryza sativa* L.), in S and SE Asian countries (Meharg and Rahman, 2003; Ninno and Dorosh, 2001). The development of rice production in the dry winter season in Bangladesh, locally known as boor, using groundwater for irrigation coupled with green revolution high yielding rice varieties (HYV)

and use of chemical fertilizers played a key role in tripling the annual rice output in Bangladesh from 9 million tons in 1970-71 to more than 25 million tons by the mid -1990's (BBS, 2004), making Bangladesh self sufficient in this staple food grain (Baffes and Goutam, 1996). Arsenic- contaminated groundwater has been used extensively to irrigate paddy rice, particularly during the dry season, with 75% of the total cropped area given to rice cultivation in Bangladesh (Meharg and Rahman, 2003). Dry season irrigation with groundwater has enabled expansion of rice production, greatly improving food security and economic opportunity for farm households (Hossain *et al.*, 2003). A large number of shallow tube-wells (STWs) and deep tube-wells (DTWs) have been installed to irrigate about 4.3 million hectares of crop land which contributes to the food grain production of the country significantly (Rashid *et al.*, 2004). Groundwater provides the main household water supply for drinking, cooking and other household purposes in As-affected regions. Besides drinking contaminated water, the population in these areas is also exposed to As by consuming food grown or cooked using contaminated water. The large diameter tube wells that have been installed extensively in As-affected areas of Bangladesh provide agricultural irrigation. In most cases, testing has shown that these wells produce water that high concentration of As. So, it is probable that many tonnes of As are being delivered with groundwater to As-affected areas of Bangladesh and being absorbed by agricultural lands. The combination of high As concentrations in soils and the use of contaminated irrigation water is likely to result in elevated concentrations of As in agricultural crops grown in these areas (Abedin *et al.*, 2002b). In Bangladesh and other areas of southern Asia, once well water is brought to the surface for irrigation, it is commonly distributed through a network of channels and then used to fill rice fields (Hossain *et al.*, 2008; Roberts *et al.*, 2007). With a complete understanding of arsenic behavior within this context, it may be possible to design land-based arsenic removal schemes to reduce arsenic loading to field soils. Broadly, arsenic retention and transport depend on pH, arsenic speciation, redox conditions, mineralogy, and aqueous chemistry (Smedley and Kinniburgh, 2002). Arsenic concentration distributions in flowing water are determined by the extent of oxidation, adsorption, and precipitation reactions, relative to flow rates, particle settling capacities, and (re) release of arsenic from soils (Cadwalader *et al.*, 2011; Ciardell *et al.*, 2008; Dixit and Hering, 2003; Langner *et al.*, 2001; Saha *et al.*, 2006; Smedley and Kinniburgh, 2002). Redox cycling of iron largely impacts arsenic solubility through reductive dissolution of Fe (III) hydroxides, which may concomitantly release arsenic to solution and oxidative precipitation of

dissolved Fe(II) , which forms Fe(III) hydroxides that may scavenge arsenic from solution (Smedley and Kinniburgh, 2002). Phosphate (Darland and Inskeep, 1997), Silica acid (Waltham and Eick, 2002), Carbonate (Appelo *et al.*, 2002) and natural organic matter (Redman *et al.*, 2002) may each compete with arsenic for sorption and co-precipitation sites, limiting arsenic removal from flowing water and enhancing arsenic transport. As a result of these (and) other processes, the distribution of arsenic species along surface flow systems may be heterogeneous (Frau and Arda, 2003; Wilkie and Hering, 1998). In southern Asia, dissolved arsenic concentrations in irrigation water flowing through distribution channels generally decrease with distance from wells (Hossain *et al.*, 2008; Roberts *et al.*, 2007), but arsenic may be further transported in suspended colloids (Robert *et al.*, 2007). Across fields, where irrigation water flows much more slowly than in channels, the extent of arsenic transport has been inferred by decreases in arsenic concentration in rice field soil. (Dittmar *et al.*, 2007; Hossain *et al.*, 2008; Norra *et al.*, 2005; Stroud *et al.*, 2011a) and standing irrigation water (Roberts *et al.*, 2007; Stroud *et al.*, 2011a). Overall, rice fields tend to be a sink of arsenic may potentially be lost from fields due to irrigation-water infiltration along field boundaries (Neumann *et al.*, 2009), pore water cycling (Robert *et al.*, 2011), desorption from soils (Saha *et al.*, 2006), monsoonal flood water retreat (Roberts *et al.*, 2010; Saha and Ali, 2007), and uptake into rice (Stroud *et al.*, 2011b). Long term irrigation with arsenic contaminated groundwater is likely to increase its concentration in crops (Ullah, 1998; Huq *et al.*, 2003).

Survey on paddy soil throughout Bangladesh showed that arsenic concentration were higher in agricultural soils of these areas where shallow tube-wells (STWs) have been in operation for longer period of time and arsenic contaminated underground water from those STWs have been irrigated to the crop fields (Meharg and Rahman, 2003). The agricultural soil of arsenic non contaminated areas of Bangladesh contain 4.0 to 8.0 mg of As/kg while that irrigated with arsenic contaminated groundwater, contain up to 83 mg of As/kg (Ullah, 1998 Alam and Sattar, 2000; Williams *et al.*, 2006). But, the maximum acceptable concentration of arsenic in agricultural soil is 20 mg/kg (Kabata-Pendias and Pendias, 1992). Arsenate is the dominant As species in aerobic soils, whereas arsenite dominates under anaerobic conditions such as paddy soil (Masscheleyn *et al.*, 1991; Marin *et al.*, 1993; Onken and Hossner, 1995, 1996; Smith *et al.*, 1998). Arsenate is an analogue of phosphate, competing for the uptake carriers in the root plasmalemma

(Meharg and Macnair, 1992; Meharg and Hartley- Whitaker, 2002). For paddy rice, one of the most common aquatic crop plants, iron plaque is commonly formed on root surface and may subsequently affect As dynamics in the rhizosphere and As accumulation by rice plants (Liu *et al.*, 2004 a, 2004b).

The formation of iron plaque on rice roots is thought to be facilitated by the release of oxygen and oxidants into the rhizosphere (Armstrong, 1964, 1967, Chen *et al.*, 1980, Taylor and Crowder, 1983, Taylor *et al.*, 1984). Iron oxides are generally considered to have great adsorption capacity for inorganic anions, especially for arsenate and phosphate, and possible capacity to oxidize arsenate (Meng *et al.*, 2002). Due to the ubiquity of iron plaque on rice roots and the potential As sequestration in iron plaque, Chen *et al.* (2005) investigated the direct role of iron plaque in As (both arsenite and arsenate) uptake into rice roots. Their results demonstrated that the presence of iron plaque enhanced arsenite and decreased arsenate uptake. Fe-oxide minerals strongly impact As dynamics in flooded rice culture (Takahashi *et al.*, 2004, Leoppert *et al.*, 2005), primarily because of their impact on As solubility, retention and release. The reduced conditions of flooded rice culture are conducive to the biological reduction of Fe^{3+} to Fe^{2+} , the dissolution of soil Fe oxides, and the increased solubility of soil Fe, predominantly as Fe^{2+} . However the rice root surface is much more highly oxidized than the surrounding bulk soil, due to radial loss of O_2 from aerenchyma structures in the plant root (Colmer, 2003). As a result, Fe-oxide plaque are formed on the roots of plant in aquatic environments, including rice, resulting from the oxidation of Fe^{2+} to Fe^{3+} and the precipitation of Fe^{III} oxides on the root surface (Chen *et al.*, 1980; Taylor *et al.*, 1984; Meharg, 2004). Thus rice plants are able to modify their environment by the formation of an Fe-oxide plaque at the rice-root surface, even in highly reduced soils (Meng *et al.*, 2002; Liu *et al.*, 2005; Chen *et al.*, 2005). Iron oxides have strong binding affinities for arsenate (As^{V}) and arsenite (As^{III}) (Raven *et al.*, 1998). The Fe-oxide plaque serves as a buffer against As solubility because of its strong adsorption of As but also as a buffer that favors a high concentration of potentially available As in the immediate vicinity of the root (Zhang *et al.*, 1998; Liu *et al.*, 2004a,b). Thus depending on specific localized conditions, Fe-oxide plaque, can serve as either a source or sink for As. Hossain *et al.* (2009) demonstrated that the application of ferrous iron to pot-culture rice plants could minimize the As uptake and increase grain yields, due to the formation of iron plaque in

rhizosphere that immobilized soluble arsenic (As). Paddy rice oxygenates its rhizosphere, resulting in the formation of an iron (Fe) oxyhydroxide plaque (Armstrong, 1964). Fe plaque is composed dominantly of ferrihydrite goethite and siderite (Hansel *et al.*, 2001). The Fe hydroxide in soil and solution have a very strong binding affinity for arsenate (Meng *et al.*, 2002; Liu *et al.*, 2004a, b), and a possible capacity to oxidize arsenite to arsenate. Iron plaque may be a barrier or a buffer to uptake of As (Liu *et al.*, 2004a, b). The effect of Fe plaque on plant uptake of contaminants or nutrients may depend on the amount of Fe plaque on the root surface (1989; Zhang *et al.*, 1998). In rice plants, Fe plaque can be formed both under natural and laboratory conditions (Chen *et al.*, 1980; Greipsson and Crowder, 1992; Greipsson, 1994, 1995). It may be important for the development of practical approaches to reducing As accumulation in rice. The formation of Fe plaque on rice roots can be affected by a number of factors, such as soil properties (Li, 1992), water management (Lu *et al.*, 1999), iron, manganese fertilization (Shi *et al.*, 2004; Zeng *et al.*, 2001) and rice genotypes (Liu *et al.*, 2001; Zhang *et al.*, 2002).

The transfer of As from irrigation water to rice grains involves several steps. The first step, input of As with irrigation water, is difficult to quantify because of a drastic decline of As concentrations in standing water across flooded fields from initial levels as high as 800 µg/L at the irrigation well (Norra *et al.*, 2005; Dittmar *et al.*, 2007; Panaullah *et al.*, 2008). The loss is caused by co-precipitation and/or adsorption of As onto flocks of Fe oxyhydroxide formed upon oxidation of dissolved Fe (II) contained in irrigation water (Robert *et al.*, 2007). The consequence is that As concentrations in surficial soil can be as high as 70 mg/kg near the entry point of irrigation water to a rice field then decline to essentially background levels <10 mg/kg As over a distance of a few hundred meter (Dittmar *et al.*, 2007; Panaullah *et al.*, 2008). Arsenic mobilized from the soil during the monsoon contributes to the highly scattered relationship between As levels in irrigation and surficial soil (Ali *et al.*, 2003; Meharg and Rahman, 2003; Dttmar *et al.*, 2007; Saha and Ali, 2007; Robert *et al.*, 2010).

An important reason for the relatively high As accumulation by rice lies in the soil chemistry of paddy fields; the anaerobic conditions of submerged paddy soil leads to reductive dissolution of iron oxide/hydroxides and release of the adsorbed arsenate, which is mobilized as arsenite in the soil pore water (Marin *et al.*, 1993; Panaullah *et al.*, 2009;

Takahashi *et al.*, 2004; Xu *et al.*, 2008). Therefore, As bioavailability is greatly enhanced in submerged paddy soils compared with aerobic soil. Indeed, when rice was grown under aerobic soil conditions, As accumulation in shoot and grain decreased markedly (Li *et al.*, 2009; Xu *et al.*, 2008).

As accumulation in irrigated paddy soil and its transfer into rice can vary depending on soil type, crop, back-ground As concentration, As concentration of irrigation water, distance from the pump source, and depth and duration of flooding (Meharg and Rahman, 2003; Norra *et al.*, 2005; Hossain *et al.*, 2008).

Arsenic transport via surface and overland flow is a potentially significant pathway for increasing arsenic contamination in the environment. Arsenic is believed to be relatively immobile in surface water, due to its low solubility under oxic conditions (Smedley and Kinniburgh, 2002). Yet it is frequently found as a contaminant in variety of flowing surface-water settings. Across the world, above-ground transport of arsenic is threatening water quality and plant health by redistributing arsenic from sources such as irrigation water, animal wastes, pesticides, mine water and geothermal waters (Brown *et al.*, 2007; Church *et al.*, 2010; Pichler *et al.*, 2008; Roberts *et al.*, 2007; Wilkie and Hering, 1998).

In a rice-rice cropping system, irrigation water is applied during Boro rice (dry season rice), and the following Aman rice (rainy season rice) is grown with natural rainfall. Irrigation with As-contaminated ground water directly affects the immediate Boro rice (Abedin, cotter- Howells, and Meharg, 2002), and the residual As in soil may affect the Aman rice (Duxbury *et al.*, 2003).

The accumulation of As in food crops especially rice and vegetable grown in areas of Bangladesh irrigated with As-contaminated groundwater is now well documented (Alam *et al.*, 2003; Farid *et al.*, 2003; Mitra *et al.*, 2004; Williams *et al.* 2006; Smit *et al.*, 2006; Huq *et al.*, 2006; Kurosawa *et al.*, 2008; Karim *et al.*, 2008; Khan *et al.*, 2010).

Extensive use of arsenic contaminated groundwater for irrigation during the dry season threatens these benefits. Following years of irrigation with groundwater, soil arsenic concentrations have risen, and arsenic is now transferring into rice at concentrations sufficient to decreased yields and create dangerous level of arsenic in rice grains (Abedin *et al.*, 2002; Brammer and Revencroft, 2009; Stroud *et al.*, 2011a; Williams *et al.*, 2006).

Bangladesh is one of the major rice growing countries and rice is the staple food crop of the country. It is estimated that 83% of the total irrigated areas of this country are used for rice cultivation (Dey *et al.*, 1996). To acquire self-sufficiency in rice production, the high yielding varieties (HYV) of rice have been cultivated widely in the country throughout the year. The rice cultivation is solely depended on underground water, particularly in the Boro (dry) season since the sources of surface water like river, dam, pond etc. becomes dry in this season. Boro rice accounts for about 55% of the total rice production in Bangladesh, and the irrigation water needed for its cultivation is mainly extracted from shallow tube wells (STWs) (MoA, 2005). Many STWs deliver As concentrations above 50 mg/L, with an estimated 35 million people living in these affected region and 57 million people exposed to As concentrations exceeding 10 µg/L. About 86% of the total groundwater withdrawn is used for irrigating dry season crops, mainly Boro rice. Irrigation-water borne As from contaminated STWs accumulates in the soil (Panaullah *et al.*, 2003; Islam *et al.*, 2007). Irrigation is principally performed by a large number of shallow tube-wells (STWs) and deep tube-wells (DTWS). The water of shallow tube-wells (STWs) contained very high level of arsenic (Nickson *et al.*, 2000; Mac Lellan, 2002; van Geen *et al.*, 2003; Alam *et al.*, 2002).

Increasing levels of arsenic in agricultural soils from contaminated underground irrigation water, and its uptake in rice, vegetables and other crops (Meharg and Rahman, 2003; Williams *et al.*, 2006) have become a real health emergency in this region. The presence of high levels of arsenic in rice is supposed to be a health disaster in South and South-East Asia is supposed to be exposed to arsenic contamination from water and foods (Sun *et al.*, 2006).

A large population in Asia arsenic endemic areas lives on subsistence diet of rice, a cereal which is grown mainly with groundwater contaminated by high level of arsenic. Therefore, rice contains relatively higher amount of arsenic, most of which is inorganic (Meharg *et al.*, 2009; Sun *et al.*, 2008; Torres-Escribano *et al.*, 2008), compared to other agricultural products (Das *et al.*, 2004; Schoof *et al.*, 1999).

The concentration of arsenic depending on rice variety (Booth, 2008) and geographical variation (Booth, 2007; Meharg *et al.*, 2009).

The inorganic arsenic species dominates over organic arsenic species in both raw and cooked rice (Williams *et al*, 2005), which is accumulated/absorbed from paddy soil, irrigation water, and cooking water. Therefore, arsenic speciation in rice grain is influenced by its speciation in soil and water. In addition, the amount of arsenic absorbed by the cooked rice from cooking water and, the dietary intake of arsenic in human body are depended on the type of rice and the way the rice is cooked. (Musaiger and D' Souza, 2008; Ohno *et al*, 2009; Rahman *et al*, 2006; Signes *et al.*, 2008a; Signes *et al.*, 2008b). Considering the high concentration of arsenic (mainly inorganic arsenic) in rice grain, cooking method and high consumption rate, rice is revealed to be a major threat to health of the people of arsenic endemic S and SE Asian countries, including Bangladesh (Rahman and Hasegawa, 2011).

Concentration of As in rice range from 160 to 580 $\mu\text{g/kg}$ from the Samta village of Jessore district (Alam *et al.*, 2002). Meharg and Rahman (2003) reported As levels in excess of 1830 $\mu\text{g/kg}$ in rice from districts in northern Bangladesh. Duxbury *et al.*, (2003) reported that in mean As concentration for Boro (winter season) rice (183 $\mu\text{g/kg}$) was 1.5 times higher than Aman (monsoon season) rice (117 $\mu\text{g/kg}$), based on 150 paddy rice samples from different districts (Barisal, Comilla, Dinajpur, Rangpur, and Rajshahi) of Bangladesh. The mean As level in rice grain was reported as 136 $\mu\text{g/kg}$ (range 40-270 $\mu\text{g/kg}$) from Chandpur and Jamalpur districts of Bangladesh (Das *et al.*, 2004). Williams *et al.* (2005) reported that the mean As concentration in 15 Bangladeshi rice samples collected from the wholesale market was 130 $\mu\text{g/kg}$. Based on the investigation on 330 Boro and Aman rice samples of Bangladesh, Williams *et al.* (2006) found that the highest mean As levels for Aman rice were 360, 240 and 220 $\mu\text{g/kg}$ from Satkhira, Chuadanga, Brahmanbaria, and Chandpur districts, respectively, and for Boro rice, the values were 510, 380 and 320 $\mu\text{g/kg}$, for Faridpur, Satkhira, and Chuadanga districts respectively.

Contamination of rice by As has several sources: pollution of paddy soils due to base and precious metal mining (Liao *et al.*, 2005; Zhu *et al.*, 2008), irrigation of paddies with arsenic-contaminated groundwater (Meharg and Rahman, 2003; Williams *et al.*, 2006) and the use of organoarsenical pesticides (Williams, 2007). Arsenic levels in rice grain are problematic even where soil As is at background levels.

Furthermore, irrigation with arsenic contaminated water can lead to a gradual increase in grain As concentration (Lu *et al.*, 2009).

Rice, unlike other cereals, is cultivated in flooded soils, where anaerobic conditions together with excessive water lead to the mobilization of As and, consequently, As elevated accumulation in the plant (Xu *et al.*, 2008). Arsenite, the most toxic form of arsenic, has high water solubility and soil mobility and is thus efficiently absorbed by rice roots, reaching the grains (Ma *et al.*, 2008).

Rahman *et al.* (2007b) evaluated the effects of soil arsenic concentrations on chlorophyll contents, growth and yield of five popular and widely cultivated Boro rice (*Oryza sativa* L.) varieties in Bangladesh. They reported that arsenic toxicity affects the photosynthesis which ultimately results in the reduction of rice growth and yield.

Large variations in grain As concentration of Bangladeshi rice varieties sampled from rice fields have been observed (Duxbury *et al.*, 2003; Zavala and Duxbury, 2008). Market basket surveys also revealed similar variability (Williams *et al.*, 2005, 2006). Some of this variability has been explained by differences in groundwater irrigation levels of As (Williams *et al.* 2005) and baseline soil As concentrations (Lu *et al.*, 2009). Norton *et al.* (2009b) indicated that variation in rice grain As concentration in Bangladesh was largely controlled by rice genetics.

Rice grain can accumulate relatively large amounts of As even from soil not contaminated by As (Daum *et al.*, 2001). Soil factors that have been reported to regulate As bio-availability to plants include: pH and redox status (Marin *et al.*, 1993), clay content (Sheppard, 1992), existence of poorly crystalline iron oxide (Bogdan and schenk, 2009; Takahashi *et al.*, 2004). These soil properties vary both at both local and regional scales in rice growing areas of Bangladesh (Brammer, 2009).

Ahmed *et al.* (2010) identified rice varieties with low grain As concentration suited to specific or broad growing environments in Bangladesh. Abedin *et al.* (2002) evaluated the effect of different concentrations of arsenite and arsenate on seed germination and early seedling growth of eight rice varieties. They reported that dry season varieties had more tolerance to arsenite or arsenate than the wet season varieties. The accumulation of arsenic in rice is viewed as a newly recognized disaster for South-East Asia, where rice is a staple food (Meharg, 2004). Abedin *et al.* (2002a) observed the accumulation of arsenic up to 92.0 mg/kg in rice straw through a green house pot experiment.

Meharg and Rahman (2003) have reported accumulation of 1.7 mg/kg of arsenic in three rice samples from Bangladesh. Alam and Rahman (2003) on the basis of their study in 21 field sites observed that the accumulation of arsenic in rice plant varied with different varieties of rice. Rice grain has been reported to accumulate arsenic up to 2.0 mg/kg by Islam *et al.* (2004) in their study in Gangetic flood plains of Bangladesh. Williams *et al.* (2006) in their study found that the predominant species of arsenic in rice was arsenite (As^{III}) followed by arsenate (As^{V}) with dimethyl arsenic acid (DMA^{V}) being a minor component. Rahman *et al.* (2007b) reported that regardless of rice varieties, accumulation of arsenic was 28 and 75 folds higher in root than that of shoot and raw rice grain, respectively.

The accumulation of high levels of arsenic in the rice straw is a potential threat to cattle that consume the contaminated straw and this indirectly to human health, through presumably contaminated bovine meat and milk (Abedin *et al.*, 2002a, Rahman *et al.*, 2008a).

Abedin *et al.* (2002) and Meharg and Rahman (2003) have reported about the rice samples with arsenic accumulation much above the WHO recommended permissible level of 1.0 mg/kg. Higher amount of arsenic was reported to accumulate in the root of the rice plant as compared with other parts (Norra *et al.*, 2005; Rahman *et al.*, 2007a; Bhattacharya *et al.*, 2009).

It has been established that rice accumulates high concentration of arsenic in its grain compared to other cereal crops (Williams *et al.*, 2007). The arsenic in rice grain is present primarily as inorganic arsenic (arsenite and arsenate) and dimethylarsenic acid (DMA) (Williams *et al.*, 2005; Meharg *et al.*, 2008; Norton *et al.*, 2009a,b). It has been proposed that rice accumulates higher concentrations of arsenic due to its cultivation in anaerobic condition, where arsenic is more available (Xu *et al.*, 2008). Not only the accumulation of arsenic in rice grains a major concern, but rice growing in arsenic contaminated environments can have reduced yields (Panaullah *et al.*, 2009).

Widespread use of arsenic contaminated ground water for irrigation in rice field could elevate its concentration in surface soil and eventually into rice plant and rice grain (Abedin *et al.*, 2002; Rahman *et al.*, 2007 a, b). Arsenic uptake and accumulation in rice plant from irrigation water and contaminated soil might depend on cultivars (Xie and Huang, 1998; Meharg and Rahman, 2003). The availability of arsenic to the rice plant

might also be subjected to the geographical location, soil properties, redox condition and cropping season (Meharg and Rahman, 2003).

The As content of lowland or paddy-rice grain is generally much higher than that of upland cereal crops (Schoof *et al.*, 1999; Williams *et al.*, 2007) because of the relatively high availability of soil As under reduced conditions. A global normal range of 0.80 to 0.2 mg As/kg has been suggested for rice (Zavala and Duxbury, 2008), but values as high as 1.8 mg As/kg have been found in Bangladesh rice (Meharg and Rahman, 2003).

In greenhouse pot experiments with higher concentration of arsenic in soil, different rice varieties have showed significant differences in the accumulation of arsenic in straw, husk and grain parts (Rahman *et al.*, 2007b; Alam *et al.*, 2003). Analyzing the two widely cultivated rice varieties in Bangladesh, Rahman *et al.* (2007b) reported that the BRRI dhan 28 and BRRI hybrid dhan 1 had difference in the amount of arsenic accumulation (0.5 ± 0.0 and 0.6 ± 0.2 mg/kg dry weight of arsenic, respectively). Rahman *et al.* (2008b) by studying five different hybrid as well as non hybrid rice samples concluded that the arsenic translocation from root to shoot (straw) and husk was higher in the hybrid variety BRRI dhan 1 as compared to those of non-hybrid varieties (BRRI dhan 28, BRRI dhan 29, BRRI dhan 35 and BRRI dhan 36). Presence of stable genetic difference in arsenic by rice plant was suggested by Norton *et al.* (2009a). They also identified a number of local cultivars with low arsenic accumulation in grain. Azad *et al.*, (2009) observed an increase in the grain arsenic uptake of transplanted Aman rice with the increase of arsenic treatment in soil and found that 30-35 mg/kg arsenic containing soil produced rice grain with arsenic levels exceeding the WHO recommended permissible limit of 1.0 mg/kg.

Rice growing in the anaerobic situation was found to score the highest amount of As among all grain crops (Marin *et al.*, 1993). Boro is exposed to As caused by both soil and irrigation water, whereas the Aman rice is exposed to As through the natural soil As in addition to the buildup of As over time due to the use of contaminated irrigation water (Duxbury and Panaullah, 2007). Duxbury and Panaullah also assessed that As accumulation in soil could be increased at the rate of 1 mg/kg crop⁻¹ through the use of 1.5 m of irrigation water containing 0.13 mg As/L, resulting in no net loss of As from the soil environment, it has a strong residual effects on following crops (Khan *et al.*, 2010). Dittmar *et al.*, (2010) also found that annually there was an estimated 4.4 ± 0.4 kg ha⁻¹a⁻¹

As deposited through irrigation water. In the top 40 cm soil, the mean As accumulation over three years were recorded to $2.4 \pm 0.4 \text{ kg ha}^{-1}\text{a}^{-1}$, implying that there was an average loss of As is 2.0 kg ha^{-1} .

In the arsenic affected areas of Bangladesh, majority of the residents depends on rice for their caloric intake (about 70% of total) suggesting that rice is an important dietary source of arsenic for Bangladesh population (Bae *et al.*, 2002). The average daily consumption of rice by a Bangladeshi male adult is high 453 gm milled rice per person per day (Bangladesh Agricultural Research Council, unpublished food and nutrition data, 2007), providing more than 70% of daily calorie intake (Ninno and Dorosh, 2001). Thus, contaminated rice may represent a significant pathway of As consumption by humans, in addition to drinking water (Duxbury *et al.*, 2003; Meharg and Rahman, 2003).

Due to the large volumes of irrigation water required, as wells the cost of highly technical treatment options, there are currently no practical methods for large-scale removal of arsenic from these systems. (Brammer, 2009; Brammer and Ravenscroft, 2009). Whereas numerous studies have investigated technology based arsenic removal from drinking water (e.g. Garelick *et al.*, 2005) and streams (e.g. Vaclavikova *et al.*, 2008), there has been less work investigating options that maximize arsenic removal from water flowing across soils - a task needed to prevent current and impending environmental and food security risks (Brammer, 2009; Duxbury *et al.*, 2003; Stone, 2008).

This study would help to recognize the rice varieties which are resistant to arsenic phytotoxicity that would have significant impact on agriculture, environment and public health. This study would also help to evaluate the severity of human health risk from arsenic toxicity through water-soil-plant system.

However, to date, most of the published reports focused mainly on the uptake and accumulation of arsenic in the rice plants irrigated of arsenic contaminated water and soil through green house pot experiment (Abedin *et al.*, 2002a, b; Rahman *et al.*, 2004, 2008b). The information on actual scenario of the accumulation of arsenic grown in the agricultural fields is quite inadequate. Little research has so far been conducted on methods for reducing impact of irrigation with As contaminated ground water on rice production and accumulation of As in grain and straw. There has been only limited

investigation of spatiotemporal distributions of arsenic in flowing irrigation water in Bangladesh. Limited literatures are available about arsenic accumulation in different rice varieties.

Therefore, a detailed information is needed for the conclusive assessment on arsenic availability in soil and its accumulation into rice and to find out the rice varieties which are resistance to the arsenic phytotoxicity. In particular, the effect of As contaminated pump distance from paddy field and elevation of paddy field from mean sea level is needed to be investigated.

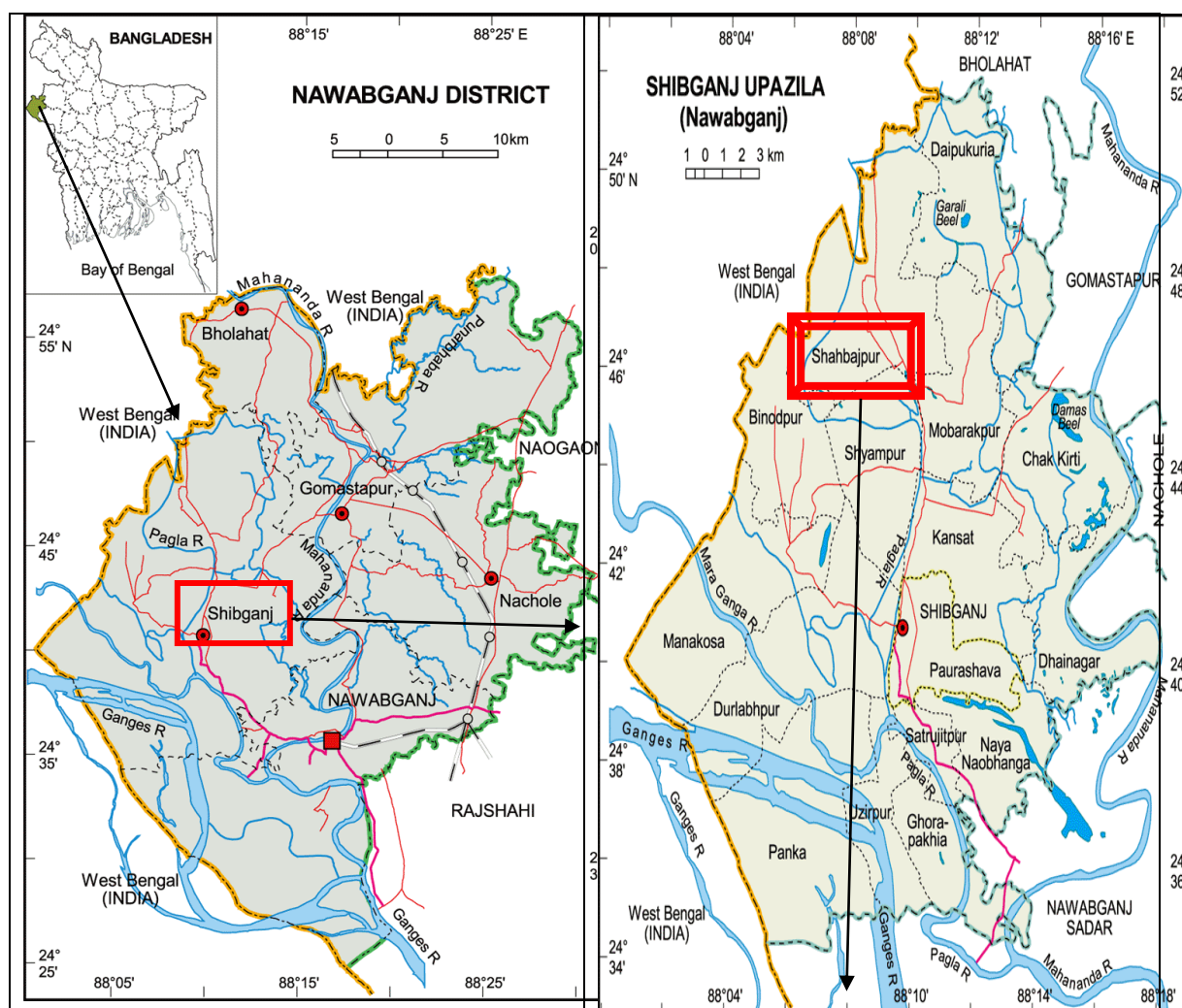
Thus, the present study was carried out to find the fate of arsenic contaminated irrigation water in an actual field situation considering the following specific objectives:

- i) to determine the effects of As contaminated irrigation water on HYV Boro rice and their residual effects on the following Aman rice
- ii) to evaluate the effect of pump distance on arsenic accumulation into drainage sediment, paddy soil, rice straw and grain
- iii) to investigate the effect the paddy field elevation on arsenic accumulation into paddy soil, rice straw and grain
- iv) to determine the seasonal effect on arsenic accumulation into different varieties of rice plants.

4.2 Materials and Methods

4.2.1 Experimental Site

This experiment was conducted in an actual paddy field at Mandal para village of Shahbajpur union parishad under Shibganj Upazila, Chapai Nawabganj District in Rajshahi Division of north-western Bangladesh.



Paddy Plots at Different Distances from Arsenic Contaminated STW Irrigation Pump

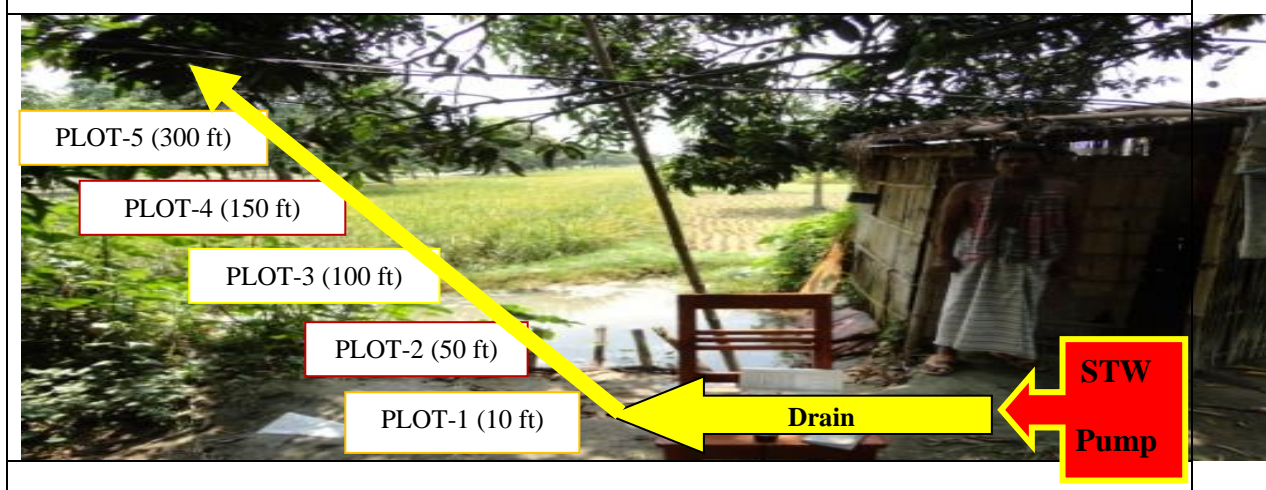


Plate 4.1 Experimental Plots at Shahbajpur Union under Shibganj Upazila of Chapai Nawabganj District in Bangladesh

4.2.1.1 Geographical Location of the Experimental Plots

The experimental plots were situated within 24°47'15.9" to 24°47'16.2" N latitude and 088°06'52.7" to 088°06'54.9" E longitudes.

The irrigation pump and experimental plots location and elevation are given in the **Table 4.1** below

Table 4.1 Irrigation Pump and Experimental Plots Location and Elevation

Irrigation Pump		Experimental Plots		
Geographical Location	Depth of pump (ft)	Distance from the Pump (ft)	Geographical Location	Elevation from mean sea level (ft)
24°47'16.00" N and 088°06'54.9" E	95	10	24°47'15.9"-24°47'16.2" N and 088°06'54.7"-088°06'54.9" E	65.54 ± 0.09
		50	24°47'15.9"-24°47'16.2" N and 088°06'54.2"-088°06'54.4" E	65.38 ± 0.08
		100	24°47'15.9"-24°47'16.2" N and 088°06'53.7"-088°06'53.9" E	65.15 ± 0.13
		150	24°47'15.9"-24°47'16.2" N and 088°06'53.2"-088°06'53.4" E	64.95 ± 0.09
		300	24°47'15.9"-24°47'16.2" N and 088°06'52.7"-088°06'52.9" E	64.47 ± 0.14

4.2.1.2 Topography of the Study Area

Chapai Nawabganj district has an area of 170.55 km² (BBS, 2011). Many rivers flow over this area. The main rivers are the Ganges and Mahanda. Most of the lands of this area are plain land with many small ponds and water reservoirs. But, recently, the geography has changed due to the corrosion and erosion by the river Padma (**Ganges**). Overload of river sediment caused by **Farrakka** barrage eroded the river banks and create a large area of land full of sand which almost looks like small dessert in this area. Based on formation of soil, Chapai Nawabganj can be divided into two different parts 1. Barendra area and 2. Diyar area.

4.2.1.2.1 Barendra Area

The east part of the river Mohananda is known as Barendra area. Barendra area is one of the oldest well known areas not only in Bangladesh but also all over the subcontinent. Barendra area was formed during the formation of the triangle of Bengal. Chapai Nawabganj sadar, part of Gomostapur upozilla and Nachol constitutes the Barendra area. The main crop of this area is the rice.

4.2.1.2.2 Diyar Area

The region in the river Padma basin is known as diyar. The area formed for the continuous change of path of the river Padma. The soils of this area are very fertile and people can cultivate multiple times a year of different types of crops. Main crops are rice, wheat, melons - most famous corn is Kalai.

4.2.1.3 Rivers and Waters of the Study Area

4.2.1.3.1 Padma River

The Ganges river originated from Himalaya and flows through India. This river then entered into Bangladesh at Shibganj and takes the name Padma. The Farakkha barrage was build just before it entered into Bangladesh which decreased the water level to Padma. But still in rainy season, this becomes very dangerous as the water level grows high and floods the area nearby.

4.2.1.3.2 Mahananda River

Mahananda River entered to this district through Bholahat upozilla and flows through the district and finally falls to the river Padma at Godagari, Rajshahi district. Nawabganj town is situated on the bank of this river and the economy of this district is driven by this river too.

4.2.1.3.3 Pagla

The Pagla River also flows from India and enters into Bangladesh in this district at Tattipur, Moraganga (dead Ganges) (Taru, 2012). After flowing a few miles, it mixes with the river Mahananda.

4.2.1.3.4 Punarbhaba River

The Punarbhaba River flows through Dinajpur and Naogoan of Bangladesh and then enters into Chapai Nawabganj.

4.2.1.4 Climate of the Study Area

Chapai Nawabganj is very close to the big city Rajshahi and the climates of both districts are very close. Under Köppen climate classification, Rajshahi has a tropical wet and dry climate. The climate of Rajshahi is generally marked with monsoons, high temperature,

considerable humidity and moderate rainfall. The hot season commences early in March and continues till the middle of July. The maximum mean temperature observed is about during the months of April, May, June and July and the minimum temperature recorded in January is about 7 to 16 °C (45 to 61 °F). The highest rainfall is observed during the months of monsoon. The annual rainfall in the district is about 1,448 millimeters (57.0 inch) (www.wikipedia, weather report).

4.2.2 Selection of Irrigation Pump

On the basis of national screening program report of DPHE, Bangladesh STWs were tested at field for selecting highest arsenic contaminated irrigation pump.

4.2.2.1 Screening Period

STWs were tested on January to April, 2012.

4.2.2.2 Arsenic Test Method

Analytical test strips method was applied for arsenic test at field.

4.2.2.3 Arsenic Test Procedure

Zinc and sulfuric acid were added to water. Then the concentration of arsenic was measured by visual comparison with mercury (II) bromide analytical test strip.

4.2.3 Soil Condition

The experiment was conducted in Gangetic soil condition. The properties of soil are given in **Table 4.2** and **Table 4.3**.

Table 4.2 Soil Conditions of Boro Paddy Field

Soil parameters	Values
Total Nitrogen	$0.06 \pm 0.02\%$
Available P	18.12 ± 0.04 ppm
Available K	0.25 ± 0.03 mol/kg
Available S	22.4 ± 0.05 ppm
Available Z	0.4 ± 0.04 ppm
p ^H	7.92 ± 0.03
Organic matter	1.23 ± 0.05
Background total arsenic (As).	9.40 ± 0.05 ppm

Table 4.3 Soil Conditions of Aman Paddy Field

Soil parameters	Values
Total Nitrogen	$0.06 \pm 0.02\%$
Available P	28.32 ± 0.04 ppm
Available K	0.17 ± 0.03 mol/kg
Available S	15.77 ± 0.05 ppm
Available Z	0.81 ± 0.04 ppm
p ^H	8.2 ± 0.03
Organic matter	1.26 ± 0.05
Background total arsenic (As).	19.40 ± 0.05 ppm

4.2.4 Rice Variety

4.2.4.1 Boro Rice Variety

BRRI dhan-36 and local rice variety Somsa were cultivated during Boro season for this experiment.

4.2.4.2 Aman Rice Variety

Local rice variety Mowka and Shorna were cultivated during Aman season for this experiment.

4.2.5 Seedling Transplantation

Boro and Aman rice seedlings were transplanted on 03 February, 2012 and 25 July, 2012 respectively. Thirty-five (35) days old seedlings were uprooted carefully from the seedbed in the morning and four seedlings for each hill were transplanted on the same day in the experimental field. The seedlings which died within 6 days of transplantation were discarded and new seedlings were replaced.

4.2.6 Intercultural Application

4.2.6.1 Fertilizer Application

To support the plant growth, urea, triple super phosphate (TSP), murate of potash (MP) and gypsum fertilizer were applied for nitrogen, phosphorus, potassium, and sulfur, respectively. The first split (one third of the dose) of urea and full doses of all other fertilizers were incorporated into the soil by hand before two days of seedling transplantation. The second and third splits of urea were applied after 30 (maximum tillering stage) and 70 (panicle initiation stage) days of transplantation, respectively.

4.2.6.2 Arsenic Source

Arsenic contaminated STW ground water was the arsenic source in this experiment. The selected irrigation pump water arsenic concentration was 0.5 mg/L.

4.2.6.3 Irrigation

Same shallow tube-well (STW) was used for irrigation during Boro and Aman season irrigation in this experiment. After transplantation of rice seedlings, 3-4 cm water above soil level was maintained throughout the growth period. Irrigation was stopped before 10 days of harvest.

4.2.7 Samples Collection and Preservation

4.2.7.1 Soil Samples Collection and Preservation

Soil samples were collected from 0 – 15 cm depth in 15 cm² area by composite sampling from the fields irrigated with arsenic contaminated water and transferred to airtight polyethylene bags. The samples were immediately air dried at room temperature after collection. Finally, the samples were dried in the Hot Air Oven at 60°C for 72 h and were stored in airtight polyethylene bags at room temperature with proper labeling.



Plate 4.2 Sample Collection and Preservation

4.2.7.2 Rice Plant Samples Collection and Preservation

The rice plants were cut at 4 cm above the soil. Boro and Aman season rice grain were harvested at their maturity stage (120 days after transplantation) on 16 May, 2012 and 27 November, 2012 respectively. Then the collected samples (straw and grain) from each season were tagged properly and sun dried for 3 days and then keeping the samples on a table.

4.2.8 Total Arsenic Measurement Methods

Soil, rice straw and grain samples were digested separately following the heating block digestion procedure (Rahman *et al.*, 2007c). Rice straw and grain samples were digested by $\text{HNO}_3\text{-HClO}_4$ and soil samples by $\text{HNO}_3\text{-H}_2\text{SO}_4\text{-HClO}_4$ for measuring arsenic concentrations in hydride generation atomic absorption spectrophotometer.

4.2.8.1 Sample Digestion

- i) At first, the oven dried samples were ground and passed through 2.0 mm pore sized sieve to get homogenized representative powder sample.
- ii) Then about 0.5 g of the sample was taken into clean dry digestion tubes and 5 ml of concentrated nitric acid (HNO_3) was added to it. The mixture was allowed to stand over night under fume hood.
- iii) In the following day, the digestion tubes were placed on a heating block and heated at 60°C for 2 h.
- iv) The tubes were then allowed to cool at room temperature.
- v) Then about 2 ml of concentrated perchloric acid (HClO_4) was added to the plant samples.
- vi) For the soil samples 3 ml of concentrated sulfuric acid (H_2SO_4) was added in addition to 2 ml of concentrated perchloric acid (HClO_4).
- vii) Then the tubes were heated at 160°C for about 4–5 h.
- viii) The heating was stopped when the dense white fume of perchloric acid (HClO_4) was emitted.
- ix) The content was then cooled, diluted to 25 ml with de-ionized water.
- x) Filtered through Whatman No. 42 filter papers for soil samples and Whatman No. 41 for plant samples and finally stored in polyethylene bottles.

All glassware and plastic bottles were previously washed with 2% HNO_3 followed by rinsing with de-ionized water and drying.

4.2.8.2 Arsenic Analysis from Soil and Rice Samples

The total arsenic of the digested soil, rice straw and grain samples were analyzed by flow injection hydride generation atomic absorption spectrophotometer (FI-HG-AAS, Perkin Elmer A Analyst 400) using external calibration through arsenate as standard (Welsch *et al.*, 1990). For each sample three replicates were taken and the mean values were obtained on the basis of calculation of those three replicates.



Plate 4.3 Arsenic Measurement at BCSIR Lab, Dhaka

4.2.9 Soil Sample Analysis Methods

Chemical properties of initial composite soil sample were analyzed. The chemical properties included soil total N, available P, exchangeable K, available S, available Zn contents, pH and organic matter.

4.2.9.1 Total Nitrogen (N)

The micro-Kjeldahl method was used for estimating total nitrogen of soil. The soil was digested with H_2O_2 and concentrated H_2SO_4 in presence of a catalyst mixture (K_2SO_4 : $CuSO_4 \cdot 5H_2O$:Se in the ratio of 10:1:0.1) and the nitrogen in the digest was

determined by distillation with 40% NaOH followed by titration of distillate trapped in H_3BO_3 with 0.01N H_2SO_4 (Page *et al.*, 1982).

4.2.9.2 Available Phosphorus (P)

Following the Olsen *et al.* (1954) method available phosphorus was extracted from the soil with 0.5 M NaHCO_3 solution, pH 8.5. Phosphorus in the extract was then determined by developing blue colour with reduction of phosphomolybdate complex and the colour intensity was measured colorimetrically at 660 nm wavelength (Page *et al.*, 1982). The P concentration of extract was calculated by fitting the absorbance reading to the standard curve.

4.2.9.3 Exchangeable K

According to Page *et al.* (1982) method exchangeable K was determined on 1N NH_4OAc (pH 7.0) extract of the soil by using flame photometer.

4.2.9.4 Available Sulphur (S)

Available S content of soil was measured by extracting the soil with CaCl_2 (0.15%) solution as explained by Page *et al.* (1982). The extractable S was estimated by developing turbidity by adding acid seed solution (20 ppm S as K_2SO_4 in 6N HCl) and BaCl_2 crystals. The intensity of turbid was estimated by spectrophotometer at 420 nm wavelength.

4.2.9.5 Available Zn

DTPA extraction method (Hunter, 1984) was applied for measuring available Zn content in soil.

4.2.9.6 Soil pH

A glass electrode pH meter was used for measuring soil pH, the soil-water ratio being maintained at 1:2.5 (Jackson, 1962).

4.2.9.7 Organic Matter Content

Wet oxidation method of Walkey and Black (1934) was applied for determining organic carbon in soil volumetrically. The organic matter content was calculated by multiplying the percent organic carbon by 1.73 (Van Bemmelen factor).

4.2.10 Statistical Analysis of Experimental Data

The analysis of variance (ANOVA) was done following the F-statistics. Duncan's multiple range test (DMRT) was used for mean comparisons of the plots at 5 % level of probability by SPSS, version 15.0 for windows software. Pearson's correlation was also carried out to find out the correlation among arsenic concentration in irrigation water, drainage sediment, irrigated field soil, different parts (straw, grain) of the rice plant, and distance of arsenic contaminated pump during Boro season and elevation of paddy field during Aman season. Graphical statistical analyses were done with the help of Microsoft Excel software.

4.3 Results

4.3.1 Ground Water Arsenic Contamination and Arsenicosis Patients in Shibganj

665 arsenicosis patients were lived in Shibganj area of Chapai Nawabganj district. All unions had arsenicosis patients, except Daipukuria. Highest arsenicosis patients (210) were found in Shahbajpur union. About 19.02 % HTWs of Shibganj upazila were found arsenic contaminated. HTWs of all unions were found arsenic contaminated and % of arsenic contaminated HTWs ranged from 1.19 to 49.59. Highest % of arsenic contaminated HTWs (49.3) was found in Satrajitpur union. 17.74 % of HTWs were arsenic contaminated in Shahbajpur union, which had highest arsenicosis patients (**Table 4.1** in Appendix-3). Highest arsenic contaminated (0.5 mg/L) STW irrigation water pump was found in Mandal para village of Shahbajpur union (Field survey, 2012).

4.3.2 Effect of Pump Distance on Arsenic Accumulation into Boro Rice in Dry Period

Distance between paddy field and arsenic contaminated pump (STW) had effect on arsenic accumulation in drainage sediment, paddy field soil and rice plants during Boro season in the study area of Shibganj, Chapai Nawabganj in Bangladesh.

4.3.2.1 Arsenic Accumulation in Drainage Sediment

Arsenic accumulation in drainage sediment significantly ($p < 0.01$) affected by arsenic contaminated pump distance. Levels of arsenic concentration in drainage sediment were decreased with increasing pump distance (**Figure 4.1.1**). The highest arsenic concentration in drainage sediment (42.28 ± 1.17 ppm) and lowest arsenic concentration

in drainage sediment (21.89 ± 1.55 ppm) were observed at 10 ft and 300 ft distance, respectively (**Table 4.5** in appendix-3). A strong negative ($r = -0.944$) significant ($p < 0.01$) correlation was observed between arsenic level in drainage sediment and distance of arsenic contaminated STW from paddy field (**Figure 4.1.2**).

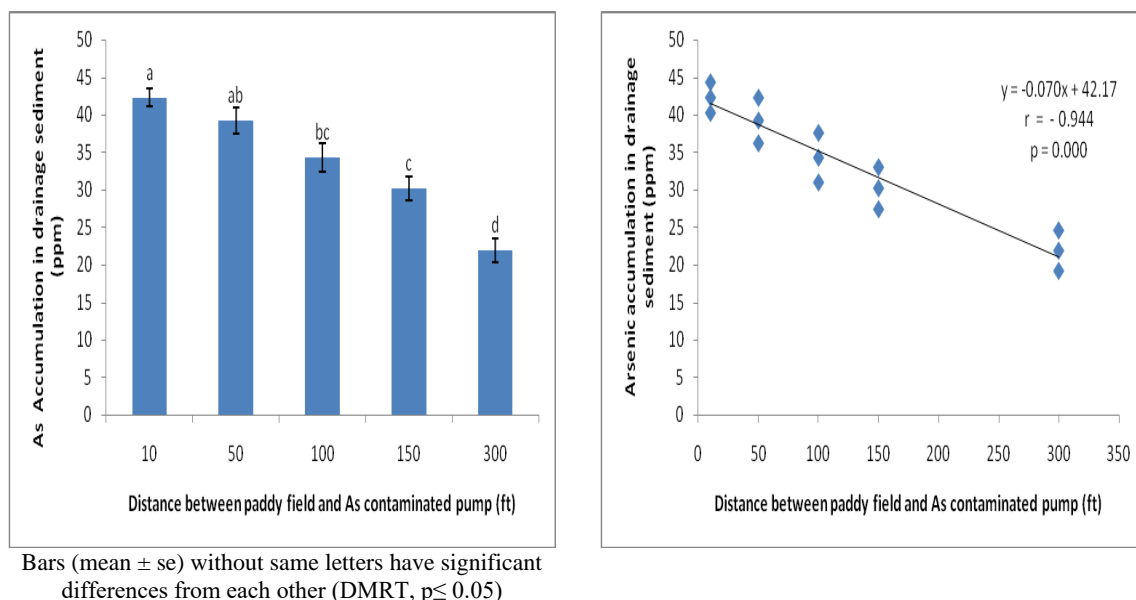


Figure 4.1.1 Effect of pump distance on arsenic accumulation in drainage sediment

Figure 4.1.2 Correlation between pump distance and arsenic accumulation in drainage sediment

4.3.2.2 Arsenic Accumulation in Paddy Field Soil

Arsenic accumulation in paddy field soil significantly ($p < 0.01$) affected by arsenic contaminated STW distance. Soil arsenic concentrations were decreased significantly ($p \leq 0.05$) with increasing paddy field distance from pump (**Fig 4.2.1**). The highest level of arsenic in soil (19.84 ± 0.05 ppm) and the lowest level of arsenic in soil (19.37 ± 0.05 ppm) were observed at 10 ft and 300 ft distance, respectively (**Table 4.5** in appendix-3). A strong negative ($r = -0.915$) significant ($p = 0.000$) correlation was found between soil arsenic level and distance of arsenic contaminated pump from paddy field (**Figure 4.2.2**).

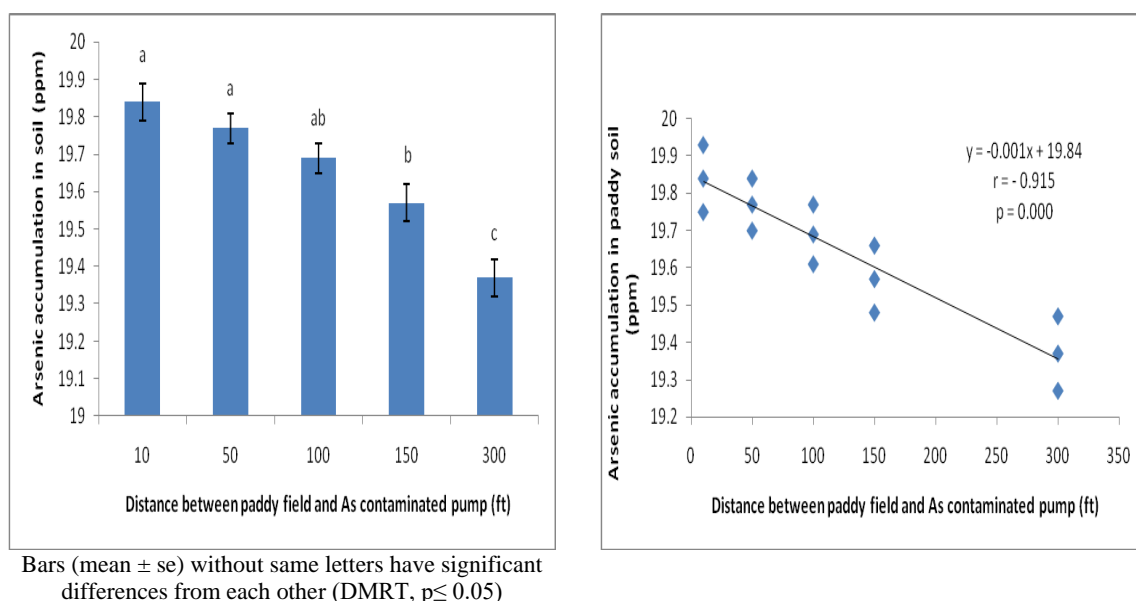


Figure 4.2.1 Effect of pump distance on arsenic accumulation in paddy soil

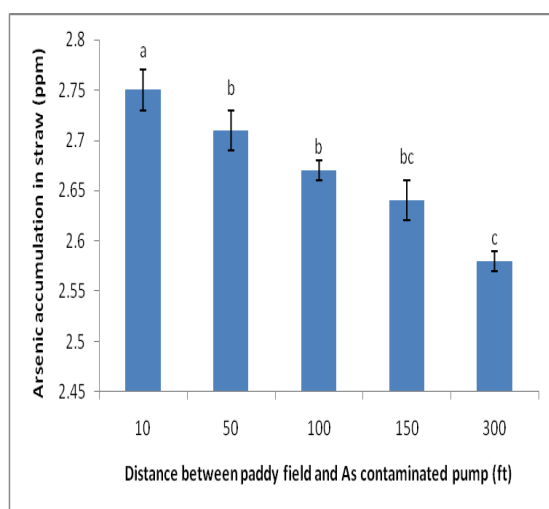
Figure 4.2.2 Correlation between pump distance and arsenic accumulation in paddy soil

4.3.2.3 Arsenic Accumulation in Local Rice, Somsri

Arsenic concentration in local rice variety named Somsri affected by arsenic contaminated STW distance from paddy field during Boro season in Shibganj area of Chapai Nawabganj district in Bangladesh.

4.3.2.3.1 Arsenic Accumulation in Rice Straw

Arsenic accumulation in rice straw was significantly ($p < 0.01$) affected by arsenic contaminated pump distance. Levels of arsenic concentration in straw were decreased significantly ($p \leq 0.05$) with increasing distance of paddy field from arsenic contaminated STW (**Figure 4.3.1**). The highest concentration of arsenic in straw (2.75 ± 0.2 ppm) and the lowest concentration of arsenic in straw (2.58 ± 0.01 ppm) were observed at 10 ft and 300 ft distance, respectively (**Table- 4.5** in appendix-3). A strong negative ($r = -0.860$) significant ($p = 0.000$) correlation was found between straw arsenic concentration and distance of arsenic contaminated STW from paddy field (**Fig 4.3.2**).



Bars (mean \pm se) without same letters have significant differences from each other (DMRT, $p \leq 0.05$)

Figure 4.3.1 Effect of pump distance on arsenic accumulation in rice straw

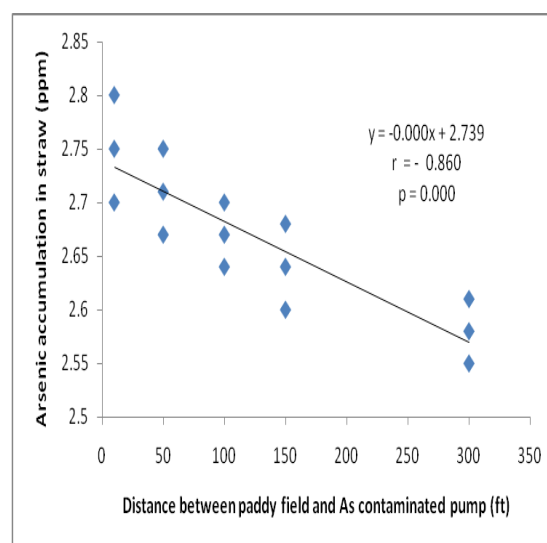
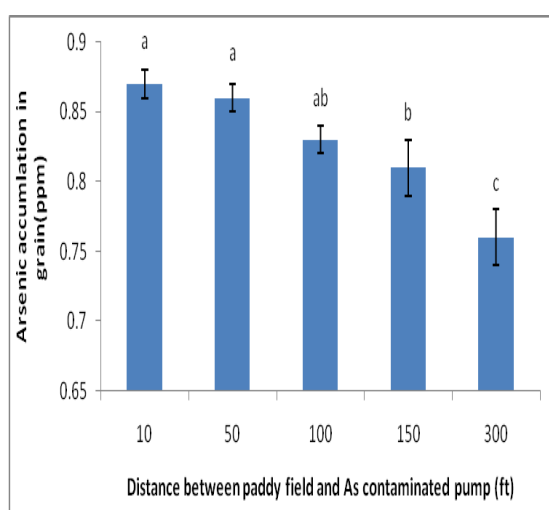


Figure 4.3.2 Correlation between pump distance and arsenic accumulation in rice straw

4.3.2.3.2 Arsenic Accumulation in Rice Grain

Arsenic accumulation in rice grain significant ($p < 0.01$) affected by distance of arsenic contaminated STW from paddy field. Grain arsenic levels were decreased significantly ($p \leq 0.05$) with increasing pump distance (**Figure 4.4.1**). The highest grain arsenic concentration (0.87 ± 0.01 ppm) and the lowest grain arsenic concentration (0.76 ± 0.02 ppm) were observed at 10 ft and 300 ft distance, respectively (**Table 4.5** in Appendix-3). A strong negative ($r = -0.885$) significant ($p = 0.000$) correlation was observed between grain arsenic level and arsenic contaminated pump distance from paddy field (**Figure 4.4.2**).



Bars (mean \pm se) without same letters have significant differences from each other (DMRT, $p \leq 0.05$)

Figure 4.4.1 Effect of pump distance on arsenic accumulation in rice grain

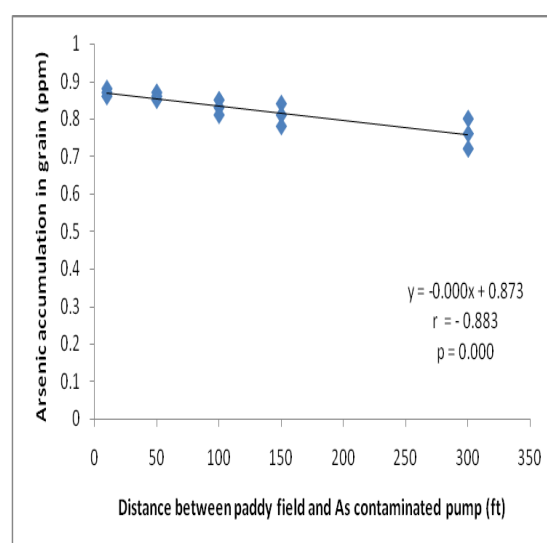


Figure 4.4.2 Correlation between pump distance and arsenic accumulation in rice grain

4.3.3 Effect of Boro Season Residual Arsenic on Arsenic Accumulation During Aman Season.

Residual arsenic from Boro season arsenic contaminated irrigation water affected soil and rice plants during Aman season in Shibganj area of Chapai Nawabganj, severe arsenic contaminated district of Bangladesh.

4.3.3.1 Residual Arsenic Accumulation in Aman Paddy Soil

Residual arsenic accumulation in Aman paddy soil significantly ($p \leq 0.01$) affected by elevation of paddy field from mean sea level. Soil arsenic levels were decreased significantly with increasing paddy field elevation (**Table 4.6** in Appendix-3). The highest arsenic concentration in soil (10.07 ± 0.40 ppm) and the lowest arsenic concentration in soil (8.05 ± 0.17 ppm) were observed at 64.47 ± 0.14 ft and 65.54 ± 0.09 ft elevated paddy field. A strong significant ($p = 0.000$) negative ($r = -0.992$) correlation was found between soil residual arsenic and paddy field elevation from mean sea level during Aman (rainy season) (**Figure 4.5**).

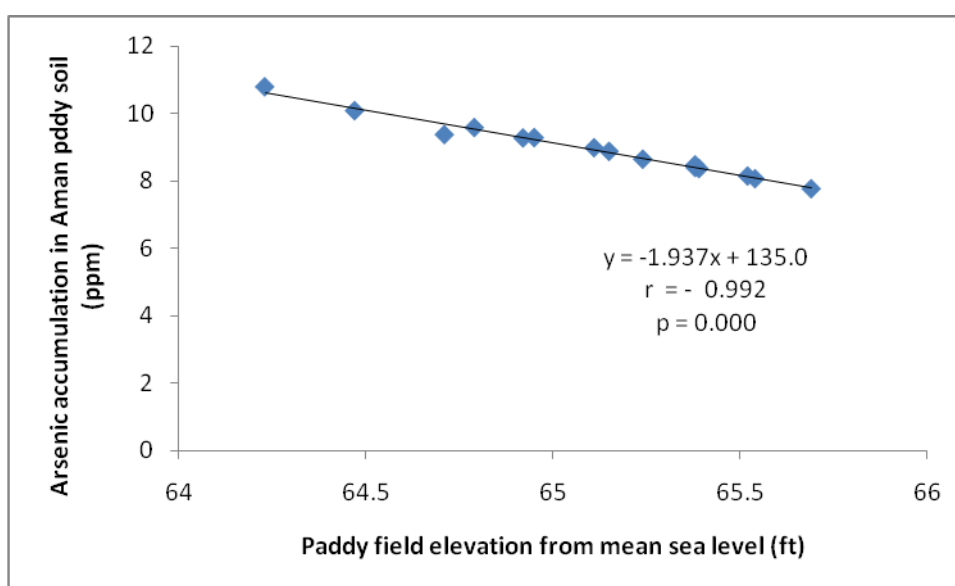


Figure 4.5 Correlation between paddy field elevation and arsenic accumulation in Aman season soil

4.3.3.2 Residual Arsenic Accumulation in Shorna, Local Aman Rice Variety.

Residual arsenic accumulation in Aman (rainy season) rice plants named Shorna was also affected by elevation of paddy field from mean sea level.

4.3.3.2.1 Residual Arsenic Accumulation in Shorna Straw

Residual arsenic accumulation in straw of Shorna rice plant significantly ($p \leq 0.01$) affected by paddy field elevation from mean sea level. Levels of straw arsenic decreased significantly ($p \leq 0.01$) with increasing paddy field elevation. The highest concentration of straw arsenic (1.76 ± 0.1 ppm) and the lowest arsenic concentration of straw arsenic were observed at 64.47 ± 0.1 ft and 65.54 ± 0.09 ft elevated paddy field from mean sea level (**Table 4.6** in Appendix-3). A significant ($p = 0.000$) strong negative ($r = -0.982$) correlation was observed between straw arsenic and elevation of paddy field during Aman (rainy season) (**Figure 4.6**).

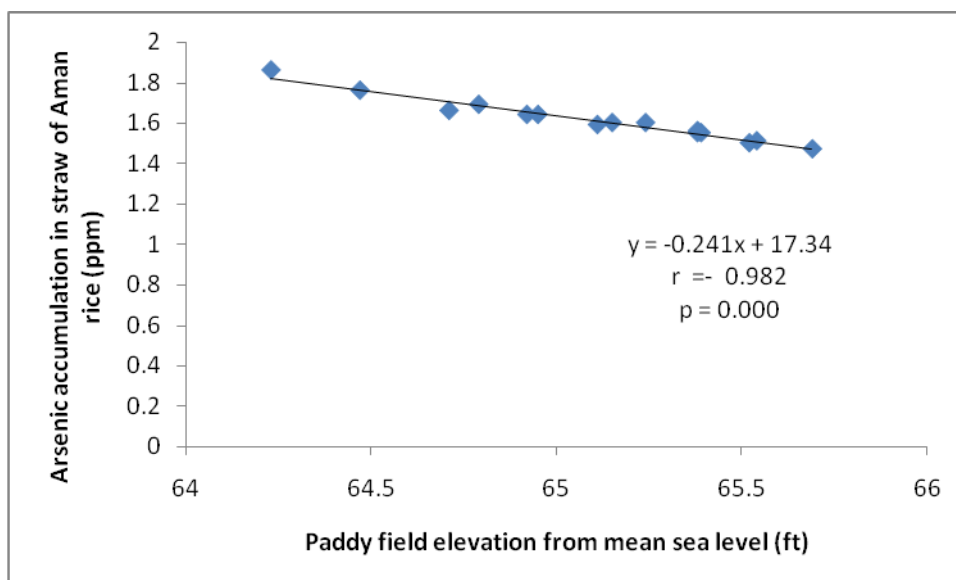


Figure 4.6 Correlation between paddy field elevation and arsenic accumulation in straw of Aman rice

4.3.3.2.2 Residual Arsenic Accumulation in Shorna Grain.

Residual arsenic accumulation in grain of Shorna was significantly ($p \leq 0.01$) affected by paddy field elevation from mean sea level. Levels of grain arsenic significantly ($p \leq 0.01$) decreased with increasing paddy field elevation. The highest concentration of grain arsenic (0.1 ± 0.01 ppm) and the lowest concentration of grain arsenic (0.07 ± 0.01 ppm) were found at 64.47 ± 0.14 ft and 65.54 ± 0.09 ft elevated paddy field (**Table 4.6** in Appendix-3). A significant strong negative ($r = -0.982$) correlation ($p = 0.000$) was observed between grain arsenic accumulation and paddy field elevation during Aman (rainy season) (**Figure 4.7**).

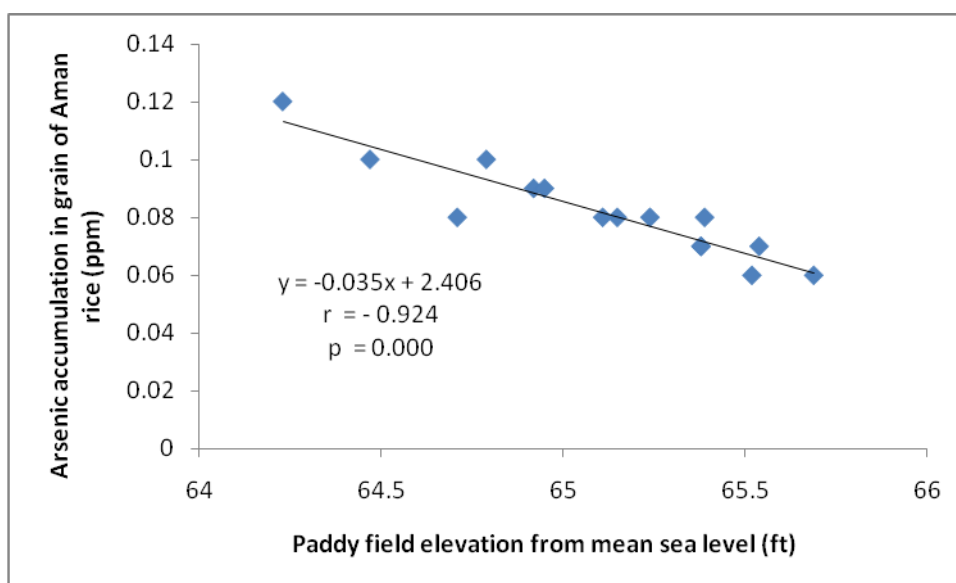
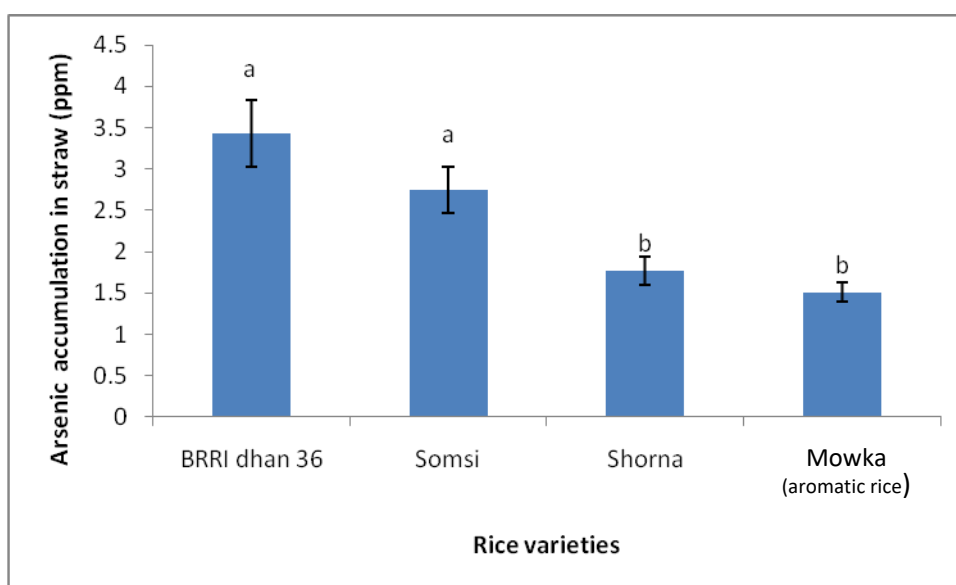


Figure 4.7 Correlation between paddy field elevation and arsenic accumulation in grain of Aman rice

4.3.4 Arsenic Accumulation into Different Varieties of Boro (dry season) and Aman (rainy season) Rice

4.3.4.1 Arsenic Accumulation in Straw

Straw of BRRI dhan-36 of Boro season accumulated highest arsenic (3.43 mg/kg) and straw of Mowka of Aman season accumulated lowest arsenic (1.51 mg/kg) among the varieties of Boro and Aman rice (**Table 4.8** in Appendix-3). Boro (dry season) rice straw accumulated more arsenic than Aman (rainy season) rice straw (**Figure 4.8**).

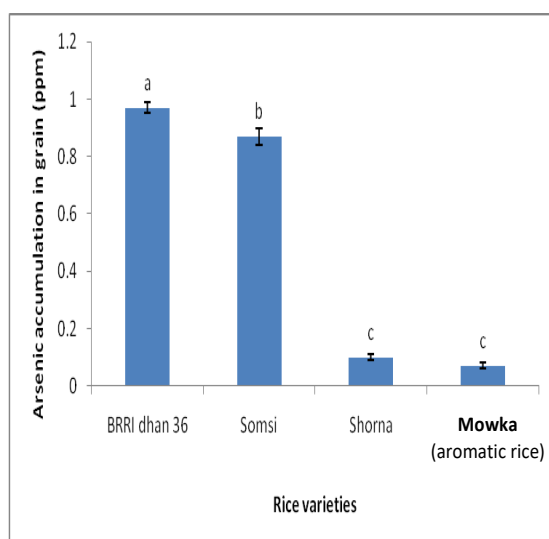


Bars (mean \pm se) without same letters have significant differences from each other (DMRT, $p \leq 0.05$)

Figure 4.8 Arsenic accumulation into straw of Boro and Aman rice varieties

4.3.4.2 Arsenic accumulation in Grain

Grain of BRRI dhan-36 of Boro season accumulated highest arsenic (0.97 mg/kg) and grain of Mowka of Aman season accumulated lowest arsenic (0.07 mg/kg) among the varieties of Boro and Aman rice (**Table 4.8** in Appendix-3). Boro (dry season) rice grain accumulated more arsenic than Aman (rainy season) rice grain (**Figure 4.9**).



Bars (mean \pm se) without same letters have significant differences from each other (DMRT, $p \leq 0.05$)

Figure 4.9 Arsenic accumulation into grain of Boro and Aman rice varieties

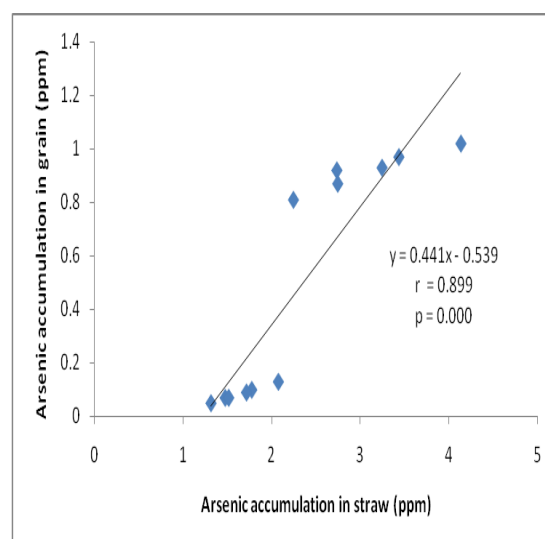


Figure 4.10 Correlation between arsenic accumulation in straw and grain of rice

4.4 Discussion

4.4.1 Arsenic Accumulation during Boro Season

In this experiment rice was cultivated with arsenic contaminated (0.5 mg/L) STW ground water at plots of different distances from the pump during Boro (dry) season. The paddy plots were irrigated with muddy drainage system which influenced arsenic accumulation in drainage sediments, paddy field soil and finally in rice plants.

4.4.1.1 Arsenic in Irrigation Water, Drainage Sediment and Paddy Field Soil

The input of arsenic to soil from the irrigation groundwater is difficult to quantify. Groundwater of most arsenic affected areas in S and SE Asia is rich in iron (Gurung *et al.*, 2005; Postma *et al.*, 2007), which is oxidized upon exposure to the air and is then precipitated as iron-hydroxides in the rhizosphere. Arsenate has high binding affinity to these precipitated iron-hydroxides. Hsu (2012) found extremely high concentration of As in the Fe flock which indicated that ground water was the major source of As. Fine Fe

oxide particles are known to be important colloidal media for the migration of contaminants in soil & aquifers, and thus most As in irrigation ground water is transported in water-soluble and adsorbed forms through paddy fields (Hossain *et al*, 2007; Khan *et al*, 2009). Arsenic in reduced groundwater delivered from a tube-well is gradually adsorbed by iron hydroxides which are precipitated as the water becomes aerated while flowing along irrigation channels (Brammer H, 2009). Polizzotto *et al*. (2013) reported that as flowing water levels rose, arsenic contaminations were elevated at field inlets and decreased with distance across field, but under subsequent static conditions, concentrations dropped and were less variable.

Saha & Ali (2007) clearly showed enrichment of As in the top soil of rice fields irrigated with As contaminated groundwater. They revealed significant spatial and temporal variations of As concentrations in rice fields. In the present study, arsenic in irrigation water is likely to get oxidized and adsorbed and co-precipitated (with iron present in groundwater) on to soil as As contaminated irrigation water travels through the rice field. Arsenic accumulation in drainage sediment significantly (DMRT, $p < 0.05$) affected by arsenic contaminated pump distance (**Figure 4.1.1**). Drainage sediment arsenic decreased significantly ($r = -0.944$, $p < 0.01$) with increasing arsenic contaminated pump distance (**Figure 4.1.2**). Hossain *et al*. (2008) reported that rice fields in Bangladesh irrigated with wells containing up to 130 mg As/L accumulated up to 60 mg/kg As in soil. The As concentration of irrigation water in the distribution channel continuously decreased with distance from the pump and similarly the Fe concentration decreased with pump distance indicating that As was co-precipitated with Fe over time & distance. An almost identical trend was noticed with the P concentration of water. Hossain (2005) also reported that arsenic concentrations in irrigation channel at a 4-ha site on the Ganges floodplain near Faridpur, Bangladesh, decreased from 136 mg/kg at the well-head to 68 mg/kg at the end of a 100 m distribution channel, with most of the loss occurring in the first 30 m. At another site approximately 70 km away, on the old Meghna Estuarine Floodplain near Munshiganj, Robert *et al*. (2007) reported that As concentrations decreased uniformly by 21% between the well-head level of 397 mg/kg and the end of a 152 m irrigation channel (i.e. by a broadly similar total amount to that reported by Hossain, 2005). The total As concentration irrigation channel did not decrease significantly during fast flow while during slow water flow the As concentration along with Fe & P decreased markedly with

increasing distance from the water inlet. Paddy fields in Bangladesh are irrigated every 2-3 days via an inlet leading to a network of channels across the paddy and good mixing of standing water. Arsenic concentrations in standing water in paddy fields were reduced considerably from initial levels of as high as 800 $\mu\text{g/L}$ at the irrigation well by co-precipitation and adsorption of arsenic onto flocs of the Fe oxyhydroxide that were formed upon oxidation of the dissolved Fe (II) in irrigation groundwater. One consequence was that the As concentrations were as high as 70 mg/kg in the surficial soils close to the inlet of irrigation ground water to a rice field, but declined to approximately background levels of <5–10 mg/kg over a distance of several hundred meters (Takahashi *et al.*, 2004; Dittmar *et al.*, 2007; Panaullah *et al.*, 2009). In the present study, a gradient in drainage sediment, As from $\sim 42.28 \pm 1.17$ mg/kg near to the As contaminated irrigation pump and 21.89 ± 1.55 mg/kg at the far side (**Table 4.5** in Appendix- 3) were found, which is in good agreement with the previous studies. Arsenic in the irrigation water was dominated by arsenite. Arsenite oxidation in the standing water may be catalyzed by microbial, surface and photochemical pathways (Roberts *et al.*, 2007). The As concentration in soil is related to the geological substratum from which the soil is formed. A rather wide range of As concentrations has been found in soils around the world, with an average of 5-10 mg/kg in uncontaminated soils (O' Neill, 1995). Arsenic concentrations in the top soil increased significantly after irrigation and cropping of Boro rice (**Table 4.5** in Appendix -3). Islam *et al.* (2005) also concluded that the level of As in the rice field would increase by $0.50 \text{ mg/kg year}^{-1}$ when 0.10 mg As/L is added in irrigation water to Boro rice. Evidence from Bangladesh and West Bengal (Miah *et al.*, 2005; Norra *et al.*, 2005) indicates that virtually all the As added to rice paddies by irrigation stays in the top soil. That is because soils used for paddy cultivation are deliberately puddled to hold water on the surface and they develop a compacted, impervious plough pan (Brammer, 1996). Panaullah *et al.* (2009) calculated that 96 % of the added As at their Faridpur, Bangladesh study site was retained in the top soil at the end of the irrigation season. Alam & Sattar (2000), Das *et al.*, (2004), Norra *et al.*, (2005 b) and Sarkar *et al.* (2012) all found a direct correlation between irrigating with arsenic-contaminated water and elevated soil arsenic levels. Arsenic accumulation in paddy soil significantly (DMRT, $p \leq 0.05$) affected by pump distance (**Figure 4.2.1**). Soil arsenic decreased significantly ($r = - 0.915$, $p = 0.000$) with increasing pump distance (**Figure 4.2.2**). Ahmed *et al.* (2011) observed negative correlations between soil As & distance

from STW ($r = -0.201$, $p < 0.01$). Input of As into rice field soil decreased significantly with increasing distance from the irrigation water inlet. (Dittmar *et al.*, 2006; Roberts *et al.*, 2006). Hsu (2012) also reported that the total arsenic content in soils depended significantly on the distance from the inlet of the irrigation ground water ($r = -0.91$, $p < 0.01$). Stroud *et al.* (2011a) found a significant gradient in soil As concentration depending on the distance from the inlet of high As irrigation water, a feature that is characteristic of As input from irrigation water (Lu *et al.*, 2009) and is caused by the co-precipitation and setting out of As (V) with iron oxides/ hydroxides and adsorption of As to the soil surface (Hossain *et al.*, 2008). Hossain *et al.* (2008) found that the As concentration of surface soil decreased with distance from the STW, with a few exception. They also found significant and positive correlation of soil As with total soil Fe, poorly crystalline Fe-oxide and total free Fe-oxide which suggested a probable role of soil Fe-oxides in As retention and mobility. Studies on pure system suggest that As has a high affinity for oxidic surfaces, although reactivity of oxides may vary considerably, depending on pH, charge density, and soil solution composition (Smith *et al.*, 1995). The amorphous Fe-oxides which have bond strongly to As, may have behaved as strong As scavengers (Hsu *et al.*, 2012). Pananullah *et al.* (2009) reported that deposition of As from irrigation water was non-uniform due to its adsorption on freshly precipitating iron oxides as reduced irrigation water oxidizes during its flow in irrigation channels and across fields. Over time, these processes created a gradient in soil As from ~ 70 mg/kg near the well to ~ 10 mg/kg at the far side of the command area. Consequently, buildup of As in soil within a command area was heterogeneous. Stroud J L *et al.*, (2011a) found that three sites which had irrigated with high As tube-well water for 7-18 years also had relatively high As concentration in the topsoil (>10 mg As/kg soil) compared to baseline values of 6.6 ± 0.10 mg/kg for the paddy soils developed from the Holocene sediment in the Jessore and Faridpur regions of Bangladesh (Lu *et al.*, 2009). Moreover, that sites showed a significant gradient in soil As concentration depending distance from the inlet of irrigation water. Casentini *et al.* (2011) observed a weakly decreasing trend of arsenic accumulation in the soil of irrigated fields with increasing distance from the irrigation device. Dittmar *et al.* (2007) reported that soil As concentration within individual fields varied between 11 and 35 mg/kg. Their site had been irrigated for about 15 years with an assumed annual application of 1000 mm of water. In one field studied in detail, As concentrations decreased from 23.0 mg/kg near the field inlet to 11.3 mg/kg at the far side

of the ca 0.3 ha field. In the present study, arsenic (As) concentrations was found 19.84 ± 0.05 mg/kg in the surficial soils to the inlet of irrigation groundwater to the rice field, but decline to 19.37 ± 0.05 mg/kg over a distance of 300 ft (**Table 4.5**- in Appendix-3), which is in good agreement with the previous studies. A significant positive correlation ($r = 0.989$, $p \leq 0.01$) was found between drainage sediment arsenic and paddy field soil arsenic (**Table 4.9** in Appendix -3). The soil pH, ranging from 5.6 to 6.5 was not related to the distance to the inlet of the irrigation groundwater. The pH was higher in the Fe flock than in the soil. The general pattern of increasing As concentrations in soil pore water with increasing % As (III) is consistent with a reductive mobilization of As in anaerobic soils (Masscheleyn *et al.*, 1991; Takahashi *et al.*, 2004; Xu *et al.*, 2008; Li *et al.*, 2009). Thus procedures that can be used to prevent development of excessive anaerobic conditions in paddy soils may be effective in decreasing As mobility and bioavailability to paddy rice (Duxbury and Panaullah, 2007; Xu *et al.*, 2008; Li *et al.*, 2009). Soil As concentration was positively correlated with As loading factors (years of irrigation \times water As concentration) suggesting that soil As levels will continue to increase over time (Ahmed Z. U. *et al.*, 2011). The background level of As in the surface soil of this experiment was 9.40 mg As/kg, higher than the world As level, 5 mg/kg (Mandal and Suzuki, 2002). Arsenic concentration decreased with soil depth up to 45 cm showing a possibility of As build up in the area for the irrigated Boro rice (summer rice) cultivation. (Hossain *et al.*, 2008). Arsenic concentrations were higher in soils where arsenic contaminated tube-wells are used to irrigate paddies (Duxbury *et al.*; 2003; Meharg and Rahman, 2003; Williams *et al.*; 2006). In this study, the highest drainage soil arsenic concentration was 42.28 ± 1.17 mg/kg (**Table 4.5** in Appendix- 3), agrees well with the highest value (44.4 mg/kg) reported by Adomako E. E. *et al.* (2009). There is a lack of regulatory standard for As concentration in agriculture soil of Bangladesh. Regulatory standards for soils As in other countries vary considerably and many are not recent. Canada instituted a human health standard of 12 mg As/kg and an Environmental health standard of 17 mg/kg for agricultural land (Canadian Council of Ministers of the Environment, 2001). The UK has soil As guideline values of 32 and 43 mg/kg for residential land and vegetable gardens (allotments), respectively (Environmental Agency, 2009). China is the only country that specifically addresses soil used for paddy rice and has limits of 25 and 30 mg As/kg for paddy and upland crop soils, respectively (State Environmental Protection Administration of China, 2008). In the present experiment, the

accumulation of arsenic in paddy soil ($19.37 \pm 0.5 - 19.84 \pm 0.5$ mg/kg) (**Table 4.5** in Appendix-3) was lower than the maximum acceptable limit for agricultural soil of 20 mg/kg as recommended by European Community (Rahman *et al.*, 2007b). The highest content of arsenic in the soil of West Bengal (19.4 mg/kg) was reported by Roy Chowdhury *et al.* (2005). Several reports have been also published about arsenic accumulation of soil due to irrigation with arsenic contaminated groundwater in west Bengal (Chakraborty *et al.*, 2002; Bhattacharya *et al.*, 2010b). Dahal *et al.* (2008) calculated a mean As deposition of 4.2 mg/kg soil per year. In Bangladesh, the soil contained a lower amount of arsenic than the average value (10 mg As/kg) in the areas where arsenic concentration was very low in ground water (Huq *et al.* 2003). The highest content of arsenic in a soil of India was 19.4 mg As/kg, irrigated with water containing 0.12 mg As/L, (Roy chowdhury *et al.*, 2005). Similarly, Farid *et al.*, 2003) found the highest content of arsenic in a soil of Bangladesh with 40 mg As/kg in comparison with an arsenic concentration of 0.689 mg As/L in irrigation water. The arsenic contents measured in the soils of study area found low in comparison to the soils of previous studies (**Table 4.5** in Appendix –3). Rahman *et al.* (2010) reported that total As in soil ranged from 4 to 68 mg As/kg. They reported that concentrations of As in the surface soil (0-15 cm) were considerably greater than in the sub-surface soil layers. This is in agreement with findings of Saha and Ali (2007), reported greater As in the top soil (0-15 cm) than in the deeper layers and suggested that this may be due to oxidation, adsorption and co-precipitation of the water As to the surface soil. They reported that remobilization of As in surface soil due to reductive dissolution of As bearing iron-oxyhydroxides (FeOOH) causes leaching to deeper layer. Contrasting results has also been reported by Alam & Sattar (2000), who found that subsurface layers contained more arsenic than surface soil. Soil arsenic is the major source for the arsenic uptake of crops. (Huang *et al.*, 2006). Besides its natural origin, various anthropogenic activities like mining, smelting, coal burning, irrigation with arsenic contaminated water and application of wastes, animal manures and arsenic-containing pesticides and herbicides may also contribute arsenic to soil (Dutre *et al.*, 1998; Flynn *et al.*, 2002; Mandal and Suzuki, 2002; Alam *et al.*, 2003; Warren *et al.*, 2003; Baroni *et al.*, 2004). There is a high possibility of increase of arsenic contamination in soil in near future if the trend of using large amount of arsenic contaminated groundwater for irrigation continues. Similar prediction was earlier given by Das *et al.* (2004) and Bhattacharya *et al.* (2010a). However, many studies document

varying rates of arsenic accumulation in irrigated soils. Factors which have been shown to influence arsenic retention in soil and soil water include: soil texture (Duxbury and Panaullah, 2007) relatively high phosphorous (P) and low iron (Fe); distance from the irrigation inlet (Hossain, 2005; Duxbury and Panaullah, 2007; Stroud *et al.*, 2011b); field to field water distribution method (Duxbury & Panaullah, 2007) and arsenic leaching out during monsoon flooding (Pal *et al.*, 2009, Roberts *et al.*, 2011). Taken together, these studies suggest that there are considerable differences in soil properties at regional local and even within tube-well areas that affect arsenic accumulation and availability. The As content in the surficial soil was higher closer to the irrigating inlet, indicating that most of the arsenic in paddy fields were from the ground water. The extremely high concentration of As in the Fe flock indicated that groundwater was the major source of As. The use of irrigation water from a arsenic contaminated STW left its chemical imprint on the drainage sediment, indicating that irrigation with As contaminated groundwater lead to loading of As to drainage sediment.

Thus we may conclude that fields in different parts of a STW pump command area receive different amounts of arsenic according to their distance from pump and the rate of As loss in distribution channels. The magnitude of As contamination of irrigation water can be reduced to a safe level by applying irrigation water to the field from a distant STW or allowed to stand in a reservoir/pond for several hours before irrigating crops.

4.4.1.2 Arsenic Accumulation in Boro Rice Straw

The accumulation of arsenic in Boro rice straw was between 2.58 to 2.75 mg/kg dry weight of straw (**Table 4.5** in Appendix-3). Bhattacharya *et al.* (2010a) reported the accumulation of arsenic in straw between 1.34 to 2.13 mg/kg dry weight of straw. They also reported that in the Boro rice there was significant correlation among arsenic concentrations in paddy field soil, rice root and straw parts and between the arsenic concentrations in the rice husk and grain parts. Abedin *et al.* (2002b) observed accumulation of arsenic as high as 92 mg/kg in rice straw. Pot experiment conducted by Abedin *et al.* (2002c) showed that straw As concentration reached 91.8 mg/kg when rice grown on soil was continuously irrigated with As contaminated water. Tsutsumi (1980) found an elevated As concentration in rice straw (up to 149 mg/kg As by dry weight) when rice was grown in soil amended with sodium arsenate at different levels (0–312.5

mg/kg As). The straw As uptake of T-Aman rice was found to differ significantly from a low concentration of As in soil (5 mg/kg) and highest uptake, 2.26 ± 1.0 mg/kg As, was observed for the treatment containing 20 mg/kg As in soil (Azad *et al.*; 2009). The trend of increasing rate of arsenic accumulation in rice straw with increase in soil arsenic treatments was reported by Bhattacharya *et al.* (2013). The As concentration in the straw significantly increased as the distance from irrigation inlet decreased ($r = -0.860$, $P < 0.01$). Rice maintains relatively high redox potentials in the rhizosphere by maintaining a continuous flux of O_2 from the shoot to the roots. The release of O_2 enables the accumulation of Fe oxyhydroxides in the rhizosphere, causing Fe plaque to form around the roots; it binds arsenic (As) and thereby reduces the translocation of As to the above-ground tissues-straw, husk and grain (Liu *et al.*, 2006b; Garnier *et al.*, 2010). In this experiment, a significant positive correlation was found between paddy soil arsenic and arsenic accumulation in rice straw ($r = 0.980$, $p \leq 0.01$) (**Table 4.9** in Appendix-3). Tsutsumi *et al.* (1980) observed elevated arsenic concentrations in rice straw (up to 149 mg/kg dry weight) when rice (*Oryza sativa* L.) was grown in soil amended with arsenate at different levels (0–312.5 mg/kg). Other studies (Marin *et al.*, 1992, 1993; Rahman *et al.*, 2007 b) also reported that the increase of arsenic in straw of rice (*Oryza sativa* L.) with increasing soil arsenic concentrations. Arsenic concentration in straw increased with increasing As addition whether via irrigation water or direct addition to soil. Abedin *et al.* (2002a) also reported significant increase of arsenic concentration in rice root, straw and husk with the increase of arsenate concentration in irrigation water. They found 3.9 mg/kg arsenic in straw at the lowest arsenate treatment (0.2 mg/L), which increased progressively with increasing arsenate application and reached to 9.18 mg/kg in the highest arsenate treatment (8.0 mg/L).

The significant positive relationship between irrigation water (STW) As and rice straw As indicates that irrigation water As may be taken up directly to the rice straw (Rahman *et al.*, 2010). This is in accordance with the findings of Alam & Rahman (2004). The statutory permissible for straw fed to cattle is 0.2 mg/kg (Nicholson *et al.*, 1999). Cattle fed with highly As contaminated straw could be a direct threat to human health, because human being take beef and milk in their food. (Wang *et al.*, 2006). Rice straw is widely used as cattle feed in rural Bangladesh ((Panaullah *et al.*, 2009; Abedin *et al.*, 2002 a) and feeding As contaminated straw to cattle may result in elevated As contents in meat and

milk. Thus a high As concentration in the straw may not only pose a direct threat to animal health but also an indirect threat to human health.

4.4.1.3 Arsenic Accumulation in Boro Rice Grain

The accumulation of arsenic into rice grain is viewed as a newly recognized disaster for South-East Asia, where rice is a staple food (Meharg, 2004). There have been some reports on arsenic content in tissues of rice (Marin *et al.*, 1992; Meharg *et al.*, 2001; Abedin *et al.*, 2002a; Rahman *et al.*, 2004). Bhattacharya *et al.* (2010a) results revealed that the accumulation of arsenic in the grain of all the studied rice samples were between 0.06 and 0.78 mg/kg dry weights of arsenic, which did not exceed the WHO recommended permissible limit in rice (1.0 mg/kg) (Abedin *et al.*, 2002c; Das *et al.*, 2004; Rahman *et al.*, 2007a). In the present study, the accumulation of arsenic in the grain of Boro rice samples were between 0.76 and 0.87 mg/kg dry weights of grain, which also not exceeded the WHO recommended permissible limit in rice (**Table 4.5** in Appendix-3). Wan *et al.* (2006) found the highest As in grain reached up to 0.82 mg/kg in their study, which is very close to the present study. Rahman *et al.* (2010) reported rice grain As ranged from 0.04 to 0.65 mg/kg with a mean of 0.23 mg As/kg. The concentrations of As in the present study are similar to the findings of other surveys conducted in Bangladesh (Das *et al.*, 2004; Duxbury *et al.*, 2003; Meharg & Rahman, 2003). Khan *et al.* (2009) reported the grain As concentrations ranged from 0.37 to 0.86 µg/g and from 0.31 to 0.59 µg/g with the Faridpur -1 and Faridpur -2 soils, respectively. Rahman *et al.* (2009) reported the As content of rice samples ranged from 2 to 557 µg/kg (dry weight). The mean As concentration in rice was 143 µg/kg, with a median value of 131 µg/kg dry weight. Roychowdhury *et al.* (2003) reported the mean As content in rice of Jalangi and Domkal block from the As affected areas of West Bengal, India, were 232 and 233 µg/kg, respectively. The mean As concentration in rice found in the present study was higher when compared with the West Bengal study. From the Kolsur study (Chowdhury *et al.*, 2001) the As concentration in rice was found to be 558 µg/kg, which is also lower than detected in the present study. Das *et al.* (2004) reported the mean As concentration in rice 136 µg/kg. Williams *et al.* (2005) reported mean arsenic concentration of 0.26 µg/g in US long grain rice and 0.40 µg/g as the highest grain arsenic concentration. They also found mean arsenic concentration of 0.13 µg/g in Bangladesh rice grain. Schoof *et al.* (1999) reported 0.303 mg of As/kg dry weight in rice grain from a market basket survey (they

did not mention whether the grain was brown or polished rice). Hsu *et al.* (2012) reported that the accumulation of As in rice grains ranged from 0.29 to 0.66 mg/kg. Meharg and Rahman (2003) found 0.1 – 0.5 mg/kg As in grain of rice that were grown in North America and Taiwan in soils that were not contaminated with As. Islam *et al.* (2004) found 0.05–2.05 µg/g dry weight of arsenic in Boro rice collected from three districts of southern Bangladesh (Gopalganj, Rajbari and Faridpur). Rahman *et al.* (2006) also reported high level of arsenic in raw rice (0.57–0.69 µg/g dry weight) collected from Satkhira district, a highly arsenic contaminated area in Bangladesh. All these studies reveal the subsistence of high arsenic in Bangladesh raw rice. Meharg *et al.* (2008) showed that As accumulation to occur in a small area on the surface of the grain. Dahal *et al.* (2008) reported the mean arsenic content in rice grains (without husk) in their study was 0.18 mg As/kg. The maximum arsenic content in the rice grains was 2.05 mg As/kg in Bangladesh (Islam *et al.*, 2004) and 0.44 mg As/kg in West Bengal, India (Roychowdhury *et al.*, 2002 a). Roychowdhury *et al.* (2002b) also reported 0.23 mg/kg (mean value) of arsenic in rice grown on arsenic contaminated soil with mean concentration of 11.35 mg/kg. Das *et al.* (2004) found 0.14 mg As/kg in rice grain. Dahal *et al.* (2008) reported that the rice grains have a high resistance towards arsenic than other parts. Rice grain collected from arsenic contaminated western part of Bangladesh had arsenic levels of 0.03 – 1.84 µg/g dry weight (Meharg & Rahman, 2003). Adomako *et al.* (2009) reported that grain arsenic content for Faridpur ranged from 0.13 – 0.99 mg/kg and for Gazipur 0.02 – 0.35 mg/kg. The grain arsenic concentration in Abedin *et al.* (2002a) experiment increased significantly from 0.26 mg/kg in the control treatment to 0.74 mg/kg in the highest arsenate treatment but did not exceed the maximum permissible limit for food stuff of 1.0 mg As/kg (National Food Authority, 1993). Xie and Huang (1998) found a similar result of increasing grain arsenic concentration when paddy rice were grown on contaminated soils. The highest rice grain arsenic concentration of 0.74 mg/kg in the highest arsenate treatment is above a proposed food hygienic standard of 0.7 mg/kg (Xie and Huang, 1998). Furthermore lifetime consumption of rice and proportion of more toxic inorganic species in the grain should be taken in to consideration for realistic risk assessment (Abedin *et al.*, 2002b).

Abedin *et al.* (2002a) observed that rice grown with arsenic contaminated irrigation water produced 0.15 – 0.42 mg/kg dry weights of arsenic in rice grain (Boro rice). Meharg and

Rahman (2003) reported 0.00 – 1.8 mg/kg dry weights arsenic content in rice grains, cultivated in Bangladesh. Azad *et al.* (2009) by studying the arsenic phytotoxicity of T–Aman rice found that the uptake of arsenic by rice grains (1.3 – 1.6 mg/kg dry weights) increased considerably with increasing soil arsenic concentrations (30–50 mg/kg dry weights). It has been showed that grain arsenic concentrations increased sharply at low soil concentrations and plateauing at higher concentrations (Adomako *et al.*, 2009; Williams *et al.*, 2007; Lu *et al.*, 2009). Adomako *et al.* (2009) argued that as shoot As concentration increases, phototoxicity sets and certain cell metabolic activities are interfered with thus impeding As transfer to the grain. The As accumulation in grain was associated with soil iron redox transformation influenced by the water management (Hua *et al.*, 2013). Hua *et al.* (2011) reported that the accumulation of As in grain was positively proportional to the soil MSMA content. Abedin *et al.* (2002c) illustrated that high arsenate in irrigation water would cause the accumulation of As in grains, leading to the grain yield reduction. Bogdan *et al.* (2009) showed a significant positive influence of the total arsenic (As) content in the soil on the As content in rice. Sheppard (1992) and Xie and Naidu (2006) states that the uptake of As by rice plants mainly depends on the As availability rather than on the total As in soil. Ahmed *et al.* (2010) reported an average grain As concentration of 0.164 mg/kg. Bhattacharya *et al.* (2013) showed that the average arsenic concentrations in rice grain and soil were related to a great extent. They also found that the accumulation of arsenic in rice grain exceeded the WHO recommended permissible limit in rice (1.0 mg/kg) at the 20 mg/kg dry weight arsenic dosing in pot soil. Some of the highest grain As concentrations were detected at Nonaghata, despite the site having low As in both the soil and the irrigation water (Stroud *et al.*, 2011a). Lu *et al.* (2009) showed that grain As concentration increased with soil As concentration only in the baseline range and approached a plateau once the soil As concentration exceeds a certain level (about 10 mg/kg). An explanation is that As phytotoxicity may inhibit As accumulation in rice grain (Panaullah *et al.*, 2009). Because of the As phytotoxicity, soil pore water As concentration was found to correlate with straw As concentration, but not with grain As concentration (Khan *et al.*, 2010). Panaullah *et al.* (2009) found grain As levels up to 0.54 mg/kg at their lowest soil As level. Adomako *et al.* (2009), at 10 tube-well sites in Gazipur district where ground water has low As concentrations, found grain As levels up to 0.35 mg/kg at a maximum soil As level of 2.4 mg/kg. In the present study, soil arsenic was significantly and positively

correlated with rice grain As (**Table 4.9** in Appendix – 3). Hossain *et al.* (2008) also reported that soil As was significantly and positively correlated with rice grain As. Farid *et al.* (2005) recorded positive correlations between arsenic concentrations in grain and soil at 96 sampling points within a single tube-well site in Brahmanbaria Bangladesh. Others scholars also suggest that increased arsenic concentrations in soil (from the application of contaminated water) results in increased arsenic concentrations in grain (Williams *et al.*, 2006; Khan *et al.*, 2009; Azad *et al.*, 2009). Arsenic accumulation in Boro rice grain in the study area significantly (DMRT, $p \leq 0.05$) affected by arsenic contaminated irrigation pump distance (**Figure 4.4.1**). Arsenic concentrations of Boro rice grain were generally decreased with increasing the paddy field distance from the STW point (**Figure 4.4.2**). Hossain *et al.* (2008) also found that the arsenic concentrations of rice grain were generally decreased with the distance from the STW point. Arsenic concentrations in soil, straw and rice grain were positively and significantly correlated with each other (**Table 4.9** in Appendix-3). Hossain *et al.* (2008) also reported that the As concentrations of rice straw, husk and grain were positively and significantly correlated with each other. The As concentrations in Boro rice grain and straw were positively correlated ($r = 0.947$, $p \leq 0.01$) (**Table 4.9** in Appendix-3). Hossain *et al.* (2009) also found that the As concentrations in rice grain and straw were positively correlated. Bhattacharya *et al.* (2010a) observed significant correlation among arsenic concentrations in paddy field soil, rice root, and straw parts between the arsenic concentrations in the rice husk and grain parts in Boro rice. Smith *et al.* (2007) among others, previously reported that As concentrations in rice tissue increased in order: grain << leaf << stem << root. The strong positive relationship between grain and soil As in this study can be attributed to this variability in soil properties and crop management practices within the STW command area. Several studies indicate As enrichment of soil due to irrigation of As contaminated water (Heikens *et al.*, 2007) and the consequent effect on elevated concentrations of rice grain As (Duxbury *et al.*, 2003; Williams *et al.*, 2006). On the contrary, Van Geen *et al.* (2006) observed that despite the accumulation of As in soil and in soil water attributable to irrigation with ground water containing elevated As levels, there was no evidence of a proportional transfer to rice grains collected from the same sites. No difference in As concentration was observed for rice grains grown in fields irrigated with high & low irrigation water As in West Bengal, India (Norra *et al.*, 2005). Garnier *et al.* (2010) reported that the order of magnitude higher As

concentrations in soil water at the sites irrigated with contaminated water compared to the control sides did not result in a proportional increase in the As content of rice grains. Panaullah *et al.* (2009) also reported a limited range of 0.3 – 0.6 mg/kg for the As content of rice grown in paddies containing 10-70 mg/kg As in soil and levels as high as 2500 µg/L As in soil water. In contrast, rice grains from the greenhouse study by Xu *et al.* (2008) collected from plants grown in flooded pots, both unamended and amended with As, contained much higher levels of As in rice grains (1-2.5 mg/kg). The greenhouse study of Xu *et al.* (2008) under estimates how much As is released to soil water in actual paddy fields while at the same time overestimating the amount of As that is transferred to the rice grain (Van Geen and Duxbury, 2009; Zhao *et al.*, 2009). The grain As contamination throughout the STW area was below 1 µg/g, the maximum permissible limit (NFA, 1993; Xie and Huang, 1998). As reported by Meharg & Rahman (2003), rice grown in the regions where arsenic is building up in the soil had high As concentrations, with rice grain samples having levels above 1.7 µg/g, which is quite high in comparison with many other studies 0.18 µg/g As reported in Bangladesh (Duxbury *et al.*, 2003) and 0.32 in India (Chakraborti *et al.*, 2004). Several countries, including the UK and Australia, use a 1 µg/g limit for arsenic in food and this is often cited as a safe level for rice. This value is clearly high for Bangladesh people who eat rice generally twice daily, amounting to 400-500 gm rice daily. Although the rice grain As concentration was below 1 µg/g, still it cannot be ignored since per day consumption of As for Bangladeshi people is considerable high. Ohno *et al.* (2007) reported that average contribution to total arsenic intake from drinking water was 13%, whereas from cooked rice it was 56%, thus making it clear that rice contributed most to the daily arsenic intake. Rice and vegetables are the main consumed food of rural people living in Bangladesh. They used to take rice vegetables thrice a day (during breakfast, lunch and dinner). Williams *et al.* (2006) reported that daily consumption of rice with a total arsenic level of 0.08 mg/kg would be equivalent to 0.01 mg/L. Thus, higher accumulation of arsenic in rice grain collected from the study area is of particular concern. The grain As concentrations were below 1 µg/g, the maximum permissible limit of the Australian Food standard code (NFA, 1993), but the straw As concentrations exceeded the limit. So, considering rice grain as human food and straw as cattle feed, human beings are less exposed to arsenic and animals are more exposed to this toxic element. Food and feed standards for As have not yet been established in Bangladesh. The chemical form of As in rice grain is important, since

inorganic As forms (i.e As^{III} and As^V) are considerably more toxic than most organic As forms (e.g. monomethyl arsonic acid, MMAs^V; dimethyl arsonic acid, DMAS^V) (Meharg & Hartley Whitaker, 2002). Arsenic species have not been determined in the present study. In a previous assessment Williams *et al.* (2005) determined that 64% of European, 80% Bangladeshi and 81% Indian rice grain arsenic (As) was inorganic. The average As concentration in the rice grain in this study, 0.82 ± 0.01 mg/kg, slightly exceeded those ranging from 0.29 to 0.66 mg/kg in southwestern Taiwan (Hsu *et al.*, 2012) and ranging from 0.22 mg/kg to 0.46 mg/kg, in rice paddies that were irrigated with groundwater that contained As at four sites in Bangladesh and West Bengal, India (Stroud *et al.*, 2011a). Indeed, the effect of irrigation ground water on As toxicity may affect the safety of rice consumption. An average adult human of the study area consuming 500 g of rice daily, would ingest 0.41 ± 0.01 mg of total As only from rice per day. Although the As concentration in the rice grain was less than 1.0 mg/kg, the estimated intake of As by humans approaches the maximum tolerable daily intake of 0.126 mg inorganic As for a 60 kg adult, recommended by the WHO (1997).

4.4.2 Arsenic Accumulation during Local Rice Cultivation (Aman Season)

4.4.2.1 Residual Effects of Arsenic Accumulation on Paddy Soil

The arsenic concentration in soil is related to the geological substratum from which the soil is formed. A rather wide range of As concentrations has been found in soils around the world, with an average of 5 – 10 mg/kg in uncontaminated soils (O' Neil, 1995). Most of the studies on As accumulation in soils indicate that the use of As contaminated water for irrigation will result in the accumulation of As in soil, which has long term implications to agricultural productivity, agricultural sustainability and food quality. Anionic As species are readily adsorbed on colloidal Fe-oxide phases because they have a net positive surface charge at approximately neutral pH. Fe oxyhydroxides are therefore regarded as important As sinks in soil and water (Dittmar *et al.*, 2007; Brammer and Ravenscroft, 2009). During the monsoon flooding season, some of the accumulated As is lost from the paddy field in the flood water (Dittmar *et al.*, 2007; Roberts *et al.*, 2010). In the present study, arsenic contents in Aman (rainy season) paddy soil (measured in November, 2012) ranged from 8.05 ± 0.17 to 10.07 ± 0.40 mg/kg, which were less than Boro (dry season) paddy soil arsenic (measured in May, 2012) (**Table 4.6** in Appendix-

3). Meharg and Rahman (2003), did not consider leaching of As, yet their studies showed that there was a buildup of As in the surface horizons, dissipating rapidly with depth; i.e. that leaching losses appear to be minimal. Khan (2007) also observed a very minimum leaching of As in the undistributed soil column experiment. However, build-up of As in top soil would be a slow process since As, apart from leaching would be removed from the field through surface run-off due to occurrence of heavy monsoon rain. As observed by Dittmar *et al.* (2007), the top soil As contents of rice fields in the dry season (May 2005) increased after irrigation with As contaminated shallow tube-well water and the soil As contents (measured in December 2005) after receiving monsoon rain came down to the initial starting level (December 2004). Khan *et al.* (2010a) reported a relatively low mobility of As in paddy soil. The surface horizons provided a good sink for As that largely prevented its transport to deeper soil horizons, although the high levels of applied As and the continuous flooding throughout the 2 – year period would have maximized the potential for As solubility and As leaching. Similar As retention patterns were observed in the previous net house study with undistributed soil columns, where greater than 70% of irrigation water As was retained in the surface soil horizons (Khan *et al.*, 2009). Saha and Ali (2007) also reported that while top soil As concentrations at four widely separated in As affected districts of Bangladesh increased during dry season irrigation. They had decreased to approximately pre-irrigation levels at the end of the following monsoon season. Despite the monsoon-season losses top soil As levels at the beginning of the study seasons at all the study sites were sever time higher than in unirrigated soils, ranging between 4.15 and 12.5 mg/kg versus 1.5 and 2.5 mg/kg at two As-unaffected sites. Arsenic had accumulated in the irrigated top soils over the numbers of the years of irrigation. Roberts *et al.* (2007) reported that arsenic contents in topsoil in Bangladesh have increased significantly over the last 15 years because of irrigation with arsenic rich ground water. The increase in As concentration was higher in the top soil layer than the deeper layers (Khan *et al.*, 2009). Bhattacharya *et al.* (2010a) reported that the arsenic contents in soil, in Aman season (3.34 – 7.03 mg/kg of arsenic) were not high due to loss of arsenic by microbial biomethylation process from soil to air, uptake by plants, infiltration, surface run off during precipitation, and flooding by the river Ganges. Irrigation with ground water containing 100 µg/L As will annually supply on the order of 100 mg of As/m² of paddy. The corresponding As enrichment in paddy soil would be ~ 0.5 mg/kg per years, assuming that the flux is retained within the upper 10 cm of soil a

20% water content and a soil density of 2.5 g/cm^3 . If there is no major remobilization of As from rice paddies during the wet season decade or two of irrigation could therefore lead to significant soil enrichments, as observed in Arai-hazar and elsewhere in Bangladesh (Geen *et al.*, 2006; Meharg & Rahman, 2003; Ali *et al.*, 2003; Huq *et al.*, 2003). Norra *et al.* (2005) reported that the As concentration in the upper most soil layers of the rice paddy field (38 mg As/kg) was roughly twice as high as that in the soil of the less intensively watered wheat field (18 mg/kg) and more than 5 times higher than that of a soil (7 mg As/kg) of a rice paddy irrigated with uncontaminated water. Evidence from Bangladesh and West Bengal (Miah *et al.*, 2005; Norra *et al.*, 2005) indicates that virtually all the As added to rice paddies by irrigation stays in the top soil. That is because soils used for paddy cultivation are deliberately puddle to hold water on the surface and they develop a compacted, impervious plough pan (Brammer, 1996). Panaullah *et al.* (2009) calculated that 96% of the added As at their Faridpur, Bangladesh study site was retained in the top soil at the end of the irrigation season. Arsenic (As) added each year in irrigation water during the dry season was estimated to be exported via flow to river during deep flooding in the summer monsoon at a site in Munshiganj district (Roberts *et al.*, 2007). In this experiment, soil arsenic concentrations were decreased significantly ($r = -0.992$, $p \leq 0.001$) with increasing paddy field elevation from mean sea level (**Figure 4.5**). Ahmed *et al.* (2011) observed negative correlations between soil As and paddy field elevation ($r = -0.34$, $P < 0.001$), which is in good agreement with present study. They also observed mean soil As concentration increased by 3.8 and 6.4 mg/kg for high land and medium high land areas, respectively. The anaerobic conditions of flooded paddy soil are responsible for the reductive dissolution of Fe oxyhydroxides, and the release of the adsorbed arsenate which causes As to be mobilized as arsenite in the soil pore water (Khan *et al.*, 2009, 2010a). Accordingly the bioavailability of As in lowland paddy soils is much higher than that in upland soils. Ahmed *et al.* (2011) estimated the expected mean increase in soil As concentration for soils cropped to rice in the different land types can be made using mean values for irrigation water As concentration and years of operation of the tube-well, and assuming the addition of 1 m depth of irrigation water per rice crop, a soil bulk density of 1.2 g/cm^3 and uniform deposition of As in irrigation water. On this basis, mean soil As concentration would have increased by 3.8 and 6.4 mg/kg for high land and medium land areas, respectively. Dahal *et al.* (2008) reported that due to the high solubility of arsenic in the reduced condition (Fitz & Wenzel, 2002),

As can be carried away downstream in flood plain paddy field condition. Thus the rate of arsenic deposition from contaminated irrigation water would be higher in flat terrain soil than that in flood land soil.

Thus we may conclude that high land is safer than low land for rice cultivation in minimum soil arsenic level. The results of arsenic retention in paddy fields indicate the need for long-term and more comprehensive assessment of this topic, including potential effects of soil and irrigation water chemistry on the extent of remobilization of As during flooding. The factors contributing to seasonal gains and losses of soil As need to be investigated over the wide range of environmental and agricultural conditions under which rice is grown. Because of the interannual variability of meteorological and hydrological conditions, observations need to be made over a number of years. The fate of any As removed also needs to be investigated, including the possibility that some may be added to near surface ground water or to soils in other sites.

4.4.2.2 Residual Effects of Arsenic Accumulation on Aman Rice Straw

Some studies demonstrated the strong residual effect of soil arsenic contamination on subsequent crops (Huq *et al.*, 2003; Khah *et al.*, 2010). Rice straw is the main cattle/buffalo feed in Bangladesh (Abedin *et al.*, 2002a; Panaullah *et al.*, 2009) and feeding As-contaminated straw to cattle they result in elevated As contents in meat and milk. A high As concentration in the straw may not only pose a direct threat to animal health but also an indirect threat to human health. Arsenic concentrations in Aman rice straw were almost half compared to those found in Boro rice. Straw arsenic concentration was almost 20 times higher than the grain As concentration (**Table 4.8** in Appendix-3). Hossain *et al.* (2007) also reported that arsenic concentrations in Aman rice straw were almost half compared to those found in Boro rice. They also found the straw As concentrations was almost 15 time higher than the grain As concentrations. This is a good agreement with the present study. Arsenic concentrations in Aman rice straw were increased significantly ($r = 0.991$, $p \leq 0.05$) with increasing soil arsenic concentrations in paddy soil (**Table 4.10** in Appendix-3). Khan *et al.* (2009) reported that a greater effect of As addition on straw-As concentration compared to grain As concentration. They also observed that both soil-As and irrigation-water As concentrations contributed to straw As concentrations. Arsenic concentrations in Aman rice straw decreased significantly ($r = -$

0.982, $p \leq 0.001$) with increasing paddy field elevation from mean sea level (**Figure 4.6**). Thus we may conclude that Aman rice straw is safe for cattle feed compared to Boro rice straw. Nationwide comprehensive study should be conducted on this problem.

4.4.2.3 Residual Effects of Arsenic Accumulation on Rice Grain

Rice grain generally has lower As concentration and the concentration remains much below the maximum permissible limit of 1 mg/kg As (Schoof *et al.*, 1998). Khan *et al.* (2009) reported that the application of As in the first (Boro rice 2004) and third (Boro rice 2005) crops had residual effects on the concentrations of As in rice grain and straw of subsequent T. Aman rice crops. The grain As concentrations of both Boro and T. Aman rice were increased substantially with increasing irrigation water As concentration during the Boro season. Das *et al.* (2004) found 0.14 mg As/kg in rice grains, 0.73 mg As/kg in shoot and 2.4 mg As/kg in roots. The mean arsenic content in rice grains (without husk) in a study was 0.18 mg As/kg (Daha *et al.*, 2008). In the present study, very low arsenic contents were found in Aman rice grains (0.07 ± 0.01 to 0.1 ± 0.01 mg As/kg) compared to straws (1.51 ± 0.04 to 1.76 ± 0.1 mg As/kg) (**Table 4.6** in Appendix-3). This indicates that the rice grains have a high resistance towards arsenic than other parts. Azad *et al.* (2009) also reported that the straw accumulated twice as much As than the grain in T-Aman rice. Meharg & Rahman (2003) reported 0.06–1.8 mg/kg dry weight arsenic content in rice grains, cultivated in Bangladesh. Abedin *et al.* (2002b) observed that rice grown with arsenic contaminated irrigation water produced 0.15-0.42 mg/kg dry weights of arsenic in rice grain (brown rice). Bhattacharya *et al.* (2010a) found significant correlation between arsenic concentration in paddy field soil and rice root and between the arsenic concentrations in the rice husk and grain parts in case of Aman rice. In the present study arsenic concentrations in Aman rice grains increased significantly ($r = 0.951$, $p \leq 0.001$) with increasing soil arsenic concentrations (**Table 4.10** in Appendix-3). Azad *et al.* (2009) by studying the arsenic phytotoxicity of T-Aman rice found that with increase in soil arsenic concentrations (30-50 mg/kg dry weights), the uptake of arsenic by rice grains (1.3-1.6 mg/kg dry weight) increased considerably. It has been showed that grain arsenic concentrations increased sharply at low soil concentrations and plateauing at higher concentrations (Adomako *et al.*, 2009, Williams *et al.*, 2007, Lu *et al.*, 2009). The slopes between soil As speciation and grain were steep at total soil As concentration lower than 3.6 mg/kg and gentle at higher concentrations. Panaullah *et al.* (2009) reported

that As toxicity to rice interfered with translocation of As to the grain. They observed a linear decreased in grain As concentration from 0.57 to 0.30 mg/kg with increasing soil As in the second year of their study. In contrast, arsenic uptake into rice grain increased with increasing soil As when rice was grown more aerobically to mitigate As toxicity at this same site (Duxbury and Panaullah, 2007). Significant positive correlation ($r = 0.959$, $p \leq 0.001$) was observed between arsenic in straw and grain of Aman rice (**Table 4.10** in Appendix-3). Panaullah *et al.* (2009) also reported a positive relationship between As in rice straw and grain. Arsenic accumulation in Aman rice grains decreased significantly ($r = -0.924$, $p \leq 0.01$) with increasing paddy field elevation from mean sea level (**Figure 4.7**). Geen *et al.* (2006) reported that the As content of summer Aman rice grown at the control site (0.18 ± 0.05 mg/kg) was about a factor of two lower than for Boro rice from all sites. Aman rice grains accumulated less arsenic compared to Boro rice grains (**Table 4.7** in Appendix-3), which is in good agreement with the previous studies. Hossain *et al.* (2008) reported that residual As from previous Boro rice showed a very similar pattern in the following Aman rice, although As concentration in Aman rice grain and straw over the treatments was almost half of the As level in Boro rice grain.

Thus we may conclude that Aman rice grains are safe for consumption compared to Boro rice.

4.4.3 Arsenic Accumulation into Different Varieties of Rice During Boro and Aman Season

Meharg & Rahman (2003) found variations of arsenic concentration in different rice varieties grown in Bangladesh Rice Research Institute research station (between 0.043 and 0.206 $\mu\text{g/g}$ dry weight) and in those collected from different district in of the country (between 0.058 and 1.835 $\mu\text{g/g}$ dry weight). Zavala and Duxbury (2008) observed a significant variation in As content of the different rice varieties. Arsenic concentrations in rice straw varied from 1.51 ± 0.11 to 3.43 ± 0.40 mg/kg in different varieties of Boro and Aman rice (**Table 4.8** in Appendix-3). Rahman *et al.* (2007b) reported that with the increase of soil arsenic concentrations arsenic accumulation in straw of all five varieties increased significantly ($p < 0.05$). They found the average straw arsenic concentration in control treatment was 7.07 ± 0.82 mg/kg dry weight. Duxbury *et al.* (2003) observed 1.5 times higher As concentrations in rice grown in the winter or dry season (Boro rice) than

in the summer (monsoon) season (T. Aman rice), consistent with the use of ground water for irrigation in the Boro season but the not T. Aman season. Khan *et al.* (2010b) reported that the As concentrations in rice straw varied over season. Straws of Boro season rice varieties accumulated more arsenic than straws of Aman season varieties (**Table 4.8 in Appendix-3**). The uptake of arsenic in rice grain was found to vary with the eight different rice varieties (Bhattacharya *et al.*, 2013) This finding is consistent with earlier observations by Alam *et al.* (2003), Delowar *et al.* (2005) and Williams *et al.* (2006). Much higher arsenic accumulation ability in rice straw by hybrid rice varieties as compared to non-hybrid varieties had been for merely reported by Rahman *et al.* (2007b). Zavala and Duxbury (2008) reported total As concentration in rice varieties from 0.005 to 0.710 $\mu\text{g/g}$ dry weight in different varieties. In the present study, grain arsenic concentrations in Boro and Aman rice varieties ranged from 0.07 ± 0.01 to 0.97 ± 0.02 mg/kg (**Table 4.8 in Appendix-3**). According to Williams *et al.* (2005), rice grain As contents varied from 0.01 to 2.05 mg/kg. Norton *et al.* (2009 b) found the ranges of total grain As were 0.27 – 0.74 and 0.37 – 0.085 mg/kg, respectively, in the cities Chenzhou & Qiyang of Hunan province, China, with a significant genotypic effect ($p < 0.001$) for total grain As. Ahmed *et al.* (2010) reported that the mean grain As concentration of rice varieties grown at different sites ranged from 0.10 mg/kg for the indica variety BR 23 to 0.22 mg/kg for the admixed variety BRRI dhan 33 in the Aman-season. The mean grain As concentration in the Boro season varieties ranged from 0.32 mg/kg for BR 3 to 0.34 mg/kg for BRRI dhan 35 and the overall mean (0.29 mg/kg) was 87 % higher than that in the Aman season. Williams *et al.* (2006) reported that arsenic level ranged between 0.04 and 0.92 $\mu\text{g/g}$ dry weight (mean 0.08 – 0.36 $\mu\text{g/g}$) in dry season rice and between 0.04 and 0.91 $\mu\text{g/g}$ dry weight (mean 0.14– 0.51 $\mu\text{g/g}$ dry weight) in monsoon season rice collected from southern part of the country. Grains of Boro season rice varieties accumulated more arsenic than Grains of Aman season varieties (**Table 4.8 in Appendix-3**). Ahmed *et al.* (2010) reported that the mean grain As concentration was significantly higher in the Boro (0.290 mg As/kg) than in the Aman (0.154 mg As/kg) season ($p < 0.001$). They also reported that popular varieties BR 11 (Aman) and BRRI dhan 28 and 29 had grain As concentrations close to the mean value and were fairly stable across environments, while high yielding, short-duration Aman season varieties (BRRI dhan 32, 38 and 39) developed for intensified cropping had relatively high grain As concentration.

Seasonal differences in grain As concentration could be caused by irrigation of Boro rice with As contaminated ground water (Duxbury *et al.*, 2003; Williams *et al.*, 2006). Lower levels of grain As in varieties grown during the Aman season could also be due to a dilution of soil solution As by As free rain water (Ahmed *et al.*, 2010). Rahman *et al.* (2007a) showed significant differences in the accumulations of As in straw & grain. Low arsenic contents were found in Boro and Aman rice grains of all varieties compared to straws (**Table 4.8** in Appendix-3). The As concentration of straw was much higher than that of grain, i.e, 0.30–6.21 µg/g for straw and 0.19–71 µg/g for grain. The As concentrations in rice grain and straw were positively correlated ($r = 0.964$, $P < 0.01$) (Hossain *et al.*, 2009). Rahman *et al.* (2007b) reported that hybrid rice varieties might have higher arsenic accumulation ability and are more tolerant to arsenic phytotoxicity than those of non-hybrid varieties. Arsenic concentration in Boro and Aman rice grains of all varieties remained below the maximum permissible limit of 1 mg/kg As recommended by WHO. Wang *et al.* (2006) reported that different rice varieties show discrepancy in their ability to take up various As compounds. Ye *et al.* (2012) reported significant ($p < 0.05$) variation in As concentration among rice cultivars. The uptake and translocation of As to consumed parts of rice varies greatly with cultivar (Duxbury *et al.*, 2003; Zavala & Duxbury, 2008). Local Aman rice variety named Mowka (aromatic rice) accumulated lowest arsenic (0.07 ± 0.01 mg/kg) among all rice varieties of Boro and Aman season. Abedin & Meharg (2002) reported considerable variation in toxicity among rice varieties. Norton *et al.* (2009 b) showed that E (soil type), G (genotype) and GEI (interaction between genotype & soil type) had significant effects on total grain As concentration in an investigation of 13 rice varieties grown at six sites in three countries (Bangladesh, India and China) - the effect of soil types was the largest, followed by genotype. Ahmed *et al.* (2011) also found similar results; that As concentration in rice grain was significantly affected by E (69 % and 80 %), G (9 % & 10%) and GEI (10 % & 21 %) in two seasons, respectively, through analyzing 38 rice varieties planted at eight sites. Norton *et al.* (2009 a) found a strong genetic control over As in grain using 76 cultivars planted at two locations during the Boro season in Bangladesh. The large genotypic variation in grain As concentrations leads to the possibility of breeding new rice cultivars with low As accumulation (Meharg, 2004). Genotype of rice varieties were not investigated in the present study.

Thus we may conclude that Aman season rice varieties are safer than Boro season rice varieties for consumption. The effect of genotype on arsenic uptake into rice plants need to be investigated for discovering arsenic resistance rice varieties in Bangladesh.

4.5 Conclusions

- Arsenic accumulation in drainage sediments, paddy field soil, rice straw, and grain were decreased significantly ($p \leq 0.01$) with increasing arsenic contaminated STW irrigation pump distance from paddy field.
- Less arsenic accumulations in soil, rice straw and grain were observed at paddy field far away from irrigation pump compared to nearer one.
- Significant positive correlations were observed among arsenic in drainage sediments, paddy field soil, rice straw and grain.
- The trend of arsenic accumulation into rice was irrigation water>drainage sediments>paddy field soil>root> straw> grain.
- Rice straw accumulated more arsenic than grain.
- Highest arsenic accumulation in rice straw exceeded the maximum permissible limit for cattle feed.
- Arsenic accumulation in paddy soil, rice straw and grain had significant ($p \leq 0.01$) negative correlation with paddy field elevation during Aman (rainy) season.
- High land accumulated lowest arsenic than medium and low land.
- Dry season (Boro) rice varieties accumulated more arsenic than rainy season (Aman) rice varieties.
- Local non- hybrid Aman season rice variety named Mowka (aromatic rice) accumulated lowest arsenic (0.07 ± 0.01 mg/kg) in grain.
- Boro season Hybrid rice variety named BRRI dhan 36 accumulated highest arsenic in grain.
- Highest arsenic accumulation in rice grain (0.97 ± 0.01) not exceeded the maximum permissible limit of 1 mg/kg As recommended by WHO.
- Aman season rice varieties are safer than Boro for consumption.

4.6 Recommendations

- National survey should be conducted on STWs for identifying the arsenic contaminated irrigation pump in Bangladesh.
- National standard for permissible level of arsenic in irrigation water for rice cultivation should be established urgently.
- Surface water and rain water should be prioritized for rice cultivation.
- Arsenic contaminated STW irrigation pump should be set at safe distance from paddy field.
- Rice cultivation should be practiced in aerobic soil conditions for less arsenic contamination.
- Rice cultivation in upland is suggested for less arsenic contamination.
- Severe arsenic contaminated irrigation water should be reserved in a reservoir first before irrigating to paddy field.
- More Aman (rainy season) rice should be cultivated than Boro (dry season) in arsenic contaminated area for less arsenic rice.
- Local non-hybrid rice varieties should be cultivated for less arsenic contamination.

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Appendices

Appendix 1

Table 2.2 Effect of arsenic amended irrigation water on chlorophyll contents of BR-11 rice leaves.

Arsenic added in water (mg/L)	Chlorophyll contents (mg/g)	
	Chlorophyll- <i>a</i>	Chlorophyll- <i>b</i>
0.0	20.27 ± 1.70ab	17.00 ± 1.84b
0.1	24.84 ± 0.39a	21.57 ± 0.73a
0.5	20.18 ± 1.14ab	15.53 ± 0.47bc
1	16.11 ± 2.68bc	12.29 ± 2.04cd
2	15.90 ± 2.97bc	12.16 ± 2.04cd
4	10.91 ± 0.66c	08.46 ± 0.03d

Values (mean±SE) having without same letters in a column do not differ significantly at $p \leq 0.05$ by DMRT

Table 2.3 Effect of arsenic amended irrigation water on growth of BR-11 rice

Arsenic added in water (mg/L)	Plant height (cm)	Tillers/Plant (no.)	Panicles/Plant (no.)	Panicle length (cm)	1000-grain weight (gm)
0	95.35±1.78a	24.00±2.65a	16.00±0.00a	24.20±0.69a	18.84±0.94a
0.1	91.17±2.00a	18.33±2.60ab	10.67±3.18a	22.80±0.50ab	17.81±0.06a
0.5	96.64±0.73a	20.67±2.33ab	15.67±0.67a	22.37±0.67ab	14.21±1.14a
1.0	92.85±2.13a	17.67±0.67ab	13.33±1.45a	22.20±0.25ab	13.90±1.81a
2.0	88.29±6.17a	16.00±0.58b	9.67±2.33a	22.10±0.58b	13.68±2.14a
4.0	82.66±7.61a	15.00±3b	9.67±3.18a	21.83±0.84b	12.50±5.11a

Values (mean±SE) having without same letters in a column do not differ significantly at $p \leq 0.05$ by DMRT

Table 2.4 Effect of arsenic amended irrigation water on yield of BR-11 rice

Arsenic added in water (mg/L)	Grain yield (g/plant)	Straw yield (g/plant)	Percent of yield reduction over control	
			Grain	Straw
0	23.38±5.55a	31.50±4.53a	0	0
0.1	15.28±1.28ab	30.60±4.29a	-34.64	-2.85
0.5	9.84±1.31ab	39.07±4.08a	-57.91	24.03
1.0	11.06±6.22ab	34.14±3.17a	-52.69	8.38
2.0	9.81±4.64ab	29.05±11.52a	-58.04	-7.77
4.0	7.24±2.32b	27.01±6.74a	-69.03	-14.25

Values (mean ± SE) having without same letters in a column do not differ significantly at $p \leq 0.05$ by DMRT

Table 2.5 Effect of arsenic amended irrigation water on Spikelet number of BR-11 rice.

Arsenic added in water (mg/L)	Filled spikelet number	Empty spikelet number	Total spikelet number
0.0	1091.67 ± 206.10a	1083.33 ± 123.47a	2172.00 ± 332.07a
0.1	678.33 ± 78.81b	945.00 ± 120.45a	1623.33 ± 81.67a
0.5	270.00 ± 116.21c	1203.33 ± 26.82a	1473.33 ± 126.07a
1	243.33 ± 138.51c	1208.00 ± 212.28a	1451.33 ± 131.13a
2	216.33 ± 108.41c	1230.00 ± 599.88a	1446.33 ± 616.68a
4	197.33 ± 97.58c	1185.00 ± 80.47a	1382.33 ± 107.54a

Values (mean±SE) having without same letters in a column do not differ significantly at $p \leq 0.05$ by DMRT

Table 2.6 Arsenic accumulation in soil, straw and grain of BR-11 rice.

Arsenic added in water (mg/L)	Arsenic in soil (mg/kg)	Arsenic in straw (mg/kg)	Arsenic in grain (mg/kg)
0.0	3.67 ± 0.39a	1.72 ± 0.16a	0.06 ± 0.01a
0.1	6.11 ± 0.46b	2.96 ± 0.14b	0.19 ± 0.01b
0.5	7.23 ± 0.40b	3.04 ± 0.16b	0.33 ± 0.01c
1	9.85 ± 0.49c	3.48 ± 0.16bc	0.34 ± 0.0c
2	13.68 ± 0.35d	3.63 ± 0.13d	0.44 ± 0.02d
4	14.61 ± 0.24d	4.69 ± 0.14d	0.47 ± 0.03d

Values (mean±SE) having without same letters in a column do not differ significantly at $p \leq 0.05$ by DMRT

Table 2.7 Matrix of arsenic accumulation correlation coefficients (r) among water, soil, straw and grain arsenic.

		Arsenic added in water (mg/L)	Arsenic in soil (mg/kg)	Arsenic in straw (mg/kg)	Arsenic in grain (mg/kg)
Arsenic added in water (mg/L)	Pearson Correlation	1	.900**	.864**	.804**
	Sig. (2-tailed)		.000	.000	.000
Arsenic in soil (mg/kg)	Pearson Correlation	.900**	1	.919**	.943**
	Sig. (2-tailed)	.000		.000	.000
Arsenic in straw (mg/kg)	Pearson Correlation	.864**	.919**	1	.921**
	Sig. (2-tailed)	.000	.000		.000
Arsenic in grain (mg/kg)	Pearson Correlation	.804**	.943**	.921**	1
	Sig. (2-tailed)	.000	.000	.000	

** indicate correlation is significant at the 0.01 level (2-tailed). N=18.

Appendix 2

Table 3.2 Effect of soil residual arsenic on chlorophyll contents of BRRi dhan-50 leaves.

Previous season arsenic in irrigation water (mg/L)	Residual arsenic in soil (mg/kg)	Chlorophyll contents (mg/gm)	
		Chlorophyll-a	Chlorophyll-b
0.0	3.67	18.37 ± 0.02b	14.28 ± 0.10c
0.1	6.11	19.68 ± 0.33bc	16.19 ± 0.60d
0.5	7.23	20.53 ± 0.52c	11.84 ± 0.80b
1	9.85	19.94 ± 0.54bc	12.66 ± 0.74bc
2	13.68	19.90 ± 0.68bc	12.22 ± 0.46b
4	14.61	16.58 ± 0.76a	9.08 ± 0.69a

Same letters in a column did not differ significantly at $p \leq 0.05$ by DMRT

Table 3.3 Effect of soil residual arsenic on growth of BRRIdhan-50

Previous season arsenic in irrigation water (mg/L)	Residual arsenic in soil (mg/kg)	Plant height (cm)	Tillers/plant (no.)	Panicles/plant (no.)	Panicle length (cm)	1000-grain weight (gm)
0.0	3.67	71.53±2.15a	13.16 ± 1.60ab	11.33 ± 1.58a	21.60 ± 0.40a	14.12 ± 0.88a
0.1	6.11	74.60±1.63a	18 ± 2.04ab	16.00 ± 2.20a	21.96 ± 0.44a	14.77 ± 0.34a
0.5	7.23	71.07±1.45a	17.50 ± 2.66ab	13.00 ± 2.20a	21.15 ± 0.93a	13.58 ± 0.99a
1	9.85	62.10±5.30b	11.00 ± 2.81a	9.5 ± 2.97a	20.21 ± 0.81a	14.40 ± 0.15a
2	13.68	74.08±1.94a	19.16 ± 2.44b	15.16 ± 1.74a	21.75 ± 0.5a	15.07 ± 0.28a
4	14.61	72.60±1.29a	15.16 ± 3.12ab	12.83 ± 2.30a	21.74 ± 0.57a	13.99 ± 0.38a

Same letters in a column did not differ significantly at $p \leq 0.05$ by DMRT

Table 3.4 Effect of soil residual arsenic on yield of BRRi dhan-50

Previous season arsenic in irrigation water (mg/L)	Residual arsenic in soil (mg/kg)	Grain yield (gm/plant)	Straw yield (gm/plant)	Percent of yield over control	
				Grain	Straw
0.0	3.67	6.55 ± 1.02a	14.81 ± 1.98a	0	0
0.1	6.11	13.19 ± 2.39a	17.94 ± 1.58a	101.374046	21.1343687
0.5	7.23	8.46 ± 1.22a	15.21 ± 3.05a	29.1603053	2.70087779
1	9.85	9.03 ± 2.68a	12.63 ± 3.15a	37.8625954	14.7197839
2	13.68	13.25 ± 2.66a	19.13 ± 2.16a	102.290076	29.1694801
4	14.61	8.5 ± 2.69a	16.67 ± 2.76a	29.7709924	12.5590817

Same letters in a column did not differ significantly at $p \leq 0.05$ by DMRT

Table 3.5 Effect of soil residual arsenic on Spikelet number of BRRI dhan-50

Previous season arsenic in irrigation water (mg/L)	Residual arsenic in soil (mg/kg)	Filled spikelet number	Empty spikelet number
0.0	3.67	362.16 ± 58.77a	605.66 ± 99.35b
0.1	6.11	812.16 ± 149b	678.33 ± 50.02b
0.5	7.23	504.16 ± 30.99ab	632.50 ± 111.24b
1	9.85	542.66 ± 182.95ab	352.00 ± 91.00a
2	13.68	790.00 ± 155.54b	689.16 ± 75.78b
4	14.61	426.66 ± 103.71ab	728.33 ± 61.39b

Same letters in a column did not differ significantly at $p \leq 0.05$ by DMRT

Table 3.6 Arsenic accumulation in soil, straw and grain of BRRI dhan-50.

Previous season arsenic in irrigation water (mg/L)	Residual arsenic in soil (mg/kg)	Arsenic in straw (mg/kg)	Arsenic in grain (mg/kg)
0.0	3.67	2.06 ± 0.04e	0.57 ± 0.03e
0.1	6.11	1.82 ± 0.03d	0.46 ± 0.02d
0.5	7.23	1.28 ± 0.02c	0.31 ± 0.02c
1	9.85	1.15 ± 0.02b	0.28 ± 0.01bc
2	13.68	1.08 ± 0.02a	0.24 ± 0.01ab
4	14.61	1.05 ± 0.01a	0.22 ± 0.01a

Same letters in a column did not differ significantly at $p \leq 0.05$ by DMRT

Table 3.7 Matrix of arsenic accumulation correlation coefficient among soil, straw and grain arsenic.

		Arsenic concentration in soil (mg/kg)	Arsenic accumulation in straw (mg/kg)	Arsenic accumulation in grain (mg/kg)
Arsenic concentration in soil (mg/kg)	Pearson Correlation	1	.917(**)	.892(**)
	Sig. (2-tailed)		.000	.000
Arsenic accumulation in straw (mg/kg)	Pearson Correlation	.917(**)	1	.975(**)
	Sig. (2-tailed)	.000		.000
Arsenic accumulation in grain (mg/kg)	Pearson Correlation	.892(**)	.975(**)	1
	Sig. (2-tailed)	.000	.000	

** indicate correlation is significant at the 0.01 level (2-tailed). N=36

Appendix 3

Table 4.4 Ground Water Arsenic Contamination and Arsenicosis Patients in Shibganj Upazila of Chapai Nawabganj District, Bangladesh

SL no.	Union	No. of HTW Surveyed	No. of H TW Operative	No. of Arsenic Safe HTW	No. of Arsenic Contaminated HTW	% of Arsenic Contaminated HTW	Arsenicosis Patients
1	Binodpur	2662	2640	1833	814	30.33	27
2	Chak Kirti	2005	1965	1429	536	27.28	9
3	Daipukuria	1994	1928	1905	23	1.19	0
4	Durlabpur	3775	3691	3078	613	16.61	61
5	Ghorapakhia	1185	1159	897	262	22.61	12
6	Kansat	3157	3127	3052	75	2.40	1
7	Manakosa	3552	3533	2758	771	21.82	26
8	Mobarakpur	1711	1658	1576	82	4.95	5
9	Nayanaobhanga	4067	3991	2331	1660	41.59	97
10	Panka	1572	1555	1426	129	8.30	14
11	Satrujitpur	1901	1860	943	917	49.30	141
12	Shahbajpur	3555	3507	2885	622	17.74	210
13	Shibganj	4177	4113	3644	459	11.4	32
14	Shyampur	2856	2837	2370	467	16.46	16
15	Uzirpur	671	655	615	40	6.11	12
Summary		40591	39859	32281	7581	19.02	665

Source: DPHE, 2003

Table 4.5 Effect of Arsenic Contaminated Pump Distance on Arsenic Accumulation During Boro Season

Paddy field			Arsenic Accumulation (ppm)			
Distance from arsenic contaminated pump (ft)	Geographical location	Elevation from mean sea level (ft)	Drainage sediment	Paddy soil	Rice straw	Rice grain
10	N-24°47'15.9"-24°47'16.2" E-088°06'54.7"-088°06'54.9"	65.54±0.09	42.28 ± 1.17a	19.84±0.05 a	2.75±.02 a	0.87±0.01a
50	N-24°47'15.9"-24°47'16.2" E-088°06'54.2"-088°06'54.4"	65.38±0.08	39.23 ± .74ab	19.77±0.04a	2.71±.02ab	0.86± 0.01a
100	N-24°47'15.9"-24°47'16.2" E-088°06'53.7"-088°06'53.9"	65.15±0.13	34.28 ± .90bc	19.69±0.04ab	2.67±0.01b	0.83±0.01ab
150	N-24°47'15.9"-24°47'16.2" E-088°06'53.2"-088°06'53.4"	64.95±0.09	30.21 ± 1.61c	19.57 ± 0.05b	2.64±0.02bc	0.81±0.02b
300	N-24°47'15.9"-24°47'16.2" E-088°06'52.7"-088°06'52.9"	64.47±0.14	21.89 ± 1.55d	19.37 ±0.05c	2.58±0.01c	0.76± 0.02c

Table 4.6 Residual Effects of Boro Season Arsenic on Arsenic Accumulation During Aman Season

Paddy field			Arsenic in Boro paddy soil (ppm)	Arsenic accumulation during Aman season		
Distance from arsenic contaminated pump (ft)	Geographical location	Elevation from mean sea level (ft)		Soil	Rice straw	Rice grain
10	N-24°47'15.9"-24°47'16.2" E-088°06'54.7"-088°06'54.9"	65.54±0.09	19.84±0.05	8.05±0.17a	1.51±0.04 a	0.07±0.01a
50	N-24°47'15.9"-24°47'16.2" E-088°06'54.2"-088°06'54.4"	65.38±0.08	19.77±0.04	8.38±0.14ab	1.55±0.05ab	0.07±0.01a
100	N-24°47'15.9"-24°47'16.2" E-088°06'53.7"-088°06'53.9"	65.15±0.13	19.69±0.04	8.86±0.23bc	1.60± 0.04ab	0.08±0.01ab
150	N-24°47'15.9"-24°47'16.2" E-088°06'53.2"-088°06'53.4"	64.95±0.09	19.57 ± 0.05	9.27±0.17c	1.64± 0.05 b	0.09±0.01ab
300	N-24°47'15.9"-24°47'16.2" E-088°06'52.7"-088°06'52.9"	64.47±0.14	19.37 ±0.05	10.07±0.40d	1.76± 0.1c	0.1±0.01b

Table 4.7 Comparison between Arsenic accumulation during Boro and Aman season

Paddy field			As accumulation during Boro season (ppm)			As accumulation during Aman season (ppm)		
Distance from arsenic contaminated pump (ft)	Geographical location	Elevation from mean sea level (ft)	Soil	Rice straw	Rice grain	Soil	Rice straw	Rice grain
10	N-24°47'15.9"-24°47'16.2" E-088°06'54.7"-088°06'54.9"	65.54±0.09	19.84 ± 0.05 a	2.75 ±. 02 a	0.87±0.01a	8.05±0.17a	1.51±0.04 a	0.07±0.01a
50	N-24°47'15.9"-24°47'16.2" E-088°06'54.2"-088°06'54.4"	65.38±0.08	19.77 ± 0.04a	2.71 ± 0.02ab	0.86± 0.01a	8.38±0.14ab	1.55±0.05ab	0.07±0.01a
100	N-24°47'15.9"-24°47'16.2" E-088°06'53.7"-088°06'53.9"	65.15±0.13	19.69 ± 0.04ab	2.67 ± 0.01b	0.83±0.01ab	8.86±0.23bc	1.60± 0.04ab	0.08±0.01ab
150	N-24°47'15.9"-24°47'16.2" E-088°06'53.2"-088°06'53.4"	64.95±0.09	19.57 ± 0.05b	2.64±0.02bc	0.81±0.02b	9.27±0.17c	1.64± 0.05 b	0.09±0.01ab
300	N-24°47'15.9"-24°47'16.2" E-088°06'52.7"-088°06'52.9"	64.47±0.14	19.37 ± 0.05c	2.58±0.01c	0.76± 0.02c	10.07±0.40d	1.76± 0.1c	0.1±0.01b

Table 4.8 Arsenic Accumulation in Different Varieties of Rice During Boro (dry) and Aman (wet) Season

Season	Varieties	Arsenic accumulation (ppm)	
		Straw	Grain
Boro	BRRI dhan 36	3.43±0.40a	0.97±0.02a
	Somsi	2.74±0.28a	0.87±0.03b
Aman	Shorna	1.77± 0.17b	0.1±0.01c
	Moahka (aromatic)	1.51± 0.11b	0.07±0.01c

Table 4.9 Correlations among Pump Distance and Arsenic Accumulation in Drainage Sediment, Paddy Field Soil, Rice Straw and Grain during Boro Season

		Distance between paddy field and As contaminated pump (ft)	Arsenic in drainage sediment (ppm)	Arsenic in paddy soil (ppm)	Arsenic in straw (ppm)	Arsenic in grain (ppm)
Distance between paddy field and As contaminated pump (ft)	Pearson Correlation	1	-.944(**)	-.915(**)	-.860(**)	-.883(**)
	Sig. (2-tailed)		.000	.000	.000	.000
Arsenic in drainage sediment (ppm)	Pearson Correlation	-.944(**)	1	.989(**)	.970(**)	.968(**)
	Sig. (2-tailed)	.000		.000	.000	.000
Arsenic in paddy soil (ppm)	Pearson Correlation	-.915(**)	.989(**)	1	.980(**)	.979(**)
	Sig. (2-tailed)	.000	.000		.000	.000
Arsenic in straw (ppm)	Pearson Correlation	-.860(**)	.970(**)	.980(**)	1	.947(**)
	Sig. (2-tailed)	.000	.000	.000		.000
Arsenic in grain (ppm)	Pearson Correlation	-.883(**)	.968(**)	.979(**)	.947(**)	1
	Sig. (2-tailed)	.000	.000	.000	.000	

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.10 Correlations among Paddy Field Elevation and Arsenic Accumulation in Soil, Rice Straw and Grain during Aman Season

		Arsenic in Aman paddy soil (ppm)	Arsenic in straw	Arsenic in grain	Elevation from mean sea level (ft)
Arsenic in Aman paddy soil (ppm)	Pearson Correlation	1	.991(**)	.951(**)	-.992(**)
	Sig. (2-tailed)		.000	.000	.000
Arsenic in straw	Pearson Correlation	.991(**)	1	.959(**)	-.982(**)
	Sig. (2-tailed)	.000		.000	.000
Arsenic in grain	Pearson Correlation	.951(**)	.959(**)	1	-.924(**)
	Sig. (2-tailed)	.000	.000		.000
Elevation from mean sea level (ft)	Pearson Correlation	-.992(**)	-.982(**)	-.924(**)	1
	Sig. (2-tailed)	.000	.000	.000	

** Correlation is significant at the 0.01 level (2-tailed).