EVALUATION OF DIFFERENT PLANTS AS ARSENIC ACCUMULATOR FROM CONTAMINATED SOIL

BY

MD. MANIRUL ISLAM

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Approved by:

Dr. Rokeya Begum
Professor
Department of Agricultural Chemistry,
SAU, Dhaka.
Supervisor

Dr. A.F.M Jamal Uddin
Associate Professor
Department of Horticulture,
SAU, Dhaka.
Co-Supervisor

Dr. Md. Abdur Razzaque
Chairman
Examination Committee

DEPARTMENT OF AGRICULTURAL CHEMISTRY
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Bangladesh

PABX: +88029144270-9
Ext. 309 (Off.)
Fax: +88029112649
e-mail:
bioc_sau@ymail.com

Ref : **Date:**

CERTIFICATE

This is to certify that the thesis entitled, **"EVALUATION OF DIFFERENT PLANTS AS ARSENIC ACCUMULATOR FROM CONTAMINATED SOIL"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURAL CHEMISTRY**, embodies the result of a piece of bona fide research work carried out by **MD. MANIRUL ISLAM**, Registration No. **06-01896** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: June, 2013
Place: Dhaka, Bangladesh

Prof. Dr. Rokeya Begum
Department of Agricultural Chemistry
Sher-e-Bangla Agricultural University
Supervisor

DEDICATED TO

Who

Struggle with arsenic toxicity

LIST OF ACRONYMS

AEZ	Agro-Ecological Zone
Anon.	Anonymous
BBS	Bangladesh Bureau of Statistics
BCSIR	Bangladesh Council of Scientific and Industrail Research
cm	Centi-meter
CV %	Percent Coefficient of Variance
DAT	Days After Transplanting
DMRT	Duncan's Multiple Range
et al.	And others
e.g.	exempli gratia (L), for example
etc.	Etcetera
FAO	Food and Agricultural Organization
g	Gram (s)
HCl	Hydro Chloric Acid
i.e.	id est (L), that is
kg	Kilogram (s)
KI	Potassium Iodide
LSD	Least Significant Difference
m^2	Meter squares
mL	Mili Litre
M.S.	Master of Science
No.	Number
SAU	Sher-e-Bangla Agricultural University
var.	Variety
C	Degree Celsius
%	Percentage
NaOH	Sodium hydroxide
$NaAsO_2$	Sodium arsenite
GM	Geometric mean
CEC	Cation-exchange capacity
As	Arsenic
mg	Mili gram
Na_2HAsO_4	sodium arsenate
Si	Silicon
P	Phosphorus
K	Potassium
Ca	Calcium
MPL	Maximum permissible limit
HG-AAS	Hydride Generation–Atomic Absorption Spectroscopy
L	Litre
ICP-MS	Inductivity coupled plasma mass spectrometry
µg	Microgram
S-XRF	Synchrotron-based X-ray fluorescence
DMAA	dimethylarsinic acid
USA	United States of America
WHO	World Health Organization

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The Author

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EVALUATION OF DIFFERENT PLANTS AS ARSENIC ACCUMULATOR FROM CONTAMINATED SOIL

BY

MD. MANIRUL ISLAM

ABSTRACT

Three pot experiment were conducted at the Horticultural farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh to mitigate arsenic from soil using trap plant from contaminated soil during the period from July 2012 to October 2012 followings Completely Randomized Design (CRD) with three replications. Rice, arum and fern (*Pteris vittata* L.) were used in the three different experiment, respectively and each of the experiment soil was treated with defferent arsenic concentration viz. As_0, Control; As_{500}, 500 ppm; As_{1000}, 1000 ppm; As_{2000}, 2000 ppm of arsenic were exploited. Fern (*Pteris vittata* L.) can survive with a very high concentration of arsenic in soil and accumulate 23837.2, 15332.6 and 17007.1 ppm arsenic when soil treated with 2000, 1000 and 500 ppm arsenic, respectively. Whereas rice straw acumulated 206.3, 194.9, and 82.9 ppm arsenic, rice grain accumulated 61.6, 36.5, and 29.9 ppm arsenic, arum leaves acuumulated 973.5, 751.7 and 422.3 ppm arsenic and arum runner accumulated 210.4, 202.3 and 178.8 ppm arsenic when soil treated with 2000, 1000 and 500 ppm arsenic, respectively. Using *Pteris vittata* L. as a trap plant is a possible way to mitigate arsenic from soil which can keep away of arsenic pollution in food chain.

CHAPTER I

INTRODUCTION

The serious arsenic contamination of groundwater as well as soil in Bangladesh has come out recently as the biggest natural calamity in the world. The people in Bangladesh are suffering due to the arsenic contamination. Exposure to high levels of arsenic can cause cancers and other disorders. Arsenic contamination of groundwater is a serious problem in Bangladesh. The arsenic comes from arsenic rich material in the regions river systems, deposited over thousands of years along with the sands and gravels which make up the land of Bangladesh. In Bangladesh, peoples are suffering by Arsenic poisoning which is occurred not only by taking arsenic contaminated water but also by the arsenic contaminated food. Now days it suggests that widespread use of groundwater for irrigation is another route of arsenic which enter the food chain and indirectly affect human health. Duxbury *et al.* (2003), was mentioned the presence of arsenic in food chain. Food could be a way of arsenic entry into human body through water-soil-plant transfer (Duxbury *et al.*, 2003; Kurosawa *et al.*, 2008). Some of our foodstuffs are also contaminated with arsenic (*Huq et al.*, 2006; Meharg and Rahman, 2003; Das *et al.*, 2004). It might be due to the high concentrations of arsenic in the soil where the foodstuffs are grown. Arsenic contaminated water used in irrigation contaminates soils, and then uptake by plants causes arsenic contamination of the edible portions of plants, such as vegetables and rice grains. Arsenic in these contaminated foods is then consumed by humans. Due to the lack of surface water, insufficient rainfall and soluble form of arsenic increase the arsenic amount in soil. Arsenic enter the food chain by its available from and indirectly affect human health. Indirect effect of arsenic poisons is disrupting the digestive system, change in skin color, formation of skin patches, stomach pains, vomiting, delirium and gangrene.

About 33 percent of total arable land of our country is now under irrigation facilities (BBS, 1996). Most of the lands are irrigated with groundwater which comes from deep tube well, shallow tube well and hand tube well. Most groundwater used for irrigation in Bangladesh is contaminated with arsenic (Khan *et al.,* 1998). If arsenic contaminated water is used for irrigation, it may create hazard both in soil environment and in crop quality and also food is contaminated by arsenic present in soil and irrigated water. Beside the natural occurrence of arsenic, indiscriminate use of arsenical pesticides worldwide has led to extensive contamination of agricultural soils (Smith *et al.,* 1998). As the plant uptake water and nutrient, so this arsenic has come to the leaves, flowers as well as grains of the food and after consuming the arsenic contaminated food peoples are affected severely. High arsenic irrigated water and soil appears to result in higher concentration of arsenic in root, stem and leaf of rice plants (Abedin *et al.,* 2002b). Rice is very efficient in arsenic accumulation, thus posing a potential health risk to people who eat lot of rice. Arum (kochu), a green vegetable commonly grown and used almost everywhere in the country, is a very rich source of vitamin A and C. But concentration of arsenic is very high all part of the arum plant. It is found that arum (kochu) vegetable accumulate more than 150 mg kg^{-1} of arsenic from contaminated soil. (Huq *et al.,* 2006).

Arsenic contamination in soil increases day by day, it is important to remove arsenic from soil and prevent possible impacts on the ecosystems. Remove of arsenic from soil and render to enter into food chain by using bioremediation process like phytoremediation, bioreactor, composting, bioventing, bioleaching, bioagumentation, land farming are the active way to remove arsenic from soil. Phytoremediation is the use of plants to remove or render contaminants harmless in the ecosystem. Phytoremediation is the use of plants, preferably hyperaccumulators, to take up contaminants. Subsequently, the plants are harvested, transported and disposed off site (Schnoor 2002). On the other hand phytoremediation process is very easy and low cost method for farmers. Hyperaccumulates is one of the important process for phytoremediation. A hyperaccumulator is a plant capable of growing in soils with very high concentrations of metals, extracting these metals through their roots, and concentrating extremely high levels of metals in their tissues. Using of non edible plants also uptake arsenic from ground water and soil thus may help to reduce the amount of arsenic which doesn't causes any hazard to the human health. Recently, an arsenic hyperaccumulator plant species *Pteris vittata* L. (Chinese brake fern) by namewas discovered Ma *et al.,* (2001). This arsenic hyperaccumulator may offer an alternative to more traditional remediation technologies for arsenic contaminated soils.

Avaiable form of arsenic in soil and groundwater is uptake by palnt root with other nutrient which indirectly enter to vegetables, fruits, cereals etc., these fruit sell in Agora, Nandon, Minabazar and so many big shops.

Today arsenic problem is one of the concern global issues. To get rid out of this problem current research was conducted with following objectives-

i. To make comparative study on arsenic accumulation from soil by different plants

ii. To find out a suitable plant to mitigate arsenic contamination from soil

CHAPTER II

REVIEW OF LITERATURE

Arsenic problem is one of the most promising concerns not only in Bangladesh but also whole world. However, some of important and informative works so far been done in home and abroad related to this experimentation have been presented in this chapter.

Azad *et al.* (2013) conducted a green house field experiment of rice (*Oryza sativa* L) with arsenic amended irrigation water was conducted at Institute of Environmental Science of Rajshahi University to observe the trend of arsenic (As) accumulation into rice and soil. They were used sodium arsenate (Na2HAsO4) amended irrigation water (0.0, 0.1, 0.5, 1.0, 2.0 and 4.0 mgL^{-1} As) for cultivating BR-11 rice. They were found A significant ($p \leq 0.01$) increasing trend of arsenic accumulation in straw, grain and soil with increase of arsenic in irrigation water. They observed the highest level of arsenic in straw, grain and soil in the treatment of 4.0 mgL^{-1} 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.

Santra *et al.* (2013) found that tuberous vegetables accumulated higher amount of arsenic than leafy vegetables and leafy vegetables followed by fruity vegetable. The highest arsenic accumulation was observed in potato, brinjal, arum, amaranth, radish, lady's finger, cauliflower whereas lower level of arsenic accumulation was observed in beans, green chilli, tomato, bitter guard, lemon, turmeric. The major oil seed of this region is mustard and was found to accumulate arsenic in the range 0.339-0.373 mg kg^{-1}. In pulses group, pea showed the highest arsenic content of 1.30 mg kg^{-1} whereas moong (Mung bean) found the lowest value (0.314 mg kg^{-1}). The arsenic accumulation was found to be more in Boro rice than in Aman, while high yielding rice varieties were found to accumulate more arsenic than local.

Pteris vittata L. was found as the arsenic hyperaccumulating plant. It can survive with a very high concentration of arsenic in soil and accumulate 27829.7 ppm, 23274.7 ppm and 14911.0 ppm arsenic when soil treated with 4000 ppm, 2000 ppm and 1000 ppm arsenic respectively (Umma *et al.*, 2013).

The threshold amount of arsenic that leads to exceed the maximum permissible limit (MPL) in those vegetables and several water-soil-plant arsenic concentration models. Total arsenic concentrations were measured by Hydride Generation–Atomic Absorption Spectroscopy (HG-AAS) technique. Arsenic-accumulation decreased in the order: Arum > Arum leaf > Amaranth > Brinjal > Radish > Indian Spinach > Carrot > Okra. A single harvesting of 10 irrigations with water (3.0 L irrigation^{-1}) having arsenic concentrations of ≥0.45 mg L^{-1} to 0.071 m^2 area (equivalent to 1.89 kg As ha^{-1}) exceeded the MPL in vegetables (1 mg kg^{-1}, wet weight). The concentration of accumulated arsenic in the vegetables increased linearly with time and exponentially with successive harvesting. Regression analyses showed that arsenic concentration in vegetables was positively correlated with that of irrigation water and soil ($r = 0.796$ for both cases). (Islam *et al.*, 2012)

Bhattacharya *et al.* (2010a) conducted an experiment on accumulation of arsenic and its distribution in rice plant (*Oryza sativa* L.) in Gangetic West Bengal, India. Results showed that the level of arsenic in irrigation water (0.05–0.70 mg l^{-1}) was much above the WHO recommended arsenic limit of 0.01 mg l^{-1} 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^{-1}. 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^{-1}) as compared to the straw (1.18 ± 0.002–2.13 ± 0.009 mg kg^{-1}), husk (0.40 ± 0.004–1.05 ± 0.006 mg kg^{-1}), and grain (0.16 ± 0.001–0.58 ± 0.003 mg kg^{-1}) 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^{-1} dry weights of arsenic, which did not exceed the permissible limit in rice (1.0 mg kg^{-1}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).

Bhattacharya *et al.* (2010b) said that the arsenic-contaminated irrigation water (0.318–0.643 mg l^{-1}) and soil (5.70–9.71 mg kg^{-1}) 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^{-1}) and FAO permissible limit for irrigation water (0.10 mg l^{-1}). But, the levels of arsenic in soil were lower than the reported global average of 10.0 mg kg^{-1}and was much below the EU recommended maximum acceptable limit for agricultural soil (20.0 mg kg^{-1}). The total arsenic concentrations in the studied samples ranged from <0.0003 to 1.02 mg kg^{-1}. 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).

Choudhury *et al.* (2009) has been found that arsenic (As) in excess of acceptable limit in ground water in many parts of Bangladesh which is one of the densely populated countries in the world. Such kind of groundwater is extensively used for the purpose of irrigation mostly in rural areas of the country. Growth and overall yield of the crop can be affected by up taking arsenic from this contaminated water. An experimental study was conducted to observe the effects of such water on the growth and yield of red amaranth vegetable plant (*Amaranthus retroflexus* L.) and also check their rate of uptake from soil to the body of the plant. Different arsenic concentrations from 0 to 60 mg L^{-1} were mixed with water and applied to the plant in a controlled environment. The yield of amaranth was significantly reduced which was observed from the experiment. Arsenic content of root, stem and leaf was also examined with different doses and a relationship was established between doses and uptake rate.

Zhao *et al.* (2009) narrated that arsenic (As) is an element that is nonessential for and toxic to plants. Arsenic contamination in the environment occurs in many regions, and, depending on environmental factors, its accumulation in food crops may pose a health risk to humans. Arsenate is taken up by phosphate transporters. A number of the aquaporin nodulin 26-like intrinsic proteins (NIPs) are able to transport arsenite, the predominant form of As in reducing environments. In rice (*Oryza sativa*), arsenite uptake shares the highly efficient silicon (Si) pathway of entry to root cells and efflux towards the xylem. In root cells arsenate is rapidly reduced to arsenite, which is effluxed to the external medium, complexed by thiol peptides or translocated to shoots. One type of arsenate reductase has been identified, but its in planta functions remain to be investigated. Some fern species in the Pteridaceae family are able to hyper accumulate As in above-ground tissues. Hyperaccumulation appears to involve enhanced arsenate uptake, decreased arsenite-thiol complexation and arsenite efflux to the external medium, greatly enhanced xylem translocation of arsenite, and vacuolar sequestration of arsenite in fronds.

Gonzaga *et al.* (2008) evaluated arsenic removal by *Pteris vittata* and its effects on arsenic redistribution in soils. They grew *Pteris vittata* in six arsenic-contaminated soils and its fronds were harvested and analyzed for arsenic. The soil arsenic was separated into five fractions via sequential extraction. The ferns grew well and took up arsenic from all soils. Fern biomass ranged from

24.8 to 33.5 g $plant^{-1}$ after 4 months of growth but was reduced in the subsequent harvests. The frond arsenic concentrations ranged from 66 to 6,151 mg kg^{-1}, 110 to 3,056 mg/ kg^{-1}, and 162 to 2,139 mg kg^{-1} from the first, second and third harvest, respectively. *Pteris vittata* reduced soil arsenic by 6.4 to13% after three harvests. Arsenic in the soils was primarily associated with amorphous hydrous oxides (40-59%), which contributed the most to arsenic taken up by P. vittata (45-72%). It is possible to use P. vittata to remediate arsenic-contaminated soils by repeatedly harvesting its fronds.

Ma *et al.* (2008) reported that two different types of transporters mediate transport of arsenite, the predominant form of arsenic in paddy soil, from the external medium to the xylem. Transporters belonging to the NIP subfamily of aquaporins in rice are permeable to arsenite but not to arsenate. Mutation in $OsNIP_2$;1 (Lsi_1, a silicon influx transporter) significantly decreases arsenite uptake. Furthermore, in the rice mutants defective in the silicon efflux transporter Lsi_2, arsenite transport to the xylem and accumulation in shoots and grain decreased greatly. Mutation in Lsi_2 had a much greater impact on arsenic accumulation in shoots and grain in field-grown rice than Lsi_1. Arsenite transport in rice roots therefore shares the same highly efficient pathway as silicon, which explains why rice is efficient in arsenic accumulation. Our results provide insight into the uptake mechanism of arsenite in rice and strategies for reducing arsenic accumulation in grain for enhanced food safety.

Meharg *et al.* (2008) conducted an experiment on Speciation and Localization of Arsenic in White and Brown Rice Grains. In this experiment Synchrotron-based X-ray fluorescence (S-XRF) was utilized to locate arsenic (As) in polished (white) and unpolished (brown) rice grains from the United States, China, and Bangladesh. In white rice As was generally dispersed throughout the grain, the bulk of which constitutes the endosperm. In brown rice As was found to be preferentially localized at the surface, in the region corresponding to the pericarp and aleurone layer. Copper, iron, manganese, and zinc localization followed that of arsenic in brown rice, while the location for cadmium and nickel was distinctly different, showing relatively even distribution throughout the endosperm. The localization of As in the outer grain of brown rice was confirmed by laser ablation ICP–MS. Arsenic speciation of all grains using spatially resolved X-ray absorption near edge structure (μ-XANES) and bulk extraction followed by anion exchange HPLC–ICP–MS revealed the presence of mainly inorganic As and dimethylarsinic acid (DMA). However, the two techniques indicated different proportions of inorganic: organic As species. A wider survey of whole grain speciation of white (n = 39) and brown (n = 45) rice samples from numerous sources (field collected, supermarket survey, and pot trials) showed that brown rice had a higher proportion of inorganic arsenic present than white rice. Furthermore, the percentage of DMA present in the grain increased along with total grain arsenic.

Smith *et al.* (2008) conducted an experiment an were found that the accumulation of arsenic (As) by rice (*Oryza sativa* L.) was of great interest considering the dietary intake of rice is potentially a major As exposure pathway in countries where rice is irrigated with As contaminated groundwater. A small scale rice paddy experiment was conducted to evaluate the uptake of As by rice. 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^{-1}. 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.

The vast majority (85%) of the market rice grains possessed total As levels < 150 ng g^{-1}. The rice collected from mine-impacted regions, however, were found to be highly enriched in As, reaching concentrations of up to 624 ng g^{-1}. Inorganic As (Asi) was the predominant species detected in all of the speciated grain, with Asi levels in some samples exceeding 300 ng g^{-1} (Zhu *et al.*, 2008).

Huq *et al.* (2006) narrated Arsenic contamination in groundwater in Bangladesh has become an additional concern vis-à-vis its use for irrigation purposes. Even if arsenic-safe drinking-water is assured, the question of irrigating soils with arsenic-laden groundwater will continue for years to come. Immediate attention should be given to assess the possibility of accumulating arsenic in soils through irrigation-water and its subsequent entry into the food-chain through various food crops and fodders. They analyzed arsenic content of 2,500 water, soil and vegetable samples from arsenic-affected and arsenic-unaffected areas during 1999–2004 also other sources of foods and fodders. Irrigating a rice field with groundwater containing 0.55 mg L^{-1} of arsenic with a water requirement of 1,000 mm results in an estimated addition of 5.5 kg of arsenic per ha per annum. Concentration of arsenic as high as 80 mg kg^{-1} of soil was found in an area receiving arsenic-contaminated irrigation. A comparison of results from affected and unaffected areas revealed that some commonly-grown vegetables, which would usually be suitable as good sources of nourishment, accumulate substantially-elevated amounts of arsenic. For example, more than 150 mg kg^{-1} of arsenic has been found to be accumulated in arum (kochu) vegetable.

Wei *et al.* (2006) conducted an experiment to compare the factors influencing arsenic (As) accumulation by *Pteris vittata* at two sites, one containing As along with Au mineralization and the other containing Hg/Tl mineralization. The soils above these two sites contained high As concentrations (26.8 - 2955 mg kg^{-1}). Although the As concentration, pH, soil cation exchange capacity and plant biomass differed significantly between the two sites, no differences were observed in the As concentrations in the fronds and roots, or the translocation factors, of *Pteris vittata*, suggesting that this species has consistent As hyperaccumulation properties in the field. The As concentration in the fronds was positively related to phosphorus (P) and potassium (K), but negatively related to calcium (Ca), at one site. Experiment also suggested that P, K and Ca influenced As accumulation by *Pteris vittata* in the field.

Williams *et al.* (2006) conducted an experiment which addresses the speciation and concentration of arsenic in rice, vegetables, pulses, and spices. Three hundred thirty aman and boro rice, 94 vegetables, and 50 pulse and spice samples were analyzed for total arsenic, using inductivity coupled plasma mass spectrometry (ICP-MS). The districts with the highest mean arsenic rice grain levels were all from southwestern Bangladesh: Faridpur (boro) 0.51 > Satkhira (boro) 0.38 > Satkhira (aman) 0.36 > Chuadanga (boro) 0.32 > Meherpur (boro) 0.29 μg As g^{-1}. The vast majority of food ingested arsenic in Bangladesh diets was found to be inorganic; with the predominant species detected in Bangladesh rice being arsenite (AsIII) or arsenate (AsV) with dimethyl arsinic acid (DMA) being a minor component. Vegetables, pulses, and spices are less important to total arsenic intake than water and rice. Predicted inorganic arsenic intake from rice is modeled with the equivalent intake from drinking water for a typical Bangladesh diet. Daily consumption of rice with a total arsenic level of 0.08 μg As g^{-1} would be equivalent to a drinking water arsenic level of 10 μg L^{-1}.

Al Rmalli *et al.* (2005) conducted an exprement to determine the total arsenic concentrations in a range of foodstuffs, including vegetables, rice and fish, imported into the United Kingdom from Bangladesh. The mean and range of the total arsenic concentration in all the vegetables imported from Bangladesh were 54.5 and 5–540 μg kg^{-1}, respectively. The highest arsenic values found were for the skin of Arum tuber, 540 μg kg^{-1}, followed by Arum Stem, 168 μg/kg, and Amaranthus, 160 μg kg^{-1}. Among the other samples, freshwater fish contained total arsenic levels between 97 and 1318 μg kg^{-1}. The arsenic content of the vegetables from the UK was approximately 2 to 3 fold lower than those observed for the vegetables imported from Bangladesh.

To understand the mechanisms of arsenic hyperaccumulation in Pteris vittata by comparing the characteristics of arsenic accumulation in Pteris and non-Pteris ferns seven Pteris (*Pteris vittata, Pteris Cretica Rowerii, Pteris Cretica Parkerii, Pteris Cretica Albo-lineata, Pteris Quadriavrita, Pteris Ensiformis* and *Pteris Dentata*) and six non-Pteris (Arachnoides simplicor, Didymochlaena truncatula, Dryopteris atrata, Dryopteris erythrosora, Cyrtomium falcatum, and Adiantum hispidulum) ferns were exposed to 0, 1 and 10 mg L^{-1} arsenic as sodium arsenate for 14-d in hydroponic systems. As a group, the Pteris ferns were more efficient in arsenic accumulation than the non-Pteris ferns, with *Pteris vittata* being the most efficient followed by *Pteris cretica*. When exposed to 10 mg L^{-1} As, arsenic concentrations in the fronds and roots of *Pteris vittata* were 1748 and 503 mg kg^{-1}. Though not all Pteris ferns were efficient in accumulating arsenic, none of the non-Pteris ferns was an efficient As accumulator (the highest concentration being 452 mg kg^{-1}. Their research confirms that the ability of *Pteris vittata* to translocate arsenic from the roots to the fronds (73–77% As in the fronds), reduce arsenate to arsenite in the fronds (>50% AsIII in the fronds), and maintain high concentrations of phosphate in the roots (48–53% in the roots) all contributed to its arsenic tolerance and hyperaccumulation (Luongo and Ma, 2005).

Williams *et al.* (2005) found that USA long grain rice had the highest mean arsenic level in the grain at 0.26 μg As g^{-1} ($n = 7$), and the highest grain arsenic value of the survey at 0.40 μg As g^{-1} . The mean arsenic level of Bangladeshi rice was 0.13 μg As g^{-1} ($n = 15$). The main As species detected in the rice extract were AsIII, DMAV, and AsV. In European, Bangladeshi, and Indian rice 64 ± 1% ($n = 7$), 80 ± 3% ($n = 11$), and 81 ± 4% ($n = 15$), respectively, of the recovered arsenic was found to be inorganic. In contrast, DMAV was the predominant species in rice from the USA, with only 42 ± 5% ($n = 12$) of the arsenic being inorganic.

Das *et al.* (2004) stated that arsenic contaminating groundwater in Bangladesh is one of the largest environmental health hazards in the world. Because of the potential risk to human health through consumption of agricultural produce grown in fields irrigated with arsenic contaminated water, they have determined the level of contamination in 100 samples of crop, vegetables and fresh water fish collected from three different regions in Bangladesh. 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^{-1}. However, rice plants, especially the roots had a significantly higher concentration of arsenic (2.4 mg kg^{-1}) compared to stem (0.73 mg kg^{-1}) and rice grains (0.14 mg kg^{-1}). Arsenic contents of vegetables varied; those exceeding the food safety limits included Kachu sak (Colocasia antiquorum) (0.09–3.99 mg kg^{-1}, n=9), potatoes (Solanum tuberisum) (0.07–1.36 mg kg^{-1}, n=5), and Kalmi sak (Ipomoea reptoms) (0.1–1.53 mg kg^{-1}, n=6). Lata fish (Ophicephalus punctatus) did not contain unacceptable levels of arsenic. These results indicate that arsenic contaminates some food items in Bangladesh.

Meharg (2004) conducted an experiment on arsenic in rice – understanding a new disaster for South-East Asia. Their finding that arsenic is sequestered in iron plaque on root surfaces in plants, regulated by phosphorus status, and that there is considerable varietal variation in arsenic sequestration and subsequently plant uptake, offers a hope for breeding rice for the new arsenic disaster in South-East Asia – the contamination of paddy soils with arsenic.

Das *et al.* (2003) found that elevated levels of arsenic contents in tube well water (range: 0.29-0.71 ppm, n = 6), soils (range: 5.64-29.47 ppm DW, n =16), human scalp hair, (range: 2.41-14.91 ppm DW, n = 8), kachu (range: 0.11-3.89 ppm FW, n = 5), water spinach (range: 0.091-2.032 ppm FW, n =6), potato (range: 0.02-1.86 ppm FW, n = 5), danta stem (1.41 ppm FW, n=1) and paddy rice (range: 0.02-3.40 ppm DW, n = 13). No significant level of arsenic was found in balsam apple (n=5), pointed gourd (n=6), ladies finger (n=1) jute leave (n=1) and catfish (n=4). Extensive withdrawal of arsenic contaminated groundwater contaminates surface soils and plants and thus affects the food chain.

Duxburya *et al.* (2003) was conducted an experiment to determine the total arsenic content of 150 paddy rice samples collected from Barisal, Comilla, Dinajpur, Kaunia, and Rajshahi districts, and from the BRRI experimental station at Rajshahi city in the boro and aman seasons of 2000 was determined by hydride generation-inductively coupled plasma emission spectroscopy (ICP). They found that arsenic concentrations varied from 10 to 420 μg kg^{-1} at 14% moisture content. Rice yields and grain arsenic concentrations were 1.5 times higher in the boro (winter) than the summer (monsoon) season, consistent with the much greater use of groundwater for irrigation in the boro season. Mean values for the boro and aman season rice were 183 and 117 μg kg^{-1}, respectively. The variation in arsenic concentrations in rice was only partially consistent with the pattern of arsenic concentrations in drinking water tube wells. There was no evidence from yield or panicle sterility data of arsenic toxicity to rice. Processing of rice (parboiling and milling) reduced arsenic concentrations in rice by an average of 19% in 21 samples collected from households. Human exposure to arsenic through rice would be equivalent to half of that in water containing 50 μg kg^{-1} for 14% of the paddy rice samples at rice and water intake levels of 400 g and 4 L cap^{-1} day^{-1}, respectively.

Meharg and Rahman (2003) conducted an experiment on Arsenic Contamination of Bangladesh Paddy Field Soils: Implications for Rice Contribution to Arsenic Consumption. They said that arsenic contaminated groundwater is used extensively in Bangladesh to irrigate the staple food of the region, paddy rice (*Oryza sativa* L.). To determine if this irrigation has led to a buildup of arsenic levels in paddy fields, and the consequences for arsenic exposure through rice ingestion, a survey of arsenic levels in paddy soils and rice grain was undertaken. Survey of paddy soils throughout Bangladesh showed that arsenic levels were elevated in zones where arsenic in groundwater used for irrigation was high, and where these tube-wells have been in operation for the longest period of time. Regression of soil arsenic levels with tube-well age was significant. Arsenic levels reached 46 µg g^{-1} dry weights in the most affected zone, compared to levels below 10 µg g^{-1} in areas with low levels of arsenic in the groundwater. Arsenic levels in rice grain from an area of Bangladesh with low levels of arsenic in groundwater and in paddy soils showed that levels were typical of other regions of the world. Modeling determined, even these typical grain arsenic levels contributed considerably to arsenic ingestion when drinking water contained the elevated quantity of 0.1 mg L^{-1}. Arsenic levels in rice can be further elevated in rice growing on arsenic contaminated soils, potentially greatly increasing arsenic exposure of the Bangladesh population. Rice grain grown in the regions where arsenic is building up in the soil had high arsenic concentrations, with three rice grain samples having levels above 1.7 µg g^{-1}.

Arsenic (As) finds its way into soils used for rice (*Oryza sativa*) cultivation through polluted irrigation water, and through historic contamination with As-based pesticides (Abedin *et al.,* 2002a). As is known to be present as a number of chemical species in such soils, so they investigate how these species were accumulated by rice. As species found in soil solution from a greenhouse experiment where rice was irrigated with arsenate contaminated water were arsenite, arsenate, dimethylarsinic acid, and monomethylarsonic acid. The short-term uptake kinetics for these four As species were determined in 7-d-old excised rice roots. High-affinity uptake (0–0.0532 mm) for arsenite and arsenate with eight rice varieties, covering two growing seasons, rice var. Boro (dry season) and rice var. Aman (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 was 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 monomethylarsonic acid, and limited uptake of dimethylarsinic acid.

Abedin *et al.* (2002b) conducted an experiment to examine the effects of 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^{-1} applied regularly throughout the 170=day post-transplantation growing period until plants were ready for harvesting. Increasing the concentration of arsenate in irrigation water significantly decreased plant height, grain yield, the number of filled grains, grain weight, and root biomass, while the arsenic concentrations in root, straw, and rice husk increased significantly. Concentrations of arsenic in rice grain did not exceed the food hygiene concentration limit (1.0 mg of As kg^{-1} dry weight). The concentrations of arsenic in rice straw (up to 91.8 mg kg^{-1} for the highest As treatment) were of the same order of magnitude as root arsenic concentrations (up to 107.5 mg kg^{-1}), suggesting that arsenic can be readily translocated to the shoot. While not covered by food hygiene regulations, rice straw is used as cattle feed in many countries including Bangladesh. The high arsenic concentrations may have the potential for adverse health effects on the cattle and an increase of arsenic exposure in humans via the plant–animal–human pathway. Arsenic concentrations in rice plant parts except husk were not affected by application of phosphate. As the concentration of arsenic in the rice grain was low, arsenic speciation was performed only on rice straw to predict the risk associated with feeding contaminated straw to the cattle. Speciation of arsenic in tissues (using HPLC–ICP-MS) revealed that the predominant species present in straw was arsenate followed by arsenite and dimethylarsinic acid (DMAA). As DMAA is only present at low concentrations, it is unlikely this will greatly alter the toxicity of arsenic present in rice.

As concentrations in soils are important for defining whether a soil is polluted. Arsenic concentrations in 441 taxonomically and geographically representative surface soils were determined using EPA Method 3052 (HCl-HNO_3-HF digestion). Cumulative distribution plots indicate that As concentrations follow a log-normal distribution and depend on soil type. Sample geometric mean (GM) (the exponential mean of the log-transformed distribution) As concentrations (mg kg^{-1}) generally follow the soil taxonomic order of Histosols (2.35) > Inceptisols (0.98), Mollisols (0.72) ≥ Ultisols (0.51) ≥ Alfisols (0.39), and Entisols (0.36) > Spodosols (0.18). The highest As concentrations were found in soils that occur exclusively or prevalently in wetlands, such as Hemists (3.16–9.44), Saprists (0.15–11.7), Aquents (0.10–50.6), Aquolls (0.03–3.34), and Aquepts (0.03–38.2). Both linear and multiple regressions indicate soil properties (clay, pH, cation-exchange capacity [CEC], organic C, and total Al), especially total Fe and P, are important factors affecting natural background concentrations of As in Florida soils. Arsenic release from bedrock (limestone) and As bioaccumulation by aquatic organisms are possible explanations for relatively high As in those wetland soils. The use of a single regulatory value criterion for As contamination in soil cannot provide an adequate assessment given the natural variation in soil As. Baseline soil-As concentration, which was defined as 95% of the expected range of background As concentrations in different soil categories, is necessary for properly assessing potential As contamination (Chen *et al.*, 2002).

Roychowdhury *et al.* (2002) found that the individual food composite and food groups containing the highest mean arsenic concentrations (µg/kg) are potato skin (292.62 and 104), leaf of vegetables (212.34 and 294.67), arum leaf (331 and 341), papaya (196.50 and 373), rice (226.18 and 245.39), wheat (7 and 362), cumin (47.86 and 209.75), turmeric powder (297.33 and 280.9), cereals and bakery goods (156.37 and 294.47), vegetables (91.73 and 123.22), spices (92.22 and 207.60) and miscellaneous items (138.37 and 137.80) for the Jalangi and Domkal blocks, respectively. Arsenic is absorbed by the skin of most of the vegetables. The arsenic concentration in fleshy vegetable material is low (mean 2.72 μg kg^{-1}, n=45). Higher levels of arsenic were observed in cooked items compared with raw.

The interactions of arsenate and phosphate on the uptake and distribution of As and phosphorus (P), and As speciation in P. vittata was investigated by Wang *et al.* (2002). In an 18-d hydroponic experiment with varying concentrations of arsenate and phosphate, *Pteris vittata* accumulated As in the fronds up to 27,000 mg As kg^{-1} dry weight, and the frond As to root As concentration ratio varied between 1.3 and 6.7. Increasing phosphate supply decreased As uptake markedly, with the effect being greater on root As concentration than on shoot As concentration. Increasing arsenate supply decreased the P concentration in the roots, but not in the fronds. Presence of phosphate in the uptake solution decreased arsenate influx markedly, whereas P starvation for 8 d increased the maximum net influx by 2.5-fold. The rate of arsenite uptake was 10% of that for arsenate in the absence of phosphate. Neither P starvation nor the presence of phosphate affected arsenite uptake. Within 8 h, 50% to 78% of the As taken up was distributed to the fronds, with a higher translocation efficiency for arsenite than for arsenate. In fronds, 49% to 94% of the As was extracted with a phosphate buffer (pH 5.6). Speciation analysis using high-performance liquid chromatography-inductively coupled plasma mass spectroscopy showed that 85% of the extracted As was in the form of arsenite, and the remaining mostly as arsenate. We conclude that arsenate is taken up by *Pteris vittata* via the phosphate transporters, reduced to arsenite, and sequestered in the fronds primarily as Arsenic (III).

Barrachinaa *et al.* (1995) conducted an experiment to find out the response of tomato (*Lycopersicum esculentum*) plants to different levels of arsenic (As) in nutrient solution and investigated the processes of uptake, distribution and accumulation of As and the effect of arsenite on yield and plant growth (plant height, diameter of stem, stem and root length, fresh and dry weight of root, stems, leaves, and fruit). The experiment was performed at three levels of As: 2, 5 and 10 mg L^{-1} [added as sodium arsenite ($NaAsO_2$)] in a nutrient solution, together with the corresponding control plants. Arsenic uptake depended on the arsenic concentration in solution and arsenic content in the roots increased as the time of treatment increased. The most important finding was the high toxicity of arsenite to roots. The concentration in stems, leaves, and fruit was correlated with the As level in the nutrient solution. Although the arsenic level of 10 mg L^{-1} damaged the root membranes, resulting in a significant decrease in the upward transport of As. Arsenic exposure resulted in a drastic decrease in plant growth parameters (e.g., maximum decrease of 76.8% in leaf fresh weight) and in tomato fruit yield (maximum reduction of 79.6%).

Arsenic uptake and concentration in shoot and root increased upon increased DMAA (Dimethyl arsenic acid) concentration in solution. Upon uptake, DMAA was readily translocated to the shoot. At the two higher rates of DMAA application (0.8 and 1.6 mg As L^{-1}), Pn and photosynthetic capacity were significantly decreased in response to tissue As concentration. Leaf area and dry matter production were also significantly reduced at the two higher rates of DMAA. At the lower rate (0.2 mg As L^{-1}) of DMAA application, there was no significant reduction in Pn or growth. Dimethyl arsenic acid application did not affect nutrient allocation within the rice plant at concentration levels used in this study (Marin *et al.,* 1993).

Arsenic contamination of soil and water can result from several anthropogenic activities, such as: pesticide use/production, mining, smelting, combustion and sewage/solid waste (O'Neill 1990).

CHAPTER III

MATERIALS AND METHOD

The experiment was conducted under pot culture at Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka-1207 to study the As uptake by different three plant species. Experiment was done during the period from July 2012 to October 2012. Location of the site is 23^0 77′ N latitude and $90^0$33' E longitude at an altitude of 8.6 meter above the sea level in Agro-Ecological Zone of "Madhupur Tract", AEZ No. 28 (Anon., 2004). This chapter includes the information regarding methodology that was used in execution of the experiment. It contains a short description of the materials used for the experiment, treatments of experiment, Parameters, data collection procedure, Chemical analysis and statistical analysis etc.

3.1. Design and layout of the experiment

Three single factorial pot experiment was carried out in a Completely Randomized Design (CRD) with four levels of arsenic. Three pots for each levels of treatment and 12 (4×3) pots were used in each of the experiment. Each pot was 35 cm (14 inches) in diameter and 30 cm (12 inches) in height and four kg soil is used in each of the pot.

3.2. Treatments of the experiment

Experiment 1

Rice plant was grown in different concentrated arsenic treated soil.

Experiment 2

Arum plant was grown in different concentrated arsenic treated soil.

Experiment 3

Fern species (*Pteris vittata*) plant was grown in different concentrated arsenic treated soil.

Plant were grown in soil treated with different concentration of arsenic each of the experiment. For these three experiment treatments were as follows

Treatments	Symbol used
Control (As)	As_0
500ppm (As)	As_{500}
1000ppm (As)	As_{1000}
2000ppm (As)	As_{2000}

3.3. Pot preparation

A ratio of 1:1 well rotten cow dung and soil were mixed and pots were filled 15 days before transplanting. All 36 pots were filled in July 2012. The pot was hand-weeded and removes stubbles. No tilling was performed prior to transplanting. No chemical fertilizer use for growing. The pot was hand-weeded approximately every two weeks as needed, and it was watered daily with spray irrigation. No additional fertilizers or soil amendments were added during the growing seasons.

3.4. Transplanting of seedlings

Took three weeks mature ferns species (*Pteris vittata)*, 30 days rice seedlings and three weeks arum seedlings and transplanted this species in July 2012. One hill or plant was placed in each pot. Plants were tagged by using plastic-coated tags as treatment wise to collect data smoothly.

3.5. Application of the treatments

Arsenic was applied in the form of Arsenic trioxide (As_2O_3) which was purchased from Loba Chemie Pvt. Ltd., India. As per analysis of molecular weight 1.32 g of As_2O_3 contains 1 gm As hence following amounts of As_2O_3 viz. 0 g, 2.64 g 5.28 g and 10.56 g, for control, 500 ppm, 1000 ppm and 2000 ppm respectively for the treatments. Apply arsenic directly into pot and then mix with soil by using site.

3.6. Intercultural operations

Weeding was done for all pots when required, to keep the plant free from weeds. Spray irrigation was done for all pots when required, frequency of watering depended upon the moisture status of the soil.

3.7. Parameters

Different data were collected from each pot in respect of the following parameters:

Experiment 1:

a) Leaf number hill^{-1}
b) Plant height
c) Leaf area
d) Number of tiller hill^{-1}
e) Number of panicle hill^{-1}
f) Grain number panical^{-1}
g) Plant leaf biomass hill^{-1}
h) Grain biomass hill^{-1}
i) Arsenic accumulation (ppm)

Experiment 2:

a) Leaf number plant^{-1}
b) Plant height
c) Number of runner plant^{-1}
d) Number of sucker plant^{-1}
e) Leaf area
f) Plant leaf biomass plant^{-1}
g) Runner biomass plant^{-1}
h) Arsenic accumulation (ppm)

Experiment 3:

a) Number of leaves plant^{-1}
b) Plant height
c) Leaf area
d) Leaf biomass plant^{-1}
e) Arsenic accumulation (ppm)

3.8. Collection of experimental data

3. 8.1. Measurement of plant height

Plant height of each plant of each pot was measured in cm by using meter scale and mean was calculated.

3.8.2. Number of leaves

Number of leaves per hill or plant was recorded by counting all the leaves of each plant and each pot and mean was calculated.

3.8.3. Leaf area measurement

Leaf area was measured by non-destructive method using CL-202 Leaf Area Meter, (USA). Mature leaf were measured all time and expressed in cm^2.

3.8.4. Different parts of plant biomass

Plant biomass was measured by using precision balance after drying in mg.

3.9. Chemical analysis

After harvesting plant samples were collected and dried in an oven. Dried samples were then grounded by using mortor and pastle machine. Then samples were prepared for chemical analysis. Arsenic accumulation on different plants were measured by Atomic Absorption Spectrophotometer in Bangladesh Council of Scientific and Industrail Research (BCSIR) laboratory.

3.10. Preparation of plant sample for arsenic analysis

Plant samples were prepared by dry oxidation method using muffle furnace. After first 0.5g for each plant species was taken in a 50 mL beaker and placed it into the furnace. Then the temperature was raised upto 300℃ , 400℃ and 500℃ gradually and the samples were kept at least one hour in the above temperature. Finally, the temperature was raised upto 550℃ and samples were kept there for 1 day.

After heating, 1:1 nitric acid and distilled water solution was added in sample. Then this sample solution was taken into hot plate and heated to get a clear solution. If clear solution was not found but samples almost dried then it was isoled at room temperature and more 1:1 nitric acid and distilled water solution was added. Again it was kept on hot plate and heated for 4-8 hours. thus the sample was prepared.

3.11. 50 time dilution

At first 50 mL volumetric flask was taken and washed it by distilled water, then 5mL concentrated HCl was taken because it is one kind of reducing agent which helps to turn arsenic into arsenic trioxide. Little bit of distilled water was added in HCl. Then 1mL solution from each plant sample was taken in different volumetric flask, done this very carefully because to avoid bubble into the solution.

Then 1gm KI was added in 150 mL distilled water. After made this solution 1gm was taken in 50 mL volumetric flask contsining plant sample and mixed with distilled water upto 50 mL. The solution turned into yellow color. Another volumetric flask was taken to make blank solution, where contain only HCl, KI and distilled water for arsenic analysis.

3.12. 1000 time dilution

At first 500 mL volumetric flasks was taken and washed it by distilled water. Then 2mL solution was taken into flask and mixed with distilled water up to 500 mL. After mixing water it was shaked very carefully. Then it was taken into 250 mL volumetric flask and again mixed distilled water upto 250 mL. 5 mL solution was taken into 25 mL volumetric flask added 2.5mL HCl and 2.5 mL KI. Then it was mixed with distilled water and shaked very smoothly until turn it into yellow color. Standard solution arsenic was added with HCl 2, 5, 10, 15, 20 ppb respectively.

3.13. 5000 time dilution

Same process of 1000 time dilution here only changes the amount of sample and chemical reagent. Like sample was taken 4 mL, HCl in 5 mL, KI in 5 mL and 5000 mL volumetric flask. Solution was taken into 25 mL volumetric flask and taken 5 mL in flask.

3.14. Statistical analysis

Collected data were statistically analyzed using MSTAT-C computer package programmed. Mean for every treatments were calculated and the analysis of variance for each one of the characters was performed by F–test (Variance Ratio). Difference between treatments was evaluated by Duncan's Multiple Range (DMRT) test at 5% level of significance (Gomez and Gomez, 1984).

CHAPTER IV

RESULT AND DISCUSSION

Three research works was conducted to identify the arsenic problem in soil and mitigation of arsenic from soil by using two edible plants and one non-edible plant, the experimental soil was treated with different concentration of arsenic and in this chapter the findings of the conducted research work have been presented and discussed in this chapter. Some of the data have been presented in tables and others in figures for ease of discussion, comprehension and understanding. A summary of the analysis of variances in respect of all the parameters have been shown in appendices. Results have been presented and discussed, and possible interpretations are given under the following heads.

4.1. Experiment 1: Rice plant was grown in 0, 500, 1000, 2000 ppm concentrated arsenic treated soil.

4.1.1. Leaf Number hill^{-1}

Rice leaf number hill^{-1} was significantly affected by arsenic treatments (Appendix I). Rice Leaf number hill^{-1} showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig. 1). Maximum number of leaves (51.3) was recorded from control (As_0) whereas the minimum plant number of leaves (2.6) was found from 2000 ppm (As_{2000}) arsenic treatment at 60 DAT. The present study identified that with number of leaves hill^{-1} was reduced with the increase of arseic concentration.

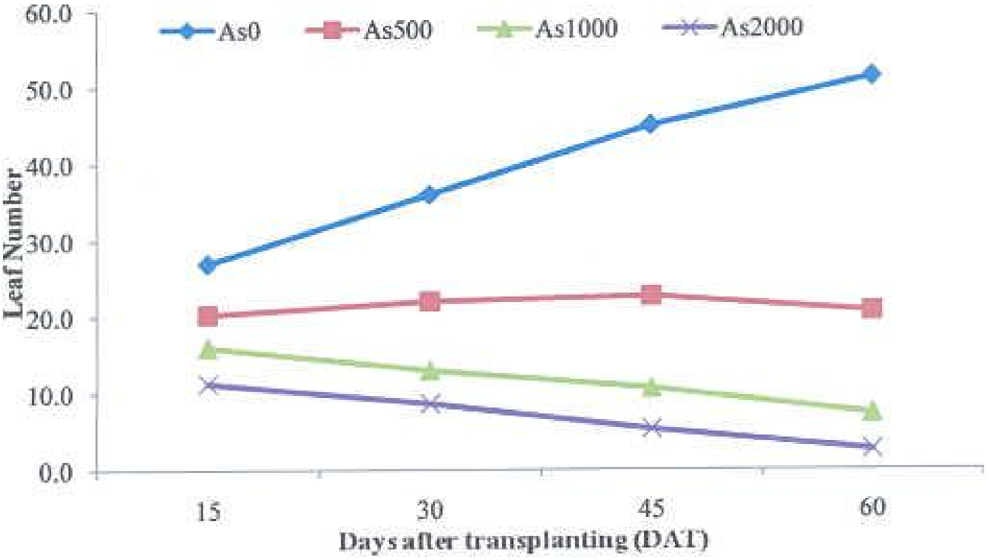

Fig.01. Effect of arsenic concentrations on rice plant leaf number at different days after transplanting (DAT)

4.1.2. Plant height

Plant height was significantly affected by arsenic treatments (Appendix II). Plant height of rice plant showed statistically significant differences among control, 500, 1000 and 2000 ppm As at 15, 30, 45 and 60 DAT (Fig. 2). Maximum plant height (92.3 cm) was recorded from control (As_0) whereas the minimum plant height (6.7 cm) was found from 2000 ppm (As_{2000}) arsenic treatment at 60 DAT. The present study indicated that plant height is reduced with the increase of arsenic concentration. Increasing the concentration of arsenate in irrigation water significantly decreased plant height. (Abedin et al., 2002b).

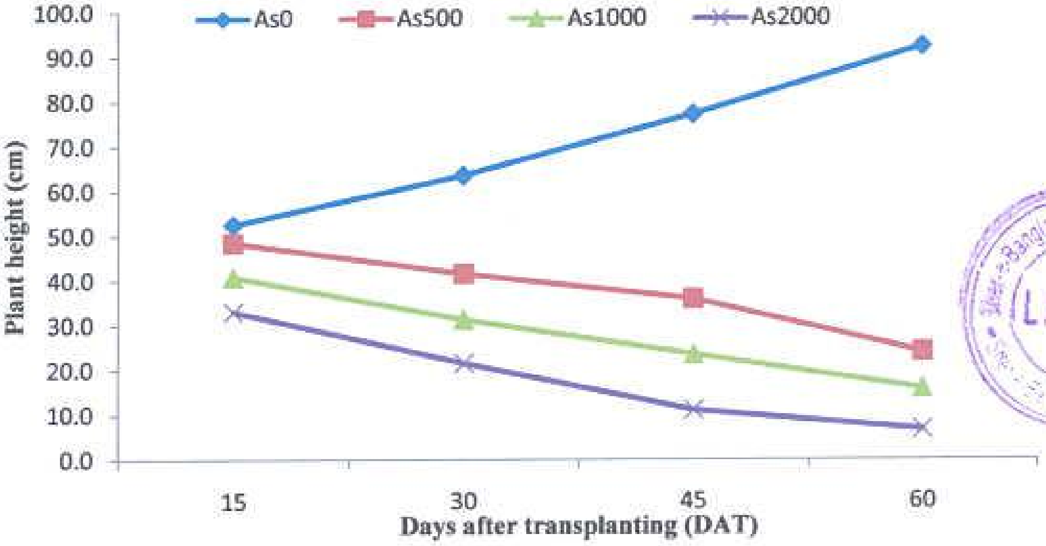

Fig.02. Effect of arsenic concentrations on rice plant height at different days after transplanting (DAT)

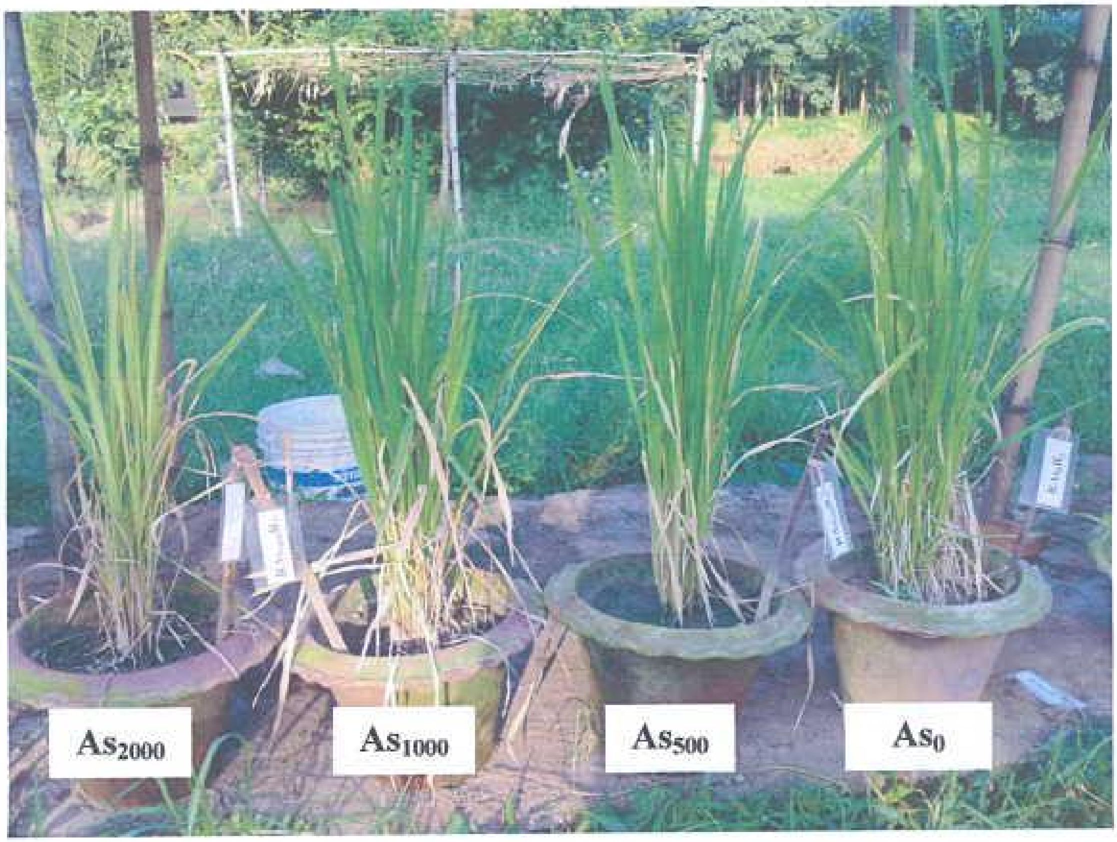

Plate 1. Rice plant growth variation with different arsnic concentration on soil.

4.1.3. Leaf area

Leaf area (cm^2) of rice plant was significantly affected by arsenic treatments (Appendix III). Leaf area of rice plant showed statistically significant differences among control, 500, 1000 and 2000 ppm As at 15, 30, 45 and 60 DAT (Fig. 3). The higher leaf areas (20.27 cm2) were recorded from control (As_0) whereas the lower leaf areas (8.1cm^2) were found from 2000 ppm (As_{2000}) arsenic treatment compared to other As treatment (Fig:03) at 15, 30, 45 and 60 DAT. The present study identified that nember leaf area of rice was reduced with the increase of arsenic concentration. Leaf area and dry matter production were also significantly reduced at the two higher rates of dimethylarsenic acid (DMAA). (Marin *et al.*, 1993).

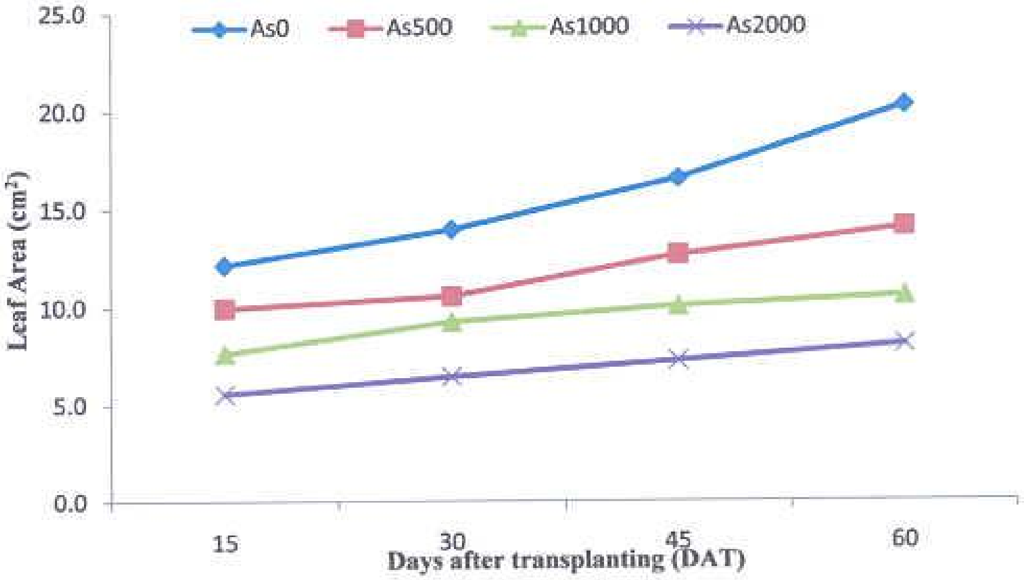

Fig.03. Effect of arsenic concentrations on rice plant leaf area (cm^2) at different days after transplanting (DAT)

4.1.4. Number of tiller at 30 days

The number of tiller per hill of rice varied significantly among the different concentration of arsenic (Appendix IV). The number of tiller per hill ranged from 4.0 to 24.0 among the different arsenic concentration (Table 1). The maximum number of tiller per hill (24.0) was produced in control (As_0). The minimum number of tiller per hill (4.0) was found in 2000ppm arsenic concentration (As_{2000}). In the present investigation it was observed that the contrrol treated rice plant gave maximum number of tiller per hill. Azad *et. al.* (2012) found that the tiller number of BR-11 rice were decreased significantly ($p \leq 0.05$) with increase of arsenic (As) concentration in irrigation water.

4.1.5. Number of panicle

Number of panicle per hill of rice plant varied significantly among the different concentration of arsenic (Appendix IV). Number of panicle of rice plant ranged from 2.9 to 21.4 among the different arsenic concentration (Table 1). The maximum number of panicle (21.4) was produced in control (As_0). The minimum number of panicle (2.9) was found in 2000 ppm (As_{2000}) arsenic concentration. The present study indicated that number of panicle per hill of rice plant was reduced with the increase of arsenic concentration.

4.1.6. Filled Grain

The number of filled grain number of rice varied significantly among the different concentration of arsenic (Appendix IV). The filled grain number ranged from 43.67 to 525.3 among the different arsenic concentration (Table 1). The maximum number of filled grain (525.3) was produced in control (As_0). The minimum number of filled grain (43.67) was found in 2000ppm (As_{2000}) arsenic concentration. In the present investigation it was observed that the control treated rich plant given maximum number of filled grain. Abedin *et al.* (2002b) increasing the concentration of arsenate in irrigation water significantly decreased the number of filled grains.

4.1.7. Unfilled Grain

The number of unfilled grain number of rice varied significantly among the different concentration of arsenic (Appendix IV). Unfill number grain ranged from 39.3 to 214.0 among the different arsenic concentration (Table 1). The maximum number of unfilled grain (214.0) was produced in 1000ppm (As_{1000})

arsenic concentration which was statistically simillar to 500ppm (As_{500}) arsenic concentration (193.3) followed by 2000 ppm As concentration (130.7). The minimum number of unfilled grain number (39.3) was found in control (As_0).

4.1.8. Plant leaf biomass (mg)

Different arsenic concentration showed significant variation in terms of rice plant leaf biomass (Appendix V). Plant leaf biomass showed statistically significant differences among control, 500, 1000 and 2000 ppm As. Maximum plant leaf biomass (405.5 mg) was recorded from control (As_0) whereas the minimum plant leaf biomass (101.8 mg) was found from 2000 ppm (As_{2000}) arsenic which is statistically simillar to 1000ppm (As_{1000}) arsenic treatment (Table 1). The present study identified that plant leaf biomass was reduced with the increase of arsenic concentration. Plant biomass varied due to the variation of the concentration of arsenic (Wei *et al.*, 2006).

4.1.9. Arsenic concentration in the 0.5 mg sample of straw

Different concentrations of As treatment significantly influenced the arsenic concentration in straw from contaminated soil (Appendix V). Rice straw contained maximum amount of arsenic (1019.0 ppb) from 2000 ppm As (As_{2000}) treated soil while minimum (0.06 ppb) amount of arsenic was obtained from control; (As_0) (Table 2). The present study identified that arsenic content was increased with the increase of arsenic concentration. The concentrations of arsenic in rice straw are up to 91.8 mg kg^{-1} for the highest arsenic treatment. (Abedin *et al.*,2002b)

4.1.10. Total arsenic accumulation by straw

The total arsenic accumulation on rice plant leaves varied significantly among the different concentration of arsenic (Appendix V). Plant from 2000 ppm (As_{2000}) treated soil was accumulated maximum amount of arsenic (206.3 ppm) which was statistically simillar to 1000 ppm (As_{1000}) arsenic treatment while control (As_{00}) was obtained minimum (0.03 ppm) amount of arsenic (Table 2).

The present study showed that total arsenic accumulation is increased with the increase of arsenic concentration. The concentrations of arsenic in rice straw are up to 91.8 mg kg^{-1} for the highest arsenic treatment. (Abedin *et al.*, 2002b)

4.1.11. Grain biomass

Different arsenic concentration showed significant variation in terms of rice plant grain biomass (Appendix VI). Maximum grain biomass (210.1 mg) per hill was recorded from control (As_0) which was statistically similar to 500 ppm (As_{500}) arsenic whereas the minimum grain biomass (141.1mg) was found from 2000 ppm (As_{2000}) arsenic (Table 2). The present study identified that grain biomass reduced with the increase of arsenic concentration. Plant biomass varied due to the variation of the concentration of arsenic (Wei *et al.*, 2006).

4.1.12. Arsenic accumulation on 0.5 mg tested grain sample

Different arsenic concentrations significantly influenced the arsenic accumulation on 0.5 mg tested grain of rice plant (Appendix VI). Plant from 2000 ppm (As_{2000}) treated soil plants was uptook maximum amount of arsenic (218.0 ppb) while control (As_{00}) was obtained minimum (0.02 ppb) amount of arsenic (Table 2). The present study identified that arsenic accumulation was increased with the increase of arsenic concentration. Rice grain uptake up to 624 ŋg g^{-1}. (Zhu *et al.*, 2008).

4.1.13. Arsenic accumulation on grain on total biomass

The total arsenic accumulation on rice plant grain on total biomass varied significantly among the different concentration of arsenic (Appendix VI). Plant from 2000 ppm (As_{2000}) treated soil plant was uptook maximum amount of arsenic (61.6 ppm) while control (As_{00}) was obtained minimum (0.01 ppm) amount of arsenic (Table 2). The present study identified that total arsenic accumulation on grain increased with the increase of arsenic concentration. Rice grain uptake up to 624 ŋg g^{-1}. (Zhu *et al.*, 2008).

Table 1. Rice plant as affected by different of arsenic concentrations to different attributes

Treatments	Number tiller at 30 DAT	Number of panicle	Grain Number		Plant leaf biomass (mg)	Grain biomass (mg)
			Filled	Unfilled		
As_0	24 a	21.4 a	525.3 a	39.3 c	405.5 a	210.1 a
As_{500}	11.7 b	9.9 b	369.7 b	193.3 a	233.0 b	222.7 a
As_{1000}	9.3 c	7.1 c	119.7 c	214 a	153.4 c	165.5 b
As_{2000}	4 d	2.9 d	43.67 d	130.7 b	101.8 c	141.1 c
LSD	1.2	1.9	25.9	60	57.9	15.8
CV%	4.9	9.5	4.9	20.8	12.9	4.2

Table 2. The arsenic content in leaf, grain and total accumulation by rice pant as affected by different arsenic concentration

Treatments	Arsenic concentration in 0.5 mg straw sample (ppb)	Total accumulation of As by plant straw (ppm)	Arsenic accumulation on 0.5 mg sample grain (ppb)	Arsenic accumulation on grain on total biomass (ppm)
As_0	0.06 d	0.03 b	0.02 d	0.01 d
As_{500}	171.5 c	82.9 b	67.1 c	29.9 c
As_{1000}	626.2 b	194.9 a	110.4 b	36.5 b
As_{2000}	1019.0 a	206.3 a	218.0 a	61.6 a
LSD	206.7	71.3	0.006	6.6
CV%	21.9	26.5	0.0	9.2

4.2. Experiment 2: Arum plant was grown in different concentrated arsenic treated soil.

4.2. 1. Leaf number

Arum leaf number per plant was significantly affected by arsenic treatments (Appendix VII). Leaf number of arum showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig.04). Maximum leaf number (14.0) was recorded from control (As_0) whereas the minimum plant leaf number (5.7) was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. The present study identified that plant leaf number was reduced with the increase of arsenic concentration.

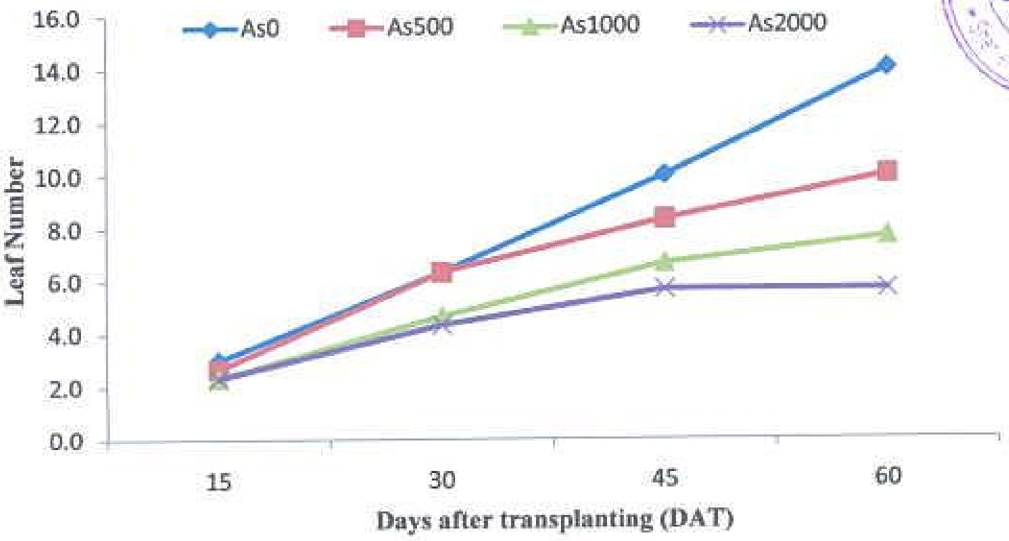

Fig.04. Effect of arsenic concentrations on arum leaf number at different days after transplanting (DAT)

4.2. 2. Number of leaves (Newly emerged) at 30 DAT

Newly emerged number of leaves per plant at 30 DAT of arum was significantly affected by arsenic treatments (Appendix IX). Newly emerged number of leaves at 30 DAT showed statistically significant differences among control, 500, 1000 and 2000 ppm. The highest newly emerged number of leaves at 30 DAT (3.6) was observed in 500 ppm (As_{500}) arsenic which are statistically similar to control (As_0) and the lowest newly emerged number of leaves at 30 DAT (2.0) was found in 2000 ppm arsenic (As_{2000}) which is statistically similar to1000 ppm arsenic treatment (As_{1000}) (Table.3).

4.2.3. Number of leaves (Died) at 30 DAT

Died number of leaves per plant at 30 DAT of arum was significantly affected by arsenic treatments (Appendix IX). Died number of leaves at 30 DAT showed statistically significant differences among control, 500 ppm, 1000 ppm and 2000 ppm. The highest died number of leaves (3.6) was observed in 2000 ppm (As_{2000}) arsenic which is statistically similar to 1000 ppm arsenic (As_{1000}) and 500 ppm arsenic (As_{500}) whereas the lowest died number of leaves (1.3) was found in control (As_0) (Table. 3). The present study identified that number of died leaves was increased with the increase of arsenic concentration.

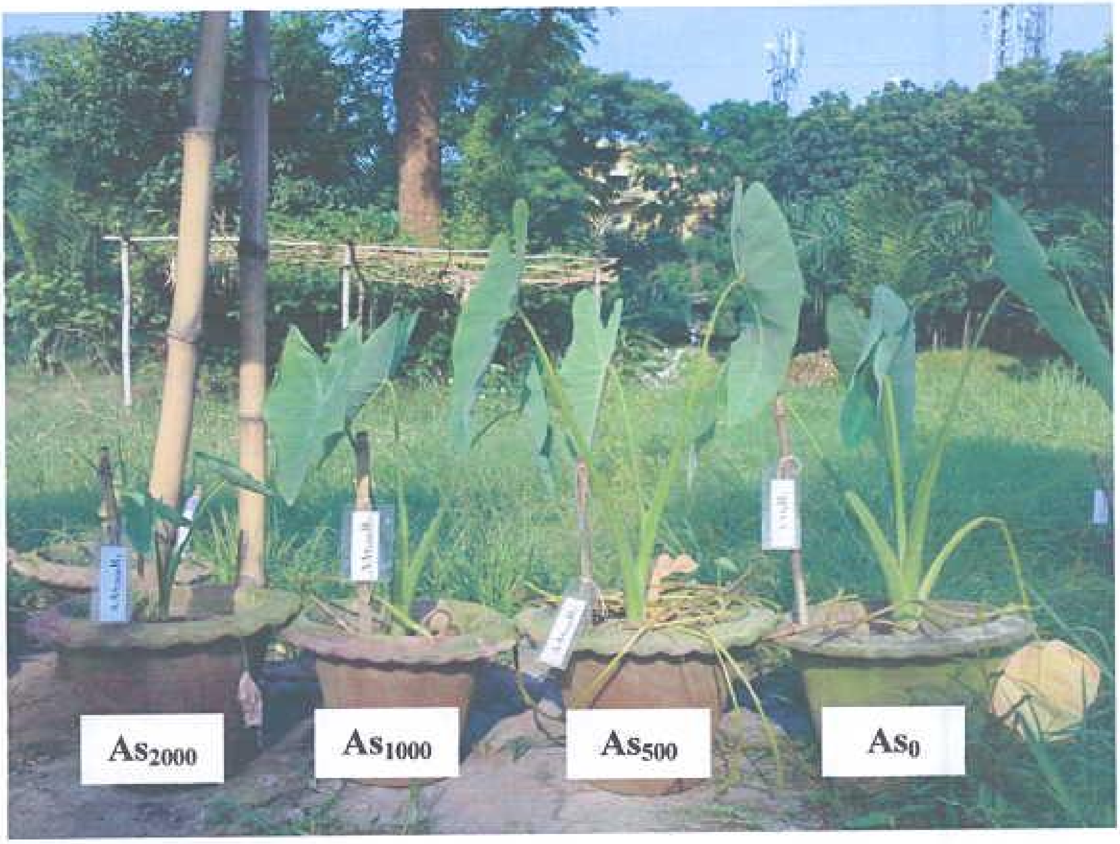

Plate 2. Arum plant growth variation with different arsnic concentration on soil.

4.2. 4. Plant height

Plant height (cm) was significantly affected by arsenic treatments (Appendix VIII). Plant height of arum showed statistically significant differences among control, 500, 1000 and 2000 ppm As at 15, 30, 45 and 60 DAT (Fig.04). Maximum plant height (62.0 cm) was recorded from control (As_0) whereas the minimum plant hieght (31.0 cm) was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. The present study identified that plant height was reduced with the increase of arsenic concentration. Increasing the concentration of arsenate in irrigation water significantly decreased plant height. (Abedin *et al.*, 2002b)

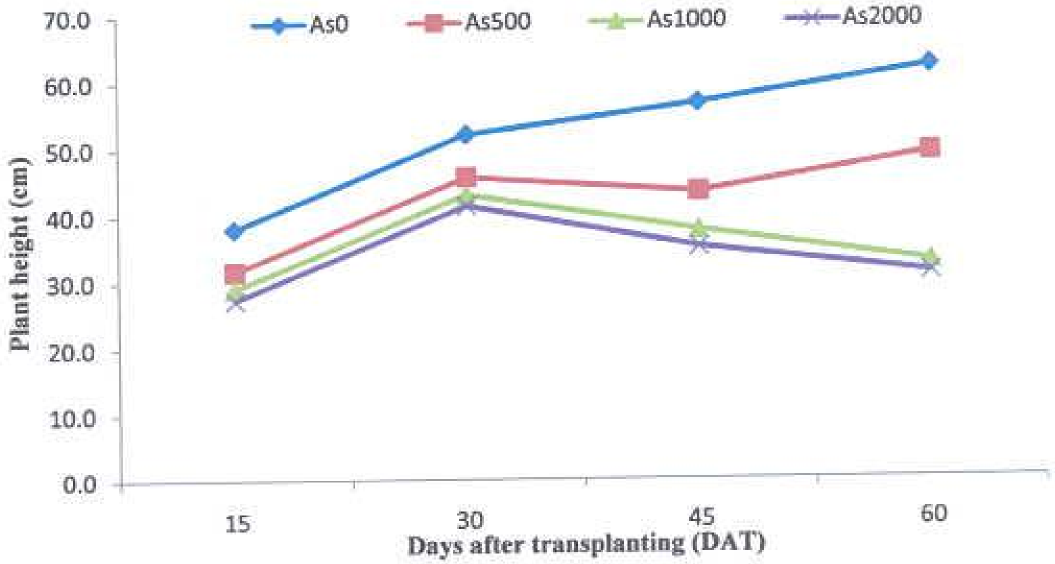

Fig.05. Effect of arsenic concentrations on arum plant height at different days after transplanting (DAT)

4.2. 5. Number of Runner

Number of runner per plant of arum was significantly affected by arsenic treatments (Appendix X). Number of runner showed statistically significant differences among control, 500, 1000 and 2000 ppm at 30 and 45 DAT (Fig. 06). Maximum number of runner of arum (6.6) was recorded from control (As_0) whereas the minimum number of runner (1.3) was found from 2000 ppm (As_{2000}) arsenic at 45 DAT. The present study identified that number of runner was reduced with the increase of arsenic concentration.

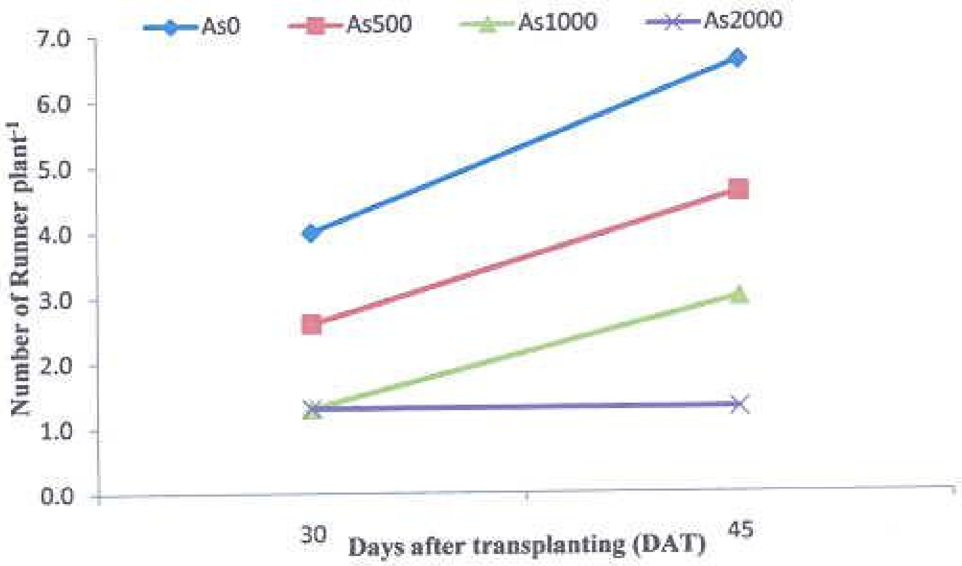

Fig.06. Effect of arsenic concentrations on numbers of runner of arum at different days after transplanting (DAT).

4.2. 6. Number of sucker

Different arsenic concentration showed significant variation in terms of number of sucker of arum (Appendix IX). Number of sucker of arum showed statistically significant differences among control, 500, 1000 and 2000 ppm. Maximum number of sucker (5.3) was recorded from control (As_0) whereas the minimum number of runner (1.3) was found from 2000 ppm (As_{2000}) arsenic (Table 3) which is statistacally similar to1000 ppm arsenic (As_{1000}). The present study identified that number of sucker was reduced with the increase of arsenic concentration.

4.2.7. Leaf area

Leaf area (cm^2) of arum was significantly affected by arsenic treatments (Appendix XI). Leaf area showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig.07). The highest leaf area (85.6 cm^2) was recorded from control (As_0) whereas lowest leaf area (34.1cm^2) was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. The present study identified that leaf area was reduced with the increase of arsenic concentration.

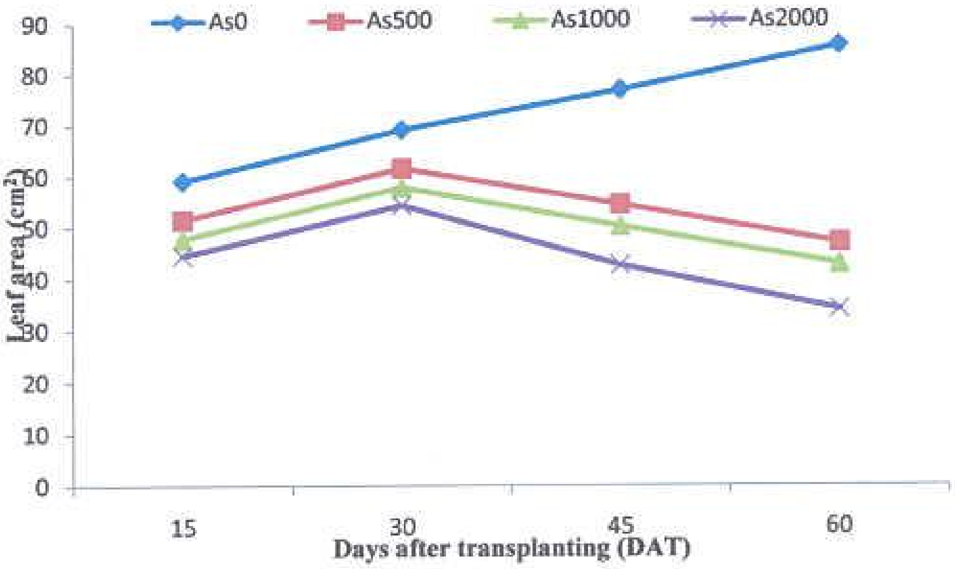

Fig.07. Effect of arsenic concentrations on arum leaf area (cm^2) at different days after transplanting (DAT).

4.2.8. Plant leaf biomass

Different arsenic concentrations showed significant variation in terms of plant leaf biomass of arum (Appendix IX). Plant leaf biomass showed statistically significant differences among control, 500, 1000 and 2000 ppm. Maximum plant leaf biomass (1.6 gm) was recorded from control (As_0) whereas the minimum plant leaf biomass (0.7 gm) was found from 2000 ppm (A_{2000}) arsenic (Table 3). The present study identified that plant leaf biomass was reduced with the increase of arsenic concentration. Plant biomass varied due to the variation of the concentration of arsenic (Wei *et al.*, 2006).

4.2.9. Arsenic accumulation on 0.5 mg tested sample of leaves

Different concentrations significantly influenced the arsenic accumulation arum leaves (Appendix XII). Maximum arsenic accumulation (1359.5 ppb) was recorded from 2000 ppm arsenic (A_{2000}) whereas the minimum arsenic accumulation (0.2 ppb) was found from control (As_0) (Table 4). This result indicated that arsenic accumulation on leaves was increase with the increase of arsenic concentration. It is found that arum (kochu) vegetable accumulate more than 150 mg/kg of arsenic from contaminated soil (Huq *et al.*, 2006).

4.2.10. Total arsenic accumulation on plant leaves

Total arsenic accumulation on plant leaves varied significantly among the soil treated with different arsenic concentrations (Appendix XII). Maximum arsenic accumulation on plant leaves (973.5 ppm) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation of leaves (0.3 ppm) was found from control (As_{00}) which is samilar to 500 ppm (As_{500}) arsenic treatment (Table 4). Arsenic accumulation of kochu plants grown at 2000 ppm arsenic treated soil was found that 973.5 ppm i.e., 973.5 mg kg^{-1}. Tani *et al.*, (2012) analyzed the different food crops at Jessore district in Bangladesh and found that kochu leaves uptook 1000 $\mu g\ kg^{-1}$ (1 mg kg^{-1}) dry weight. On the other hand, it was found that kochu (kochu) vegetable accumulate more than 150 mg/kg of arsenic from contaminated soil. (Huq et al., 2006). But current study indicated that kochu leaves uptook 973.5 mg kg^{-1} arsenic which was much more than the previous. It may be due the high level of arsenic in soil. So, if the arsenic level in soil increased the uptake ability of the kochu plant also increased.

4.2.11. Runner biomass

Different arsenic concentration showed significant variation in terms of plant runner biomass (Appendix XII). Maximum plant runner biomass (361.6 mg) was recorded from control (A_0) whereas the minimum plant leaf biomass (90.4 mg) was found from 2000 ppm (As_{2000}) arsenic which is statistacally similar to 1000 ppm (As_{1000}) arsenic (Table 4). The present study identified that plant runner biomass was reduced with the increase of arsenic concentration. Plant biomass varied due to the variation of the concentration of arsenic (Wei *et al.*, 2006).

4.2.12. Arsenic accumulation on 0.5 mg tested sample of runner

Arsenic accumulation on 0.5 mg tested sample of runner varied significantly among the soil treated with different arsenic concentrations (Appendix XIII). Maximum arsenic accumulation on 0.5 mg tested sample of runner (1166.8 ppb) was recorded from 2000 ppm treatment (A_{2000}) whereas the minimum arsenic accumulation on 0.5 mg tested sample of runner (0.1 ppb) was found from control (As_{00}) arsenic treatment (Table 4). The present study identified that arsenic accumullation was increase with the increase of arsenic concentration. It is found that arum (kochu) vegetable accumulate more than 150 mg/kg of arsenic from contaminated soil (Huq *et al.*, 2006).

4.2.13. Total arsenic accumulation on runner

Total arsenic accumulation on runnar of arum varied significantly among the soil treated with different arsenic concentrations (Appendix XIII). Maximum arsenic accumulation on runner of (210.3 ppm) was recorded from 2000 ppm arsenic (A_{2000}) which is statistacally similar to 500 ppm (As_{500}) arsenic and

1000 ppm (As_{1000}) arsenic whereas the minimum arsenic accumulation on runner (0.04 ppm) was found from control (As_{00}) (Table 4). Arsenic accumulation of kochu plants grown at 2000 ppm arsenic treated soil was found that 210.3 ppm i.e., 210.3 mg kg^{-1}. Similarly it was found that kochu (kochu) vegetable accumulate more than 150 mg kg^{-1} of arsenic from contaminated soil (Huq *et al.*, 2006). Many countries including Bangladesh have no legislation on arsenic Maximum Permissible Limit (MPL) but MPL of total arsenic in fresh vegetables in Bangladesh is considered as 1.0 mg kg^{-1} (wet weight), based upon the maximum recommended value (1.0 mg kg^{-1}, wet weight) of arsenic in food (Ahmed, 2000).

Table 3. Arum responses to arsenic concentrations at different attributes

Treatments	Number of leaves at 30 DAT		Number of sucker	Plant leaf biomass (g)	Runner biomass (mg)
	Newly Emerged	Died			
As_0	3.3 a	1.3 b	5.3 a	1.6 a	361.6 a
As_{500}	3.6 a	2.3 ab	3.3 b	1.1 b	226.0 b
As_{1000}	2.3 b	3.0 a	2.0 c	1.0 b	135.6 c
As_{2000}	2.0 b	3.6 a	1.3 c	0.7 c	90.4 c
LSD	0.7	1.3	0.9	197.2	67.7
CV%	13.1	26.01	16.6	8.7	16.7

Table 4. The arsenic content in leaves, runner and total accumulation by arum plant as affected by different arsenic concentration

Treatments	Arsenic accumulation on 0.5 mg tested sample of leaves (ppb)	Total arsenic accumulation on plant leaves (ppm)	Arsenic accumulation on 0.5 mg tested sample of runner (ppb)	Total arsenic accumulation on runner (ppm)
As_0	0.2 d	0.3 c	0.1 d	0.04 b
As_{500}	370.5 c	422.3 c	398.3 c	178.8 a
As_{1000}	718.0 b	751.7 b	745.8 b	202.3 a
As_{2000}	1359.5 a	973.5 a	1166.8 a	210.4 a
LSD	0.002	160.3	26.5	97.8
CV%	0.0	12.6	2.2	30.1

4.3. Experiment 3: Fern (*Pteris vittata* L.) plant was grown in different concentrated arsenic treated soil.

4.3.1. Leaf number

Leaf number per plant was significantly affected by arsenic treatments (Appendix XIV). Leaf number of fern species (*Pteris vittata*) showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig. 08). The highest leaf number (40.7) was observed in control (As_0) and the lowest leaf number (14.0) was found in 2000 ppm (As_{2000}) arsenic at 60 DAT. The present study identified that plant leaf number was reduced with the increase of arsenic concentration.

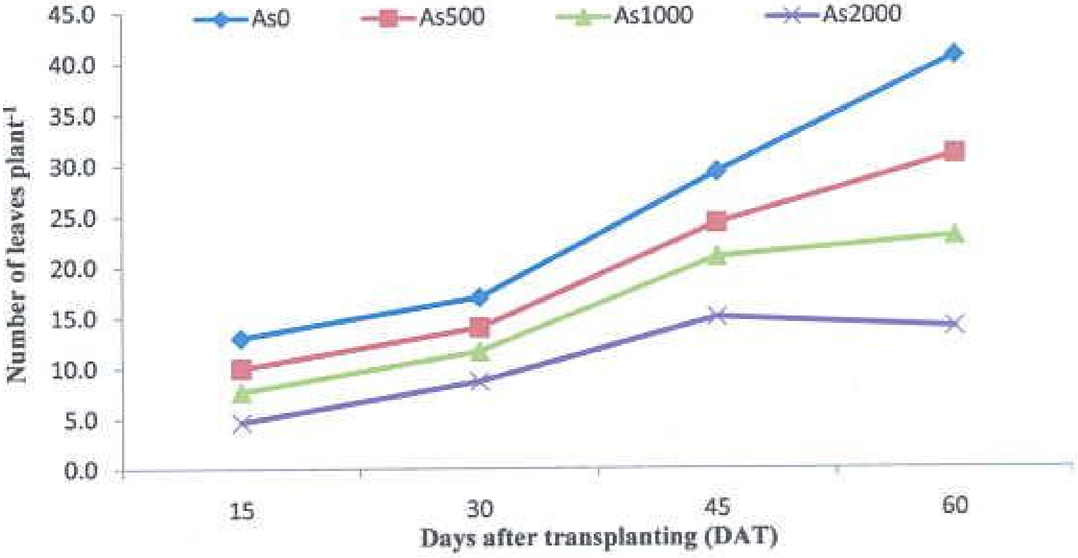

Fig.08. Effect of arsenic concentrations on plant leaf number at different days after transplanting (DAT)

4.3.2. Number of leaves (Newly Emerged) at 30 DAT

Newly emerged number of leaves at 30 DAT of fern (*Pteris vittata*) was significantly affected by arsenic treatments (Appendix XV). Newly emerged number of leaves at 30 DAT of fern species showed statistically significant differences among control, 500, 1000 and 2000 ppm (Table. 5). The highest newly emerged leaves (12.6) was observed in control (As_0) and the lowest newly emerged leaves (8.3) was found in 2000 ppm (As_{2000}) arsenic which is statistically similar to1000 ppm (As_{1000}) arsenic (Table. 5). The present study identified that newly emerged leaves number was reduced with the increase of arsenic concentration.

4.3.3. Number of leaves (Died) at 30 DAT

Number of died leaves at 30 DAT of fern was significantly affected by arsenic treatments (Appendix XV). Number of died leaves at 30 DAT of fern species (*Pteris vittata*) showed statistically significant differences among control, 500, 1000 and 2000 ppm (Table. 5). Highest died number of leaves (4.6) was observed in 2000 ppm (As_{2000}) arsenic which is statistacally similar to 1000 ppm arsenic (As_{1000}) and lowest died number of leaves (1.6) was found in control (As_0) (Table. 5). The present study identified that died number of leaves was increase with the increase of arsenic concentration.

Plate 3. Fern (*Pteris vittata*) plant growth variation with different arsnic concentration on soil

4.3.4. Plant height

Plant height was significantly affected by arsenic treatments (Appendix XVI). Plant height of ferns (*Pteris vittata*) showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig. 09). The highest plant height (60.0 cm) was observed in control (As_0) and the lowest (39.3cm) was found in 2000 ppm (As_{2000}) arsenic concentration at 60 DAT. The present study identified that plant height was reduced with the increase of arsenic concentration.

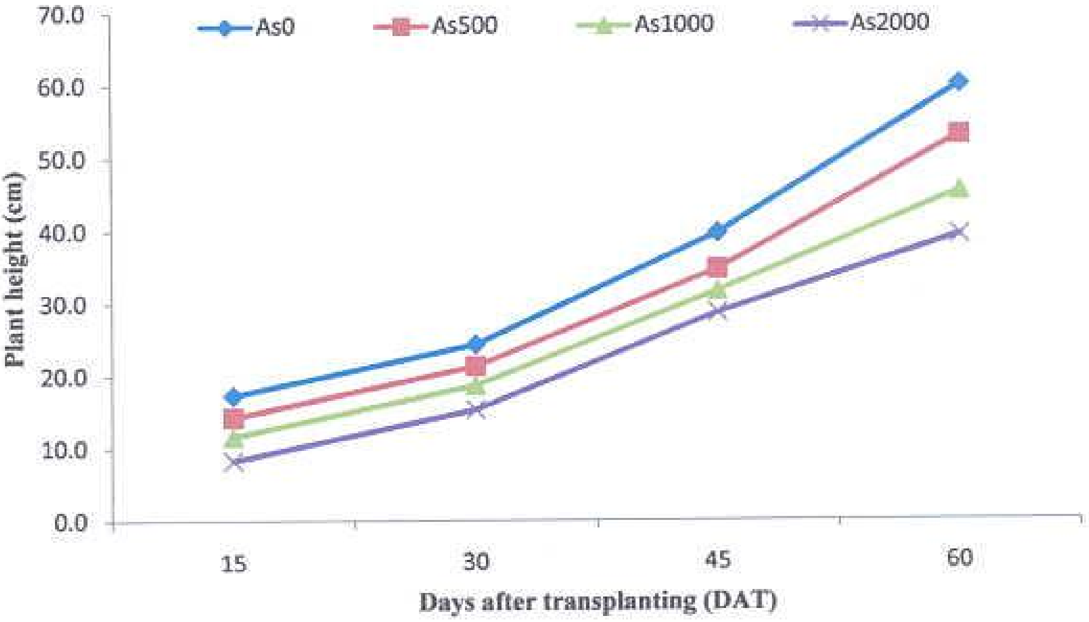

Fig.09. Effect of arsenic concentrations on plant height at different days after transplanting (DAT)

4.3.5. Leaf area

Leaf area (cm^2) of fern (*Pteris vittata*) was significantly affected by arsenic treatments (Appendix XVII). Leaf area of fern species (*Pteris vittata*) showed statistically significant differences among control, 500, 1000 and 2000 ppm at 15, 30, 45 and 60 DAT (Fig. 10). The highest leaf area (35.0 cm^2) was observed in control (As_0) and the lowest (26.0 cm^2) leaf area was found in 2000 ppm (As_{2000}) arsenic at 60 DAT. The present study identified that leaf area of fern was reduced with the increase of arsenic concentration.

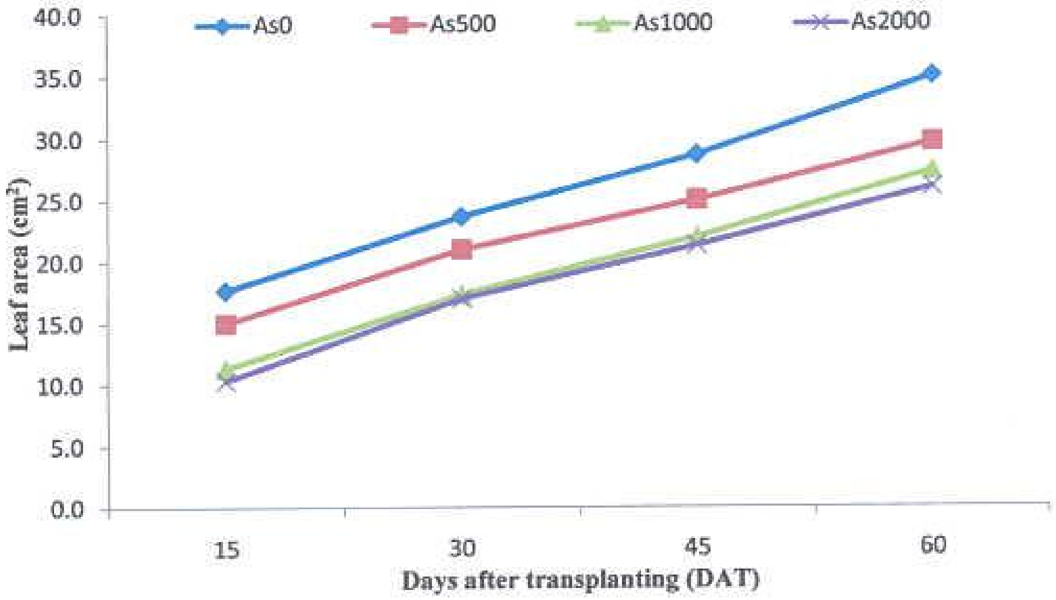

Fig.10. Effect of arsenic concentrations on plant leaf area at different days after transplanting (DAT)

4.3.6. Plant leaf biomass

Different arsenic concentration showed significant variation in terms of plant leaf biomass of fern (*Pteris vittata*) (Appendix XV). Plant leaf biomass of fern (*Pteris vittata*) showed statistically significant differences among control, 500, 1000 and 2000 ppm (Table. 5). Maximum plant leaf biomass (2.5 gm) was recorded from control (As_0) whereas the minimum plant leaf biomass (1.6 gm) was found from 2000 ppm (A_{2000}) arsenic (Table 5). The present study identified that plant leaf biomass was reduced with the increase of arsenic concentration. Plant biomass varied due to the variation of the concentration of arsenic (Wei *et al.*, 2006).

4.3.7. Arsenic accumulation in 0.5 mg dried leaf sample (ppb)

Different concentrations significantly influenced the arsenic accumulation from contaminated soil in terms of plant leaf biomass of fern (*Pteris vittata*) (Appendix XVIII). Maximum arsenic accumulation on leaf (35974.0 ppb) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation on leaf (0.7 ppb) was found from arsenic control (As_0) (Table 5). This result indicated that ferns grown with 2000 ppm arsenic treated soil accumulated maximum amount of arsenic. Wang *et al.* (2002) also found that *Pteris vittata* accumulated arsenic in the fronds up to 27,000 mg As kg^{-1}(ppm) dry weight.

4.3.8. Total arsenic accumulation on plant leaves (ppm)

Total arsenic accumulation on plant leaves of *Pteris vittata* varied significantly among the soil treated with different arsenic concentrations (AppendixXVIII). Maximum arsenic accumulation on leaf (23837. 2ppm) was recorded from 2000 ppm (A_{2000})arsenic whereas the minimum arsenic accumulation on leaf (0.9 ppm) was found from arsenic control (As_0) (Table 5). Soil treated with 2000 ppm arsenic hastened the amount of arsenic uptake. Wang *et al.* (2002); Umma *et al.* (2013) also found that *Pteris vittata* accumulated arsenic in the fronds up to 27,000 mg As kg^{-1}(ppm) dry weight

4.3.9. Arsenic accumulation by total plant leaves biomass

Arsenic accumulation by total plant leaves biomass was varied significantly among the soil treated with different arsenic concentrations (XIX). Maximum arsenic accumulation on leaves biomass (38.13 mg) was recorded from 2000 ppm (A_{2000})arsenic whereas the minimum arsenic accumulation on leaf (0.002 mg) was found from arsenic control (As_0) (Table 5).

Table 5. Fern responses of arsenic concentrations to different attributes

Treatments	Number of leaves at 30 DAT		Plant leaf bio-mass (g)	Arsenic accumula-tion on 0.5 mg tested sample (ppb)	Total arsenic accumulation on plant leaves (ppm)	Arsenic accumulation by total plant leaves biomass (mg)
	Newly Emerged	Died				
As_0	12.6 a	1.6 c	2.5 a	0.7 d	0.9 d	0.002 c
As_{500}	10.3 b	3.3 b	1.9 c	1840 c	17007.1 b	32.3 b
As_{1000}	8.7 c	4.0 ab	2.1 b	16977 b	15332.6 c	32.2 b
As_{2000}	8.3 c	4.6 a	1.6 d	35974 a	23837.2 a	38.1 a
LSD0.05	1.5	0.7	1.5	88.1	44.8	2.9
CV%	7.3	10.9	0.04	0.3	0.14	5.7

CHAPTER V

SUMMARY AND CONCLUSION

The agricultural irrigation system of Bangladesh is completely reliant on groundwater. Large amounts of As and other heavy metals are added to agricultural soils due to the irrigation which is ultimately entering into the food chain causing a very alarming health as well as biodiversity depletion threat. Hence, an attempt was made to explore a sustainable way to address the arsenic pollution problem employing trap plant. To find out the trap plant work at the Horticulture field, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period from July 2012 to October 2012. Each of the experiment included different three species viz. Fern (*Pteris vittata* L.), Rice and Arum and soil was treated with different arsenic concentration viz. Control, 500 ppm, 1000 ppm and 2000 ppm. The one factor experiment was laid out in Completely Randomized Design (CRD) with three Replications. The summary of the results and conclusion have described in this chapter.

In the experiment with rice the maximum leaf number (51.3) was recorded from control (As_0) whereas the minimum plant leaf number (2.6) was found from 2000 ppm (As_{2000}) arsenic treatment at 60 DAT. Maximum plant height (92.3 cm) was recorded from control (As_0) whereas the minimum plant height (6.7 cm) was found from 2000 ppm (As_{2000}) arsenic treatment at 60 DAT. Highest leaf area (20.27 cm^2) was recorded from control (As_0) whereas lowest leaf area (8.1cm^2) was found from 2000 ppm (As_{2000}) arsenic treatment at 60 DAT. The maximum number of tiller per hill (24.0) was produced in control (As_0) and minimum number of tiller per hill (3.9) was found in 2000 ppm arsenic concentration (As_{2000}). The maximum number of panicle (21.5) per hill was produced in control (As_0) and minimum number of panicle (2.9) per hill was found in 2000 ppm (As_{2000}) arsenic concentration. The maximum number of filled grain (525.3) per hill was produced in control (As_0) and minimum number of filled grain (43.7) per hill was found in 2000 ppm (As_{2000}) arsenic concentration. The maximum number of unfilled grain (214.0) per hill was produced in 1000 ppm (As_{1000}) arsenic concentration and minimum number of unfilled grain number (39.3) per hill was found in conrol. Maximum plant leaf biomass (405.5 mg) per hill was recorded from control (As_0) whereas the minimum plant leaf biomass (57.9 mg) was found from 2000 ppm (As_{2000}) arsenic. On 0.5 mg tested sample of straw, plant from 2000 ppm (As_{2000}) treated soil was uptaken maximum amount of arsenic (1019.0 ppb) while control (As_0) was obtained minimum (0.06 ppb) amount of arsenic. Total plant straw from 2000 ppm (As_{2000}) treated soil was accumulated maximum amount of arsenic (206.3 ppm) while control (As_0) was obtained minimum (0.03ppb) amount of arsenic. Maximum grain biomass (210.1 mg) was recorded from control (As_0) whereas the minimum grain biomass (141.1 mg) was found from 2000 ppm (As_{2000}) arsenic. On 0.5 mg tested grain sample, plant from 2000 ppm (As_{2000}) treated soil plants was accumulated maximum amount of arsenic (218.0 ppb) while control (As_{00}) was obtained minimum (0.02 ppb) amount of arsenic. Total grain from 2000 ppm (As_{2000}) treated soil plant was uptook maximum amount of arsenic (61.6 ppm) while control (As_{00}) was obtained minimum (0.01 ppm) amount of arsenic.

In the experiment with arum the maximum leaf number (14.0) per plant was recorded from control (As_0) whereas the minimum plant leaf number (5.7) per plant was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. The highest newly emerged number of leaves (3.6) was observed in 500 ppm (As_{500}) arsenic and the lowest newly emerged number of leaves (2.0) was found in 2000 ppm arsenic (As_{2000}). The highest died number of leaves at (3.6) was observed in 2000 ppm (As_{2000}) arsenic and 500 ppm arsenic (As_{500}) and the lowest died number of leaves at (1.3) was found in control (As_0). Maximum plant height (62.0 cm) was recorded from control (As_0) whereas the minimum plant hieght (31.0cm) was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. Maximum number of runner of (6.6) was recorded from control (As_0) whereas the minimum number of runner (1.3) was found from 2000 ppm (As_{2000}) arsenic at 45 DAT. Maximum number of sucker (5.3) was recorded from control (As_0)

whereas the minimum number of runner (1.3) was found from 2000 ppm (As_{2000}) arsenic. The highest leaf area (85.6 cm^2) was recorded from control (As_0) whereas lowest leaf area (34.1cm^2) was found from 2000 ppm (As_{2000}) arsenic at 60 DAT. Maximum plant leaf biomass (1.6 gm) per plant was recorded from control (As_0) whereas the minimum plant leaf biomass (0.7mg) was found from 2000 ppm (A_{2000}) arsenic. On 0.5 mg tested sample of leaf, maximum arsenic accumulation (1359.5 ppb) was recorded from 2000 ppm arsenic (A_{2000}) whereas the minimum arsenic accumulation (0.2 ppb) was found from control (As_0). Maximum total arsenic accumulation on leaves (973.5 ppm) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation (0.3 ppm) was found from control (As_0). Maximum plant runner biomass (361.6 mg) was recorded from control (A_0) whereas the minimum plant leaf biomass (90.4 mg) was found from 2000 ppm (As_{2000}) arsenic. Maximum arsenic accumulation on 0.5 mg tested sample on runner (1166.8 ppb) was recorded from 2000 ppm treatment (A_{2000}) whereas the minimum arsenic accumulation (0.1 ppb) was found from control (As_0) arsenic. Maximum total arsenic accumulation on runner (210.3 ppm) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation on runner (0.04 ppm) was found from control (As_0).

In experiment with fern (*Pteris vittata*) the highest leaf number (40.7) per plant was observed in control (As_0) and lowest leaf number (14.0) was found in 2000 ppm (As_{2000}) arsenic at 60 DAT. The highest newly emerged leaves (12.6) were observed in control (As_0) and the lowest newly emerged leaves (8.3) were found in 2000 ppm (As_{2000}) arsenic. The highest number of dead leaves (4.6) was observed in 2000 ppm (As_{2000}) arsenic. The highest number of died leaves (4.6) was observed in 2000 ppm (As_{2000}) arsenic and the lowest died number of leaves (1.6) was found in control (As_0). The highest plant height (60.0 cm) was observed in control (As_0) and the lowest (39.3cm) was found in 2000 ppm (As_{2000}) arsenic concentration at 60 DAT. The highest leaf area (35.0 cm^2) was observed in control (As_0) and the lowest (26.0 cm^2) leaf area was found in 2000 ppm (As_{2000}) arsenic at 60 DAT. Maximum plant leaf biomass (2.5 gm) per plant was recorded from control (As_0) whereas the minimum plant leaf biomass (1.5 gm) per plant was found from 2000 ppm (A_{2000}) arsenic. Maximum arsenic accumulation on 0.5 mg tested sample of leaf (35974.0 ppb) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation (0.7 ppb) was found from arsenic control (As_0). Maximum arsenic accumulation on leaves biomass (38.13 mg) was recorded from 2000 ppm (A_{2000})arsenic whereas the minimum arsenic accumulation on leaf (0.002 mg) was found from arsenic control (As_0). Maximum total arsenic accumulation on leaf (23837.2 ppm) was recorded from 2000 ppm (A_{2000}) arsenic whereas the minimum arsenic accumulation on leaf (0.9 0 ppm) was found from arsenic control (As_0).

CONCLUSION

From the above results it was observed that non-edible fern (*Pteris vittata* L.) bears maximum arsenic uptake from soil. So *Pteris vittata* L. is the excellent trap plant for arsenic accumulate from soil and it can stay alive when soil arsenic concentration is 2000 ppm. So, the result of present study offers a good scope for mitigation of arsenic from soil by using this method. It was also observed that edible plant rice and arum (kochu) both accumulated arsenic from soil.

Suggestions:

Considering the findings of the present research, advance studies in the subsequent areas may be suggested:

I. Further research could be conducted with *Pteris vittata* L. and other plant for checking out arsenic uptake from soil.

II. Further research could be conducted incorparating *Pteris vittata* L. with edible crops to protect arsenic from entering into the food chain.

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APPENDICES

Experiment 1:

Appendix I. Analysis of variance of the data on leaf number at different DAT of rice

Source of Variation	Degrees of freedom (df)	Mean square of leaf number at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	9.3	7	2.3	1.8
Arsenic concentrations	3	133.1*	432.9*	930.9*	1442.1*
Error	6	1.1	2.6	3.9	3.9

*: Significant at 0.05 level of probability

Appendix II. Analysis of variance of the data on plant height at different DAT of rice

Source of Variation	Degrees of freedom (df)	Mean square of plant height at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	10.5	8.2	4.8	22.6
Arsenic concentrations	3	222.7*	964.3*	2475.4*	4579.7*
Error	6	3.1	4.4	2.2	6.6

*: Significant at 0.05 level of probability

Appendix III. Analysis of variance of the data on leaf area at different DAT of rice

Source of Variation	Degrees of freedom (df)	Mean square of leaf area at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	5.7	5.7	5.7	5.7
Arsenic concentrations	3	24.4*	29.1*	46.9*	83.9*
Error	6	0	0	0	0

*: Significant at 0.05 level of probability

Appendix IV. Analysis of variance of the data on number of tillar (at 30 days), number of panicle, filled grain number and nufilled grain number of rice

Source of Variation	Degrees of freedom (df)	Mean square of			
		Number of tillar	Number of panicle	Filled Grain Number	Unfilled Grain number
Replication	2	18.7	15.1	250.3	394.3
Arsenic concentrations	3	215.5*	189.5*	148838.1*	18466.2*
Error	6	0.3	0.9	169.0	901.9

*: Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data on plant leaf biomass, arsenic accumulation (on 0.5 mg tested sample of straw) and total arsenic accumulation on straw

Source of Variation	Degrees of freedom (df)	Mean square of		
		Plant leaf biomass	Arsenic accumulation (on 0.5 mg tested sample of straw)	Total arsenic accumulation on straw
Replication	2	5686.6	26991.6	6849.9
Arsenic concentrations	3	52930.4*	577424.2*	17870.1*
Error	6	838.2	10702	1272.3

*: Significant at 0.05 level of probability

Appendix VI. Analysis of variance of the data on plant grain biomass, arsenic accumulation (on 0.5 mg tested sample of grain) and total arsenic accumulation on grain

Source of Variation	Degrees of freedom (df)	Mean square of		
		Grain biomass	Arsenic accumulation (on 0.5 mg tested sample of grain)	Total arsenic accumulation on grain
Replication	2	228.1	103.0	50.5
Arsenic concentrations	3	4359.1*	19034.6*	1136.4*
Error	6	62.8	0.0	10.9

*: Significant at 0.05 level of probability

Experiment 2:

Appendix VII. Analysis of variance of the data on leaf number at different DAT of arum

Source of Variation	Degrees of freedom (df)	Mean square of leaf number at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	0.08	1.1	0.08	0.5
Arsenic concentrations	3	0.3*	3.4*	10.9*	38.4*
Error	6	0.3	0.08	0.3	0.3

*: Significant at 0.05 level of probability

Appendix VIII. Analysis of variance of the data on plant height at different DAT of arum

Source of Variation	Degrees of freedom (df)	Mean square of plant height at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	6.3	6.2	12.5	11.1
Arsenic concentrations	3	65.9*	65.9*	279.2*	614.3*
Error	6	0.5	0.4	0.4	0.7

*: Significant at 0.05 level of probability

Appendix IX. Analysis of variance of the data on newly emerged number (At 30 DAT), died number (At 30 DAT), number of sucker and plant leaf biomass of arum

Source of Variation	Degrees of freedom (df)	Mean square of			
		Newly emerged number	Died number	Number of sucker	Plant leaf biomass
Replication	2	0.6	1.4	0.2	27056.6
Arsenic concentrations	3	1.9*	2.9*	9.3*	420451.8*
Error	6	0.1	0.5	0.2	9742.6

*** : Significant at 0.05 level of probability**

Appendix X. Analysis of variance of the data on number of runner at different DAT of arum

Source of Variation	Degrees of freedom (df)	Mean square of number of runner at	
		30 DAT	45 DAT
Replication	2	2.5	3.1
Arsenic concentrations	3	4.9*	15.6*
Error	6	0.1	0.6

*** : Significant at 0.05 level of probability**

Appendix XI. Analysis of variance of the data on leaf area at different DAT of arum

Source of Variation	Degrees of freedom (df)	Mean square of leaf area at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	3.2	4.2	2.8	2.8
Arsenic concentrations	3	117.2*	120.9*	653.5*	1558.1*
Error	6	0.2	0.1	0.1	0.1

*** : Significant at 0.05 level of probability**

Appendix XII. Analysis of variance of the data on runner biomass, arsenic accumulation (on 0.5 mg tested sample of leaves) and total arsenic accumulation on leaves of arum

Source of Variation	Degrees of freedom (df)	Mean square of		
		Runner biomass	Arsenic accumulation (on 0.5 mg tested sample of leaves)	Total arsenic accumulation on leaves
Replication	2	1149.2	2964.0	37353.7
Arsenic concentrations	3	42903.8*	749283.6*	230741.1*
Error	6	1149.2	0.0	6435.7

*** : Significant at 0.05 level of probability**

Appendix XIII. Analysis of variance of the data on arsenic accumulation (on 0.5 mg tested sample of runner) and total arsenic accumulation on runner of arum

Source of Variation	Degrees of freedom (df)	Mean square of	
		Arsenic accumulation (on 0.5 mg tested sample of runner)	Total arsenic accumulation on runner
Replication	2	3441.9	1435.1
Arsenic concentrations	3	654652.8*	15211.9*
Error	6	176.3	2396.2

*** : Significant at 0.05 level of probability**

Experiment 3:

Appendix XIV. Analysis of variance of the data on leaf number at different DAT of fern (*Pteris vittata*)

Source of Variation	Degrees of freedom (df)	Mean square of leaf number at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	8.1	8.1	18.1	72.3
Arsenic concentrations	3	37.4*	37.4*	108.5*	387.6
Error	6	0.2	0.2	1.5	8.0

*** : Significant at 0.05 level of probability**

Appendix XV. Analysis of variance of the data on newly emerged number (At 30 DAT), died number (At 30 DAT), and plant leaf biomass of fern (*Pteris vittata*)

Source of Variation	Degrees of freedom (df)	Mean square of		
		Newly emerged number	Died number	Plant leaf biomass
Replication	2	0.7	0.5	449.2
Arsenic concentrations	3	11.8*	4.9*	444866.8*
Error	6	0.5	0.1	0.5

*** : Significant at 0.05 level of probability**

Appendix XVI. Analysis of variance of the data on plant height at different DAT of fern (*Pteris vittata*)

Source of Variation	Degrees of freedom (df)	Mean square of plant height at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	7.5	7.5	1.5	9.3
Arsenic concentrations	3	44.1*	44.1*	66.0*	243.1*
Error	6	1.6	1.6	2.2	7.8

*** : Significant at 0.05 level of probability**

Appendix XVII. Analysis of variance of the data on leaf area at different DAT of fern (*Pteris vittata*)

Source of Variation	Degrees of freedom (df)	Mean square of leaf area at			
		15 DAT	30 DAT	45 DAT	60 DAT
Replication	2	14.0	10.7	5.2	3.0
Arsenic concentrations	3	34.3*	30.3*	33.6*	47.2*
Error	6	2.3	1.6	1.4	1.9

*** : Significant at 0.05 level of probability**

Appendix XVIII. Analysis of variance of the data on arsenic accumulation (on 0.5 mg tested sample of leaves) and total arsenic accumulation on leaves of fern (*Pteris vittata*)

Source of Variation	Degrees of freedom (df)	Mean square of	
		Arsenic accumulation (on 0.5 mg tested sample of leaves)	Total arsenic accumulation on leaves
Replication	2	11659.8	17528.8
Arsenic concentrations	3	686455068.2*	131222594.9*
Error	6	1945.0	503.3

*** : Significant at 0.05 level of probability**