### PERFORMANCE OF RICE CULTIVARS TO DIFFERENT CONCENTRATIONS OF ARSENIC AT SEEDLING STAGE

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### PERFORMANCE OF RICE CULTIVARS TO DIFFERENT CONCENTRATIONS OF ARSENIC AT SEEDLING STAGE

BY

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# CERTIFICATE

This is to certify that the thesis entitled "PERFORMANCE OF RICE CULTIVARS TO DIFFERENT CONCENTRATIONS OF ARSENIC AT SEEDLING STAGE" 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 Nur Alam Shopon, Registration number: 11-04571 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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



Dated: December, 2017 Dhaka, Bangladesh **Prof. Dr. Md. Abdur Razzaque** Department of Agricultural Chemistry Sher-e-Bangla Agricultural University Dhaka-1207 "A single tear caused by the remberance of ALLAH brings a comfort to the heart that nothing in the dunya can match"

DEDICATED TO My Beloved Father & Mother The person who taught me that "Always Trust Your Struggle"

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December, 2017 SAU, Dhaka The Author

#### PERFORMANCE OF RICE CULTIVARS TO DIFFERENT CONCENTRATIONS OF ARSENIC AT SEEDLING STAGE

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#### ABSTRACT

A two factorial tray experiment was conducted in the net house of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, during November 2017 to January 2018, to study the performance of rice cultivars to different concentrations of arsenic at seedling stage. Factor A: different doses of arsenic [As<sub>0</sub> = No arsenic added (control), As<sub>1</sub> = 3 ppm arsenic, As<sub>2</sub> = 6 ppm arsenic,  $As_3 = 9$  ppm arsenic and  $As_4 = 12$  ppm arsenic (soil water basis)] and Factor B: different rice cultivars  $[T_1 = BINA dhan-8, T_2 =$ BINA dhan-10,  $T_3$  = BINA dhan-14,  $T_4$  = BINA dhan-18,  $T_5$  = BR 3,  $T_6$ = BR 14, T<sub>7</sub> = BR 16, T<sub>8</sub> = BRRI dhan28, T<sub>9</sub> = BRRI dhan29, T<sub>10</sub> =BRRI dhan36,  $T_{11}$  = BRRI dhan45,  $T_{12}$  = BRRI dhan47,  $T_{13}$  = BRRI dhan50,  $T_{14} = BRRI$  dhan55,  $T_{15} = BRRI$  dhan58,  $T_{16} = BRRI$  dhan59,  $T_{17}$  = BRRI dhan60 and  $T_{18}$  = BRRI dhan61]. Different growth and yield parameters varied significantly due to difference in the doses of arsenic. Arsenic toxicity adversely affects all the growth and yield related attributes of all rice cultivars. BINA dhan-18 produced highest seedling height and BRRI dhan29 produced the lowest. BRRI dhan-61 produced highest shoot weight and BR 3 produced the lowest. BINA dhan-18 produced maximum root length and BRRI dhan45 produced the lowest. BRRI dhan29 produced maximum root weight and BR 3 produced the minimum. BRRI dhan29 produced maximum total dry matter and BR 3 produced minimum. BRRI dhan29 contained maximum N in shoot and BINA dhan-10 contained the minimum. BRRI dhan61 contained maximum P in shoot and BR 3 contained the minimum. BRRI dhan61 contained maximum K in shoot and BR 3 contained the minimum. BRRI dhan29 contained maximum N in root and BINA dhan-8 contained the minimum. BRRI dhan61 contained maximum P in root and BR 3 contained the minimum. BRRI dhan61 contained maximum K in root and BR 3contained the minimum.

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# Acronyms

Abbreviation	Full word
%	Percent
@	A the rate of
°C	Degree Celsius
AEZ	Agro Ecological Zone
As	Arsenic
BBS	Bangladesh Bureau of Statistics
BARI	Bangladesh Agricultural Research Institute
cm	Centimeter
$CuSO_4.5H_2O$	Green vitriol
CV%	Percentage of Coefficient of Variance
DAS	Days after sowing
DI	Di-Ionized
DMRT	Duncan's Multiple Range Test
dS/m	Deci Semens/Meter
EC	Electrical conductivity
e.g	As for example
et al	And others
G	Gram
i.e	That is
Κ	Potassium
Kg	Kilogram
KCl	Potassium chloride
LSD	Least significance difference
m	Meter
MoP	Murate of potash
Ν	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
NS	Not significance
OM	Organic matter
Р	Phosphorus
pН	Hydrogen ion concentration
ppb	Parts per billion
ppm	Parts per million
SAU	Sher-e-Bangla Agricultural University
TSP	Triple super phosphate
Zn	Zinc



# Chapter I INTRODUCTION

#### **CHAPTER I**

#### **INTRODUCTION**

Rice (Oryza sativa L.) belongs to the cereal crops under Poaceae family and is one of the world's most widely consumed grains. It is the staple food and the driving force of Bangladesh agriculture (MoFDM, 2012). Presently, it occupies first position which covers about 80% of arable land in Bangladesh agriculture whereas it occupies third position in rice area and fourth position in rice production of the world (BRRI, 2012). Rice is the staple dietary item for the people and per capita rice consumption is about 166 kg/year (BBS, 2010). It alone provides 76% of the calorie intake and 66% of total protein requirement (Bhuiyan et al., 2002). The total rice production in our country is about 34.00 million tons to feed her 149.69 million people (Mondal and Choudhury, 2014). BBS (2010) reported that the population will have possibly increased to 230 million by the year 2030 which need more cereal crops for meet their demand. Therefore, it is essential to increase the production of rice to need the demand of excess food of us. Growth and development of the crops including rice depend on environmental factors such as atmosphere, temperature, light, humidity, nutrients etc. If deficiency or toxicity of any component of the environment that deleterious effect on the crops. Many abiotic factors such as heat, cold, drought, salinity and heavy metal contamination reduce the growth and development of the crops. Arsenic contamination is a one kind of heavy metal stress with generally alters the morpho-physiology, yield contributing characters and yield of agricultural crops. So many authors have been reported that arsenic in general, is accumulated mainly in the root system, to a lesser extent in the above ground parts of plant. It inhibits the growth together with fresh and dry biomass accumulation (Stoeva et al., 2003) and causes physiological

disorders (Wells and Gilmor, 1997), as well as reduction of the crop productivity (Stepanok, 1998). Long-term exposure to inorganic arsenic, mainly through drinking of contaminated water, eating of food prepared with this water and eating food irrigated with arsenic-rich water, can lead to chronic arsenic poisoning. Rai et al. (2015) reported that accumulation of arsenic (As) in grain is a serious concern worldwide which affects nutritional status in rice grain and is associated with higher rates of skin, bladder, and lung cancers, and heart disease. In Bangladesh researches have been held to combat against drought and salinity. Various cultivars, tolerant to these stresses have been improved. But arsenic tolerant variety for rice has yet to be developed. Even there is a scarcity of information about sensitivity of rice towards arsenic contamination. Singh et al. (2006) stated that arsenic reduces the rate of photosynthesis by damage mechanism of different cell organelles such as plasma membrane, chloroplast which causing electrolyte leakage and an increase in malondialdehyde, a product of lipid peroxidation, pointing to the role of oxidative stress in As toxicity. Nevertheless, there is significant evidence that exposure of plants to inorganic arsenic does result in the generation of reactive oxygen species (ROS), which is linked with arsenic valence change, a process that occurs readily in plants (Flora, 1999). ROS has the capacity to directly damage proteins, amino acids and nucleic acids and also cause peroxidation of membrane lipids (Dat et al., 2000). To combat these effects, enzymatic and nonenzymatic antioxidants are mobilized to quench ROS. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) etc. and nonenzymatic antioxidants include glutathione and ascorbate (Dat et al., 2000). Large numbers of studies indicated that low concentrations of arsenic stimulated the growth of plants; but excessive arsenic did harm to plants (Han et al., 2002). Hossain et al. (2008) stated that almost all the

growth parameters (plant height, number of effective tillers, filled grains panicle<sup>-1</sup> and 1000-grain weight) decreased significantly with increasing arsenic levels, and unfilled grains panicle<sup>-1</sup> increased significantly. Onken and Hossner (1996) reported that the rice yield reduction by 66% when mean soil solution As concentration was raised to 1.5 mg As L<sup>-1</sup>. Moreover, As in lower concentrations increased grain yield and straw yield; this was also supported by the yield-contributing characteristics of rice (Islam *et al.*, 2004). Therefore, it suggest that arsenic reduces the yield of rice along with creates numerous diseases in human and animal health.

Being an agro-based country most of the people of Bangladesh depend on agriculture for their livelihood. Nature plays pivotal role in Bangladesh agriculture where some calamities and contaminations are inevitable. Arsenic, a naturally occurring metalloid, is very mobile in the environment. It enters into farming systems through a variety of means which include natural geochemical processes (Smedley and Kinniburgh, 2002), use of arsenic-based pesticides, mining operations, irrigation with arsenic contaminated groundwater, and also fertilization with municipal solid wastes (Meharg et al., 2009). For Bangladesh perspective, ground water contamination of arsenic is a pressing issue for various districts. Up to various level most of the districts have arsenic contaminated ground water and 17 of them have exceeded the safety level of WHO recommendation for safety including Chandpur, Comilla, Noakhali, Feni, Munshiganj, Brahmanbaria, Faridpur, Madaripur, Laksmipur, Gopalganj, Shariatpur, Narayanganj, Narail, Satkhira and Chapainawabganj (Haque et. al., 2007). Therefore, it is suggesting that arsenic contamination is gradually increasing both in area and groundwater in our country.

However, to my knowledge no study has elicited whether, responses of rice plant to different concentration of arsenic in Bangladesh.

#### **Detailed research objectives:**

The work has been designed and planned with the following objectives:

- To find out the toxic effects of arsenic on growth and vigor of some rice cultivars in Bangladesh.
- To screen out the cultivars on the basis of resistance and susceptible by analyzing As and other essential cation uptake including N, P and K in both root and shoot of rice seedlings.



# Chapter II REVIEW OF LITERATURE

#### **CHAPTER II**

#### **REVIEW OF LITERATURE**

Arsenic is one of the major abiotic stresses, which harmfully effects crop growth and yield and nutrient contents. Cultivated rice under saline condition faces different types of stress i.e. ion toxicity and the other arises from low water availability. The effects of arsenic on crop growth have been carried out by a large number of scientists at home and in abroad. But the physiological aspects of growth, yield and nutrient contents with growth analysis on various crops to identify the cause of yield reduction because of increasing salinity of soils has not yet been done at appropriate level. Growth and yield of rice plants are greatly influenced by the environmental factors i.e. air, day length or photoperiod, temperature, variety and agronomic practices like transplanting time, spacing, number of seedlings, depth of planting, fertilizer management etc. and abiotic stresses like salinity, drought, flood, contamination by heavy metals etc. Yield and yield contributing characters of rice are greatly influenced by different levels of contamination by heavy metals like Arsenic, Cadmium, Lead etc. Arsenic is one the most pervasive and toxic heavy metals in several parts of the world. It is one of the main pollutants in rice fields near industrial areas and highly toxic to plant growth and development. But the available relevant review associated to arsenic reduction in rice is very limited in the context of Bangladesh as well as the World. In this section attempt has been made review some of the available information on the soil arsenic and its effects on growth, nutrient contents and yield components of rice.

Arsenic, a metalloid and naturally prevailing component, is one of the most plentiful components in the earth's crust and is found throughout our environment. Arsenic can affix to very small particles in the air, stay in the air for many days, and travel long extents. Arsenic is firstly applied as an insecticide and herbicide or preservatives for wood due to its germicidal power and resistance to rotting and decay respectively. Arsenic is also applied in medicine, electronics, and industrial manufacturing (Nriagu and Azcue, 1990).

Arsenic is one of the noxious environmental pollutants which has recently attracted attention because of its chronic and epidemic effects on human health through widespread water and crop contamination due to the natural release of this noxious component from aquifer rocks in Bangladesh (Fazal *et al.*, 2001; Smith *et al.*, 2000; Ahmed, 2000 and Hopenhayn, 2006), West Bengal of India (Chakraborti and Das, 1997; Banerjee, 2000). Geogenic contamination of arsenic in aquifer rocks has also been noted in Thailand (Visoottiviseth *et al.*, 2002), Vietnam, Inner Mongolia, Greece, Hungary, USA, Ghana, Chile, Argentina and Mexico (O'Neill, 1995; Smedley and Kinniburgh, 2002).

Arsenic and its compounds are flexible and cannot be destroyed in the environment noted by Ferguson (1990). However, interaction with oxygen or other molecules present in air, water, or soil, as well as with bacteria that live in soil or sediment can induce arsenic to change form, attach to different particles, or separate from these particles. Elevated concentrations of arsenic have firstly resulted from natural sources, such as erosion and leaching from geological formations or anthropogenic sources. In addition, arsenic use for industrial purposes, mining activities, metal processing, and pesticides and fertilizers are other major sources of contamination. Gibb *et al.* (2011) and Argos *et al.* (2010) reported that many common arsenic compounds can dissolve in water, thus arsenic can pollute lakes, rivers, or underground water by dissolving in rain, snow, or

through discarded industrial wastes. Therefore, arsenic contamination in groundwater is a major public health threat worldwide. In addition, the effect of chronic arsenic exposure from ingested arsenic polluted food and water or inhaled polluted air has been investigated in various countries and found to be associated with detrimental health effects such as hyperpigmentation, keratosis, various types of cancer and vascular diseases. Arsenic is a crystal-shaped metalloid component which is brittle in nature and grayish white in color. It is a naturally occurring poisonous chemical compounds and widely distributed in the soil profile as component of minerals and found in nominal amounts in all organisms. Arsenic can be found as a compound of oxygen, chlorine, sulfur, carbon, hydrogen, lead, mercury, gold and iron. There are as many as 150 species of arsenic-bearing minerals that exist on the earth. However, only few of them are regarded as arsenic ore, because the amount of arsenic is higher in these compounds and also they are more available. These compounds are realgar or arsenic disulphide (AsS), orpiment or arsenic trisulphide  $(As_2S_3)$  and arsenopyrite or ferrous arsenic sulphide (FeAsS) (Nordstrom, 2002). Mandal and Suzuki (2002) noted that the terrestrial abundance of arsenic is approximately 1.5-3.0 mg/kg. Colbourn et al. (1975) observed that the amount of arsenic in soil varies considerably from country to country from 0.1 to 50 mg/kg with an average concentration of about 5-6 mg/kg. Arsenic may derive in soils from parent material (Tanaka, 1988), but in soils, it present in higher concentrations than those in rocks (Peterson et al., 1981). Mandal and Suzuki (2002) reported that unpolluted soils normally contain 1.0-40.0 mg/kg of arsenic with the lowest concentrations in sandy soils and those originated from granites, whereas larger concentrations are found in alluvial and organic soils. In soils, the concentration of arsenic is mostly present in sulphide ores of metals including sodium, copper, lead, silver and gold (BGS, 1999).

Hughes (2002) reported that arsenic is detected in natural and anthropogenic sources. It occurs naturally in rocks and soil, water, air, plants and animals. Volcanic activity, erosion of rocks and minerals, and forest fires are natural sources. Arsenic materializes naturally in soil, water, air, plants and animals. There are two forms of arsenic: organic and inorganic. Both the forms are easily absorbed, but the inorganic form is more harmful. It gathers in body organ, which is classified as a carcinogen and may affect different chemical and metabolic processes in the body.

Lu (1990) reported that arsenic used in industrial process, is used to produce antifungal wood preservatives conduct to soil contamination. Incineration of preserved wood products and pressure treated with chromate copper arsenate were found to be the source of environmental arsenic contamination. Arsenic is used in sheep dips, glass industries, pharmaceuticals, antifouling paints, leather preservatives, poison baits and arsenic containing pigments, and are also applied in optical industries and microelectronics. EPA (1983) noted that methylated arsenic is a minor component in the air of suburban, urban and industrial areas, and that the major inorganic portion of air is composed of the trivalent and pentavalent compounds. USDA (1970) noted that arsenic compounds are used in insecticides and pesticides because of its germicidal power. The inorganic arsenic compounds firstly sodium arsenite have been widely applied as a weed killer, and selective soil sterilant. Arsenic presents mainly as inorganic species, but it also can attach to organic material in soils (BGS, 1999; Mandal and Suzuki, 2002). Arsenic may assemble in soils through the use of arsenical pesticides, herbicide, fertilizer etc. Inorganic arsenic may be transformed to arsenic compounds by soil microorganisms (Wei et al., 1991). The total amount of arsenic in soils

and its chemical formula has an important influence on plant, animal and human health (Nriagu and Lin, 1994). Accumulation of arsenic can cause harmful effects to plants and go through into the human food chain. Arsenic retention and released by sediments depends on the chemical properties of the sediments, especially on the amount of iron and aluminium oxides and hydroxides (BGS, 1999). The amount of sedimentary iron is an crucial factor that impacts arsenic retention in sediments (Mandal and Suzuki, 2002).

Akter et al. (2005) noted that once arsenic compound is absorbed, it is generally processed via the liver's metabolic pathway, and then transformed into many different types of inorganic and organic species including arsenite  $(As^{3+})$ , arsenate  $(As^{5+})$ , dimethylarsinate (DMA), and mono-methyl-arsonate (MMA). Inorganic arsenic and organic arsenic are assimilated quickly into the blood and circulated to the human gastrointestinal tract. Organic arsenic types are generally regarded innocuous since they are poorly absorbed into living cells. In contrast, inorganic arsenics are highly reactive and influence a series of intercellular reactions (Drobna et al., 2010). Bhattacharya et al. (2002) reported that groundwater is a main source of drinking water, and elevated concentration of arsenic in groundwater has been held by various negative health effects in human. Arsenic in drinking water is one of the most notable environmental causes of cancer. In 1963, WHO has recommended limits to the maximum concentrations of arsenic in drinking water and their recommendation was of 50  $\mu$ g/L, but after new evidence associate with low arsenic concentrations for cancer risk. WHO further minimized their recommendation to 10 µg/L in 1992 (WHO, 2001). Chakraborti et al. (2001) noted that polluted used to cultivate rice and vegetables for human utilization is an important pathway of arsenic

ingestion. Le et al. (1994) reported that some crustaceans hold arsenobetaine and some seaweed contains arsenosugar, but seafood usually contains organic arsenic compounds that are less toxic than inorganic counterparts. Groundwater contamination is one of the major pathways of human subjection to inorganic arsenic and the risk of arsenic contamination is normally much higher in groundwater than that in surface water (Argos et al., 2012). Chowdhury et al. (2000) noted the elevated concentrations of arsenic in groundwater of Bangladesh, Vietnam (Berg et al., 2001), China (Lianfang and Jianzhong, 1994), Taiwan (Chen et al., 1994), Argentina (Smedley et al., 2005), and Canada (Grantham and Jones, 1977). Smokers have a significantly higher total urinary arsenic concentration than non-smokers do because some chemicals in cigarettes compete with different enzymes or co-factors responsible for the arsenic methylation process (Tseng, 2005). Ferreccio et al. (2000) found that cigarette smoking and ingesting arsenic in drinking water had a symbiotic effect. Cigarette smokers exposed to excessive amount of arsenic in drinking water (200  $\mu$ g/L) had a higher risk of blood cancer than smokers exposed to low concentrations of arsenic did (Morales et al., 2000 and Steinmaus et al., 2003). Cosmetics are also considered as an unlikely source of arsenic exposure and as a simple impurity, but are a leading cause of high exposure among many individuals. Assessing the amount of dermal absorption from a single component in a cosmetic product is composite and depends on many factors such as the concentration of arsenic in the product, the amount of product used, the duration of time left on the skin and the presence of emollients and penetration enhancers in the cosmetic products (Hostynek, 2014).

EPA (2004) noted that human subjection to arsenic through the air generally occurs at very small amount ranging from 0.4 to 30  $ng/m^3$ . They also estimated that about 40 to 90 ng of arsenic per day are typically ingested by humans. In unpolluted areas, around 50 ng or less arsenic is ingested per day. FAO (2007) noted that the problem originated from the arsenic-rich bed-rock of the Brahmaputra river basin that strains drinking water pumped to the surface over millions of tube wells. High concentrations of arsenic enter the food chain via soaking up by crops from roots to straw and grain polluted from irrigated water. Duxbury and Panaullah (2007) noted that the most severe effects have been found in Bengal Delta region in Bangladesh and West Bengal, India where the groundwater has been extensively spread to supply drinking and irrigation water. Around 30 million people drink water from arsenic polluted Shallow Tube Wells (STWs) and approximately 900000 STWs are used in irrigation 2.4 million out of 4 million ha land under irrigation in Bangladesh, mainly rice fields. Also, about 95% of the groundwater withdrawn is used for irrigation. It was also evaluated that water pumping from shallow aquifers for irrigation adds one thousand ton of arsenic per year to the cultivable soil in Bangladesh, mainly in the rice fields. Mirdar-Ul-Haq et al. (2005) noted that there are many factors responsible for heavy metal such as arsenic contamination in soils are long term usage of sewage or effluents for irrigation purposes which in turn will have adverse effects on plants, animals and human health. Islam and Islam (2007) noted that arsenic is poison. It is a notable health risk to millions of people over the world when it is there in food and drink. It is highly noxious at higher doses but chronic exposure to lower levels increases the risk of cancer of skin, lungs, kidney, bladder, liver, colon, pulmonary disease, prostrate; cardiac disease, cardiovascular disease, diabetes; diseases of arteries and capillaries; infertility, increased sensitivity to

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Hepatitis B infection and other ailments. Visible symptoms to the arsenic poisoning can be thickening and discoloration of skin, diarrhea, nausea, stomach pain, vomiting, paralysis and blindness.

A test was conducted by Duxbury et al. (2002) to determine the concentration of arsenic in rice grain of 150 samples collected from different districts of Bangladesh including Barisal, Rajshahi, Comilla, Dinajpur and Rangpur. Arsenic concentration was found in the range from 0.01 to 0.041 mg/kg in dry weight. As expected boro rice grain contained higher arsenic concentration (mean value 183 µg/kg dry weight) compared to aman rice (mean value 117 µg/kg dry weight). Chakraborti et al. (2001) noted that 90% inorganic and 10% organic arsenic present in rice. Irrespective of chemical root forms arsenic concentration was 10.5 mg/kg in the 0.05 mg/L treatment, which increased to 212.7 mg/kg in the 0.8 mg/L treatment (Marin et al., 1992). This arsenic accumulation by green algae from irrigation water may cause lower arsenic accumulation by rice plant which would be helpful for the people of Bangladesh (Huq et al., 2001). A test was conducted by Das et al. (2003) for arsenic in rice grown land on the soils adjacent to water source polluted with arsenic. They found highest concentration of arsenic accumulated by roots of rice followed by shoot and grain (0.23 ppm). Yan Chu (1994) observed a relationship between concentration of arsenic in soil solution and rice examined to quantify the effect of arsenic level uptaken by rice. The regression equation found the amount of arsenic present in the rice plant, Y, and the amount of arsenic in aqueous solution, X, to be: Y = 0.042X-0.0413. Xie and Huang (1998) reported that arsenic accumulation is influenced by concentration of arsenic in soil and nutrient media which increased greatly with increasing levels of arsenic. Heitkemper et al. (2001) found that rice grain has lower

concentrations of arsenic and the concentrations remain much below than maximum permissible limit of 2 mg/kg.

Rice is highly efficient in taking up arsenic, because it is grown in waterflooded condition which reduces the binding of arsenic by soil. This makes arsenic more vulnerable to rice. The semi-aquatic nature of rice plant and grain give the options to uptake arsenic from available sources. It is fact that arsenic is naturally occurring substance and because it is present in both soil and water, so it is going to get through into food. Rice uptakes more arsenic than other crops from the soil. It is because of the way rice is grown. Rice is grown in flooded soil which charges the soil readily releasing arsenic. The flooded soil is strongly reduced and anaerobic. By this situation arsenic is readily available to rice roots. Moreover, a higher amount of water is used for irrigated rice than is used for aerobic culture. A different variety of rice varies in arsenic tolerance. Major affected varieties develop straight head diseases, empty panicle at maturity stage (Brammer, 2008). Duxbury and Panaullah (2007) noted that rice production decreased from 8.9 t/ha at 26.3 ppm soil arsenic to 3 t/ha at 57.5 ppm arsenic. The results highlighted that the practical limit for rice production might lie between 30-50 mg/kg soil arsenic. Abedin et al. (2002a) noted that irrigation water polluted with arsenic decrease seed germination, plant height, root growth and yield of rice. In Bangladesh, groundwater of 58 out of 64 districts is polluted by arsenic in different concentrations. Arsenic concentration exceeds 1.00 mg per liter of water observed in 17 districts including Noakhali, Chandpur, Comilla, Feni, Munshiganj, Faridpur, Madaripur, Narayenganj, Brahmanbaria, Laksmipur, Gopalganj, Shariatpur, Narail. Satkhira and Chapainawabganj. Huq and Joardar (2008) noted that the yield of BRRI dhan29 and BRRI dhan28 decreased 16% from the control to higher dose

(2.0 mg/L). Higher level of arsenic negatively affected the nutrient uptake in rice and nutrient content except Nitrogen. Nitrogen content is positively correlated with arsenic level. Higher amount of nutrient uptake and nutrient content was found in BRRI dhan48 and flooding increased higher nutrient content and uptake in rice. This study suggests the possible management of moisture content and considering less arsenic susceptible variety, which might decrease the toxic effects of arsenic on nutrient uptake (Bhattacherjee *et al.*, 2014).

Abedin et al. (2002b) noted that increasing in the content of arsenic in irrigation water led to increasing arsenic content in rice and consequent decrease in plant yield. Islam et al. (2004) noted that household survey on dietary habits represent women consumed on an average 3.2 liter of water, 1.2 kg of cooked rice and 43 g of dry weight of curry per day. The total ingestion rates varied from 33.1-129.3  $\mu$ g/day (mean 64.5  $\mu$ g/day) and the results indicated that the major route of arsenic in Bangladesh is rice followed by carry and water. Khan et al. (2010) observed that the mobility of applied arsenic and the likely continued detrimental gathers of arsenic within the roots zone. Arsenic level present in irrigation water or in soil resulted in reduced of yield from 20-73% in boro rice and 9-79% in Transplanted Aman rice, the later indicating the high residual effect of arsenic on later grown crops. The concentration level of arsenic in rice grain (0.23-0.80  $\mu/g$ ), straw (2.63-12.51  $\mu/g$ ) and husk (1.21-2.47  $\mu/g$ ) increased with increasing extra arsenic. In lab experiment, the growth of rice was inhibited when soil contained less than 16 mg As/kg, and highly toxicity symptoms were found when soil contained 61 mg As/kg. Wang et al. (2010) noted that arsenic is one of the most vital contaminants as noxious component especially inorganic arsenic and it has chronic poisoning effect on human body. Very few studies have shown that rice is

much more efficient accumulator of arsenic into its grain and straw than other cereal crops, and rice consumption constitutes a large proportion of dietary intake of arsenic. The total arsenic level in rice differs from 0.005-0.712 mg/kg. The amount of inorganic arsenic in rice ranges from 10-91% of arsenic. Bhattacharya *et al.* (2010) noted that the arsenic uptake in rice differs from different rice varieties; the highest accumulation was recorded in white Minikete  $(0.32\pm0.006 \text{ mg/kg})$  and IR 50  $(0.30\pm0.001 \text{ mg/kg})$  rice varieties and highest was found in the Jaya rice variety  $(0.14\pm0.002 \text{ mg/kg})$ . In rice, highest arsenic accumulation found in the straw part  $(0.90\pm0.019-1.65\pm0.021 \text{ mg/kg})$  in contrast with the accumulation in husk  $(0.31\pm0.011-0.86\pm0.016 \text{ mg/kg})$  and grain  $(0.15\pm0.002-0.31\pm0.005 \text{ mg/kg})$  part. For the rice sample concentration of arsenic in the grain did not exceed the WHO recommended limit in rice (1.0 mg/kg).

Duel and Swoboda (1972) and Jacobs et al. (1970) found that displacement of soil phosphate-biarsenate at low soil arsenic concentration increased the availability of phosphate to the plant resulting in the increase of plant growth. Jahan et al. (2003); Rahman et al. (2004); Xie and Huang (1998) noted that plant height and biomass production decreased with the increase of arsenic concentration. Reduction in growth of rice in terms of tillering, plant height and biomass production is due to the result of arsenic phytotoxicity at high arsenic concentration. Schoof et al. (1999) observed that rice has more inorganic arsenic concentration than most other food, and consequently, diet that rely more on rice may contain the most inorganic arsenic. Van Geen et al. (2006) noted that the health risk due to ingestion of arsenic contained in rice therefore appear to be dwarfed in countries such as Bangladesh. Several studies observe that rice (Oryza sativa L.) in different growth stage accumulates arsenic

in different levels but at maturing stage uptakes maximum amount significantly than the other stages (Wang *et al.*, 2006). Islam *et al.* (2005) and Delowar *et al.* (2005) noted that rice plants accumulate about 2 mg/kg which is much more the permissible limit of 1.0 mg/kg, according to the WHO recommendation. Mehrag and Rahman (2002) observed that the contribution to total arsenic intake from drinking water was 12%, whereas from cooked rice, it was 54%, thus making it clear that rice contributed most into the daily arsenic uptake.

Dahal et al. (2008) noted that significant presence of arsenic polluted water on alkaline soils and arsenic uptake in agricultural plants at field level in Nepal. He noted his study by giving the average arsenic content of edible plant material (dry weight) in the order of onion leaves (0.56 mg As kg-1>onion bulb (0.44 mg As kg-1>cauliflower (0.34 mg As kg-1>rice (0.17 mg As kg-1>brinjal (0.08 mg As kg-1>potato (<0.02 mg As kg-1 indicating that in Nepal, onion leaves had highest and rice (fourth in order of concentration) As uptake. Dittmar et al. (2010) investigated that concentration of arsenic in straw and grain were raised in the field and maximum near the irrigation water inlet tank, where arsenic concentration in both irrigation water and soil were maximum. On the basis of a recently published scenario of long duration accumulation of arsenic at the study site, it was thought that, under unchanged irrigation practice, arsenic concentration in grain increase from currently mean approximately from 0.15 mg/kg to 0.25-0.58 mg/kg by the year 2050. This translates to 1.5-3.8 times higher intake of arsenic by the local population by rice. Begum et al. (2008) investigated that the grain yield of boro rice was lessen by 20.8% for 15 ppm arsenic and 64.8% due to 30 ppm arsenic treatments. Such reductions of straw yield were 21.3% and 65.4% with these two treatments of arsenic respectively. Residual effect

of arsenic was also significant and negative in T. Aman rice. The grain arsenic concentrations in all cases were below 1 ppm, and the straw arsenic content was well above 1 ppm. The concentrations of arsenic in both straw and grain were higher in boro rice than in T. Aman rice. Hossain (2005) noted that yield reduction more than 42% and 60% for two popular rice varieties (BRRI Dhan28 and Iratom-24) when 20 mg/kg of arsenic was applied to soil, in contrast to the control. Delowar et al. (2005) noted the span of arsenic accumulation in rice plant and its effects on growth and yield of rice. Arsenic concentrations in rice soils (irrigated with 0, 2.5, 5, 10, 15 and 20 mg/L of arsenic water) were 0-0.18, 0-0.96 and 0-0.28 mg/kg at tillering, heading and ripening stages. Arsenic accumulation in rice grains from soil, water and arsenic accumulation varied significantly in two studied rice varieties. The concentrations in rice grain were 0-0.08 and 0-0.16 mg/kg dry weight in rice varieties BRRI dhan28 and Iratom-24 respectively. The growth and yield of rice plants were reduced greatly with increased doses of arsenic but the grain production was not affected. Among the different yield components, the number of tillers per pot, number of effective tillers per pot and grain yield per pot reduced significantly with the increased dose (20 mg/L) of arsenic applied. Reduction of yield of more than 40% and 60% for BRRI dhan28 and Iratom-24 was found with 20 mg/L of arsenic in contrast to control. Straw yield reduction was also significantly higher for both of rice varieties with the 20 mg/L arsenic treatment.

Jahiruddin *et al.* (2004) found the effects of arsenic contamination on crop yield and arsenic accumulation under control condition. The levels of applied arsenic on soils were 0, 5, 10, 15, 20, 30, 40 and 50 ppm, and that of irrigation water arsenic were 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2 ppm. The effect of applied arsenic (plus 2.7 ppm soil arsenic) was

examined directly on boro rice (BRRI dhan29) and its residual effect on T. Aman rice (BRRI dhan28). The pots for both the crops got an equal amount of fertilizers. They observed that the grain protein was adversely affected due to arsenic contamination. 41% grain yield reduction for 10 mg/kg addition of arsenic to BAU farm soil. Kang et al. (1996) noted that increasing the level of arsenic decreased plant height, number of effective tiller, dry weight of above ground parts and 1000-grain weight. Yield decreased from 46.7 g/pot with the lowest rate of arsenic to 16.9 g with the highest rate. Arsenic contents were higher in roots than in stems plus leaves or in grain, but in all parts the content increased as soil arsenic increased. The contents of arsenic in stems and leaves were more closely bind to soil total and available arsenic than those of roots or grain. Shah et al. (2004) noted that the level of arsenic in soil with concentration above 20 ppm may affect rice yield of Bangladesh variety. Abedin and Mehrag (2002) noted that the increased levels of soil arsenic resulting from long-term use of arsenic polluted ground water for irrigation in Bangladesh may reduce seed germination and seedling establishment of rice, the main food crop of country. A study of germination on rice seeds and a short-term toxicity experiment with different concentrations of arsenite and arsenate on rice seedlings were conducted. Percentage (%) of germination over control treatment decreased significantly with increasing concentrations of arsenite and arsenate. Arsenite was found to be more harmful than arsenate for rice seed germination. There were varietal differences among the test varieties in response to arsenite and arsenate exposure. The performance of the dry season cultivar Purbachi was the best cultivar among the cultivars. Germination of Purbachi was not reduced up to 4 mg/L arsenite and 8 mg/L arsenate treatment. Root tolerance index (RTI) and relative shoot height (RSH) for rice seedlings decreased with increasing concentrations of arsenite and arsenate.

Reduction of RTI caused by arsenate was higher than that of arsenite. In general, dry season varieties have more tolerance to arsenite or arsenate than the wet season varieties.



# Chapter III MATERIALS & METHODS

#### **CHAPTER III**

#### **MATERIALS AND METHODS**

The present deals with a brief description about the materials and methods those were used when researching and writing this paper. It demotes the key methods, use of different parameters to correlate with establishing rice plant. It also covers the data collection procedure, source of data and ways of data were analyzed.

#### **3.1 Conduction of experiment**

#### 3.1.1 Site of experiment

The experiment was conducted under tray-culture at the net house and the agro-environmental chemistry laboratory of the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka-1207 during the Boro season of 2017-18 to evaluate the performance of rice cultivars to different concentrations of arsenic at seedling stage.

#### 3.1.2 Climate

The climate was sub-tropical humid and was characterized by high temperature accompanied by moderately high rainfall during Aus season (April-September) and low temperature in Aman season (October-March). Geographically, the net house stands at 23°41' N latitude and 90°22' E longitude at an altitude of 8.6 meter above the sea level.

#### 3.1.3 Description of the soil

The soil used in tray was collected from the experimental field of Sher-e-Bangla Agricultural University (SAU), Dhaka. The topography of the land was medium high and the soil was collected from 0-15 cm depth. After collecting the soil, it was sun dried and ground well. Then the soil debris was removed by sieving and the soil was put into plastic tray after mixing with fertilizer. The soil of this experiment was sandy loam in texture.

#### **3.1.4 Preparation of Tray**

An amount of 4 kg soil was taken in a series of trays. The required number of tray 5, having 40 inches length, 30 inches wide and 8 inches depth were collected from the local market and cleaned before use. There were altogether 18 small plots measuring 6 inches length and 4 inches wide, comprising 18 rice cultivars in those small polts with one arsenic treatment. For others arsenic treatments, another 4 trays like first one were to be made with respective arsenic concentrations (Appendix IV). Water was added to the tray to bring the soil up to saturation. Arsenic was also added at the final tray preparation at desire rate of treatment. Every tray was filled up with muddy soil like seedbed.

#### 3.1.5 Treatments of the experiment

Five rates of arsenic *viz.* 0, 3, 6, 9, and 12 ppm arsenic (on soil water basis). The source of arsenic was Sodium Arsenate (Na<sub>2</sub>HAsO<sub>4</sub>.7H<sub>2</sub>O). There were two factors of the experiment with 3 replications.

- I. Factor A: Different doses of arsenic,
  - $A_0 = No arsenic added (control)$
  - $A_1 = 3$  ppm arsenic (soil water basis)
  - $A_2 = 6$  ppm arsenic (soil water basis)
  - $A_3 = 9$  ppm arsenic (soil water basis)
  - $A_4 = 12$  ppm arsenic (soil water basis)

#### **II.** Factor B: Different rice cultivars

 $T_1 = BINA dhan-8$ 

 $T_2 = BINA dhan-10$  $T_3 = BINA dhan-14$  $T_4 = BINA dhan-18$  $T_5 = BR 3$  $T_6 = BR \ 14$  $T_7 = BR \ 16$  $T_8 = BRRI dhan 28$  $T_9 = BRRI dhan 29$  $T_{10} = BRRI dhan36$  $T_{11} = BRRI dhan45$  $T_{12} = BRRI dhan47$  $T_{13} = BRRI dhan50$  $T_{14} = BRRI dhan55$  $T_{15} = BRRI dhan58$  $T_{16} = BRRI dhan59$  $T_{17} = BRRI dhan60$  $T_{18} = BRRI dhan61$ 

#### 3.1.6 Design and layout of the experiment

The experiment was laid out in Completely Randomized Design (CRD) with three replications (appendix IV). There were 270 small plots all together with the given factors.

#### 3.1.7 Seed collection

The selected rice cultivars were collected from BINA (Bangladesh Institute of Nuclear Agriculture) Mymensigh and BARI (Bangladesh Agricultural Research Institute) Gazipur, Dhaka.

#### 3.1.8 Sterilization of seed

Seeds were surface sterilized with 1% sodium hypochloride solution prior to germination test. Distilled water containing glass vials for rinsing seed was sterilized for 20 minutes in an autoclave at 121±1°C and at 15 bar air pressure.

## 3.1.9 Application of fertilizer

All the trays received fertilizers according to BRRI's recommended fertilizer dose (BRRI, 2017). The amounts of nitrogen, phosphorus, potassium and sulphur required for each tray were calculated as per their rates of application. Except nitrogen, full dose of P and K were added at the time of final tray preparation. Nitrogen was added after germination and 15 days after germination.

#### **3.1.10 Raising of seedlings**

The seedlings were raised at the wet seed bed in tray. The seeds were sprouted by soaking for 72 hours. The sprouted seeds were sown uniformly in the well prepared seed bed in  $12^{\text{th}}$  December 2017.

## **3.1.11 Intercultural operations**

Weeding of soils around the seedlings were done when felt necessary. Top dressing of urea was done when felt necessary. At the germination stage, the trays were covered with net to protect the grains from the attack of birds. Observation was regularly made. All the stages of plants and plants response as per treatments were observed carefully.

#### **3.1.12 Weeding and Irrigation**

Weeding and irrigation were done as per required. There was no extra water at the germination stage, and after germination stage water was kept as per seedling requirement.

# **3.1.13 Plant protection**

As the trays were in net house, Birds did not harm. There was not found any other insect pests except rat. For this reason, rodenticides were used to control rat.

# 3.2.1 Sampling and data collection

Data were collected from the experiment on different growth stages which were done under the following heads as per experimental requirements and average values were recorded for analysis.

# 3.2.2 Shoot and root length

From the two plots of each treatment, 5 seedlings were selected randomly and uprooted very carefully so that no harms were occurred into root zone. It was washed thoroughly with tap water. Shoot and root length were measured with centi-meter scale and preserved for data analysis.

# 3.2.3 Shoot and root biomass

The collected seedlings for shoot and root length were preserved for shoot and root biomass. A cleaned and sharp scissor was used to cut the root and shoot from seedlings. It was then sun dried and oven dry for 72 hours at 115 °C. Then the dry weights were taken and average values were recorded for data analysis.

# 3.2.4 Seedling height

From the two plots of each treatment, 5 seedlings were selected randomly and the height was measured with scale. Average values were recorded.

# **3.2.5** Number of root per seedling

For number of root per seedling, 5 seedlings were selected from each treatment and the selected seedlings were uprooted carefully with root. Then it was washed thoroughly in tap water. Roots were counted and preserved for data analysis.

## **3.2.6 Total dry matter per seedling**

Five seedlings of each treatment were selected for total dry matter content of seedling. The oven dried weights were stored for final data analysis.

#### **3.3 Chemical Analysis of shoot and root**

# 3.3.1 Arsenic determination

**Information of the sample**: Plant digest samples, pH less than 2 with HCl 5mL/L.

**Sample storage**: The samples were stored in a refrigerator in less than 4 °C.

**Instrument**: Flame Atomic Absorption Spectrophotometer with HVG, Ar gas (99.99%) as carrier of the samples, HCl 5M and 0.4% NaBH<sub>4</sub> as reagent for HVG. The flow rate of sample was 5mL/min.

**Reagents used**: (i) Conc.  $HNO_3$  (ii) KI (iii) Conc. HCl (iv) 1000 ppm standard solution of As (v) De-ionized water (vi) 5M HCl (vii) NaBH<sub>4</sub>

#### **Preparation of the reagents**

**Preparation of KI (20% w/v):** 20 g KI was taken in 100 mL volumetric flask then dissolved in water and marked up to volume.

**Preparation of NaBH**<sub>4</sub> solution (0.4% w/v): 2.5 g Sodium Hydroxide and 2.0 g Sodium Boro-hydrate were mixed in 500 mL volumetric flask and marked up to volume with Di-Ionized water.

**Preparation of calibration standard from 1000 ppm standard solution of As:** 1 mL of As (1000 ppm standard solution) was taken in 100 mL volumetric flask and then mark up with Di-Ionized water. Then 1mL from 10 ppm solution was taken in 100 mL volumetric flask and marked up to volume with DI water. Then dilutions were as follows from the 100 ppb solution:

0 mL= 100 mL water (o ppb, blank) 2.5 mL= 100 mL water (2.5 ppb) 5 mL= 100 mL water (5 ppb) 10 mL= 100 mL water (10 ppb)

After that, 40 mL from each solution was taken in individual 50 mL volumetric flask and then 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added to each and left to dark for 15 minutes.

**Preparation of 5M HCl:** 200 mL DI water was taken in a volumetric flask and then 208 mL of HCl (37%) was added and volume was marked up to with DI water.

**Preparation of blank:** 40 mL DI water was taken in 50 mL volumetric flask and 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added and left to dark for 15 minutes.

**Treatment and preparation of sample:** 1 g well-mixed sample was taken in a beaker. About 10 mL conc.  $HNO_3$  was added. The sample was covered with a watch glass and heated on hot plate at 90 ° to 95 °C until

the volume reduced to 15-20 mL. The beaker was removed and allowed to cool. The beaker walls and watch glass were washed down with DI water when necessary filter or centrifuge the sample to remove silicates or other insoluble material. Then the final volume to 50 mL was with diluent. After that, 40 mL of this was taken in 50 mL volumetric flask and 4 mL of 37% HCl and 2 mL of freshly prepared 20% (w/v) KI were added and left to dark for 15 minutes.

**Procedure:** For shoot and root arsenic analysis, 1 mL of each samples were taken in a 100 mL conical flask and 50 mL of 0.5 mol/L NaHCO<sub>3</sub> solution was added. Then the whole materials were shaken for 1 hr in a "to and fro" horizontal shaker and after completion of shaking, the suspensions were filtered through Whatman filter paper No. 42. The filtered were collected for arsenic analysis with Atomic Absorption Spectrophotometer coupled with Hydride Vapor Generator (HVG) unit after reducing with 2 mL of 10% KI solution and 2 mL of 35% HCl, NaBH<sub>4</sub> solution and 4 mol/L HCl solution separately from three containers were allowed passing to a mixing manifold by a peristaltic pump. From the mixing manifold by argon (inert gas), carrier, AsH<sub>3</sub> (arsine) generated in the reaction loop. The arsenic was then atomized in a flame of air-acetylene and the direct arsenic concentrations in the sample were measured.

## 3.3.2 Nitrogen determination

The Macro Kjeldahl method was used to determine the total Nitrogen in root, shoot and grain of plant samples. Three steps were followed in this method. Here is given below:- A. Digestion: In this step the organic nitrogen was converted to ammonium sulphate by sulphuric acid and digestion accelerators (Catalyst Mixture) at a temperature of 360-440° C.

$$\mathbf{N} + \mathbf{H}_2 \mathbf{SO}_4 = (\mathbf{N}\mathbf{H}_4)_2 \mathbf{SO}_4$$

B. Distillation: In this step, the solution was made alkaline from the distillation of ammonia. The distilled ammonia was received in boric acid solution.

 $(NH_4)_2SO_4 + NaOH = Na_2SO_4 + NH_3 + H_2O$  $NH_3 + H_3BO_3 = (NH_4)_2BO_3 + H_2O$ 

C. Titration: To determine the amount of NH<sub>3</sub>, ammonium borate was titrated with standard sulfuric acid.

$$(NH_4)_2BO_3 + H_2SO_4 = (NH_4)_2SO_4 + H_2O$$

**Reagents**: 4% Boric Acid solution, Mixed indicator (Bromocresol green and Methyl red), 4% Sodium Hydroxide solution, Standard Sulphuric Acid solution and 0.05 N Na<sub>2</sub>CO<sub>3</sub> solution.

**Procedure:** About 0.25 g of oven dried grain sample was weighed and then taken into a 250 ml Kjeldahl flask. Then 5 g catalysts mixer ( $K_2SO_4$ :CuSO\_4.5H<sub>2</sub>O: Ratio=100:1) was added in to flask. Then about 25 ml concentrated H<sub>2</sub>SO<sub>4</sub> was also added o the flask. The flask was heated until the solution become clear and then allowed to cool. After digestion, 40% NaOH was added o the conical flask and attached quickly to the distillation set. Then the flask was heated continuously. In the meantime, 25 ml of 4% boric acid solution and 2-4 drops of mixed indicator was added into the receiver conical flask. The distillate was then titrated with standard H<sub>2</sub>SO<sub>4</sub> taken from a burette until the green color completely turns to pink. The same procedure was followed for a blank sample. The result was calculated using the following formula-

#### $N = (T-B) \times N \times 1.4/S$

Where, T= Titration value for sample (ml), B= Titration value for blank (ml), N= Normality of  $H_2SO_4$ , S= Weight of the sample, 1.4= Factor

## **3.3.3 Determination of Potassium**

The amount of potassium was determined from the plant extract with the help of a flame photometer.

**Preparation of primary potassium standard solution (1000 ppm):** 1.918 g potassium chloride was taken in a 1L volumetric flask. About 200-300 mL distilled water was added and the flask was shaked thoroughly until a clear solution was obtained. The volume was made up to the mark with distilled water. Thus, 1000 ppm K solution was prepared.

**Preparation of secondary potassium solution (100 ppm and 10 ppm):** About 10 mL of the 1000 ppm K solution was taken in a 100 mL volumetric flask. The volume was made up to mark with distilled water and shaked thoroughly. Thus, 100 ppm K solution was prepared. From 100 ppm solution, 10 mL was taken in a 100 mL volumetric flask. The volume was made up to the mark with distilled water and shaked thoroughly. Thus, 10 ppm solution was obtained.

**Preparation of potassium standard series solution:** A series of standard solution containing 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm and 6 ppm were prepared by pipetting 10 mL, 20 mL, 30 mL, 40 mL, 50 mL and 60 mL of 10 ppm K solution in six different 100 mL volumetric flask respectively. The volume was made up to the mark by distilled water and shaked thoroughly. Then, the reading (% emission) were taken from flame emission spectrophotometer and a standard curve was prepared

from the reading taken. Plant samples were taken in volumetric flask and volume was made up to the mark by distilled water. Then the samples reading were taken and concentrations were calculated from the standard curve.

#### **3.3.4 Determination of Phosphorus**

The amount of phosphorus was determined by ascorbic acid blue color method with the help of spectrophotometer.

#### **Reagents required**

- A. Mixed reagent: 12.0 g ammonium molybdate (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>.4H<sub>2</sub>O was dissolved in 250 mL distilled water. About 0.2908 g antimony potassium tartarate K<sub>2</sub>Sb<sub>2</sub>(C<sub>4</sub>H<sub>2</sub>O<sub>6</sub>)<sub>2</sub>.3H<sub>2</sub>O was dissolved in 1000 mL H<sub>2</sub>SO<sub>4</sub>. Two solutions were mixed together and volume was made up to 2000 mL with distilled water and stored in a pyrex bottle in a dark cool place.
- B. Color developing reagent: 0.53 g ascorbic acid was added to 100 mL of the mixed reagent.
- C. Standard Phosphorus solution (100 ppm): 0.439 g potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) was weighed into a 1L volumetric flask. About 500 mL distilled water was added and shaked the contents until the salt dissolved. Then the volume was made up to 1L with distilled water.

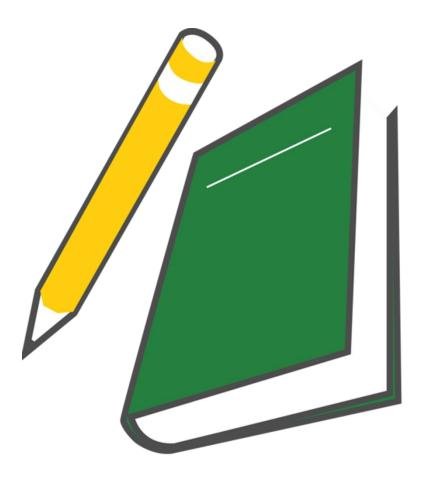
# Procedure

A. Color development: About 20 mL of the extract was pipetted out in a 100 mL volumetric flask. About 20 mL color developing reagent was added slowly and carefully to prevent the loss of sample due to excessive foaming. After the evolution of  $CO_2$  had ceased, the flask was shaked gently to mixed the contents. The volume was made up to the mark by adding distilled water.

B. Preparation of working standard P solution: About 20 mL of the standard P solutions (100 ppm) was pipetted to a 1L volumetric flask and volume was made up to the mark by distilled water. This solution contained 2 ppm P. About 0, 5, 10, 15, 20 and 25 mL aliquot were pipetted out from 2 ppm solution in 100 mL volumetric flask respectively. About 20 mL color developing reagent was added to each flask, mixed and volume was made with distilled water. These solutions gave 0, 0.1, 0.2, 0.3, 0.4 and 0.5 ppm of P solution respectively. The solution was allowed to stand for 15 minutes and then color intensity (% absorbance) was measured at 660 nm. A standard curve was prepared from the spectrophotometer reading and concentrations of plant samples were calculated from the curve.

#### **3.4 Statistical Analysis**

The data from rice seedilng samples were compiled and tabulated in proper form and were subjected to statistical analysis. The computer package MSTAT-C program developed by Russel (1986) was used to analysis of variance. The mean differences among the treatments were adjusted by least significant difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).



# Chapter IV RESULTS & DISCUSSION

## **CHAPTER IV**

## **RESULTS AND DISCUSSION**

The present chapter deals with the result of the experiment as influenced by the effect of eighteen rice cultivars under different arsenic stress condition and their interaction on morphological characters and nutrient contents are presented in table 1-4. The analysis of variance and other necessary information have been presented in Appendices I to VIII. Results and discussion of the experiment are given below.

#### 4.1 Effect of Arsenic on the morphological characters of rice cultivars

#### **4.1.1 Seedling Height**

Seedling height was significantly varied with the arsenic treatment. Arsenic treatment,  $As_0$  showed highest height 13.14 cm and  $As_4$  showed lowest height 7.19 cm (Fig. 1). Plant height was affected due to application of Arsenic (Holmgren *et al.* 1993 and Das *et al.* 1997).

It was found that seedling height was significantly varied with the cultivars. Highest seedling height 13.23 cm was recorded from BINA dhan-18 and the height of BINA dhan-14, 12.94 cm was statistically similar. BRRI dhan29 showed lowest seedling height 8.14 cm (Table 1). Shamsuddin *et al.*, (1988) showed that plant height differed due to varietal variation.

Interaction effect of cultivars and arsenic treatment showed significant variation on seedling height. The highest seedling height (16.63 cm) was observed from BINA dhan-8 at  $As_0$  arsenic treatment which was statistically similar with the seedling height (16.23 cm) of same cultivar at  $As_1$  arsenic treatment. The lowest seedling height (5.23 cm) was

observed from BRRI dhan29 at  $As_0$  arsenic treatment which was statistically similar with the seedling height (5.57 cm) of BRRI dhan58 at same arsenic treatment (fig. 2). Hossain *et al.*, (2008) found that plant height significantly varied with different concentrations of Arsenic.

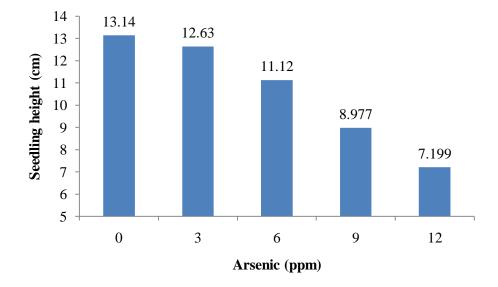


Figure 1: Effect of arsenic on seedling height

## 4.1.2 Shoot weight

Shoot weight rice seedlings were greatly influenced due to arsenic treatment (fig. 3). The result revealed that the arsenic treatment  $As_0$  produced highest shoot weight (0.34 g) and arsenic treatment  $As_4$  produced lowest shoot weight (0.20 g).

Effect of cultivars on shoot weight of rice seedlings were also significantly varied (table 1). The highest shoot weight was recorded from BRRI dhan61 (0.393 g) and followed by BRRI dhan29 (0.390 g), BINA dhan-18 (0.371 g) and BRRI dhan28 (0.370 g). The lowest shoot weight was recorded from BR 3 (0.182 g). This result was in agreement with the finding of Patel (2000) who reported that yield performance varied with variety.

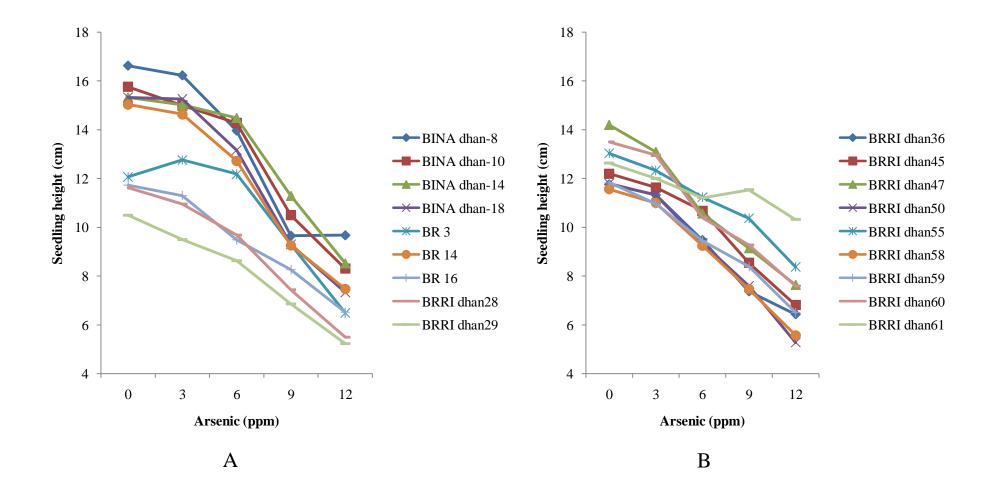


Figure 2: Interaction effect of arsenic and cultivar on seedling height

Cultivar	Shoot height	Shoot weight	Root length
	(cm)	(g)	(cm)
BINA dhan-8	12.08 C	0.264 EF	4.507 K
BINA dhan-10	12.77 B	0.232 GHI	5.625 C
BINA dhan-14	12.94 AB	0.251 FG	5.035 H
BINA dhan-18	13.23 A	0.371 A	6.532 A
BR 3	10.56 F	0.182 K	6.232 B
BR 14	11.83 CD	0.211 IJ	5.561 CD
BR 16	9.459 H	0.207 J	4.554 JK
BRRI dhan28	9.044 IJ	0.370 A	5.468 D
BRRI dhan29	8.144 K	0.393 A	5.197 EFG
BRRI dhan36	9.277 HIJ	0.242 FGH	5.290 EF
BRRI dhan45	9.973 G	0.225 HIJ	3.875 L
BRRI dhan47	10.93 EF	0.312 C	5.055 GH
BRRI dhan50	9.073 HIJ	0.346 B	4.681 IJ
BRRI dhan55	11.07 E	0.208 J	4.741 I
BRRI dhan58	8.971 J	0.288 D	5.279 EF
BRRI dhan59	9.429 HI	0.287 DE	3.948 L
BRRI dhan60	10.74 EF	0.303 CD	5.151 FGH
BRRI dhan61	11.54 D	0.3907 A	5.303 E
LSD	0.392	0.022	0.142
Level of significanc	*	*	*
CV%	15.12	12.53	13.86

Table 1: Effect of cultivar on seedling shoot height (cm), shoot weight (g) and root length (cm).

The interaction effect between cultivars and arsenic treatment was also significant (fig. 4). The highest shoot weight was found from BRRI dhan61 (0.483 g) at As<sub>0</sub> arsenic treatment. The lowest shoot weight was found from BRRI dhan55 (0.129 g) at As<sub>4</sub> arsenic treatment. Similarly Begum *et al.*, (2008) showed that the shoot weight of Boro rice was reduced by 21.0 % for 15 ppm Arsenic treatment and 65.2 % due to 30 ppm Arsenic.

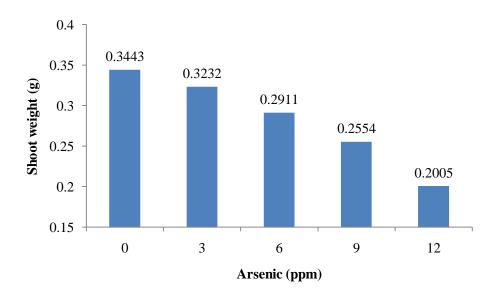


Figure 3: Effect of arsenic on shoot weight (g)

## 4.1.3 Root length

The root length of rice seedlings were greatly influenced due to arsenic treatment (fig. 5). The longest root (6.15 cm) was found at  $As_0$  treatment. The smallest root (3.79 cm) was found at  $As_4$  treatment. The result revealed that increasing arsenic level decreased root length.

Due to cultivars, the root length was also significantly influenced (table 1). The longest root length was found from BINA dhan-18 (6.53 cm) whereas the smallest root was found from BRRI dhan45 (3.87 cm) followed by BRRI dhan59 (3.94 cm).

Interaction effect between cultivars and arsenic treatment on root length was also significantly influenced (fig. 6). The longest root (8.44 cm) was found from BINA dhan-18 at  $As_0$  arsenic treatment whereas the smallest root (3.05 cm) was found from BRRI dhan45 at  $As_4$  arsenic treatment and followed by BRRI dhan59 (3.07 cm) at same arsenic treatment.

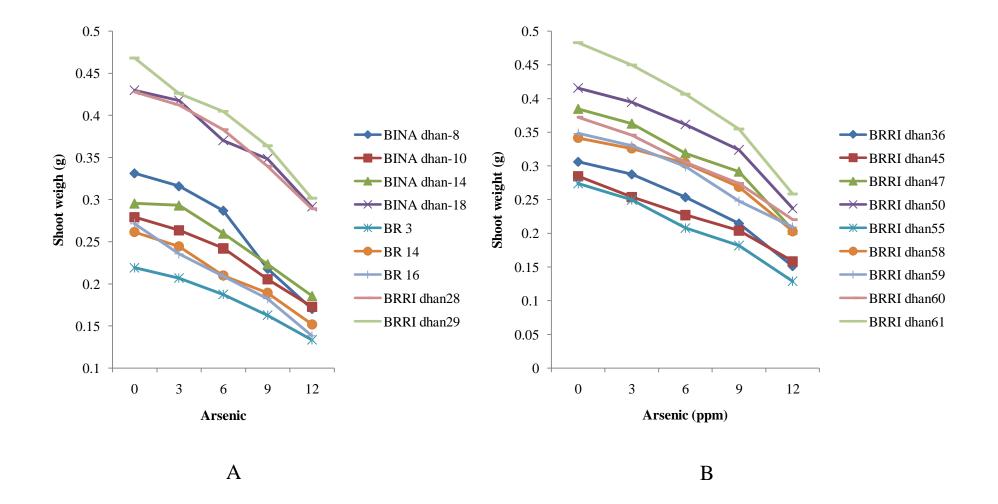


Figure 4: Interaction effect of arsenic and cultivar on shoot weight (g)

Cultivar	Root weight (g)	Total dry	Nitrogen (%) in
		matter (g)	shoot
BINA dhan-8	0.079 EFGH	0.343 CDEF	0.554 H
BINA dhan-10	0.072 GH	0.305 DEF	0.609 FGH
BINA dhan-14	0.074 GH	0.325 DEF	0.741 CDE
BINA dhan-18	0.114 ABC	0.475 ABC	0.782 CD
BR 3	0.061 H	0.243 F	0.605 GH
BR 14	0.071 GH	0.284 EF	0.658 EFG
BR 16	0.074 FGH	0.282 EF	0.717 DE
BRRI dhan28	0.116 AB	0.487 AB	0.744 CDE
BRRI dhan29	0.124 A	0.517 A	0.954 A
BRRI dhan36	0.071 GH	0.314 DEF	0.651 EFGH
BRRI dhan45	0.088 DEFG	0.314 DEF	0.715 DE
BRRI dhan47	0.092 CDEFG	0.405 ABCDE	0.661 EFG
BRRI dhan50	0.098 BCDE	0.445 ABCD	0.935 A
BRRI dhan55	0.082 DEFGH	0.291 EF	0.764 CD
BRRI dhan58	0.087 DEFG	0.376 ABCDE	0.824 BC
BRRI dhan59	0.084 DEFG	0.371 BCDEF	0.703 DEF
BRRI dhan60	0.097 BCDEF	0.400 ABCDE	0.815 BC
BRRI dhan61	0.102 ABCD	0.493 AB	0.893 AB
LSD	0.023	0.143	0.096
Level of significance	*	*	*
CV%	11.46	12.47	8.75

Table 2: Effect of cultivar on root weight (g), Total dry matter (g) and Nitrogen (%).

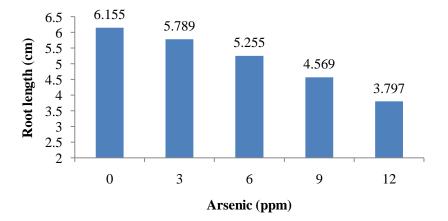


Figure 5: Effect of arsenic on root length (cm)

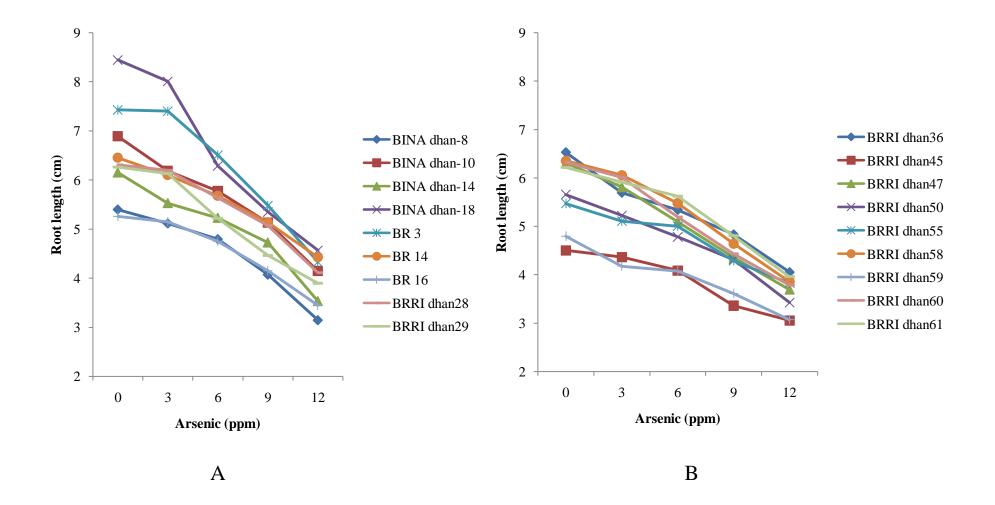


Figure 6: Interaction effect of arsenic and cultivar on root length (cm)

#### 4.1.4 Root weight

Root weight of rice seedling was greatly influenced due to arsenic treatment (fig. 7). The highest root weight (0.111 g) was recorded from  $As_0$  arsenic treatment whereas the lowest root weight (0.063 g) was recorded from  $As_4$  arsenic treatment. This result revealed that increasing arsenic level decreased root biomass.

Root weight of rice seedling was also greatly influenced due to cultivar (table 2). The highest root weight (0.126 g) was recorded from BRRI dhan29. BRRI dhan28 (0.116 g), BINA dhan-18 (0.114 g) and BRRI dhan61 (0.102 g) showed statistically similar result with the BRRI dhan29. The lowest root weight (0.061 g) was found from BR 3. Similar results were also reported by Halim *et al.* (1988).

Interaction effect between cultivars and arsenic treatment was also significant (fig. 8). The highest root weight (0.152 g) was recorded from BRRI dhan29 at  $As_0$  arsenic treatment, and this cultivar also showed statistically similar root weight (0.143 g) at  $As_1$  arsenic treatment. The lowest root weight (0.044 g) was recorded from BR 3 at  $As_4$  arsenic treatment.

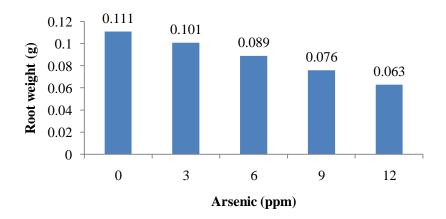


Figure 7: Effect of arsenic on root weight (g)

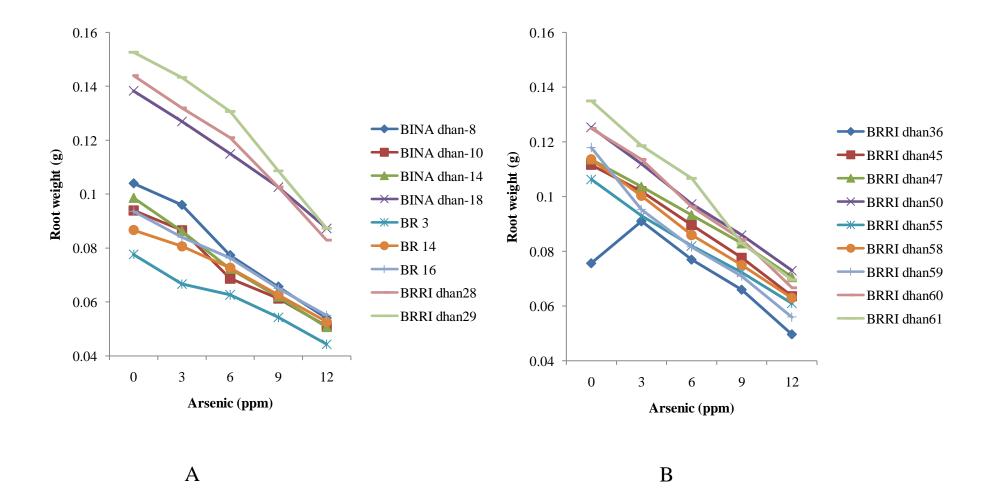


Figure 8: Interaction effect of arsenic and cultivar on root weight (g)

#### 4.1.5 Total dry matter

Total dry matter of rice seedling was greatly influenced due to arsenic treatment (fig. 9). The highest total dry matter (0.456 g) was found at  $As_0$  arsenic treatment whereas total dry matter (0.425 g) was statistically similar at  $As_1$  arsenic treatment. The lowest total dry matter (0.263 g) was found at  $As_4$  arsenic treatment whereas total dry matter (0.332 g) was statistically similar at  $As_3$  arsenic treatment. From the findings, it was clear that increasing arsenic concentration decreased total dry matter content.

Total dry matter of rice seedling was also significant due to cultivar (table 2). The highest total dry matter (0.517 g) was recorded from BRRI dhan29. BRRI dhan61 (0.493 g) showed statistically similar result followed by BRRI dhan28 (0.487 g), BRRI dhan50 (0.445 g), BRRI dhan47 (0.407 g), BRRI dhan60 (0.400 g) and BRRI dhan58 (0.376 g). The lowest total dry weight (0.243 g) was recorded from BR 3. BR 16 (0.282 g) showed statistically similar result followed by BRRI dhan55 (0.291 g).

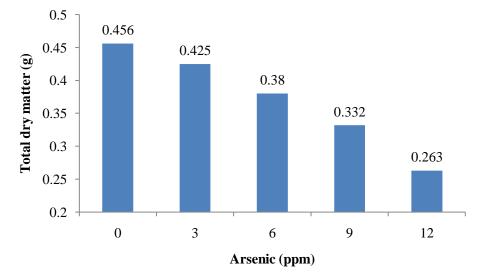


Figure 9: Effect of arsenic on total dry matter (g)

Interaction effect between arsenic and cultivars was also significant (fig. 10). The highest total dry matter (0.621 g) was found from BRRI dhan29 at  $As_0$  arsenic treatment and followed by BRRI dhan61 (0.618 g) at same arsenic treatment. The lowest total dry matter (0.178 g) was found from BR 3 at  $As_4$  arsenic treatment and followed by BRRI dhan55 (0.190 g) at same arsenic treatment.

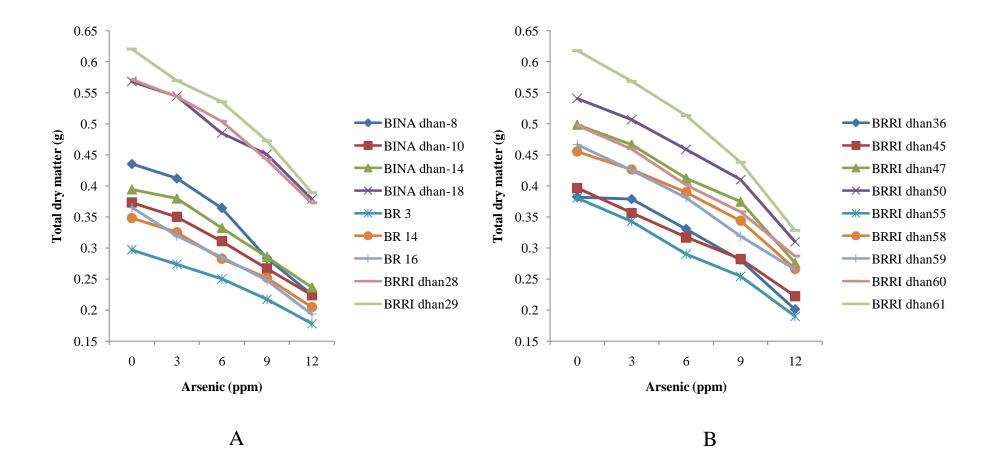


Figure 10: interaction effect between arsenic and cultivar on total dry matter (g)

#### 4.2 Effect of Arsenic on the chemical composition of rice cultivars

#### 4.2.1 Nitrogen content in shoot

Nitrogen content in rice seedling shoot showed statistically significant difference due to arsenic treatment (fig. 11). The highest nitrogen content (1.029%) was found at  $As_0$  arsenic treatment whereas the lowest nitrogen content (0.333%) at  $As_4$  arsenic treatment. Khatik and Dikshit (2001) reported that the increasing concentration of arsenic in soil caused decreased the uptake pattern nitrogen in rice seedling.

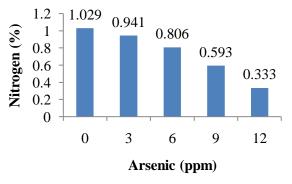


Figure 11: Effect of arsenic on Shoot Nitrogen (%)

Nitrogen content in rice seedling shoot was statistically significant due to cultivar (table 2). The highest nitrogen content (0.954%) was found from BRRI dhan29 and followed by BRRI dhan50 (0.935%) and BRRI dhan61 (0.893%) showed statistically similar result. The lowest nitrogen content (0.554%) was found from BINA dhan-8 and BINA dhan-10 (0.609) showed statistically similar result.

Interaction effect between arsenic and cultivar was also significant on nitrogen content in rice seedling (fig. 12). The highest nitrogen content (1.311%) was found from BRRI dhan50 at  $As_0$  arsenic treatment and followed by BRRI dhan29 (1.263%) at same arsenic treatment. The lowest nitrogen content (0.109%) was found from BINA dhan-8 at  $As_4$  arsenic treatment. The result revealed that increasing arsenic concentration decreased nitrogen uptake in rice seedling.

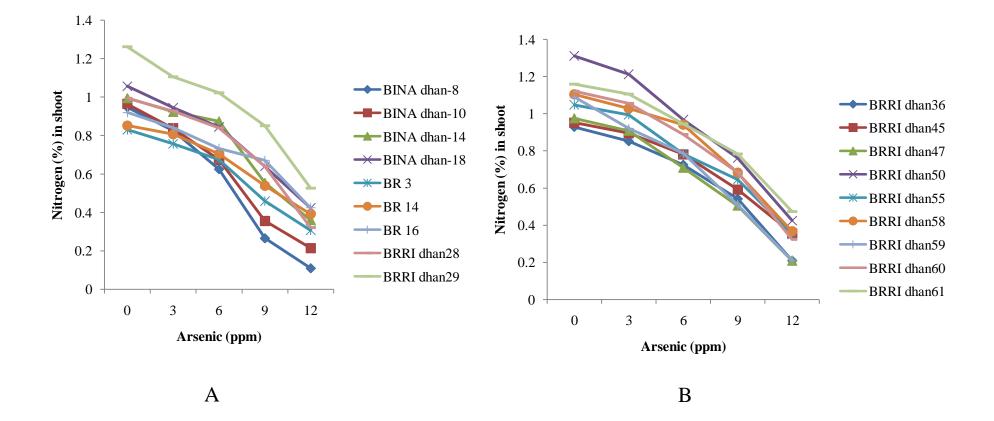


Figure 12: Interaction effect between arsenic and cultivar on Nitrogen (%) in shoot

#### 4.2.2 Arsenic content in shoot

Arsenic content in rice seedling shoot showed statistically significant due to application of arsenic (fig. 13). The maximum arsenic content (1.083 ppm) was found in As<sub>4</sub> arsenic treatment whereas the minimum arsenic content (0 ppm) was found in As<sub>0</sub> arsenic treatment. The result revealed that increasing arsenic concentration increased uptake of arsenic content in rice seedling shoot. Zahida zia *et al.*, (2017) reported that increasing arsenic concentration increased arsenic uptake in rice plants.

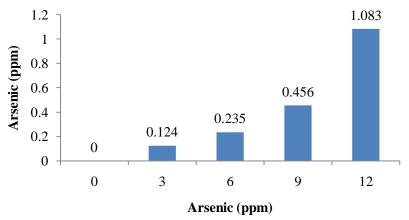


Figure 13: Effect of arsenic on arsenic content in shoot

Effect of cultivar on arsenic content in rice seedling shoot was also significant (table 3). The highest arsenic content (0.583 ppm) in rice seedling shoot was recorded from BRRI dhan50 and BRRI dhan55 (0.548 ppm) was showed statistically similar result. The lowest arsenic content (0.204 ppm) in rice seedling shoot was recorded from BINA dhan-18. BINA dhan-14 (0.261 ppm) was statistically similar and followed by BRRI dhan61 (0.266 ppm).

Interaction effect of arsenic and cultivar on arsenic content in rice seedling shoot was also significant (fig. 14). The highest arsenic content (1.629 ppm) was found from BRRI dhan50 at  $As_4$  arsenic treatment. All the eighteen rice cultivars weren't uptake any arsenic at  $As_0$  arsenic treatment.

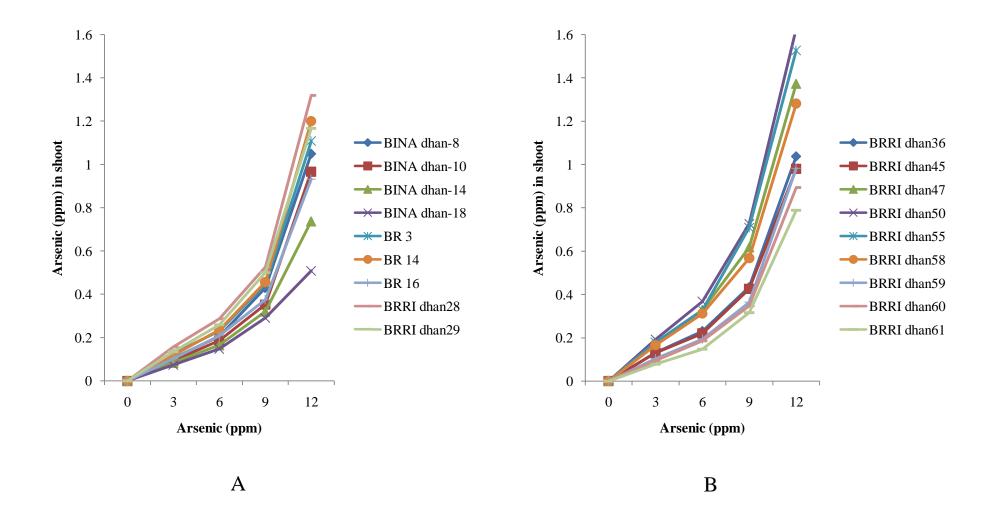


Figure 14: Interaction effect of arsenic and cultivar on arsenic content in shoot

#### 4.2.3 Phosphorus content in shoot

Effect of arsenic on phosphorus content in rice seedling shoot was significant (fig. 15). The maximum phosphorus content (0.827%) in rice seedling shoot was recorded from  $As_0$  arsenic treatment. The minimum phosphorus content (0.120%) was recorded from  $As_4$  arsenic treatment.

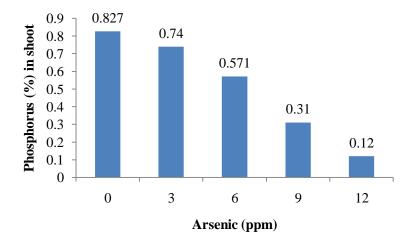


Figure 15: Effect of arsenic on Phosphorus (%) in shoot

Effect of cultivar on phosphorus content in rice seedling shoot was also significant (table 3). The highest phosphorus content (0.643%) in rice seedling shoot was found in BRRI dhan61. The lowest phosphorus content (0.395%) was found in BR 3 and followed by BR 16 (0.406%) and BR 14 (0.424%).

The interaction effect of arsenic and cultivar on phosphorus content in rice seedling shoot was also significant (fig. 16). The maximum phosphorus content (0.993%) in rice seedling shoot was recorded from BRRI dhan29 and followed by BRRI dhan61 (0.986%) at  $As_0$  arsenic treatment, phosphorus content (0.961%) in BRRI dhan50 was statistically similar. The minimum phosphorus content (0.055%) was recorded from BRRI dhan36 at  $As_4$  arsenic treatment. BR 16 (0.078%) showed statistically similar result at  $As_4$  arsenic treatment.

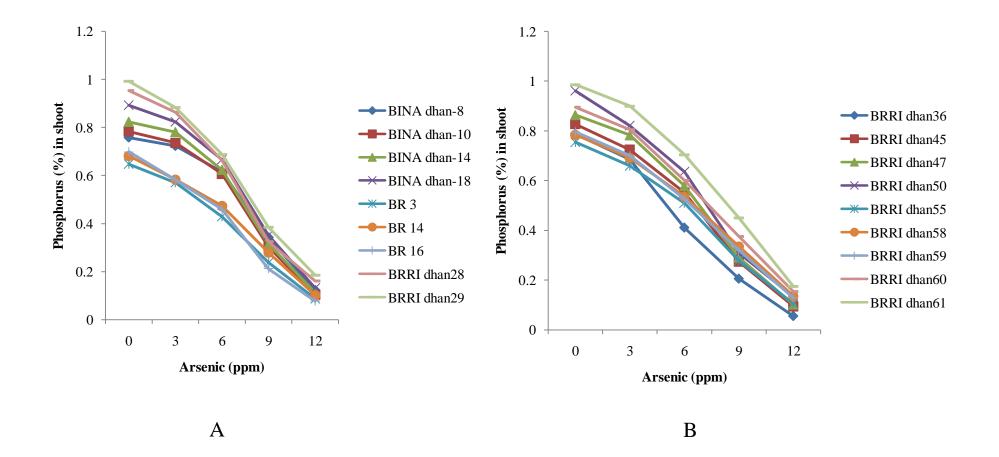


Figure 16: Interaction effect between arsenic and cultivar on phosphorus (%) in shoot

Table 3: Effect of cultivar on Arsenic (ppm), Phosphorus (%) and Potassium (%) in rice seedling shoot

Cultivar	Arsenic	Phosphorus	Potassium (%)
	(ppm)	(%)	
BINA dhan-8	0.358 CDEF	0.512 DEF	0.636 F
BINA dhan-10	0.319 DEF	0.507 DEF	0.767 E
BINA dhan-14	0.261 EF	0.532 CDE	0.866 BC
BINA dhan-18	0.204 F	0.574 BCD	0.911 AB
BR 3	0.382 BCDE	0.395 H	0.446 H
BR 14	0.402 BCDE	0.424 H	0.513 G
BR 16	0.323 DEF	0.406 H	0.606 F
BRRI dhan28	0.457 ABCD	0.593 ABC	0.644 F
BRRI dhan29	0.412 ABCDE	0.627 AB	0.820 CD
BRRI dhan36	0.366 CDEF	0.428 GH	0.544 G
BRRI dhan45	0.351 CDEF	0.494 EFG	0.548 G
BRRI dhan47	0.499 ABC	0.523 DEF	0.621 F
BRRI dhan50	0.583 A	0.571 BCD	0.889 B
BRRI dhan55	0.548 AB	0.461 FGH	0.534 G
BRRI dhan58	0.465 ABCD	0.496 EFG	0.643 F
BRRI dhan59	0.329 CDEF	0.494 EFG	0.615 F
BRRI dhan60	0.304 DEF	0.565 BCD	0.785 DE
BRRI dhan61	0.266 EF	0.643 A	0.955 A
LSD	0.172	0.068	0.212
Level of	*	*	*
significance			
CV%	11.69	9.50	9.69

# **4.2.4 Potassium content in shoot**

Effect of arsenic on potassium content in rice seedling shoot was statistically significant (fig. 17). The highest potassium content (1.051%) in rice seedling shoot was found at As<sub>0</sub> arsenic treatment. The lowest potassium content (0.232%) in rice seedling shoot was found at As<sub>4</sub> arsenic treatment. The result revealed that increasing arsenic concentration decreased potassium uptake in rice seedling shoot.

The effect of cultivar on potassium content in rice seedling shoot was significant (table 9). The highest potassium content (0.955%) in rice seedling shoot was found from BRRI dhan61 and BINA dhan-18 (0.911%) showed statistically similar result. The lowest potassium content (0.446%) in rice seedling shoot was found from BR 3.

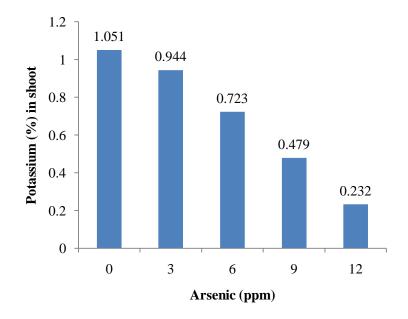


Figure 17: Effect of arsenic on potassium (%) in shoot

The interaction effect between arsenic and cultivar on potassium content in rice seedling shoot was also significant (fig. 18). The highest potassium content (1.481%) was recorded from BRRI dhan61 at  $As_0$ arsenic treatment whereas the lowest potassium content (0.108%) was recorded from BR 3 at  $As_4$  arsenic treatment.

#### **4.2.5** Nitrogen content in root

Nitrogen content in rice seedling root showed statistically significant difference due to arsenic treatment (fig. 19). The highest nitrogen content (0.681%) was found at As<sub>0</sub> arsenic treatment whereas the lowest nitrogen

content (0.220%) at  $As_4$  arsenic treatment. Khatik and Dikshit (2001) reported that the increasing concentration of arsenic in soil caused decreased the uptake pattern nitrogen in rice seedling.

Nitrogen content in rice seedling root was statistically significant due to cultivar (table 4). The highest nitrogen content (0.631%) was found from BRRI dhan29 and followed by BRRI dhan50 (0.619%). The lowest nitrogen content (0.367%) was found from BINA dhan-8 and BR 3 (0.401%) showed statistically similar result.

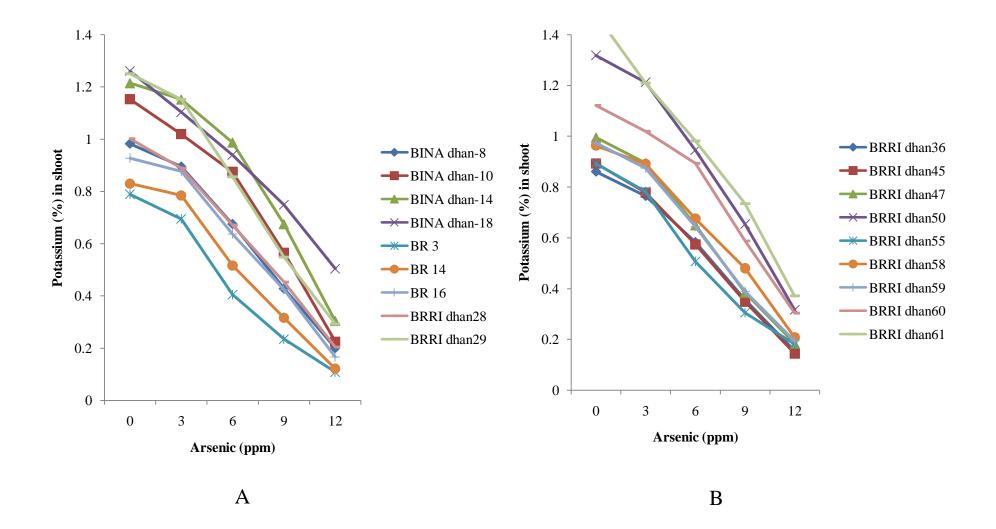


Figure 18: Interaction effect between arsenic and cultivar on potassium (%) in shoot

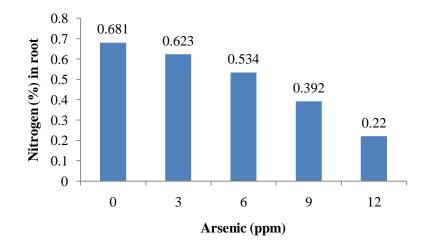


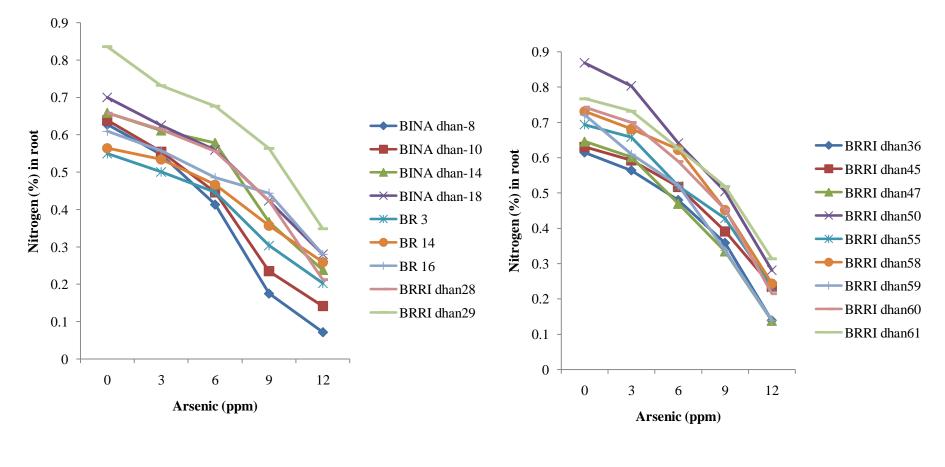
Figure 19: Effect of arsenic on Nitrogen (%) in root

The interaction effect between arsenic and cultivar was also significant on nitrogen content in rice seedling root (fig. 20). The highest nitrogen content (0.868%) in rice seedling root was found from BRRI dhan50 at As<sub>0</sub> arsenic treatment and followed by BRRI dhan29 (0.836%) at same arsenic treatment. The lowest nitrogen content (0.072%) in rice seedling root was found from BINA dhan-8 at As<sub>4</sub> arsenic treatment. The result revealed that increasing arsenic concentration decreased nitrogen uptake in rice seedling.

## 4.2.6 Arsenic content in root

Arsenic content in rice seedling root showed statistically significant due to application of arsenic (fig. 21). The maximum arsenic content (1.353 ppm) was found in  $As_4$  arsenic treatment whereas the minimum arsenic content (0 ppm) was found in  $As_0$  arsenic treatment. The result revealed that increasing arsenic concentration increased uptake of arsenic content in rice seedling shoot.

Effect of cultivar on arsenic content in rice seedling root was also significant (table 4). The highest arsenic content (0.729 ppm) in rice seedling root was recorded from BRRI dhan50 and BRRI dhan55 (0.686



А

В

Figure 20: Interaction effect between arsenic and cultivar on Nitrogen (%) in root

ppm) was showed statistically similar result. The lowest arsenic content (0.255 ppm) in rice seedling root was recorded from BINA dhan-18. BINA dhan-14 (0.326 ppm) was statistically similar and followed by BRRI dhan61 (0.332 ppm).

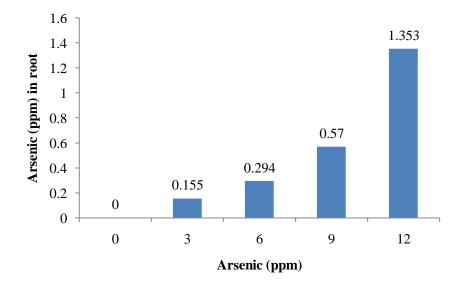


Figure 21: Effect of arsenic on Arsenic (ppm) content in root

Interaction effect of arsenic and cultivar on arsenic content in rice seedling root was also significant (fig. 22). The highest arsenic content (2.036 ppm) in rice seedling root was found from BRRI dhan50 at  $As_4$  arsenic treatment. All the eighteen rice cultivars weren't uptake any arsenic at  $As_0$  arsenic treatment.

## 4.2.7 Phosphorus content in root

Effect of arsenic on phosphorus content in rice seedling root was significant (fig. 23). The maximum phosphorus content (0.935%) in rice seedling root was recorded from  $As_0$  arsenic treatment. The minimum phosphorus content (0.136%) in rice seedling root was recorded from  $As_4$  arsenic treatment.

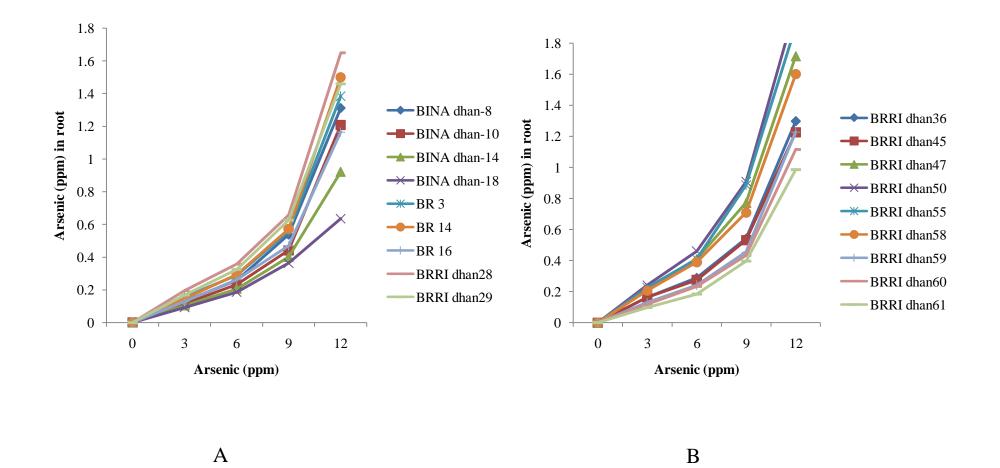


Figure 22: Interaction effect between arsenic and cultivar on arsenic (ppm) in root

Effect of cultivar on phosphorus content in rice seedling root was also significant (table 4). The highest phosphorus content (0.726%) in rice seedling root was found in BRRI dhan61 and followed by BRRI dhan29 (0.709%). The lowest phosphorus content (0.446%) in rice seedling root was found in BR 3 and followed by BR 16 (0.459%).

The interaction effect of arsenic and cultivar on phosphorus content in rice seedling root was also significant (fig. 24). The maximum phosphorus content (1.122%) in rice seedling root was recorded from BRRI dhan29 and followed by BRRI dhan61 (1.114%) at As<sub>0</sub> arsenic treatment, phosphorus content (1.086%) in rice seedling root from BRRI dhan50 was statistically similar at same arsenic treatment. The minimum phosphorus content (0.062%) in rice seedling root was recorded from BRRI dhan36 at As<sub>4</sub> arsenic treatment. BR 16 (0.088%) showed statistically similar result at As<sub>4</sub> arsenic treatment.

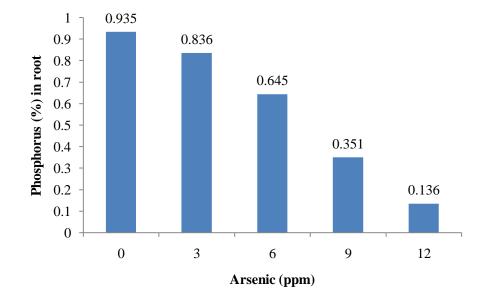


Figure 23: Effect of arsenic on Phosphorus (%) in root

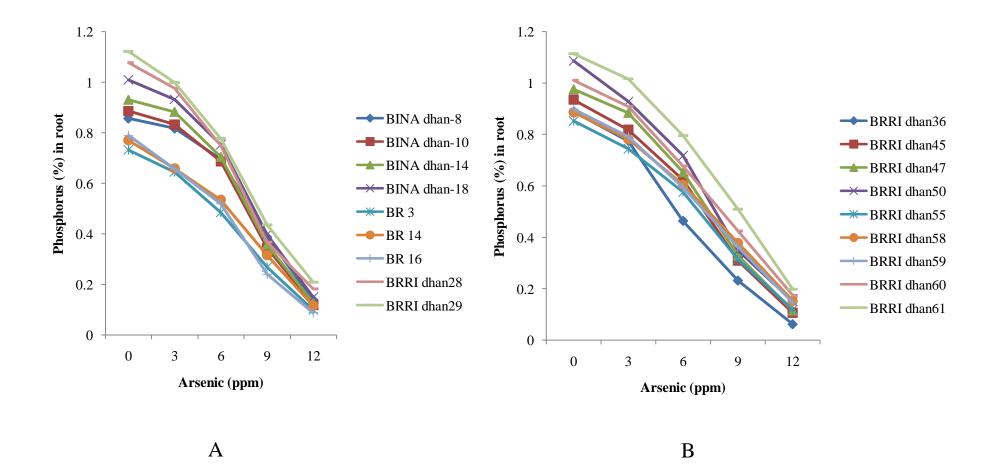


Figure 24: Interaction effect between arsenic and cultivar on phosphorus (%) in root

### 4.2.8 Potassium content in root

Effect of arsenic on potassium content in rice seedling root was statistically significant (fig. 25). The highest potassium content (0.790%) in rice seedling root was found at  $As_0$  arsenic treatment. The lowest potassium content (0.174%) in rice seedling root was found at  $As_4$  arsenic treatment. The result revealed that increasing arsenic concentration decreased potassium uptake in rice seedling root.

The effect of cultivar on potassium content in rice seedling root was significant (table 4). The highest potassium content (0.718%) in rice seedling root was found from BRRI dhan61 and BINA dhan-18 (0.685%), BINA dhan-14 (0.651%) showed statistically similar result. The lowest potassium content (0.335%) in rice seedling root was found from BR 3 and BR 14 (0.386%) showed statistically similar result.

The interaction effect between arsenic and cultivar on potassium content in rice seedling root was also significant (fig. 26). The highest potassium content (1.113%) in rice seedling root was recorded from BRRI dhan61 at  $As_0$  arsenic treatment whereas the lowest potassium content (0.081%) in rice seedling root was recorded from BR 3 at  $As_4$  arsenic treatment.

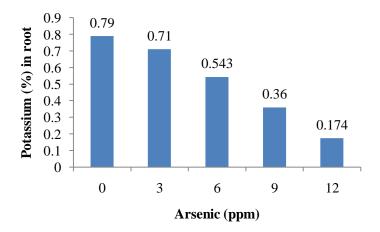


Figure 25: Effect of arsenic on potassium (%) in root

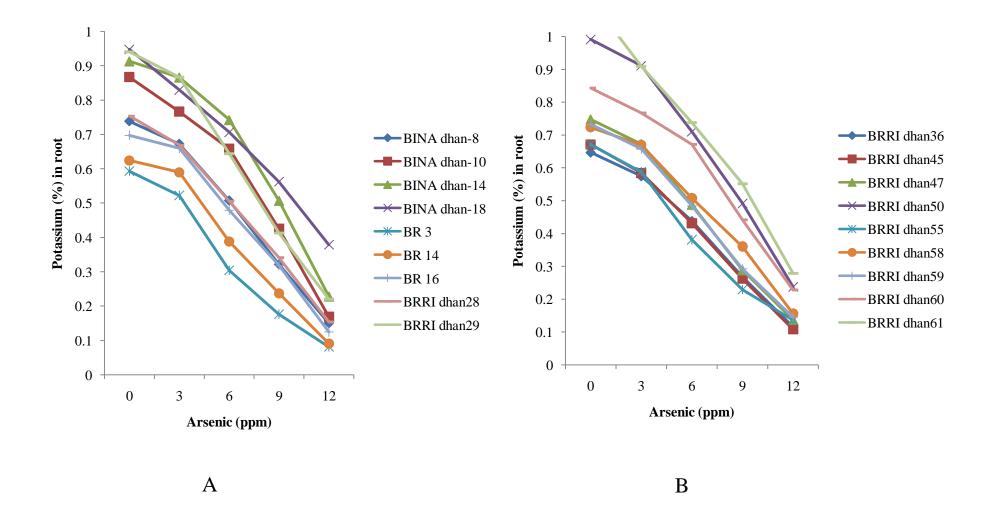
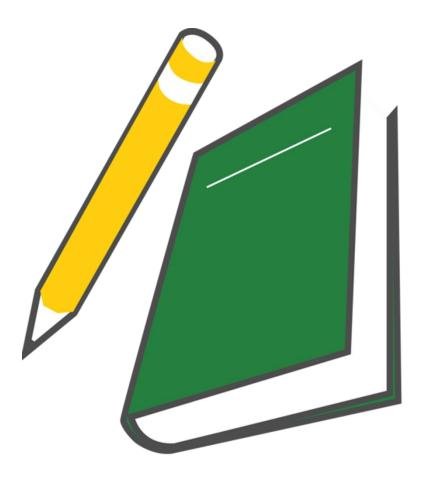


Figure 26: Interaction effect between arsenic and cultivar on Potassium (%) in root

Table 4: Effect of cultivar on Nitrogen (%), Arsenic (ppm), Phosphorus (%) and Potassium (%) in rice seedling root

Cultivar	Nitrogen (%)	Arsenic (ppm)	Phosphorus (%)	Potassium (%)
BINA dhan-8	0.367 H	0.4477CDEF	0.579 BCD	0.478 EF
BINA dhan-10	0.403 FGH	0.3998DEF	0.573 CDE	0.577 D
BINA dhan-14	0.491 DEF	0.326 EF	0.601 BCD	0.651 ABCD
BINA dhan-18	0.518 CDE	0.255 F	0.648 ABC	0.685 AB
BR 3	0.401 GH	0.478 BCDE	0.446 G	0.335 H
BR 14	0.436 EFGH	0.502 BCDE	0.479 FG	0.386 GH
BR 16	0.475 DEFG	0.404 DEF	0.459 G	0.456 EFG
BRRI dhan28	0.493 DE	0.572 ABCD	0.670 AB	0.484 E
BRRI dhan29	0.631 A	0.515 BCDE	0.709 A	0.616 BCD
BRRI dhan36	0.431 EFGH	0.458 CDEF	0.484 EFG	0.409 EFGH
BRRI dhan45	0.473 DEFG	0.438 CDEF	0.558 CDEF	0.412 EFGH
BRRI dhan47	0.438 EFGH	0.623 ABC	0.591 BCD	0.467 EF
BRRI dhan50	0.619 AB	0.729 A	0.646 ABC	0.668 ABC
BRRI dhan55	0.506 CDE	0.686 AB	0.521 DEFG	0.401 FGH
BRRI dhan58	0.546 ABCD	0.581 ABCD	0.561 CDEF	0.483 E
BRRI dhan59	0.465 DEFG	0.411 DEF	0.558 CDEF	0.462 EFG
BRRI dhan60	0.540 BCD	0.380 DEF	0.639 ABC	0.590 CD
BRRI dhan61	0.591 ABC	0.332 EF	0.726 A	0.718 A
LSD	0.088	0.212	0.093	0.078
Level of Significance	*	*	*	*
CV%	3.75	4.69	2.50	4.25



# Chapter V SUMMARY & CONCLUSION

#### **CHAPTER V**

### SUMMARY AND CONCLUSION

The two factorial experiment was laid out in a Completely Randomized Design (CRD) with three replications. Factor A: different doses of arsenic  $[As_0 = No \text{ arsenic added (control)}, As_1 = 3 \text{ ppm arsenic}, As_2 = 6 \text{ ppm arsenic}, As_3 = 9 \text{ ppm arsenic and } As_4 = 12 \text{ ppm arsenic (soil water basis)}] and Factor B: different rice cultivars [T<sub>1</sub>= BINA dhan-8, T<sub>2</sub> = BINA dhan-10, T<sub>3</sub> = BINA dhan-14, T<sub>4</sub> = BINA dhan-18, T<sub>5</sub> = BR 3, T<sub>6</sub> = BR 14, T<sub>7</sub> = BR 16, T<sub>8</sub> = BRRI dhan28, T<sub>9</sub> = BRRI dhan29, T<sub>10</sub> = BRRI dhan36, T<sub>11</sub> = BRRI dhan45, T<sub>12</sub> = BRRI dhan47, T<sub>13</sub> = BRRI dhan50, T<sub>14</sub> = BRRI dhan55, T<sub>15</sub> = BRRI dhan58, T<sub>16</sub> = BRRI dhan59, T<sub>17</sub> = BRRI dhan60 and T<sub>18</sub> = BRRI dhan61].$ 

Different growth and yield parameters varied significantly due to difference in the doses of arsenic. The highest seedling height (13.14 cm) was recorded fromAs<sub>0</sub> treatment and the lowest (7.19cm) from As<sub>4</sub> treatment. The highest shoot weight (0.34 g) was observed from As<sub>0</sub> treatment and the lowest (0.20 g) from As<sub>4</sub> treatment. The longest root (6.15 cm) was recorded from As<sub>0</sub> treatment and the smallest (3.79 cm) from As<sub>4</sub> treatment. The highest root weight (0.111 g) was recorded from As<sub>0</sub> treatment and the lowest (0.063 g) from As<sub>4</sub> treatment. The highest total dry matter (0.456 g) was found from As<sub>0</sub> treatment and the lowest (0.263 g) from As<sub>4</sub> treatment.

The highest nitrogen content (1.029%) in shoot was found from As<sub>0</sub> treatment and the lowest (0.333%) from As<sub>4</sub> arsenic treatment.The maximum arsenic content (1.083 ppm) in shoot was found from As<sub>4</sub> treatment and the minimum (0 ppm) from As<sub>0</sub> treatment. The maximum

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phosphorus content (0.827%) in shoot was recorded from  $As_0$  treatment and the minimum (0.120%) from  $As_4$ treatment. The highest potassium content (1.051%) in shoot was found from  $As_0$  treatment and the lowest (0.232%) from  $As_4$  treatment.

The highest nitrogen content (0.681%) in root was found from  $As_0$  treatment and the lowest (0.220%) from  $As_4$ treatment. The maximum arsenic content (1.353 ppm) in root was found from  $As_4$  arsenic treatment and the minimum (0 ppm) from  $As_0$  arsenic treatment. The maximum phosphorus content (0.935%) in root was recorded from  $As_0$  treatment and the minimum (0.136%) from  $As_4$ treatment. The highest potassium content (0.790%) in root was found from  $As_0$  treatment and the lowest (0.174%) from  $As_4$  arsenic treatment.

Different growth and yield parameters varied significantly due to difference in the cultivars. The highest seedling height (13.23 cm) was recorded from BINA dhan-18 and the lowest (8.14 cm) from BRRI dhan29. The highest shoot weight (0.393 g) was observed from the BRRI dhan61 and the lowest (0.182 g) from BR 3. The longest root (6.53 cm) was recorded from BINA dhan-18 and the smallest (3.87 cm) from BRRI dhan45. The highest root weight (0.126 g) was recorded from BRRI dhan29 and the lowest (0.061 g) from BR 3. The highest total dry matter (0.517 g) was found from BRRI dhan29and the lowest (0.243 g) from BR 3.

The highest nitrogen content (0.954%) in shoot was found from BRRI dhan29 and the lowest (0.554%) from BINA dhan-10. The maximum arsenic content (0.583 ppm) in shoot was found from BRRI dhan50and the minimum (0.204 ppm) was from BINA dhan-18. The maximum phosphorus content (0.643%) in shoot was recorded from BRRI

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dhan61and the minimum (0.395%) from BR 3.The highest potassium content (0.955%) in shoot was found from BRRI dhan61and the lowest (0.446%) was found from BR 3.

The highest nitrogen content (0.631%) in root was found from BRRI dhan29 and the lowest (0.367%) from BINA dhan-8.The maximum arsenic content (0.729 ppm) in root was found from BRRI dhan50and the minimum (0.255 ppm) from BINA dhan-18.The maximum phosphorus content (0.726%) in root was recorded from BRRI dhan61 and the minimum (0.446%) from BR 3.The highest potassium content (0.718%) in root was found from BRRI dhan61and the lowest (0.335%) was found from BR 3.

The highest seedling height (16.63 cm) was observed from BINA dhan-8 at  $As_0$  arsenic treatment and the lowest (5.23 cm) from BRRI dhan29 at  $As_0$  arsenic treatment. The highest shoot weight (0.483 g) was found from BRRI dhan61 at  $As_0$  arsenic treatment and the lowest (0.129 g) from BRRI dhan55 at  $As_4$  arsenic treatment. The longest root (8.44 cm) was found from BINA dhan-18 at  $As_0$  arsenic treatment and the smallest (3.05 cm) from BRRI dhan45 at  $As_4$  arsenic treatment. The highest root weight (0.152 g) was recorded from BRRI dhan29 at  $As_0$  arsenic treatment and the lowest (0.044 g) from BR 3 at  $As_4$  arsenic treatment. The highest total dry matter (0.621 g) was found from BRRI dhan29 at  $As_0$  arsenic treatment and the lowest (0.178 g) from BR 3 at  $As_4$  arsenic treatment.

The highest nitrogen content (1.311%) in shoot was found from BRRI dhan50 at As<sub>0</sub> arsenic treatment and the lowest (0.109%) from BINA dhan-8 at As<sub>4</sub> arsenic treatment. The highest arsenic content (1.629 ppm) in shoot was found from BRRI dhan50 at As<sub>4</sub> arsenic treatment. All the eighteen rice cultivars weren't uptake any arsenic at As<sub>0</sub> arsenic

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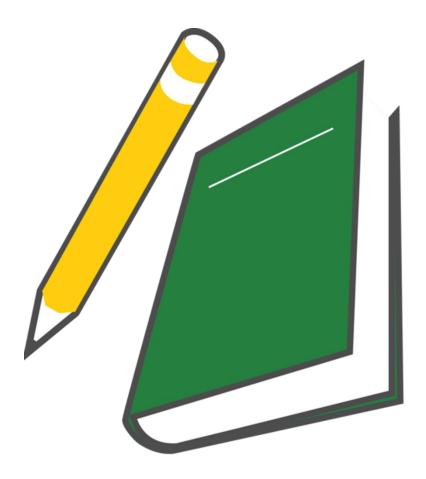
treatment. The maximum phosphorus content (0.993%) in shoot was recorded from BRRI dhan29 at As<sub>0</sub> arsenic treatment and the minimum (0.055%) from BRRI dhan36 at As<sub>4</sub> arsenic treatment. The highest potassium content (1.481%) in shoot was recorded from BRRI dhan61 at As<sub>0</sub> arsenic treatment and the lowest (0.108%) from BR 3 at As<sub>4</sub> arsenic treatment.

The highest nitrogen content (0.868%) in root was found from BRRI dhan50 at  $As_0$  arsenic treatment and the lowest (0.072%) from BINA dhan-8 at  $As_4$  arsenic treatment. The highest arsenic content (2.036 ppm) in root was found from BRRI dhan50 at  $As_4$  arsenic treatment. All the eighteen rice cultivars weren't uptake any arsenic at  $As_0$  arsenic treatment. The maximum phosphorus content (1.122%) in root was recorded from BRRI dhan29 and the minimum (0.062%) from BRRI dhan36 at  $As_4$  arsenic treatment. The highest potassium content (1.113%) in root was recorded from BRRI dhan61 at  $As_0$  arsenic treatment and the lowest (0.081%) from BR 3 at  $As_4$  arsenic treatment.

From the above results it can be concluded that,

- Arsenic toxicity adversely affects all the growth and yield related attributes of all rice cultivars.
- BINA dhan-18 produced highest seedling height and BRRI dhan29 produced the lowest. BRRI dhan-61 produced highest shoot weight and BR 3 produced the lowest. BINA dhan-18 produced maximum root length and BRRI dhan45 produced the lowest. BRRI dhan29 produced maximum root weight and BR 3 produced the minimum. BRRI dhan29 produced maximum total dry matter and BR 3 produced minimum.

- BRRI dhan29 contained maximum N in shoot and BINA dhan-10 contained the minimum. BRRI dhan61 contained maximum P in shoot and BR 3 contained the minimum.BRRI dhan61 contained maximum K in shoot and BR 3 contained the minimum.
- BRRI dhan29 contained maximum N in root and BINA dhan-8 contained the minimum. BRRI dhan61 contained maximum P in root and BR 3 contained the minimum. BRRI dhan61 contained maximum K in root and BR 3contained the minimum.



# Chapter VI REFERENCES

#### **CHAPTER VI**

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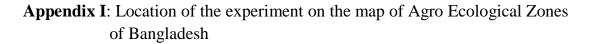
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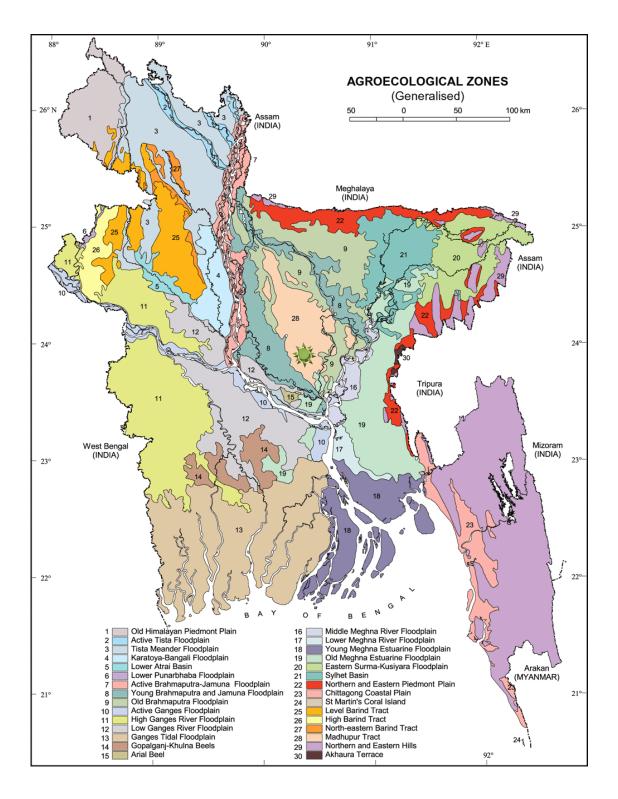
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# APPENDICES

### **APPENDICES**





Morphology	Characteristics	
Location	SAU Farm, Dhaka.	
Agro-ecological zone	Madhupur Tract (AEZ- 28)	
General Soil Type	Deep Red Brown Terrace Soil	
Parent material	Madhupur Terrace.	
Topography	Fairly level	
Drainage	Well drained	
Flood level	Above flood level	
Flood level	Above flood level	

Appendix II: Morphological characteristics of the experimental field

### (SAU Farm, Dhaka)

Appendix III: Initial physical and chemical characteristics of the soil

Characteristics	Value		
Mechanical fractions:			
% Sand (2.0-0.02 mm)	22.26		
% Silt (0.02-0.002 mm)	56.72		
% Clay (<0.002 mm)	20.75		
Textural class	Silt Loam		
pH (1: 2.5 soil- water)	5.9		
Organic Matter (%)	1.09		
Total N (%)	0.028		
Available K (ppm)	15.625		
Available P (ppm)	7.988		
Available S (ppm)	2.066		
	(SAII Farm Dhaka		

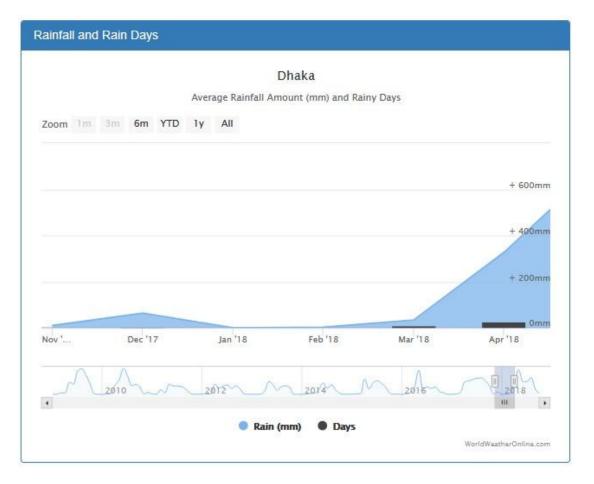
(SAU Farm, Dhaka)

### Appendix IV: Monthly weather data of Dhaka during experiment (from Nov'2017 to Mar'2018)





(Source- www.worldweatheronline.com)



(Source- www.worldweatheronline.com)

## Appendix V: Analysis of variance of the data on Height, Weight and Root length of rice seedlings

Source of	Degrees of	Mean square		
variation	freedom	Height	weight	Root
				length
Arsenic (A)	4	338.36*	0.176*	48.47*
Cultivar (B)	17	35.55*	0.071*	6.938*
Interaction (A×B)	68	1.89*	0.001*	0.246*
Error	180	0.29	0.001	0.039

\* = Significant at 5% level

**Appendix VI:** Analysis of variance of the data on Root weight and total dry matter of rice seedlings

Source of	Degrees of	Mean square		
variation	freedom	Root weight	Total dry matter	
Arsenic (A)	4	0.02*	0.314*	
Cultivar (B)	17	0.005*	0.108*	
Interaction (A×B)	68	0.001*	0.001*	
Error	180	0.001	0.001	

\* = Significant at 5% level

## **Appendix VII:** Analysis of variance of the data on N, P, K and As contents in shoot

Source of variation	Degrees of	Mean square			
	freedom	Ν	Р	K	As
Arsenic (A)	4	4.257*	4.705*	6.071*	9.85*
Cultivar (B)	17	0.190*	0.080*	0.356*	0.153*
Interaction	68	0.011*	0.005*	0.009*	0.037*
(A×B)					
Error	180	0.001	0.001	0.003	0.002

\* = Significant at 5% level

## **Appendix VIII:** Analysis of variance of the data on N, P, K and As contents in root

Source of	Degrees	Mean square			
variation	of				
	freedom	Ν	Р	K	As
Arsenic (A)	4	1.867*	6.08*	3.432*	15.40*
Cultivar (B)	17	0.083*	0.102*	0.201*	0.239*
Interaction	68	0.005*	0.007	0.005*	0.058*
(A×B)					
Error	180	0.001	0.001	0.001	0.005

\* = Significant at 5% level