

**INTERACTION EFFECTS OF ARSENIC AND PHOSPHORUS ON GROWTH,
YIELD AND ARSENIC LOADING IN BRRI dhan29**

A Thesis

BY

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YIELD AND ARSENIC LOADING IN BRRI dhan29**

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CERTIFICATE

This is to certify that the thesis entitled “**INTERACTION EFFECTS OF ARSENIC AND PHOSPHORUS ON GROWTH, YIELD AND ARSENIC LOADING IN BRRI dhan29**” 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 (M.S.) IN SOIL SCIENCE**, embodies the results of a piece of *bona fide* research work carried out by **Tahmina Yeasmin Khan, Registration. No. 11-04570**, under my supervision and guidance.

No part of this thesis has been submitted for any other degree or diploma.

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

Dated:
Dhaka, Bangladesh

Professor Dr. Mohammad Mosharraf Hossain
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Dedicated
To
My father & mother

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Author

Dated:

ABSTRACT

A pot experiment was conducted at the Soil Science Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to March 2017 to evaluate the effect of arsenic and phosphorus on the growth and yield of BBRI dhan29. A two factor factorial pot experiment was conducted which comprised with three levels of arsenic doses 0, 15 and 30 ppm and five levels of phosphorus 0, 20, 40, 60 and 80 ppm. The treatment combinations were As0P0, As0P20, As0P40, As0P60, As0P80, As15P0, As15P20, As15P40, As15P60, As15P80, As30P0, As30P20, As30P40, As30P60 and As30P80. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. Increased As showed adverse effect on plant height, effective tillers per plant, non-effective tillers per plant, filled grain per plant, unfilled grain per plant, grains per panicle, 1000-grain weight and grain & straw yield of rice. This study was made on the effect of arsenic (As) and AsXP interaction in BBRI dhan29. The highest plant height was observed from As0P80 treatment (88.2) and lowest plant height was recorded from As30P80 treated pot. The reduction of plant height due to application of arsenic and phosphorus were recorded (35.1% and 9.73%). highest effective tillers per pot (37.7), highest filled grains per panicle (97.00) and the reduction of 1000 grain weight (g) due to application of arsenic and phosphorus were also recorded (15.35% and 8.45%). The highest grain yield was observed from As0P80 treatment (80.1) and lowest grain yield was recorded from As30P80 treated pot (8.3). Furthermore, the reduction of grain yield due to application of arsenic and phosphorus were also recorded (67.89% and 17.94%). On the one hand, highest straw yield was observed from As0P80 treatment (118.07) and lowest straw yield was recorded from As30P80 treated pot (19.5). Furthermore, the reduction of straw yield due to application of arsenic and phosphorus were also recorded (65.18% and 11.40%). On the other hand, highest arsenic concentration (As conc. in ppm) in grain was observed from As30P80 treatment (1.47) and lowest arsenic concentration (As conc. in ppm) in grain was recorded from As0P20 treated pot (0.235). Furthermore, the reduction of arsenic concentration (ppm) in grain due to application of arsenic and phosphorus. The adverse effect of arsenic was further enhanced by P addition. This reaction has an implication to P fertilizer management in rice.

ABBREVIATIONS, ACRONYMS AND SYMBOLS

| | |
|-----------|---|
| AAS | Atomic Absorption Spectrophotometer |
| AEZ | Agro-ecological Zone |
| As | Arsenic |
| As (III) | Arsenite |
| As (V) | Arsenate |
| AsB | Arsenobetaine |
| AsC | Arsenocoline |
| AsS | Arsenic disulphate |
| BARC | Bangladesh Agricultural Research Council |
| BAU | Bangladesh Agricultural University |
| BGS | British geological survey |
| BRRRI | Bangladesh Rice Research Institute |
| BSMRAU | Bangabandhu Sheikh Mujibur Rahman Agricultural University |
| CEC | Cation exchange capacity |
| cfu | Colony forming unit |
| CMC | Carboxy methyl cellulose |
| CRD | Complete Randomized Design |
| CV | Co-efficient of variation |
| DMMA | Dimethylarsinic acid |
| DNA | Dioxy ribonucleic acid |
| DPHE | Directorate of public health and engineering |
| FI-HG-AAS | Flow Injection Hydride Generation Atomic Absorption Spectrophotometer |
| FW | Fresh weight |
| g | Gram |

| | |
|---------------------|--------------------------------------|
| g/cc | Gram per cubic centimeter |
| GP | Germination percentage |
| IAA | Indole-3-acetic acid |
| Kb | Kilo base pair |
| Kg | Kilogram |
| LSD | Least significant differences |
| meq | Milli equivalent |
| mg kg ⁻¹ | Milligram per kilogram |
| MMA | Monomethylarsonic acid |
| MoP | Muriate of potash |
| NA | Nutrient agar |
| PCR | Polymer chain reaction |
| PGPR | Plant growth promoting rhizobacteria |
| pH | Hydrogen ion concentrations |
| ppb | Parts per billion |
| ppm | Parts per million |
| PSI | Phosphate solubilization index |
| RCBD | Randomized Complete Block Design |
| ROL | Radial oxygen loss |
| ROS | Reactive oxygen species |
| SRDI | Soil Resources Development Institute |
| TSP | Triple Superphosphate |
| USDA | United States of America |

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CHAPTER I

INTRODUCTION

Arsenic (As), a toxic metalloid, is widely distributed in the environment, which results from both natural and anthropogenic sources (Jhao et al., 2010). It is a human toxic and carcinogenic element that exists throughout the earth's crust and can be mobilized into water and subsequently incorporated into the human food chain. Thousands of drinking wells in Bangladesh and the regions in East India, mainly West Bengal, derive water from an As-contaminated water source, resulting in wide scale exposure of the populous to As through water (Nordstrom, 2002; Rahman et al., 2014). Arsenic contaminated ground water is not just used for drinking water, but is also widely used for irrigation of crops, particularly for the staple paddy rice, which provides 73 % of calorific intake of the South and Southeast Asian diet (Ninno and Dorosh, 2001). Making this situation worse, the elevated As concentrations in the soils where rice is grown are phytotoxic and can contribute to decreased rice grain fill, contributing to lowered yield and reduced food availability (Abedin et al., 2002 and Panaullah *et al.* 2009). It is estimated that there will be about 8 million people by the year 2020, requiring 760 million tons of rice. This means that the production of rice needs to be increased by 2% per year to meet future demands. This will require double the amount of currently applied synthetic fertilizers, which is neither economically feasible nor environmentally desirable. Arsenic is a toxic pollutant released into the environment either by natural phenomena or anthropogenic activities. It is a potent human toxin, which causes many diseases such as diarrhoea, bladder cancer etc.

Arsenic contamination of groundwater is widely reported worldwide, including Argentina, Bangladesh, Bolivia, Brazil, Chile, China, Cambodia, Ghana, Greece, Hungary, India, Japan, Korea, Mexico, Mongolia, Nepal, New Zealand, Poland, Taiwan, Thailand, Vietnam , some parts of the USA and recently Iran (O'Neill, 1995; Smith et al., 2001; Meharg and Rahman,

2003; Ravenscroft et al., 2009; Smedley and Kinniburgh, 2002). Arsenic uptake and toxicity/tolerance have been well characterized in plants (Hartley-Whitaker et al., 2001). Plants can develop toxicity symptoms such as: inhibition of seed germination (Abedin and Meharg, 2002); decrease in plant height (Marin et al., 1992, Carbonel-Barrachina et al., 1995); depressed tillering (Rahman *et al.*, 2004); reduction in root growth (Abedin et al., 2002); decrease in shoot growth (Carbonel-Barrachina et al., 1998); lower fruit and grain yield (Carbonel-Barrachina et al., 1995) and reduction in photosynthesis rate (Miteva and Peycheva, 1999) while they are exposed to excess As either in soil or in solution culture.

Bangladesh is a delta of high arsenic (As) contamination in groundwater and the water being widely used for irrigation. Total As concentrations ranges from 5.0 to 33 mgkg⁻¹ with an average of 17 mgkg⁻¹ which has been reported for some soil samples in Nawabganj, Rajarampur, Jessore, Jhenidah and Comilla regions in Bangladesh (Islam et al., 2000). Arsenic uptake and accumulation by plant is significantly influenced by As concentration in the soil or growth media and substantially increased with increasing As levels in soil solution (Marin et al., 1992, 1993b). Concentrations of As were reported as 5.8 to 17.7 mgkg⁻¹ (mean 11.2 mgkg⁻¹) in soil, 1.48-17.6 mgkg⁻¹ (mean 5.88 mg kg⁻¹) in straw and 0.241-1.298 (mean 0.759 mg kg⁻¹) in rice grain (Islam et al., 2000). Rice grain generally has lower As concentration and the concentration remains below maximum permissible limit (MPL) of 1 mg Askg⁻¹. However, Xie and Huang, (1998) found higher As concentration in rice grain (husked rice) in some cultivars which exceeds 1.0 mgkg⁻¹As of MPL when grown in As contaminated soils. In today's day and age, our prime target is to enhance our agricultural output to meet the needs of the growing population but in a sustainable and eco-friendly manner. The residual toxicity and other ill effects of arsenic are rendering their use unsustainable. Several field studies conducted in Bangladesh showed that irrigation with As-rich groundwater resulted in elevated topsoil As concentrations of up to ~80 mg kg⁻¹ in the

upper paddy soil layers, whereas background soil As contents were typically $<10 \text{ mg kg}^{-1}$ (Panaullah et al., 2009). While increasing during irrigation, As concentrations in topsoil of paddy fields were reported to decrease significantly during the monsoon season in regions with pronounced monsoon flooding (Islam et al., 2005). It is hoped that the results of the research herein will provide the scientific community with a useful piece of information, being accessible for governments and policy makers concerned with the consequences of paddy irrigation with As-rich groundwater, and that the conclusions will serve as a basis for the process of mitigating further As accumulation in soils and crops.

Arsenic (As) contamination of groundwater is a severe problem in Bangladesh and it has affected at least 25 million people (Ravenscroft *et al.* 2005). Next to drinking water, rice could be a potential source of As exposure to the people living in the As affected areas of Bangladesh (Hossain *et al.* 2008 and Panaullah *et al.* 2009). Roberts *et al.* (2007) estimated that over 1000 tons of As might be transferred to arable land each year through As contaminated groundwater irrigation, creating a potential risk for future agricultural sustainability and food security of the country. People of Bangladesh not only drink the As contaminated groundwater, they also irrigate their crops (mainly rice) with it mostly in the Boro season. Long term use of As contaminated water for irrigation may result in the elevation of As concentration in soils and plants (Ullah 1998 and Haq *et al.* 2003). Normal irrigated soils in Bangladesh contain 4-8 mg As kg^{-1} while soils irrigated with As contaminated water contain up to 83 mg As kg^{-1} (Ullah 1998). As contaminated water contain up to 83 mg As kg^{-1} (Ullah 1998).

There is increasing concern worldwide regarding the contamination of soil with arsenic (As), and the potential risk to human and environmental health arising from such contamination (Smith et al., 1998). Arsenic contamination of soil can occur as a result of both natural sources and anthropic activities, including the use of arsenical pesticides and herbicides,

atmospheric deposition, mining activity, waste disposal, and other sources (Mandal and Suzuki, 2002).

Arsenate uptake and toxicity/tolerance have been well characterized in plants (Hartley-Whitaker *et al.* 2001). Plants can develop toxicity symptoms such as: inhibition of seed germination (Abedin and Meharg 2002); decrease in plant height (Marin *et al.* 1992, Carbonel-Barrachina *et al.* 1995, Abedin *et al.* 2002b and Jahan *et al.* 2003a); depressed tillering (Kang *et al.* 1996 and Rahman *et al.* 2004); reduction in root growth (Carbonel-Barrachina *et al.* 1998 and Abedin *et al.* 2002); decrease in shoot growth (Cox *et al.* 1996 and Carbonel-Barrachina *et al.* 1998); lower fruit and grain yield (Carbonel-Barrachina *et al.* 1995, Kang *et al.* 1996 and Abedin *et al.* 2002b) and reduction in photosynthesis rate (Miteva and Merakehiyska, 2002) while they are exposed to excess As either in soil or in solution culture. Arsenate the dominant form of As in aerobic conditions is taken up by plants via the phosphate transport systems because of the chemical similarity between arsenate and phosphate (Dixon 1997). Phosphorus is a chemical analogue of As (Adriano 2001) which competes with As in plant uptake (Meharg and Macnair 1992), is one of essential elements for plant growth (Raghothama 1999). The effect of P on the absorption of As in soil environments has received great attention, especially when P is used as a crop fertilizer (Peryea 1998). Phosphorus fertilizer is common in rice cultivation which might interact with As uptake (Qafoku *et al.* 1999). In rice seedlings, cultivars found to be susceptible to arsenate and become more resistant by raising level of intracellular P (Geng *et al.* 2006 and Wang and Duan 2009).

It is estimated that crop yields on around 30-40 % of the world's arable land are limited by P availability (RungeMetzger, 1995). The effect of P on the sorption/desorption of As in soil environments has received great attention, especially when P is used as a crop fertilizer (Peryea, 1998). The bioavailable fraction of As in soils to crop plants depends on different

physical and chemical properties of soils. In fact soils rich in variable charge minerals (Al or Fe oxides) do not release As easily. Only large additions of P to high anion-fixing soils or alkaline pH or Fe and Al oxide dissolution may affect As solubility (Smith et al., 1998; Violante and Pigna, 2002). However, As sensitivity is intimately linked to P nutrition in plants. In rice seedlings, even cultivars found to be susceptible to arsenate can become more resistant by raising level of intracellular P (Geng et al., 2006; Wang and Duan, 2009). Gultz et al. (2005) reported that P availability and P demand, which are plant specific, have to be taken in account to predict uptake of As by crop plants. Therefore, As toxicity in crops can be more prevalent in situation where As contamination is found coexisting with low available P.

Arsenic may enter into human body directly through drinking water and indirectly through foods, chiefly rice for Bangladeshi people. Rice covers about 75% of the total cropped areas in this country. Many areas have high groundwater and soil arsenic contents which is likely to be taken up by plants through roots and transported to the aerial parts (Jahiruddin *et al.*, 2000; Huq *et al.*, 2006; Williams *et al.*, 2006). Islam *et al.* (2005) reported 12.3 ppm mean As over 456 soil samples across the country. Phosphorus fertilization is common in rice cultivation which might interact with arsenic uptake since phosphate is an analogue of arsenate (Beever and Burns, 1980) and compete for the same sorption sites (Qafoku *et al.*, 1999). Considering the above point in view, the present study was made to evaluate the effect of arsenic and its interaction with P on yield loss and arsenic accumulation in rice. The experiment was conducted with varying doses of arsenic addition to soil having low arsenic content in order to understand the situation in high arsenic soil. Much of the research on As in grain crops have focused on rice (*Oryza sativa* L.) (Abedin et al., 2002; Williams et al., 2005; Rahman et al., 2007).

The aim of this work was to evaluate the role of arsenic (As) and phosphorus (P) fertilization on growth, yield and As uptake in rice plant.

CHAPTER II

REVIEW OF LITERATURE

2.1 Arsenic and its occurrence

The name 'Arsenic' is derived from the Greek word "*arsenikon*" meaning potent (Frost, 1984). Arabian alchemist, Geber discovered arsenic during the eighth century when he heated orpiment (As_2S_3) (Mellor, 1954). In 1775, a famous Swedish chemist, Scheele discovered arsine (AsH_3) (Nriagu, 2002), however its deadly nature was not known until the death of a chemistry professor in Munich, who inhaled a minor quantity of AsH_3 , in 1815. Arsenic is a metalloid (element that has properties of both metals and non-metals), and belongs to the Group VA in the periodic table of elements (Allen, 1989; Emsley, 1991; Smith et al., 1998). Arsenic has an atomic number 33, atomic mass $74.9216 \text{ g mol}^{-1}$ and an electronic configuration of $4s^2 3d^{10} 4p^3$ (Smith *et al.*, 1998; Grafe and Sparks, 2006). Arsenic can show either electro-positive or electro-negative valence in its compound. The valences of arsenic are 0, -3, +3 and +5. Under aerobic conditions arsenic exists as As (+5) where as reducing environment is congenial for existence of elemental Arsenic (0), Arsenite (+3) and Arsine (-3). Arsenical, both trivalent and pentavalent, are soluble over a wide pH range and are found routinely in surface as well as in ground water. Elemental arsenic is crystalline and exists in yellow, black, or grey allotropic forms (grey being most stable). The oxidation states and electron orbitals are similar between arsenic and phosphate (Tamaki and Frankenberger, 1992).

Arsenic found in environment are classified into three major groups namely inorganic arsenic compounds, organic arsenic compounds, and arsine gases. Arsenic is widely dispersed in rocks, soils, waters, air, plants, and animals (Cullen and Reimer, 1989). Although arsenic is found in the environment to a small extent in its elemental form, it occurs mostly in inorganic and organic compounds.

Inorganic arsenic is usually found to combine with other elements such as cobalt (CoAs_2), nickel (NiAs), iron (FeAs_2). However, arsenic is found concentrated in magmatic sulphides and iron ores, leading to the fact that most important ores of arsenic are arsenopyrites or mispickel (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3) (Nriagu, 1994). Organic form of arsenic can be encountered attributes to the biological methylation of inorganic arsenic by microorganism (biotransformation). Arsenic is found as methylated forms such as monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), trimethylarsine oxide (TMAO). These species are well known to be found in living organisms (Nriagu, 1994).

The primary sources of arsenic include natural processes, such as weathering of rocks, volcanic emissions and discharge from hot springs, and various anthropogenic activities like mining, smelting, and the use of As-containing pesticides, herbicides, wood preservatives, and feed additives. These contribute to the regional background base level of arsenic and to abnormal geochemical arsenic conditions in some local areas. Historically, the use of arsenic-based pesticides has led to considerable contamination of domestic and agricultural land, through their use as lawn herbicides, and insecticides for rice, orchards and cotton (Woolson et al., 1971).

2.2 Causes of arsenic contamination in Bangladesh

Presently, two hypotheses are prevailing to describe the cause (mobilization) of arsenic into groundwater. These are: i) Pyrite Oxidation and ii) Oxy-hydroxide Reduction.

2.2.1 Pyrite oxidation hypothesis

Arsenic is assumed to be present in certain sulphide minerals (pyrites) that are deposited within the aquifer sediments. Due to the lowering of water table below deposits, the newly-introduced O_2 oxidizes the arsenopyrite in the vadose zone and releases arsenic and arsenic is adsorbed on iron hydroxide. During the subsequent recharge period, iron hydroxide releases arsenic into groundwater. According to this hypothesis, groundwater contamination with

arsenic is man-made, which has a relationship with excessive groundwater withdrawal. The pyrite oxidation hypothesis came from West Bengal, a state of India bordering to Bangladesh that has similar geological and environmental factors. West Bengal has also a serious arsenic problem. In West Bengal, it is believed by several authors that the source of arsenic in groundwater is geological and the cause of contamination is pyrite oxidation. The intensive irrigation development in the country supports the pyrite oxidation hypothesis. Irrigation development in Bangladesh, using DTWs and STWs, started in the early 1960s and rapidly expanded in the early 1980s. The contribution of groundwater to total irrigated area increased from 41 percent in 1982/1983 to over 75 percent in 2004/05 with an increasing tendency in each year, while the contribution of surface water steadily declined from 59 to <25 percent over the same period (Rashid, 1997 and Rashid and Islam, 2006).

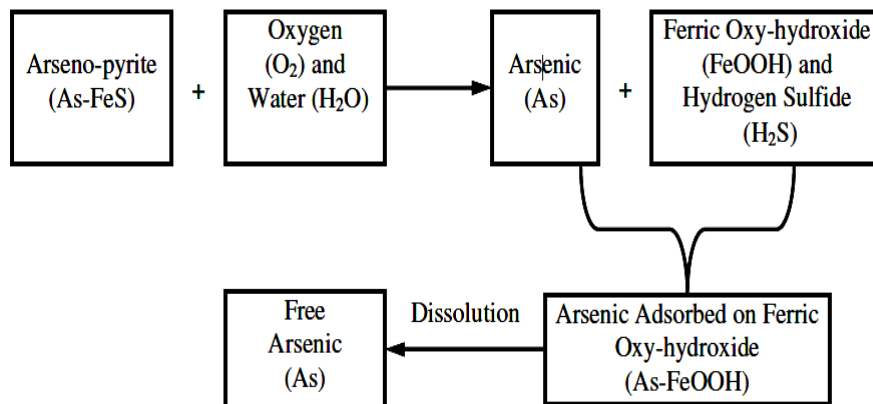


Fig. 2.1 Arsenic release according to pyrite oxidation hypothesis (Adapted from Safiuddin and Karim, 2001).

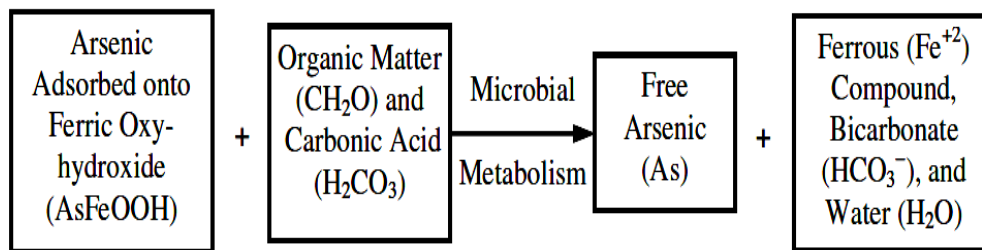


Fig. 2.2 Arsenic release according to hydroxide reduction hypothesis (Adapted from Safiuddin and Karim, 2001).

2.2.2 Oxy-hydroxide reduction hypothesis

Arsenic is assumed to be present in alluvial sediments with high concentrations in sand grains as a coating of iron hydroxide. The sediments were deposited in valleys eroded in the delta when the stream base level was lowered due to the drop in sea level during the last glacial advance. The organic matter deposited with the sediments reduces the arsenic-bearing iron hydroxide and releases arsenic into groundwater. According to this hypothesis, the origin of arsenic-rich groundwater is due to a natural process, and it seems that the arsenic in groundwater has been present for thousands of years without being flushed from the delta. This hypothesis was first proposed by Nickson et al., (1998). BGS accepted this hypothesis and considered that groundwater arsenic contamination has no relation with the excessive groundwater withdrawal.

2.3 Arsenic in the environment and human exposure

2.3.1 Arsenic in air

Arsenic is released to atmosphere from both natural and anthropogenic sources. The principal natural source is volcanoes. Man-made emission to air arises from the smelting of metals, the combustion of fuels, especially of low-grade brown coal, and the use of pesticides. Arsenic in air is present mainly in particulate forms as inorganic arsenic like arsenic trioxide (As_2O_3) (Leonard, 1991). Moreover, recent studies has shown the presence of arsine (AsO_3) and methylated arsenic (MeAsH_2 , Me_2AsH , and Me_3As) as trace component of the air sample, particularly over sites of higher biological activity (Feldmann and Grumping, 1994; Feldmann and Hirner, 1995; Hirner et al., 1998). Particulate arsenic compounds may be inhaled, deposited in the respiratory tract and absorbed into blood. Inhalation of arsenic from ambient air is usually a minor exposure route for the general population.

2.3.2 Arsenic in water

Arsenic is mainly transported in the environment by water. Arsenic is found at low concentration in natural water. The unpolluted fresh water has a level of arsenic ranging from 1-10 $\mu\text{g L}^{-1}$, rising to 100-5000 $\mu\text{g L}^{-1}$ in areas of sulphide mineralization and mining (Mandal and Suzuki, 2002). The World Health Organization (WHO, 1981) recommends that the As concentration in drinking water should not exceed 10 $\mu\text{g L}^{-1}$. However, the permissible limit of As in drinking water in many countries, including Bangladesh and the United States, is up to 50 $\mu\text{g L}^{-1}$. Higher levels of arsenic are found in groundwater sources than in surface-water sources. Arsenic in groundwater is mainly inorganic with arsenate comprising about 50% of the total (Abedin et al., 2002b).

Arsenic contamination in the groundwater has been reported from many countries, with the most severe problems occurring in Asia, namely Bangladesh (Dhar et al., 1997; Biswas et al., 1998; Chowdhury et al., 1999), West Bengal, India (Mandal et al., 1997), China (Huang et al., 1992; Liangfang and Jianghong, 1994), Vietnam (Winkel et al., 2011) and Taiwan (Chen et al., 1995). In the United States, arsenic-contaminated groundwater has been reported in New England (Peters et al., 1999), the Mid-west (Welch et al., 2000), Oklahoma (Schlottmann and Breit, 1992), Nevada (Welch and Lico, 1998) and California (Wilkie and Hering, 1998). In South East Asia, drinking (ground) water is mainly contaminated by geogenic arsenic. As a result, millions of people have been exposed to As placing their health at risk. It has been reported that approximately 42 million people are exposed to As containing potable water with a concentration of more than 50 $\mu\text{g L}^{-1}$ and more than 100 million people worldwide are affected by As contaminated water with a concentration of more than 10 $\mu\text{g L}^{-1}$.

Groundwater in Bangladesh is currently contaminated by up to 2 mg/L As with reports of widespread arsenic-related health effects on millions of people (Abedin et al., 2002b). This

problem is at its worst in the Bengal Delta region (encompassing Bangladesh and West Bengal, India), where tube-wells were installed to provide “safe” drinking water free from microorganisms causing gastrointestinal diseases, without prior knowledge of As contamination. The geochemical and hydrological conditions causing this As contamination are still being debated, but it is clear that elevated As concentrations are linked to the reducing environment developed in Holocene alluvial and deltaic deposits (BGS/DPHE, 2001). Use of this contaminated water for irrigation to crops has led to elevated concentrations of arsenic in agricultural soils.

2.3.3 Arsenic in soil

The global average concentration of As in soil is about 5 mgkg^{-1} . Uncontaminated soils typically contain $<10 \text{ mgkg}^{-1}$ total As, but the concentration can reach hundreds or thousands of mgkg^{-1} in contaminated environments (Kabata-Pendias and Pendias, 1992). Anthropogenic sources of As have elevated the background concentration of As in soils (Adriano, 2001). For example, in areas near As mineral deposits, As levels in soils may reach up to 9300 mgkg^{-1} (Ashley and Lottermoser, 1999). Depending on the nature of the geogenic and anthropogenic sources, As concentration in soils can range from <1 to $250,000 \text{ mg kg}^{-1}$. Due to soil parent material, a large fluctuation was found among countries; for example, calcareous soils can be expected to have higher levels of As than noncalcareous soils (Aichberger and Hofe, 1989). Although the dominant source of As in soils is geological, additional inputs may also be derived locally from industrial sources, such as smelting and fossil fuel combustion products and agricultural sources, namely pesticides and phosphatic fertilizers.

The soluble As concentration in soil is largely determined by redox conditions, pH, biological activity, and adsorption reactions. The adsorption and mobility of As in soil are affected more

strongly by the presence of H_2PO_2^- ion than any other anions. Arsenic is subject to both chemical and biological transformations in soils, resulting in the formation of various species. Marin et al., (1992) reported that anthropogenic activities, such as pesticide accumulation and herbicide applications, mining and irrigation with the contaminated groundwater have significantly enhanced As levels in agricultural soils in many parts of the world.

Meharg and Rahman (2003) and Loeppert et al., (2005) stated that the natural soil-As concentrations are generally less than 10 mg kg^{-1} in the surface soils of Bangladesh, but the concentrations can be significantly higher and sometimes greater than 40 mg kg^{-1} in soils that have been intensively irrigated with the As contaminated water.

Dittmar et al., (2005) reported that the highest concentration of As in soil receiving irrigation from a shallow tube well ($0.077 \text{ mg As L}^{-1}$). At this site, the sub-surface soil contained about 3 mg kg^{-1} As. Arsenic concentration in the topsoil of a paddy field has been reported to be approaching 100 mg kg^{-1} . These indicate that the As added to the soil through irrigation is mostly concentrated in the top 0-150 mm layer. This layer corresponds to the main root zone depth for most cultivated crops.

2.3.4 Arsenic in plants

Arsenic is detected in low concentrations ($< 1.5 \text{ } \mu\text{g kg}^{-1}$) in plants grown in the uncontaminated soils (McLaren et al., 2006). Several studies on the arsenic contamination in crop plants reveal an alarming situation in Bangladesh. A very extensive study conducted by Ullah et al., (2009) reported the extent and severity of arsenic poisoning in the food chain. They examined arsenic contents in soil and plant samples collected from arsenic contaminated areas under 11 districts of Bangladesh. They selected 40 plant species with majority of crop plants from these sites. Their results showed that vegetables such as amaranth, red amaranth, arum, coriander, and spinach as well as rice and wheat were found to contain arsenic in the edible parts above the upper permissible limit.

Das et al., (2004) conducted a survey among different crop plants including rice and vegetables on the contamination of arsenic. Though they did not find arsenic concentration in rice grain samples above the upper permissible limit of 1.0 mgkg^{-1} but they found that roots had a significantly higher concentration of arsenic (2.4 mgkg^{-1}) compared to stem (0.73 mgkg^{-1}) and rice grains (0.14 mgkg^{-1}).

Meharg and Rahman (2003) reported rice grain As contents ranging between 0.058 and 1.835 mgkg^{-1} in 13 rice varieties tested from Bangladesh. The comparative levels were 0.20– 0.46 mgkg^{-1} in the US and $0.063\text{-}0.20 \text{ mgkg}^{-1}$ in Taiwan varieties.

Rice has been studied substantially for the determination of their level of contamination. A number of studies analyzed together 788 rice samples (USAID, 2003; Duxbury et al., 2003; Hironaka and Ahmad, 2003; Meharg and Rahman, 2003; Shah et al., 2004). Their studies revealed that daily arsenic intakes from 84% of the Bangladeshi rice samples would exceed daily arsenic intake from water at the US and European drinking water standard of 10 ppb. This estimated data of arsenic consumption from only rice indicate that arsenic from rice alone is an important source of exposure in the Bangladeshi food system.

2.4 Arsenic chemistry in the rice rhizosphere

Arsenic chemistry in the rhizosphere is complex and is controlled by several factors (Fitz and Wenzel, 2002). Under paddy field conditions, inorganic As is inter-converted between the reduced inorganic species arsenite (As(III)) and the oxidized species arsenate (As(V)) (Marin, 1993). Soil microbes can also methylate inorganic As to produce monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMA) (Turpeinen et al., 1999). In roots, oxygen transported within the root aerenchyma is consumed by adjacent tissue cells, or diffused towards the root apex or the rhizosphere; the transfer of oxygen from aerenchyma to the rhizosphere is termed radial oxygen loss (ROL) (Colmer, 2003a). ROL can oxidize rhizosphere soil elements (e.g. Fe^{2+} to Fe^{3+}) and cause precipitation of toxic metals in

rhizosphere soil and on root surfaces (Otte et al., 1989; Smolders and Roelofs, 1996), subsequently altering rhizosphere metal mobility. Rice plants develop aerenchyma to transfer O_2 from the aerial parts of the plant to the roots, resulting in the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), and the precipitation of Fe oxides or hydroxides (Fe-plaque) on the root surfaces (Chen et al., 1995). Iron plaque can sequester metals on wetland plant roots (Hansel et al., 2001; Blute et al., 2004) and prevent translocation of As from roots to shoots (Liu et al., 2005, 2007). Therefore rhizosphere interactions play a key role in controlling As bioavailability to crop plants (Fitz and Wenzel, 2002).

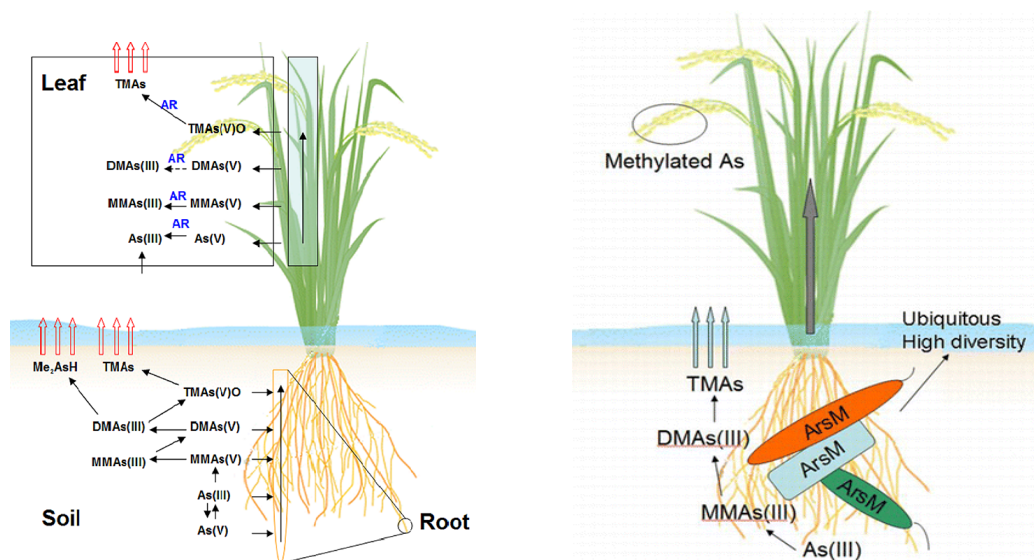


Fig. 2.3 Arsenic speciation rice rhizosphere under paddy field condition in presence of bacteria in rhizosphere

2.5 Arsenic transport and metabolism

Arsenic is taken up by plants inadvertently through the pathways of essential or beneficial nutrients and detoxified via a variety of mechanisms. Along its route from soil to plants, As interacts with a number of elements, most noticeably with iron (Fe), phosphorus (P), silicon (Si), and sulphur (S). Zhao et al., (2009) reviewed the pathways of As uptake and metabolism in plants as shown in Fig. 2.4.

2.5.1 Arsenic transport

The most common forms of As in soil solution available for plant uptake are arsenate, arsenite, MMA (Monomethylarsonic acid), and DMA (Dimethylarsenic acid). Their uptake mechanisms are described below:

2.5.1.1 Uptake of arsenate

With dissociation constants (pK_a) of 2.2, 6.97, and 11.5, most arsenic acid (H_3AsO_4) is dissociated as the oxyanions $H_2AsO_4^-$ or $HAsO_4^{2-}$ under normal pH conditions (pH 4-8), and they are the chemical analogs of corresponding phosphate ions.

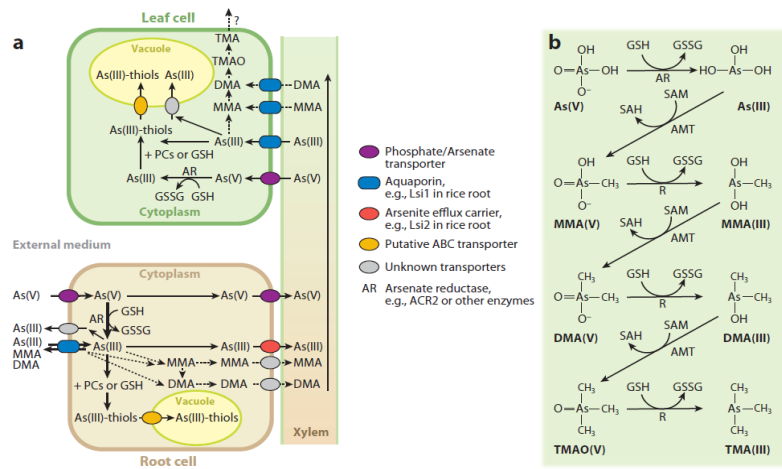


Fig. 2.4 Arsenic uptake and metabolism in plants. (a) A simplified schematic diagram of arsenic transport and metabolism in plants. The thickness of arrow lines is indicative of the relative flux. (b) The Challenger pathway of arsenic methylation in microorganisms. AR: arsenate reductase; R: reductase; AMT: arsenic methyltransferase; GSH, reduced glutathione; GSSG, oxidized glutathione; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine (Zhao et al., 2009).

Arsenate is taken up by plant roots via phosphate transporters. Physiological and electrophysiological studies proved that a potent inhibition of phosphate on arsenate uptake (Abedin et al., 2002c; Asher and Reay, 1979; Ullrich-Eberius et al., 1989) and recent reports showed that *Arabidopsis thaliana* mutants defective in phosphate transport are more tolerant to arsenate (Catarecha et al., 2007; Gonzalez et al., 2005; Wang et al., 2002).

2.5.1.2 Uptake of arsenite

In contrast to arsenate, arsenous acid (H_3AsO_3 , $\text{p}K_a = 9.2, 12.1, \text{ and } 13.4$) is mostly undissociated at normal pH conditions (>94% undissociated at $\text{pH} < 8.0$). Therefore, plant roots take up arsenite mainly as the neutral molecule $\text{As}(\text{OH})_3$. Arsenic (As) in microorganisms and mammalian tissues (Bhattacharjee and Rosen, 2007), arsenite enters plant root cells via some aquaglyceroporin channels. In higher plants, the nodulin 26-like intrinsic proteins (NIPs) are the structural and functional equivalents of the microbial and mammalian aquaglyceroporins (Wallace et al., 2006). NIPs are a subfamily of the plant major intrinsic proteins (MIPs), collectively known as aquaporins or water channels.

In rice roots, *Lsi1* (OsNIP2;1), which is highly expressed in the distal side of the plasma membranes of the exodermis and endodermis cells where *Casparian* strips are formed, is a major entry route for silicic acid (Ma et al., 2011b) and arsenite (Ma et al., 2011a); mutation in this protein resulted in a 60% loss of the arsenite influx in the short term. However, the effect of *Lsi1* mutation on As accumulation in rice shoots is relatively small over a longer growth period (Ma et al., 2011a). *Lsi2* is localized to the proximal side of the plasma membranes of the exodermis and endodermis cells, allowing solute efflux toward the stele for xylem loading (Ma et al., 2011). This process is a crucial step in the accumulation of As in rice shoot and grain; it is also the step in which Si exerts a strong inhibitory effect. Transport of Si mediated by *Lsi2* is an active process driven by the proton gradient (Ma et al., 2011). *Lsi2* has a low degree of homology (18%) with the arsenite efflux transporter ArsB in *Escherichia coli* (Ma et al., 2011).

2.5.1.3 Uptake of methylated arsenic:

MMA and DMA have lower dissociation constants than arsenite ($\text{p}K_a = 4.2$ and 6.1 , respectively). The permeability of MMA and DMA across liposomes was estimated to be 1.4

$\times 10^{-13}$ and 4.5×10^{-11} cms^{-1} , respectively (Cullen and Reimer, 1992). Undissociated pentavalent MMA and DMA are also taken up by rice aquaporin *Lsi1* (Li et al., 2009a); the uptake capacity for MMA and DMA through the rice *Lsi1* mutant was observed to decreased by 80% and 50%, respectively, compared with the wild-type rice. At pH 5.5, uptake by rice roots decreases in the order of arsenite > MMA > DMA (Abedin et al., 2002c). The substrate properties that may explain this order are: (a) the extent of dissociation within the normal pH range, and (b) the number of hydroxyl groups; formation of the hydrogen bonds between the hydroxyl group of a substrate and the aquaporin protein along the pore structure greatly facilitates the flux through the channel (Wu and Wong, 2007). In contrast to arsenite, the rice *Lsi2* is not involved in the efflux of MMA or DMA, possibly because most MMA and DMA are dissociated at the cytoplasmic pH (Li et al., 2009a). Despite its limited uptake (Abedin et al., 2002c and Raab et al., 2007), for unknown reasons, DMA is more efficiently translocated from roots to shoots (Li et al., 2009; Marin et al., 1992 and Raab et al., 2007).

2.6 Arsenic metabolism

Two aspects of As metabolism are discussed here:

2.6.1 Arsenate reduction:

The dominance of trivalent As in plant tissues is found when arsenic is supplied to the plants in the form of arsenate (Dhankher et al., 2002, Pickering et al., 2000, Xu et al., 2007, Zhao et al., 2009) indicates a higher capacity of arsenate reduction. Both roots and shoots of rice exhibit arsenate reduction activities (Duan et al., 2007), but roots may be quantitatively more important because arsenite is the main form found in the xylem sap of a number of plant species (Zhao et al., 2009). The plant homologues of the yeast arsenate reductase *Acr2p* have recently been isolated from *A. thaliana* (Dhankher et al., 2006), *Holcus lanatus* (Bleeker et al., 2006), rice (Duan et al., 2007), and *P. Vittata* (Ellis et al., 2006). The plant *ACR2* proteins are CDC25- like (cell division cycle) tyrosine phosphatases that have both phosphatase and

arsenate reductase activities; PvACR2 from *Pteris vittata* appears to be an exception with only the activity of an arsenate reductase (Ellis et al., 2006). Purified recombinant proteins of plant *ACR2s* are able to reduce arsenate in vitro using GSH and glutaredoxin as reductants. However, the role of *ACR2* remains unresolved, since there are conflicting reports on the phenotype of the *ACR2* knockout or knockdown lines of *A. thaliana* with regard to arsenate tolerance and As translocation from roots to shoots in plant (Bleeker et al., 2006; Dhankher et al., 2006). Furthermore, the As speciation in the *Arabidopsis ACR2* knockout mutants is still dominated by As (III) (Zhao et al., 2009), suggesting a functional redundancy of *ACR2*. The possible existence of other arsenate reductases or nonenzymatic reduction mechanisms warrants further investigation.

2.6.2 Arsenic methylation:

Arsenic is metabolised from inorganic to organic forms by a wide range of organisms, with limited evidence that this occurs in plants (Nissen and Benson, 1982). In a range of organisms, metabolism typically occurs through biomethylation to give MMA, DMA, tetramethylarsonium ions (TETRA) and trimethylarsonium oxide (TMAO) (Cullen and Reimer, 1989). A number of organisms have also been found in some terrestrial plants that metabolise inorganic arsenic to arsenocholine, arsenobetaine and arseno-sugars (Tamaki and Frankenberger, 1992 and Kuehnelt et al., 2000). Gosio (1897), as cited in Cullen and Reimer, (1989) was the first to establish that fungi could generate methylated As. Challenger proposed a scheme in which As(+5) was eventually transformed to TMAs (Challenger, 1951). In this scheme, As (+5) is first reduced to As (+3) then methylated, and each methylation step results in the reoxidation of the As, thus requiring a reductive step to As (+3) prior to further methylation as shown in the equation (Cullen, 1989).

2.7 Arsenic toxicity and detoxification mechanism in plants

2.7.1 Arsenic toxicity in plants

Phytotoxicity of arsenic species is affected considerably by the chemical form in which it occurs in the soil and concentration of the metalloid. Production of reactive oxygen species (ROS) like superoxide anions, singlet oxygen, hydroxyl radicals and hydrogen peroxide by plants exposed to environmental stresses causes damage to DNA, proteins and lipids (Singh et al., 2006). Plants exposed to either arsenate or arsenite produce ROS (Srivastava et al., 2007). The primary cause of As^{3+} toxicity is its interference with a variety of enzymes because it has high affinity to sulfhydryl groups found on many enzymes, whereas being a phosphate analogue, As^{5+} can substitute inorganic phosphate in a plethora of biochemical processes, thus affecting key metabolic processes in the cell. Arsenate and arsenite are also toxic to plants by disturbing central cellular functions. Arsenate is transported across the plasma membrane via phosphate co-transport systems (Ullrich-Eberius et al., 1989). Once arsenate enters inside the cytoplasm it competes with phosphate, for example replacing phosphate in ATP to form unstable ADP-As, generates reactive oxygen species (ROS) in plant tissues, induces oxidative stress such as lipid peroxidation (Ahsan et al., 2008; Mylona et al., 1998 and Hartley-Whitaker et al., 2001) and leads to the disruption of energy flows in cells (Meharg, 1994).

The primary impact of arsenic toxicity on plants is a reduction in growth (Kabata-Pendias and Pendias, 1991). The thorough review by Punz and Sieghardt (1993) outlined the important responses of plant roots to heavy metals, which can be applied to arsenic. Other responses of heavy metal: changes in root biomass - typically a reduction in weight; changes in the root system architecture increased lateral root growth leading to a compacted system; changes in growth rate; inhibition of root elongation-largely due to a disruption of cell division and mitosis. They also noted other important morphological changes, including root

discolouration, decreased root hair density, vessel diameter, and vessel number and structural changes to hypodermis, endodermis, and pericycle. Further physiological responses include damage to root cell membrane, decreased water permeability of the plasmalemma, decreased turgor or plasmolysis, reduced root respiration, reduced water uptake, and increased water flow resistance (Punz and Seighardt, 1993). At higher concentration, arsenic is toxic to most plants. It interferes with metabolic processes and inhibits plant growth and development through arsenic induced phytotoxicity (Marin et al., 1993b). Smith et al. (1998) reported that rice, bean, oats can suffer from phytotoxicity at a soil concentration of 20 mgkg^{-1} As, whereas for maize and radish this is 100 mgkg^{-1} As. Certainly, the reduction of rice plant growth, in terms of tillering, plant height, root length, shoot and root biomass production, was the ultimate result of arsenic phytotoxicity at high soil arsenic concentrations (Jahan et al., 2003; Rahman et al., 2004; Xie and Huang, 1998) though the phytotoxicity at lower soil arsenic concentrations was not significant. High As concentrations in soils can reduce crop yield, since it inhibits plant growth and under stringent conditions may be lethal to the plant (Nriagu et al., 2007).

2.7.2 Arsenic detoxification mechanism

For non-hyperaccumulator plants, As toxicity often occurs at a shoot As concentration varying between 1 and 100 mgkg^{-1} (Kabata-Pendias and Pendias, 1992), whereas the As hyperaccumulator *P. Vittata* can withstand $5000\text{--}10,000 \text{ mgkg}^{-1}$ of As in the frond tissue without suffering from toxicity (Lombi et al., 2002 and Tu and Ma, 2002). In general, a high level of tolerance to heavy metals could rely on either reduced uptake or increased plant internal sequestration, which is manifested by an interaction between a genotype and its environment (Macnair et al., 2000 and Hall, 2002). A variety of tolerance and resistance mechanisms including avoidance or exclusion, which minimizes the cellular accumulation of metals, and tolerance, which allows plants to survive while accumulating high concentrations

of metals, have been identified in plants. These include: (i) decreased uptake of As^{5+} due to suppression of the high-affinity phosphate uptake system; (ii) binding to the cell wall; (iii) detoxification of As^{5+} by reduction to As^{3+} , which subsequently complexes with thiols, particularly phytochelatins (PCs), metallothioneins, and metal-binding proteins and remains sequestered in roots; (iv) rapid efflux of As^{5+} and As^{3+} from plant roots back to the medium; (v) repair of stress-damaged proteins; and (vi) the compartmentation of metals in the vacuole by tonoplast-located transporters (Hall, 2002). Arsenic hyperaccumulator plants take up and sequester exceptionally high concentrations of arsenic in the aboveground parts and hence offer a great promise to phytoremediation of arsenic. Tolerance in these plants appears to involve increased As^{5+} uptake, decreased As^{3+} -thiol complexation and As^{3+} efflux to the external medium, greatly enhanced xylem translocation of As^{3+} , and vacuolar sequestration of As^{3+} in fronds.

2.8 Arsenic concentration in rice straw and grain

Arsenic contamination in the Bangladeshi staple food, rice, showed presence of high levels of arsenic 1700 mg kg^{-1} (Meharg and Mazibur, 2003). A greenhouse study was conducted to examine the effects of arsenic-contaminated irrigation water on the growth of rice and uptake and speciation of arsenic. Increasing the concentration of arsenate in irrigation water significantly decreased plant height, grain yield, the number of filled grains, grain weight, and root biomass, while the arsenic concentrations in root, straw, and rice husk increased significantly. Concentrations of arsenic in rice grain did not exceed the food hygiene concentration limit ($1.0 \text{ mg of As kg}^{-1}$ dry weight). The concentrations of arsenic in rice straw (up to 91.8 mg kg^{-1} for the highest As treatment) were of the same order of magnitude as root arsenic concentrations (up to 107.5 mg kg^{-1}), suggesting that arsenic can be readily translocated to the shoot (Abedin et al., 2002).

Das et al., (2004) determined the level of contamination in 100 samples of crop, vegetables and fresh water fish collected from three different regions in Bangladesh. They reported that, no samples of rice grain had arsenic concentrations more than the recommended limit of 1.0 mgkg⁻¹. Rice plants, especially the roots had a significantly higher concentration of arsenic (2.4 mgkg⁻¹) compared to stem (0.73 mgkg⁻¹) and rice grains (0.14 mgkg⁻¹).

Abedin et al. (2002a) reported that significant increase of arsenic concentration in rice root, straw and husk with the increase of arsenate concentration in irrigation water. He found 3.9 mgkg⁻¹ arsenic in straw at the lowest arsenate treatment (0.2 mgL⁻¹), which increased progressively with increasing arsenate application and reached to 91.8 mgkg⁻¹ in the highest arsenate treatment (8.0 mgL⁻¹).

Jahiruddin et al., (2004) spiked silt loam soil with As. First, a Boro rice cultivar developed by the Bangladesh Rice Research Institute "BRRI dhan29" and then an Aman cultivar "BRRI dhan3" were grown. For Boro rice, the first significant effects occurred at 10 mg kg⁻¹ soil, causing a grain yield reduction of more than 45 percent.

Abedin et al. (2002) and Meharg and Rahman (2003) have reported about the rice samples with As accumulation much above the WHO recommended permissible level (1.0 mgkg⁻¹). Meharg and Rahman (2003) reported that Bangladesh rice grain accumulate As up to 2.0 mgkg⁻¹.

Delowar et al., (2005) reported the accumulation of As in rice grain in the range 0.0-0.14 mgkg⁻¹ which was cultivated with 0.0-20.0 mgL⁻¹ As contaminated water. Arsenic accumulation in rice straw at very high levels indicates that the cattle populations are in direct threat for their health and also, indirectly, human population, via consuming contaminated bovine meat and milk (Abedin et al., 2002).

Delowar et al., (2005) and Williams et al., (2006) noticed that some of the rice varieties (IR 50, White Minikit, Red Minikit) are high accumulator of As (0.24, 0.009-0.31, 0.005 mgkg⁻¹) with respect to varieties (Nayanmani, Jaya, Ratna, Ganga-kaveri, Lal Sanna) with low accumulation(0.14, 0.002-0.20, 0.005 mgkg⁻¹).

Williams et al., (2006) reported that daily consumption of rice with a total As level of 0.08 mg kg⁻¹ would be equivalent to drinking contaminated water with As level of 0.01 mg/L. Arsenic uptake and accumulation in different rice parts found in the order roots > stem > leaf> grain (Abedin et al., 2002). 2400µgkg⁻¹ As in roots, 730 µgkg⁻¹ in stems and leaves, and 140µgkg⁻¹ in grain (Das et al., 2004 and Islam et al., 2004).

Rice grain has been reported to accumulate arsenic upto 2.0 mgkg⁻¹ by Meharg et al., (2003), Islam et al., (2004) and Delowar et al. (2005), which is much above the permissible limit in rice (1.0 mgkg) according to WHO recommendation.

Rahman et al., (2007), Abedin et al., (2002) and Das et al., (2004) reported that the concentration of arsenic in the grain of all the studied rice samples was found to be between 0.25±0.014 to 0.73±0.009 mgkg⁻¹ dry weight of arsenic, which did not exceed the permissible limit in rice (1.0 mgkg⁻¹) according to WHO recommendation.

Rahman et al., (2007a,b) conducted glasshouse and field-level experiments to investigate the concentrations of arsenic in different parts of rice plant and found that arsenic concentration in grain (0.2–0.7 mgkg⁻¹ dry weight), straw (0.9–23.7 mgkg⁻¹ dry weight), husk (0.2–1.6 mgkg⁻¹ dry weight), and root (46.3–51.9 mgkg⁻¹ dry weight).

Rahman et al., (2007a) and Tsutsumi et al., (1980) reported that 23.7 and 149 mg of As kg⁻¹ dry weight, respectively was found in rice straw when the soil arsenic concentrations were 60 and 313 mgkg⁻¹ dry weight, separately.

Abedin et al., (2002) also found 25 mg of As kg⁻¹ dry weight in rice straw when the rice was irrigated by 2 mg of As l⁻¹ water. The study revealed that the highest level of arsenic in root at 40.3 ± 57 mgkg⁻¹ dry weight As (n = 4) was located at the RS1 field with the arsenic content of 43.8 mgkg⁻¹ dry weight in topsoil.

Das et al., (2004) also stated that the highest concentrations of arsenic in rice grain (0.27 mgkg⁻¹), shoot (1.58 mgkg⁻¹), root (9.71 mgkg⁻¹), and soil (27.28 mgkg⁻¹) were found in samples collected from the irrigated fields of Kachua Upazila of Chandpur in Bangladesh.

Patel et al., (2016) found that the concentration of As in the rice grain, husk, straw and root (n = 20) was ranged from 0.17 - 0.72, 0.40 - 1.58, 2.5 - 5.9 and 204 - 354 mgkg⁻¹ with mean value of 0.47 ± 0.07, 0.83 ± 0.15, 4.2 ± 0.5 and 276 ± 21 mgkg⁻¹, respectively.

2.9 Plant responses to arsenic toxicity

2.9.1 Effect of arsenic in seed germination of rice

Marked decreases in germination percentage and shoot and root elongation were recorded with As treatments in rice seedlings. The germination of rice seed decreased significantly with the higher concentrations of As in water and the adverse effect was different among the varieties.

Abedin et al., (2002) reported that plant height ranged between 91.1 and 84.1 cm with lower ranges of arsenate doses (0-1.0 mg As L⁻¹), while with higher arsenate doses (2.0 - 8.0 mg of As L⁻¹) plant height decreased to 79.2 - 63.8 cm. Leaf number plant⁻¹ was less affected by As in water. Similar to germination of seeds, the adverse effect of As on seedling height was different among the varieties.

Carbonell et al., (1997) suggested that root length decreased with increasing arsenic concentration but the present study showed no regular pattern of variation in root length in

Barisal soil series while in Sara series an increasing trend of root length was observed up to 5 ppm showed and decreased thereafter.

The germination decreased significantly with the increase in As concentration. Root length, shoot length, root and shoot fresh weight of rice seedlings were greatly inhibited at 50 mM As (III) and 500 mM As (V) (Shri et al., 2009).

Li et al., (2007) observed that germination performance of the plant was better in lower concentrations and with the increase of concentration germination was adversely affected. At an As concentration of 0.5 mgkg^{-1} the germination energy and germination index increased by 104.33% and 101.97%, respectively.

The vitality index increased by 6.56% at an As concentration of 1 mgkg^{-1} . Germination energy, germination percentage, germination index and vitality index reduced significantly in rice, by 9.60%, 4.04%, 10.73% and 46.61%, respectively, with higher As concentration of 20 mgkg^{-1} (Li et al., 2007).

2.10 Effect of arsenic in growth of rice plant

Rahman et al., (2012) reported germination percentage (GP), germination speed (GS) and vigor index (VI) of rice varieties decreased significantly ($p > 0.01$) with increasing As(V) concentrations. Abedin and Meharg, (2002) also studied germination on rice seeds and a short-term toxicity experiment with different concentrations of arsenite and arsenate on rice seedlings. Percent germination over control decreased significantly with increasing concentrations of arsenite and arsenate. Arsenite was found to be more toxic than arsenate for rice seed germination.

Shaibur et al., (2006), conducted an experiment to observe the effect of arsenic (As) on a number of physiological and mineralogical properties of rice (*Oryza sativa* L. cv. Akihikari) seedlings. Seedlings were treated with 0, 0.5, 1.0 and 2.0 mg As L^{-1} for 14 days in a

greenhouse. Shoot dry matter yield decreased by 23, 56 and 64%; however, the values for roots were 15, 35 and 42% for the 0.5, 1.0 and 2.0 mg As L⁻¹ As treatments, respectively. Shoot height decreased by 11, 35 and 43%, while that of the roots decreased by 6, 11 and 33%, respectively. These results indicated that the shoot was more sensitive to As than the root in rice. Leaf number and width of leaf blade also decreased with As toxicity. Arsenic toxicity induced chlorosis symptoms in the youngest leaves of rice seedlings by decreasing chlorophyll content.

Delowar et al., (2005) conducted a study aims at assessing the extent of accumulation of arsenic in rice plants and its effects on growth and yield of rice. Arsenic concentrations in paddy soils (irrigated with 0, 2.5, 5, 10, 15 and 20 mgL⁻¹ of arsenic water) were 0-0.2, 0-0.95 and 0-0.27 mg kg⁻¹ at tillering, heading and ripening stages. The growth and yield of rice plants were reduced significantly with increased doses of arsenic but the grain weight was not affected.

Abedin et al., (2002) found that increasing the concentration of arsenate in irrigation water significantly decreased plant height, grain yield, the number of filled grains, grain weight, and root biomass, while the arsenic concentrations in root, straw, and rice husk increased significantly.

Azad et al., (2009) conducted a pot-culture experiment in open-field conditions in Bangladesh with highly cultivated locally transplanted aman rice cv. BR22 in arsenic (As)-amended soil (0, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mgkg⁻¹) to determine the effect of As on the growth, yield of rice. They found arsenic affect the plant height, tiller and panicle numbers, grain and straw yield of T. aman rice significantly.

Panaullah et al., (2009) reported the growth of rice plant was restrained due to the toxicity of inorganic As (iAs). Haq et al., (2006) observed different symptoms in the rice plants due to

phytotoxicity of arsenic. The symptoms were: delayed seedling emergence; reduced plant growth; yellowing and wilting of leaves; and finally, reduced grain yield. Brown necrotic spots were also found on old leaves of the plants growing at 20 and 40 mg kg⁻¹ As treatment in both BRRI dhan28 and BRRI dhan29 rice varieties.

Islam and Jahiruddin (2010) found the grain yield of rice was reduced by 20.6 % for 15 mg kg⁻¹ As treatment and 63.8 % due to 30 mgkg⁻¹ As. Such reductions for straw yield were 21.0 and 65.2 % with these two As treatments, respectively.

Khan et al., (2010) reported arsenic addition in either irrigation water or as soil-applied As resulted in yield reductions from 21 to 74 % in *Boro* rice and 8 to 80 % in *T. Aman* rice, the latter indicating the strong residual effect of As on subsequent crops.

Hossain et al., (2005) assessed effect of arsenic on growth and yield of rice. They reported that the growth and yield of rice plants were reduced significantly with increased doses of arsenic but the grain weight 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 greatly with the higher dose (20 mgL⁻¹) of arsenic applied. Yield reduction of more than 60 and 40% for Iratom-24 and BRRI dhan28, was found with 20 mg L⁻¹ of arsenic as compared to control. The reduction in straw yield was also significantly higher for both of rice varieties with the 20 mgL⁻¹ arsenic application.

Abedin et al., (2002b) reported a reduction of 20% of crop (cereal) production from approximately 20 mgL⁻¹ of arsenic in the plant body. Arsenic concentration in irrigation water (0.1 to 2.0 mgL⁻¹) and soil (5 to 50 mgkg⁻¹) could result in lower yield of a local rice variety. Rice production is reported to decrease by 10% at a concentration of 25 mg kg⁻¹ As in soil (Xiong et al., 1987).

Marin et al., (1992) found significant reduction of rice shoot height when arsenite or monomethyl arsenic acid was applied at the relatively low dose of 0.8 mg As L⁻¹.

Abedin and Meharg (2002) reported that germination and early seedling growth of rice decreased significantly with increasing concentrations of As. The inhibition was stronger in the root than in the shoot when treated with As (Wang et al., 2002). When uptake of nutrition was inhibited in roots, the growth of the whole plant was constrained, and the plant biomass decreased finally (Mitchell and Barr, 1995). The reason is that plant roots were the first point of contact for these toxic arsenic species in the nutrient media (Abedin and Meharg, 2002).

2.11 Effect of arsenic in yield of rice

Abedin et al., (2002) also observed that tillers number was reduced significantly with increase of arsenic concentration in irrigation water up to 8 mgL⁻¹. Khan et al., (2010) also found that the addition of arsenic significantly reduced tillering in rice plant.

Abedin (2002) found that presence of arsenic as arsenate at a higher concentration in irrigation water significantly reduced the 1000 grain weight. Tsutsumi (1980) also reported that arsenic could reduce 1000 grain weight.

Hossain et al., (2009) reported that grain yield of rice was decreased as the level of arsenic addition was increased, and the yield was reduced drastically with the 30 mg Askg⁻¹ addition.

Arsenic application in rice significantly reduced yield and different yield-contributing parameters including the number of panicles per plant, panicle dry weight, the number of spikelets and full grains per plant and 1000-grain weight (Vromman et al., 2013).

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to April 2017 to study the effect of different doses of arsenic on yield of rice. The details of the materials and methods have been presented below:

3.1 Description of the experimental site

3.1.1 Location

The present piece of research work in pot was conducted in the front of Soil Science department of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the site is 23° 47' N latitude and 90° 35' E longitude with an elevation of 8.2 meter from sea level.

3.1.2 Soil

The soil of the experimental area that used in the pot for rice grown belongs to “The Modhupur Tract”, AEZ 28. Pot soil was silty clay in texture. Soil pH was 5.6 and has organic carbon 0.45%. The details of the pot soil have been presented in Appendix I.

3.1.3 Climate

The geographical location of the experimental site was under the subtropical climate, characterized by three distinct seasons, winter season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October (Edriset *al.*, 1979). Details of the meteorological data of air temperature, relative humidity, rainfall and sunshine hour during the period of the

experiment was collected from the Weather Station of Bangladesh, Sher-e Bangla Nagar, Dhaka and has been presented in Appendix II.

3.2 Experimental details

3.2.1 Treatments

The experiment comprised of two factors.

Factor A: Arsenic level (3 levels):

i. As0: Control (No arsenic)

ii. As15: 15 ppm As

iii. As30: 30 ppm As

Factor B: Phosphorus (P) doses (5 levels)

i. P1: Control (no phosphorus)

ii. P2: 20 ppm P

iii. P3: 40 ppm P

iv. P4: 60 ppm P, and

v. P5: 80 ppm

There were 15 treatments combinations. The treatment combinations were As0P0, As0P20, As0P40, As0P60, As0P80, As15P0, As15P20, As15P40, As15P60, As15P80, As30P0, As30P20, As30P40, As30P60 and As30P80.

3.2.2 Experimental design and layout

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. There were 45 pots for 15 treatment combination in each of 3 replications. The 15 treatment combinations of the experiment were assigned at random in 45 pots of each replication.

3.3 Growing of crops

3.3.1 Raising seedlings

3.3.1.1 Seed collection

The seeds of the test crop i.e. BRRI dhan29 is collected from Bangladesh Rice Research Institute (BRRI), Joydevpur, Gazipur.

3.3.1.2 Seed sprouting

Healthy seeds were selected by specific gravity method and then immersed in water bucket for 24 hours and then they were kept tightly in gunny bags. The seeds started sprouting after 48 hours and were sown after 72 hours.

3.3.1.3.Preparation of seedling nursery bed and seed sowing

According to BRRI recommendation seed bed was prepared with 1 m wide seed bed adding nutrients as per the requirements of soil. Seeds were sown in the seed bed on December 8, 2016, in order to transplant the seedlings in the pot as per experimental treatment.

3.3.2 Preparation of the pot

The pot for the experiment was filled up with soil at 2 January, 2017. Weeds and stubble were removed from the soil and finally obtained a desirable tilth of soil for transplanting of seedlings.

3.3.3 Fertilizers and manure application

The fertilizers N, P, K, S, Zn and B in the form of urea, TSP, MoP, gypsum, zinc sulphate and borax, respectively were applied. The entire amount of TSP, MoP, gypsum, zinc sulphate and borax were applied during the final preparation of pot land. Urea was applied in two equal installments at tillering and before panicle initiation.

Different concentration of As was mixed the soil as per treatment. The dose and method of application of fertilizers are shown in Table 1.

Table 1. Dose and method of application of fertilizers

| Fertilizers | Dose (kg/ha) | Application (%) | | |
|-------------|--------------|-----------------|-----------------------------|-----------------------------|
| | | Basal | 1 st installment | 2 nd installment |
| Urea | 150 | 33.33 | 33.33 | 33.33 |
| TSP | 0 | 0 | - | - |
| MP | 100 | 100 | - | - |
| Gypsum | 60 | 100 | - | - |
| Borax | 10 | 100 | - | - |

Source: Anon., 2010, BRRRI, Joydevpur, Gazipur

3.3.4 Uprooting of seedlings

The nursery bed was made wet by application of water one day before uprooting of the seedlings. The seedlings were uprooted on January 8, 2017 without causing much mechanical injury to the roots.

3.3.5 Transplanting of seedlings in the pots

The rice seedlings were transplanted in the pot at 9 January, 2017 and 2 healthy seedlings were transplanted in the pot in a hill.

3.3.6 After care

After establishment of seedlings, various intercultural operations were accomplished for better growth and development of the rice seedlings.

3.3.6.1 Irrigation and drainage

Sprinkler irrigation was provided to maintain a constant level of standing water up to 6 cm in the early stages to enhance tillering and 10-12 cm in the later stage to discourage late tillering. The pot was finally dried out at 15 days before harvesting.

3.3.6.2 Gap filling

First gap filling was done for all of the pots at 10 days after transplanting (DAT) by planting same aged seedlings.

3.3.6.3 Weeding

Weeding were done to keep the pots free from weeds, which ultimately ensured better growth and development. The newly emerged weeds were uprooted carefully at tillering stage and at panicle initiation stage by manual means.

3.3.6.4 Top dressing

After basal dose, the remaining doses of urea were top-dressed in 2 equal installments in the soil.

3.3.6.5 Plant protection

Furadan 57 EC was applied at the time of final land preparation and later on other insecticides were applied as and when necessary.

3.4 Harvesting, threshing and cleaning

The rice was harvested depending upon the maturity of plant and harvesting was done manually from each pot. The harvested crop of each pot was bundled separately, properly tagged and brought to threshing floor. Enough care was taken during harvesting, threshing and also cleaning of rice seed. Fresh weight of grain and straw were recorded pot wise. The grains were cleaned and finally the weight was adjusted to a moisture content of 13%. The straw was sun dried and the yields of grain and straw pot⁻¹ were recorded and converted to t/ ha.

3.5 Data recording

3.5.1 Plant height

The height of plant was recorded in centimeter (cm) at the time of harvest. The height was measured from the ground level to the tip of the tiller.

3.5.2 Number of tillers hill⁻¹

The number of tillers hill⁻¹ was recorded at the time of harvest by counting total tillers in a hill.

3.5.3 Total tillers hill-1 (at harvest)

The total number of total tillers hill⁻¹ was counted as the number of panicle bearing and nonbearing tillers hill⁻¹. Data on total tillers hill⁻¹ were counted at harvest and value was recorded.

3.5.4 Effective tillers hill⁻¹

The total number of effective tillers hill⁻¹ was counted as the number of panicle bearing tillers plant⁻¹. Data on effective tiller hill⁻¹ were counted and value was recorded.

3.5.5 Length of panicle

The length of panicle was measured with a meter scale from 5 selected panicles and the average value was recorded.

3.5.6 Number of panicle

The number of total panicle per pot were counted.

3.5.7 Filled grain hill⁻¹

The total number of filled grain per hill were counted manually.

3.5.8 Un-filled grain hill⁻¹

The total number of unfilled grain per hill were counted manually.

3.5.9 Filled grains panicle⁻¹

The total number of filled grains was collected randomly from selected 3 panicles of a pot on the basis of grain in the spikelet and then average number of filled grains panicle⁻¹ was recorded.

3.5.10 Unfilled grains panicle⁻¹

The total number of unfilled grains was collected randomly from the same 3 panicles where filled grains were counted of a pot on the basis of no grain in the spikelet and then average number of unfilled grains panicle⁻¹ was recorded.

3.5.11 Weight of 1000 seeds

One thousand seeds were counted randomly from the total cleaned harvested seeds of each individual pot and then weighed in grams and recorded.

3.5.12 Grain yield

Grains obtained from each unit pot were sun-dried and weighed carefully. The dry weight of grains of each pot was measured and grain yield pot⁻¹.

3.5.13 Straw yield

Straw obtained from each unit pot were sun-dried and weighed carefully. The dry weight of the straw of each pot was measured.

3.6 Statistical analysis

The data obtained for different characters were statistically analyzed using Statistix 10 software to observe the significant difference among the treatments. The mean values of all the characters were calculated and factorial analysis of variance was performed. The significance of the difference among the treatment means was estimated by the Least Significant Difference Test (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

Chapter III

RESULTS AND DISCUSSION

Results of the conducted experiment are presented in this chapter. The entitled experiment were conducted and described below in detailed. The different growth, yield and nutrients concentrations parameters have been presented in the tables and figures format.

3.1 Effect of arsenic (As) and phosphorus (p) on growth and yield of BRRI dhan29

3.1.1 Plant Height

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on plant height in rice variety BRRI dhan29 (Table 3.1). It was observed that plant height of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Plant Height was affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRI dhan29 (Table 1, Fig.3.1). The highest plant height (84.6) was found in As0 treated pot and lowest plant height (54.9) was found in As30 treated pot. On the other hand, the highest plant height (53.4) was found in P0 treated pot and lowest plant height (48.2) was found in P80 treated pot. In interaction effect, highest plant height was observed from As0P80 treatment (88.2) and lowest plant height was recorded from As30P80 treated pot (51.1) (Table 3.2). Furthermore, the reduction of plant height due to application of arsenic and phosphorus were also recorded (35.1% and 9.73%) (Fig. 3.1). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in effective tillers per pot, filled grains per panicle and 1000-grain weight; these together contributed reduced grain yield. Rice grain yield was reduced by 20.6% for 15 mgkg^{-1} As treatment and 63.8% due to 30 mgkg^{-1} As. Plant height might be reduced due to increase in arsenic concentration in soil. Abedin *et al.*, (2002a) reported that As affect the root development and reduced the plant height of rice. Similar findings was also reported by some other researchers and they have also reported that plant roots are unable to accumulate the essential nutrients from soil in presence of excess arsenic because As (III) reacts with sulfhydryl groups of proteins (Speer, 1973)

causing disruption of root functions of plants (Orwick *et al.*, 1976). These results are in agreement with previous researches such as Biswas *et al.*, (2009), Abedin *et al.*, (2002) and Azad *et al.*, (2009).

Table 3.1 Effects of added arsenic (As) and phosphorus (P) on growth and yield parameters of BRRi dhan29

| Treatment (As or P) | Plant Height (cm) | Effective tillers pot ⁻¹ | Filled grains panicle ⁻¹ | 1000-grain weight (g) | Grain yield (g pot ⁻¹) | Straw yield (g pot ⁻¹) |
|---------------------|-------------------|-------------------------------------|-------------------------------------|-----------------------|------------------------------------|------------------------------------|
| As (ppm) | | | | | | |
| 0 | 84.6a | 33.60 ^a | 96.72 ^a | 24.53 ^a | 74.17 ^a | 86.00 ^a |
| 15 | 69.7b | 31.33 ^b | 92.14 ^a | 24.94 ^a | 58.86 ^b | 67.94 ^b |
| 30 | 54.9c | 23.40 ^c | 49.57 ^b | 21.11 ^b | 23.81 ^c | 29.94 ^c |
| S.E. (±) | 1.27 | 0.89 | 4.55 | 0.42 | 2.98 | 1.98 |
| P (ppm) | | | | | | |
| 0 | 53.4 | 29.67 | 82.15 | 22.58 | 58.95 | 64.91 |
| 20 | 52.7 | 29.44 | 77.38 | 22.75 | 54.09 | 66.62 |
| 40 | 51.8 | 32.00 | 71.97 | 23.52 | 48.37 | 57.51 |
| 60 | 51.1 | 27.89 | 79.43 | 24.49 | 50.92 | 58.51 |
| 80 | 48.2 | 28.22 | 86.41 | 24.28 | 49.06 | 58.92 |
| S.E. (±) | NS | NS | NS | NS | NS | NS |

S.E. = Standard error of means , NS= Non-significant, In a column, the figures having same letter do not differ significantly at 5% level by DMRT.

3.1.2 Effective tillers per pot

The concentration of arsenic (As) and phosphorus (P) showed remarkable effect on effective tillers per pot in rice variety BRRi dhan29 (Table 3.1). It was observed that effective tillers per pot of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Effective tillers per pot was affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRi dhan29 (Table 1, Fig.3.2). The highest effective tillers per pot (33.60) was found in As0 treated pot and lowest effective tillers per pot (23.40) was found in As30 treated pot. On the other hand, the highest effective tillers per pot (32.00) was found in

P40 treated pot and lowest effective tillers per pot (27.89) was found in P60 treated pot. In interaction effect, highest effective tillers per pot was observed from As0P0 treatment (37.7) and lowest effective tillers per pot was recorded from As30P80 treated pot (14.8) (Table 3.2). Furthermore, the reduction of effective tillers per pot due to application of arsenic and phosphorus were also recorded (30.35% and 7.85%) (Fig. 3.2). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in effective tillers per pot, filled grains per panicle and 1000-grain weight; these together contributed reduced grain yield. Rice grain yield was reduced by 20.6% for 15 mgkg⁻¹ As treatment and 63.8% due to 30 mgkg⁻¹ As.

3.1.3 Filled grains per panicle

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on filled grains per panicle in rice variety BRR1 dhan29 (Table 3.1). It was observed that filled grains per panicle of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Filled grains per panicle were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRR1 dhan29 (Table 1, Fig.3.1). The highest filled grains per panicle (96.72) were found in As0 treated pot and lowest filled grains per panicle (49.57) were found in As30 treated pot. On the other hand, the highest filled grains per panicle (86.41) were found in P80 treated pot and lowest filled grains per panicle (71.97) were found in P40 treated pot. In interaction effect, highest filled grains per panicle was observed from As0P20 treatment (97.00) and lowest filled grains per panicle was recorded from As30P20 treated pot (24.40) (Table 3.2). Furthermore, the reduction of filled grains per panicle due to application of arsenic and phosphorus were also recorded (48.74% and 12.39%) (Fig. 3.3). This results were found similar with the findings from Hussain *et al.*, (2005), Islam and Jahiruddin (2010). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in filled grains per panicle, filled grains

per panicle and 1000-grain weight; these together contributed reduced grain yield. Rice grain yield was reduced by 20.6% for 15 mgkg⁻¹ As treatment and 63.8% due to 30 mgkg⁻¹ As.

3.1.3 1000 grain weight (g)

Increasing soil As concentration affected the 1000 grain weight of rice in As contaminated soils. The effects of arsenic (As) and phosphorus (P) showed remarkable effect on 1000 grain weight (g) in rice variety BRRI dhan29 (Table 3.1). It was observed that 1000 grain weight (g) of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. 1000 grain weight (g) were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRI dhan29 (Table 1, Fig.3.1). The highest 1000 grain weight (g) (24.94) were found in As15 treated pot and lowest 1000 grain weight (g) (21.11) were found in As30 treated pot. On the other hand, the highest 1000 grain weight (g) (24.49) were found in P60 treated pot and lowest 1000 grain weight (g) (22.58) were found in P0 treated pot. In interaction effect, highest 1000 grain weight (g) was observed from As0P0 treatment (23.8) and lowest 1000 grain weight (g) was recorded from As30P80 treated pot (20.3) (Table 3.2). Furthermore, the reduction of 1000 grain weight (g) due to application of arsenic and phosphorus were also recorded (15.35% and 8.45%) (Fig. 3.4). Similar findings were found from some researchers, such as, Abedin (2002) found that presence arsenic at a higher concentration in irrigation water significantly reduced the 1000 grain weight of rice plant. Tsutsumi (1980) also reported that arsenic could reduce 1000 grain weight. Arsenic application in rice significantly reduced yield and different yield-contributing parameters including the number of panicles per plant, panicle dry weight, the number of spikelets and full grains per plant and 1000-grain weight (Vromman *et al.*, 2013). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in 1000 grain weight (g), 1000 grain weight (g) and 1000-grain weight;

these together contributed reduced grain yield. Rice grain yield was reduced by 20.6% for 15 mgkg⁻¹ As treatment and 63.8% due to 30 mgkg⁻¹ As.

Table 3.2 Interaction effects of added arsenic (As) and phosphorus (P) on growth and yield parameters of BRR1 dhan29

| As×P Interaction | Plant Height (cm) | Effective tillers pot ⁻¹ | Filled grains panicle ⁻¹ | 1000-grain weight (g) | Grain yield (g pot ⁻¹) | Straw yield (g pot ⁻¹) |
|------------------|-------------------|-------------------------------------|-------------------------------------|-----------------------|------------------------------------|------------------------------------|
| As0P0 | 81.7 | 35.7 | 77.3 | 23.8 | 69.0 | 99.2 |
| As0P20 | 83.7 | 33.3 | 97.0 | 23.0 | 71.2 | 101.1 |
| As0P40 | 84.8 | 32.7 | 94.0 | 22.3 | 74.9 | 108.0 |
| As0P60 | 87.9 | 32.1 | 92.5 | 22.1 | 73.6 | 108.9 |
| As0P80 | 88.2 | 29.7 | 87.8 | 22.7 | 80.1 | 118.07 |
| As15P0 | 72.8 | 23.3 | 75.7 | 21.8 | 48.2 | 61.9 |
| As15P20 | 71.5 | 22.2 | 74.8 | 21.6 | 38.2 | 54.6 |
| As15P40 | 68.2 | 21.3 | 73.7 | 24.5 | 30.8 | 49.7 |
| As15P60 | 67.9 | 20.9 | 67.5 | 22.8 | 29.6 | 41.6 |
| As15P80 | 66.6 | 17.6 | 44.1 | 21.6 | 17.5 | 39.7 |
| As30P0 | 59.1 | 21.4 | 47.7 | 21.1 | 28.2 | 42.0 |
| As30P20 | 55.4 | 17.7 | 24.4 | 22.5 | 23.0 | 34.6 |
| As30P40 | 54.1 | 16.3 | 33.7 | 21.4 | 21.2 | 28.5 |
| As30P60 | 53.5 | 15.6 | 32.1 | 21.0 | 16.8 | 20.1 |
| As30P80 | 51.1 | 14.8 | 30.5 | 20.3 | 8.3 | 19.5 |
| S.E.(±) | NS | NS | 1.08 | NS | 1.26 | 1.70 |

S.E. = Standard error of means , NS= Non-significant, In a column, the figures having same letter do not differ significantly at 5% level by DMRT.

3.1.4 Grain yield

The yield of rice variety BRR1 dhan29 were found adversely affected with different levels of soil As concentration in soils. The effects of arsenic (As) and phosphorus (P) showed remarkable effect on grain yield in rice variety BRR1 dhan29 (Table 3.1). It was observed that grain yield of rice plant decreased significantly ($p < 0.05$) with increasing soil As

concentration. grain yield were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRR1 dhan29 (Table 1, Fig.3.1). The highest grain yield (74.17) were found in As0 treated pot and lowest grain yield (23.81) were found in As30 treated pot. On the other hand, the highest grain yield (58.95) were found in P0 treated pot and lowest grain yield (48.37) were found in P40 treated pot. In interaction effect, highest grain yield was observed from As0P80 treatment (80.1) and lowest grain yield was recorded from As30P80 treated pot (8.3) (Table 3.2). Furthermore, the reduction of grain yield due to application of arsenic and phosphorus were also recorded (67.89% and 17.94%) (Fig. 3.5). These finding were in agreement with Jahiruddin *et al.* (2004) stated that the highest grain yield reduction was observed with the maximum dose of irrigation arsenic treatment. These results are in good agreement with the findings of Akter (2006). Due to arsenic toxicity in rice plant, grain yield reduction was reported by Hossain *et al.* (2005); Hossain *et al.* (2008) and Kang *et al.* (1996). Similar results were published by some another researchers, such as, Panaullah *et al.*, (2009); Carbonell-Barrachina *et al.*, (1997); Abedin and Meharg (2002); Farn (1988), Milan (1988) and Tsutsumi (1980). Another report was found from Islam *et al.*, (2004), he reported that higher doses of arsenic with irrigation water significantly reduced of plant height, panicle length, grains panicle⁻¹, grain and straw yields of rice in boro season. Abedin *et al.*, (2002) found that increasing the concentration in irrigation water significantly decreased plant height, grain yield, the number of filled grains, grain weight, and root biomass, while the arsenic concentrations in root, straw, and rice husk increased significantly.

3.1.5 Straw yield

The straw yield of rice was found negatively affected with increasing soil As concentration in soils. The effects of arsenic (As) and phosphorus (P) showed remarkable effect on straw yield in rice variety BRR1 dhan29 (Table 3.1). It was observed that straw yield of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Straw yield were

affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRIdhan29 (Table 1, Fig.3.1). The highest straw yield (86.00) were found in As0 treated pot and lowest straw yield (29.94) were found in As30 treated pot. On the other hand, the highest straw yield (66.62) were found in P20 treated pot and lowest straw yield (57.51) were found in P40 treated pot. In interaction effect, highest straw yield was observed from As0P80 treatment (118.07) and lowest straw yield was recorded from As30P80 treated pot (19.5) (Table 3.2). Furthermore, the reduction of straw yield due to application of arsenic and phosphorus were also recorded (65.18% and 11.40%) (Fig. 3.6). This might be due to the accumulation of As in rice leads to a disturbance of essential physiological processes, such as photosynthesis and respiratory activity. Recently reported with soil test-based soil arsenic concentration which could be reduced the grain yield of rice (Hossain *et al.*, 2005; and Kang *et al.*, 1996). Several studies have shown that soil arsenic concentration on irrigated rice-based cropping system may cause heavy depletion of straw yield of rice (Abedin *et al.*, 2002; Yan *et al.*, 2005; Hossain *et al.*, 2005 and Islam *et al.*, 2004). This results reflected similar correlation with the findings from some researchers, such as, Islam and Jahiruddin (2010) found that the grain yield of rice was reduced by 20.6 % for 15 mg kg⁻¹ As treatment and 63.8 % due to 30 mgkg⁻¹ As. Such reductions for straw yield were 21.0 and 65.2 % with these two As treatments, respectively. Islam *et al.*, (2004) reported that higher doses of arsenic with irrigation water significantly reduced of plant height, panicle length, grains panicle⁻¹, grain and straw yields of rice in boro season. Similar results were also found from others researchers, such as, Li *et al.*, (1996); Azad *et al.*, (2009) and Abedin and Meharg (2002).

3.1.6 Nitrogen concentration (N conc.) in straw %

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on nitrogen concentration (N conc.) in straw % in rice variety BRRIdhan29 (Table 3.3). It was observed that nitrogen concentration (N conc.) in straw % of rice plant decreased significantly ($p < 0.05$)

with increasing soil As concentration. Nitrogen concentration (N conc.) in straw % was affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRIdhan29 (Table 3.3, Fig.3.7). The highest nitrogen concentration (N conc.) in straw % (0.839) was found in As30 treated pot and lowest nitrogen concentration (N conc.) in straw % (0.572) was found in As0 treated pot. On the other hand, the highest nitrogen concentration (N conc.) in straw % (0.844) was found in P60 treated pot and lowest nitrogen concentration (N conc.) in straw % (0.588) were found in P20 treated pot. In interaction effect, highest nitrogen concentration (N conc.) in straw % was observed from As30P0 treatment (0.627) and lowest nitrogen concentration (N conc.) in straw % was recorded from As0P60 treated pot (0.423) (Table 3.4). Furthermore, the reduction of nitrogen concentration (N conc.) in straw % due to application of arsenic and phosphorus were also recorded (46.67% and 26.13%) (Fig. 3.8).

3.1.7 Nitrogen concentration (N conc.) in grain %

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on nitrogen concentration (N conc.) in grain % in rice variety BRRIdhan29 (Table 3.3). It was observed that nitrogen concentration (N conc.) in grain % of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Nitrogen concentration (N conc.) in grain % were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRIdhan29 (Table 3, Fig.3.9). The highest nitrogen concentration (N conc.) in grain % (1.520) were found in As30 treated pot and lowest nitrogen concentration (N conc.) in grain % (1.264) were found in As0 treated pot. On the other hand, the highest nitrogen concentration (N conc.) in grain % (1.644) were found in P80 treated pot and lowest nitrogen concentration (N conc.) in grain % (1.284) were found in P20 treated pot. In interaction effect, highest nitrogen concentration (N conc.) in grain % was observed from As15P40 treatment (1.64) and lowest nitrogen concentration (N conc.) in grain % was recorded from As15P20 treated

pot (0.89) (Table 3.4). Furthermore, the reduction of nitrogen concentration (N conc.) in grain % due to application of arsenic and phosphorus were also recorded (20.25% and 27.34%) (Fig. 3.7).

3.1.8 Phosphorus concentration (P conc.) in straw %

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on phosphorus concentration (P conc.) in straw % in rice variety BRRI dhan29 (Table 3.3). It was observed that phosphorus concentration (P conc.) in straw % of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Phosphorus concentration (P conc.) in straw % were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRI dhan29 (Table 3.3, Fig.3.8). The highest phosphorus concentration (P conc.) in straw % (0.410) were found in As30 treated pot and lowest phosphorus concentration (P conc.) in straw % (0.301) were found in As0 treated pot. On the other hand, the highest phosphorus concentration (P conc.) in straw % (0.410) were found in P80 treated pot and lowest phosphorus concentration (P conc.) in straw % (0.291) were found in P0 treated pot. In interaction effect, highest phosphorus concentration (P conc.) in straw % was observed from As15P80 treatment (0.137) and lowest phosphorus concentration (P conc.) in straw % was recorded from As30P0 treated pot (0.059) (Table 3.4). Furthermore, the reduction of phosphorus concentration (P conc.) in straw % due to application of arsenic and phosphorus were also recorded (46.67% and 26.13%) (Fig. 3.8).

3.1.9 Phosphorus concentration (P conc.) in grain %

The effects of arsenic (As) and phosphorus (P) showed remarkable effect on phosphorus concentration (P conc.) in grain % in rice variety BRRI dhan29 (Table 3.3). It was observed that phosphorus concentration (P conc.) in grain % of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Phosphorus concentration (P conc.) in grain % were affected markedly due to the effects of different arsenic (As) levels and phosphorus in

BRRRI dhan29 (Table 3.3, Fig.3.9). The highest phosphorus concentration (P conc.) in grain % (0.410) were found in As30 treated pot and lowest phosphorus concentration (P conc.) in grain % (0.310) were found in As0 treated pot. On the other hand, the highest phosphorus concentration (P conc.) in grain % (0.410) were found in P80 treated pot and lowest phosphorus concentration (P conc.) in grain % (0.291) were found in P0 treated pot. In interaction effect, highest phosphorus concentration (P conc.) in grain % was observed from As0P60 treatment (0.211) and lowest phosphorus concentration (P conc.) in grain % was recorded from As30P40 treated pot (0.066) (Table 3.4). Furthermore, the reduction of phosphorus concentration (P conc.) in grain % due to application of arsenic and phosphorus were also recorded (36.21% and 40.89%) (Fig. 3.9).

3.1.10 Arsenic concentration (As conc. in ppm) in straw

Arsenic (As) uptake by rice straw of rice variety BRRRI dhan29 were severely affected by increasing concentration of soil arsenic. The effects of arsenic (As) and phosphorus (P) showed remarkable effect on arsenic concentration (As conc. in ppm) in straw in rice variety BRRRI dhan29 (Table 3.3). It was observed that arsenic concentration (As conc. in ppm) in straw of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Arsenic concentration (As conc. in ppm) in straw was affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRRI dhan29 (Table 3.3, Fig.3.10). The highest arsenic concentration (As conc. in ppm) in straw (8.27) were found in As30 treated pot and lowest arsenic concentration (As conc. in ppm) in straw (2.55) were found in As0 treated pot. On the other hand, the highest arsenic concentration (As conc. in ppm) in straw (7.47) were found in P80 treated pot and lowest arsenic concentration (As conc. in ppm) in straw (4.84) were found in P40 treated pot. In interaction effect, highest arsenic concentration (As conc. in ppm) in straw was observed from As30P0 treatment (18.13) and lowest arsenic concentration (As conc. in ppm) in straw was recorded from As0P20 treated pot (3.19)

(Table 3.4). Furthermore, the reduction of arsenic concentration (As conc. in ppm) in straw due to application of arsenic and phosphorus were also recorded (224.31% and 48.50%) (Fig. 3.12). These results were in agreement with the results from Rahman *et al.*, (2007a) and Tsutsumi *et al.*, (1980). Similar results were in agreement with Islam and Jahiruddin (2010), who reported that arsenic treatment resulted in a marked decrease in arsenic concentration (As conc. in ppm) in straw, arsenic concentration (As conc. in ppm) in straw and 1000-grain weight; these together contributed reduced arsenic concentration (As conc. in ppm) in straw. Rice arsenic concentration (As conc. in ppm) in straw was reduced by 20.6% for 15 mgkg⁻¹ As treatment and 63.8% due to 30 mgkg⁻¹ As. Many scientists reported that arsenic concentration in rice grain vary widely depending on the cultivars (Marin *et al.*, 192, Heitkemper *et al.* 2001, Abedin *et al.*, 2002, Islam *et al.*, 2004 and Rahman *et al.*, 2007). Rouf *et al.*, (2011) found that the As concentrations ranged from 2.2 to 38 mg kg⁻¹ in root, 1.37 to 11.80 mg kg⁻¹ in straw, 0.28 to 1.76 mg kg⁻¹ in husk and 0.20 to 0.67 mg kg⁻¹ in grain of BRR1 dhan 33. In BR11 cultivar, As concentrations ranged from 2 to 30.0 mg kg⁻¹ in root, 2.10 to 10.30 mg kg⁻¹ in straw, 0.35 to 1.87 mg kg⁻¹ in husk and 0.17 to 0.94 mg kg⁻¹ in grain.

3.1.11 Arsenic concentration (As conc. in ppm) in grain

Grain arsenic (As) uptake by rice plant of rice variety BRR1 dhan29 greatly affected by soil As concentrations in soil. The effects of arsenic (As) and phosphorus (P) showed remarkable effect on arsenic concentration (As conc. in ppm) in grain in rice variety BRR1 dhan29 (Table 3.3). It was observed that arsenic concentration (As conc. in ppm) in grain of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Arsenic concentration (As conc. in ppm) in grain were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRR1 dhan29 (Table 3.3, Fig.3.11). The highest arsenic concentration (As conc. in ppm) in grain (0.507) were found in As30 treated pot and lowest arsenic concentration (As conc. in ppm) in grain (0.214) were found in As0

treated pot. On the other hand, the highest arsenic concentration (As conc. in ppm) in grain (0.44) were found in P40 treated pot and lowest arsenic concentration (As conc. in ppm) in grain (0.34) were found in P60 treated pot. In interaction effect, highest arsenic concentration (As conc. in ppm) in grain was observed from As30P80 treatment (1.47) and lowest arsenic concentration (As conc. in ppm) in grain was recorded from As0P20 treated pot (0.235) (Table 3.4). Furthermore, the reduction of arsenic concentration (As conc. in ppm) in grain due to application of arsenic and phosphorus were also recorded (136.91% and 20.93%) (Fig. 3.11). Similar results were found from some researchers, such as, Rahman *et al.*, (2007), Abedin *et al.*, (2002) and Das *et al.*, (2004) reported that the concentration of arsenic in the grain of all the studied rice samples was found to be between 0.25 ± 0.014 to 0.73 ± 0.009 mgkg^{-1} dry weight of arsenic, which did not exceed the permissible limit in rice (1.0 mgkg^{-1}) according to WHO recommendation. Rice grain has been reported to accumulate arsenic upto 2.0 mgkg^{-1} by Meharg *et al.*, (2003), Islam *et al.*, (2004) and Delowar *et al.* (2005), which is much above the permissible limit in rice (1.0 mgkg) according to WHO recommendation.

Table 3.3 Effects of added arsenic (As) and phosphorus (P) on nitrogen (N), phosphorus (P) and arsenic (As) concentration of BRRI dhan29

| Treatment (As or P) | N conc. (%) | | P conc. (%) | | As conc. (ppm) | |
|------------------------|--------------------|--------------------|---------------------|--------------------|--------------------|-------------------|
| | Grain | Straw | Grain | Straw | Grain | Straw |
| As (ppm) | | | | | | |
| 0 | 1.264 ^c | 0.572 ^b | 0.301 ^c | 0.107 ^a | 0.214 ^c | 2.55 ^c |
| 15 | 1.441 ^b | 0.800 ^a | 0.345 ^b | 0.076 ^b | 0.376 ^b | 6.01 ^b |
| 30 | 1.520 ^a | 0.839 ^a | 0.410 ^a | 0.085 ^b | 0.507 ^a | 8.27 ^a |
| S.E. (\pm) | 0.012 | 0.027 | 0.009 | 0.005 | 0.012 | 0.20 |
| P (ppm) | | | | | | |
| 0 | 1.291 ^c | 0.796 ^a | 0.291 ^c | 0.065 ^c | 0.43 ^{ab} | 5.03 ^b |
| 20 | 1.284 ^c | 0.588 ^b | 0.353 ^b | 0.071 ^c | 0.35 ^c | 5.27 ^b |
| 40 | 1.524 ^b | 0.773 ^a | 0.339 ^{bc} | 0.094 ^b | 0.44 ^a | 4.84 ^b |

| | | | | | | |
|----------|--------------------|---------------------|---------------------|--------------------|---------------------|-------------------|
| 60 | 1.298 ^c | 0.844 ^a | 0.367 ^{ab} | 0.118 ^a | 0.34 ^c | 5.46 ^b |
| 80 | 1.644 ^a | 0.684 ^{ab} | 0.410 ^a | 0.099 ^b | 0.37 ^{abc} | 7.47 ^a |
| S.E. (±) | 0.016 | 0.035 | 0.011 | 0.006 | 0.016 | 0.26 |

S.E. = Standard error of means , NS= Non-significant, In a column, the figures having same letter do not differ significantly at 5% level by DMRT.

Table 3.4 Interaction effects of added arsenic (As) and phosphorus (P) on nitrogen (N), phosphorus (P) and arsenic (As) concentration of BRRI dhan29

| As×P Interaction | N conc. (%) | | P conc. (%) | | As conc. (ppm) | |
|---------------------|-------------|-------|-------------|-------|----------------|-------|
| | Grain | Straw | Grain | Straw | Grain | Straw |
| As0P0 | 1.12 | 0.523 | 0.197 | 0.088 | 0.215 | 5.40 |
| As0P20 | 1.18 | 0.467 | 0.205 | 0.088 | 0.235 | 3.19 |
| As0P40 | 0.95 | 0.433 | 0.218 | 0.077 | 0.353 | 4.07 |
| As0P60 | 0.95 | 0.423 | 0.211 | 0.069 | 0.369 | 3.52 |
| As0P80 | 0.97 | 0.450 | 0.131 | 0.061 | 0.406 | 3.83 |
| As15P0 | 0.91 | 0.580 | 0.121 | 0.123 | 0.373 | 16.9 |
| As15P20 | 0.89 | 0.597 | 0.215 | 0.081 | 0.353 | 9.98 |
| As15P40 | 1.64 | 0.590 | 0.230 | 0.085 | 0.529 | 13.06 |
| As15P60 | 1.56 | 0.625 | 0.229 | 0.112 | 0.632 | 13.25 |
| As15P80 | 1.36 | 0.647 | 0.213 | 0.137 | 1.06 | 10.91 |
| As30P0 | 1.08 | 0.627 | 0.186 | 0.059 | 0.549 | 18.13 |
| As30P20 | 0.96 | 0.570 | 0.223 | 0.091 | 0.559 | 11.33 |
| As30P40 | 1.12 | 0.530 | 0.066 | 0.091 | 0.647 | 13.02 |
| As30P60 | 1.09 | 0.536 | 0.69 | 0.091 | 0.691 | 14.21 |
| As30P80 | 1.00 | 0.562 | 0.152 | 0.095 | 1.470 | 14.98 |
| | 0.653 | NS | 0.0153 | 0.010 | 0.0256 | 0.777 |

S.E. = Standard error of means , NS= Non-significant, In a column, the figures having same letter do not differ significantly at 5% level by DMRT.

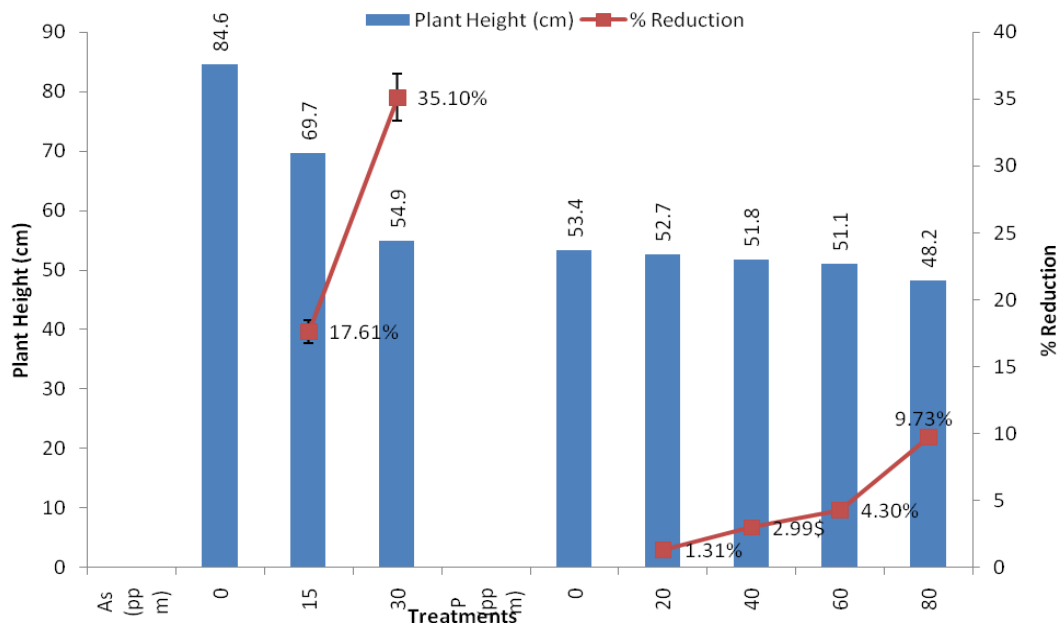


Fig.: 2.5 Effect of arsenic and phosphorus on plant height and % reduction of BRRIdhan29

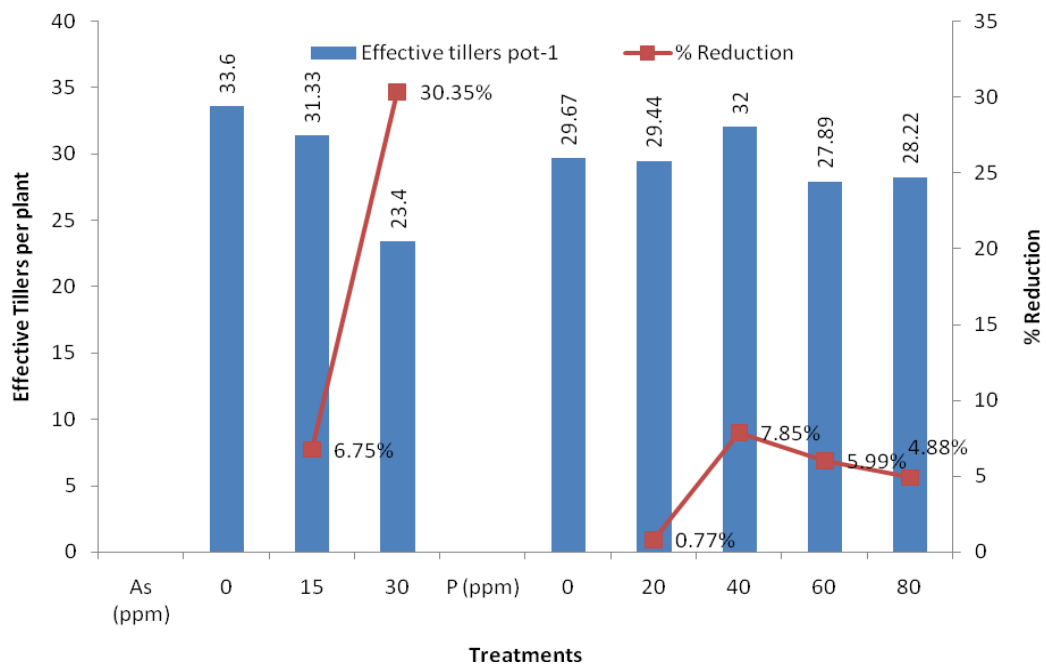


Fig.: 2.6 Effect of arsenic and phosphorus on effective tillers per pot and % reduction of BRRIdhan29

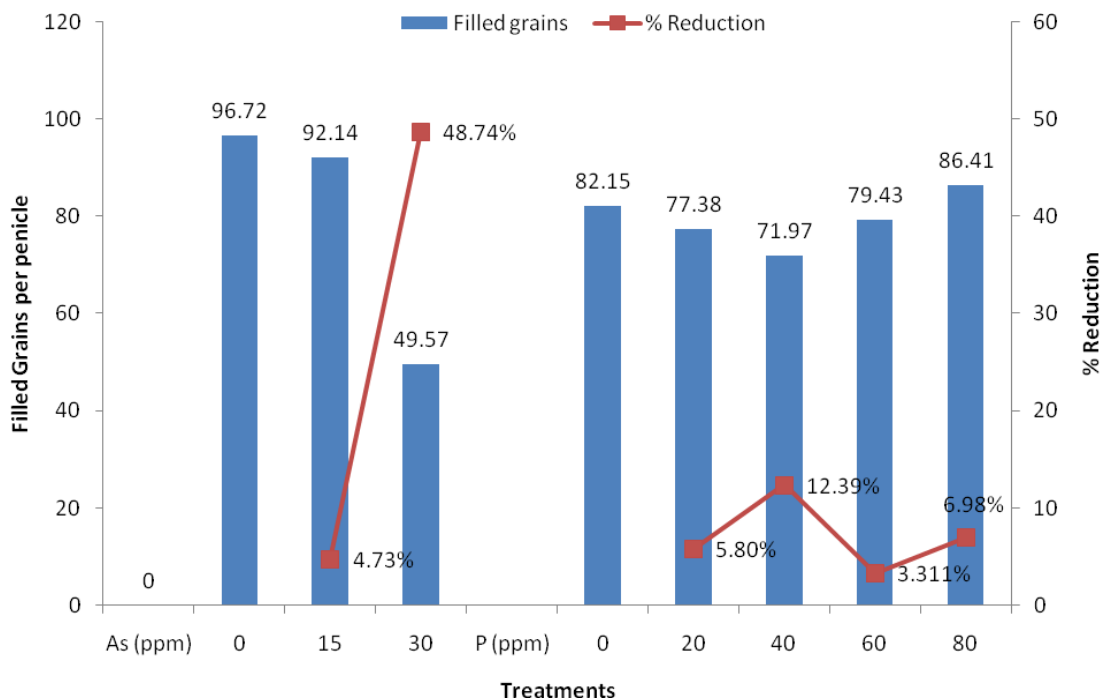


Fig.: 2.7 Effect of arsenic and phosphorus on filled grain per panicle and % reduction of BRR1 dhan29

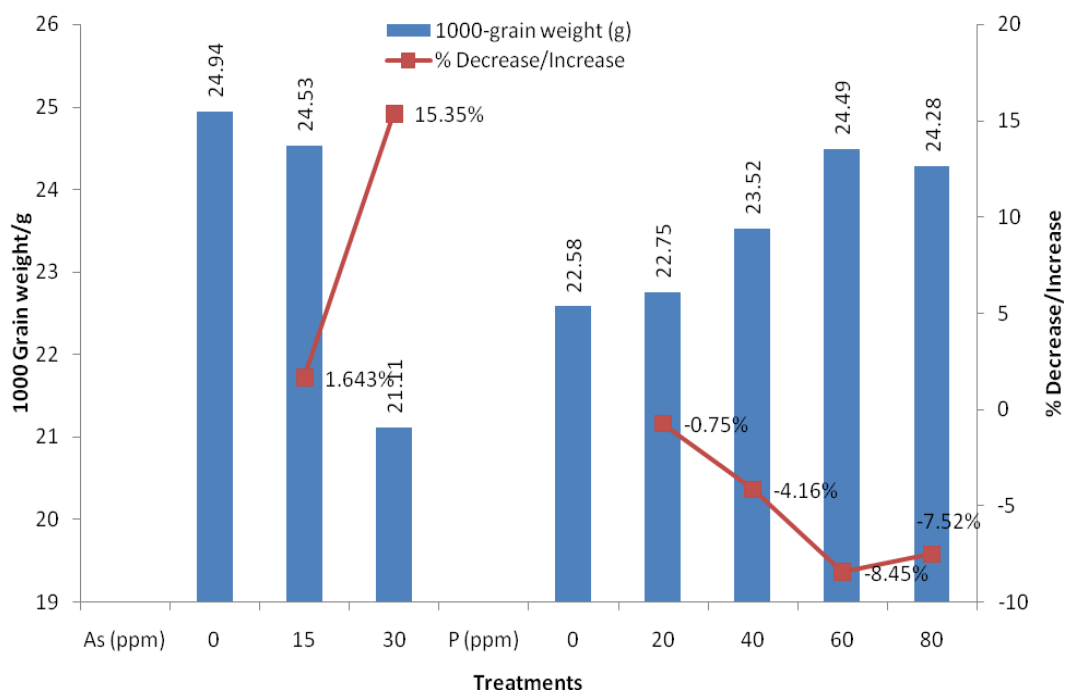


Fig.: 2.8 Effect of arsenic and phosphorus on 1000-grain weight and % reduction of BRR1 dhan29

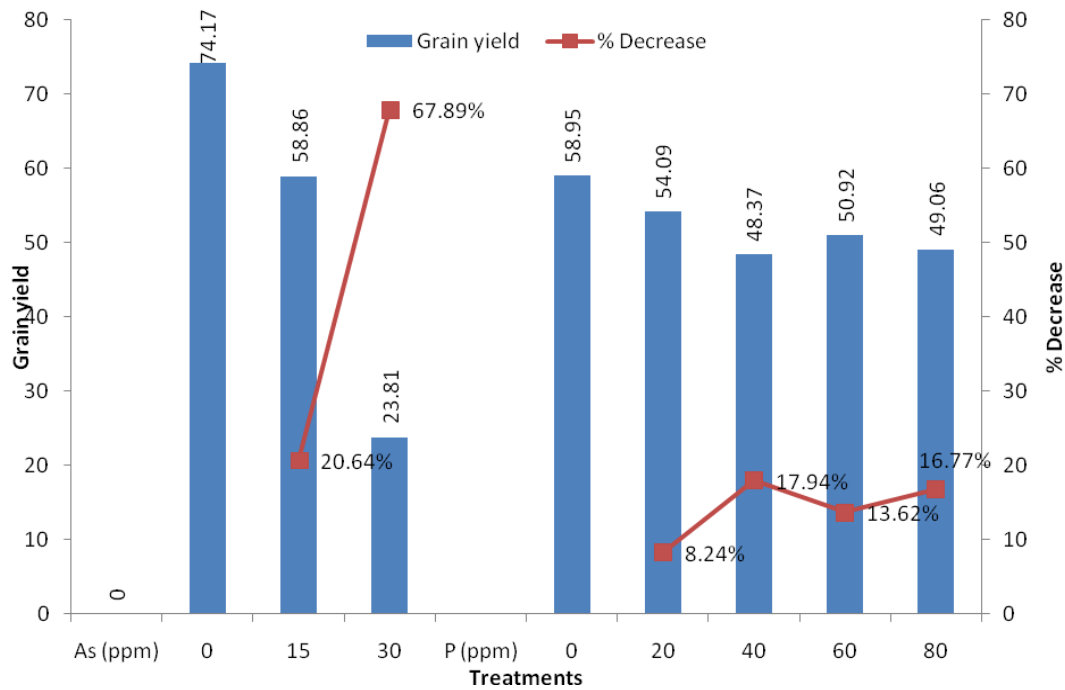


Fig.: 2.9 Effect of arsenic and phosphorus on grain yield and % reduction of BRRIdhan29

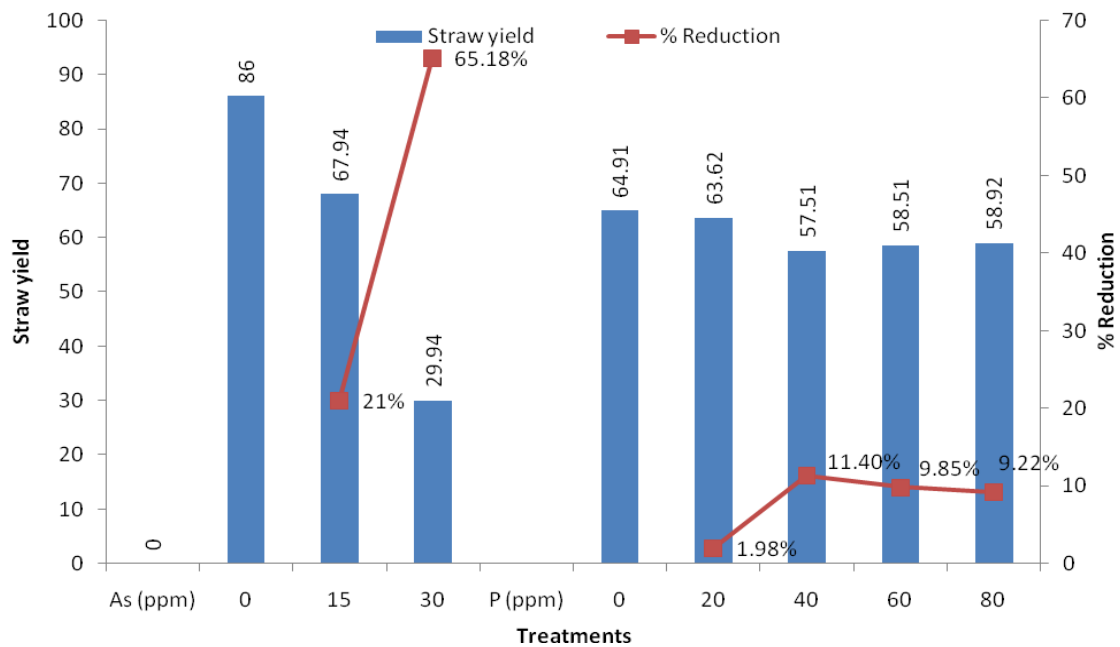


Fig.: 2.10 Effect of arsenic and phosphorus on straw yield and % reduction of BRRIdhan29

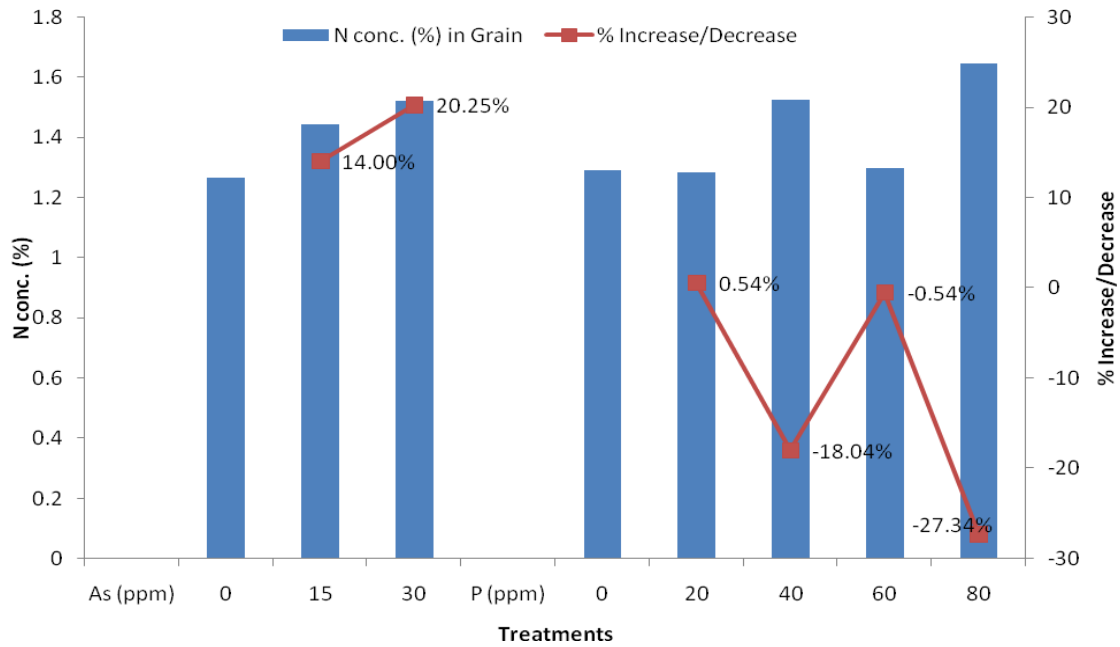


Fig.: 2.11 Effect of arsenic and phosphorus on N conc. and % reduction of BRRIdhan29

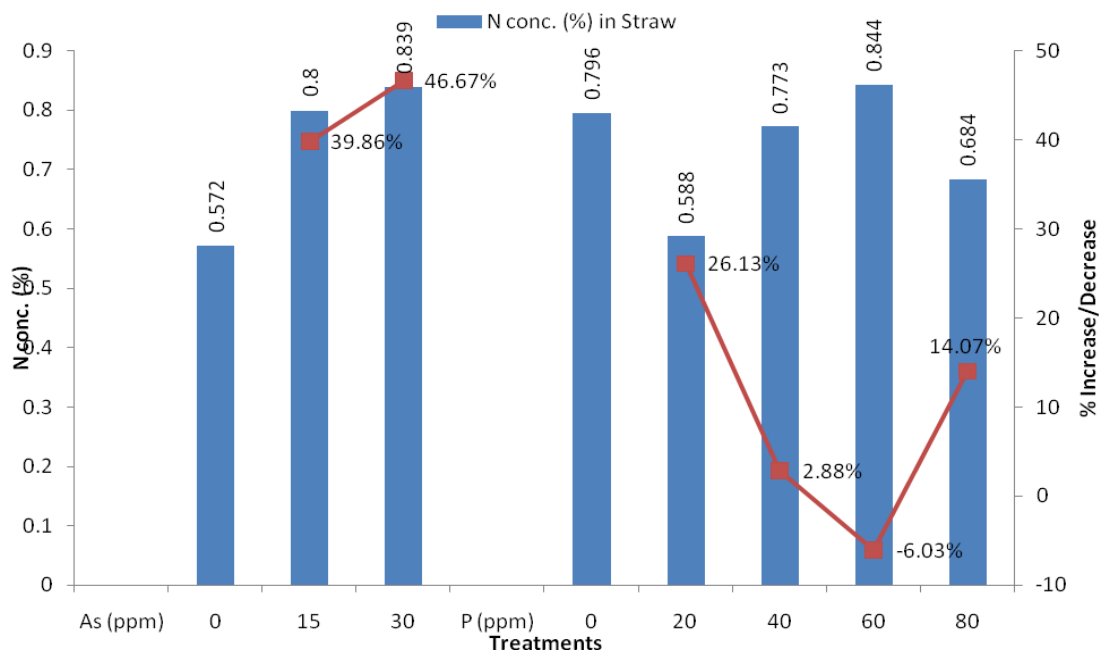


Fig.: 2.12 Effect of arsenic and phosphorus on N conc. and % reduction of BRRIdhan29

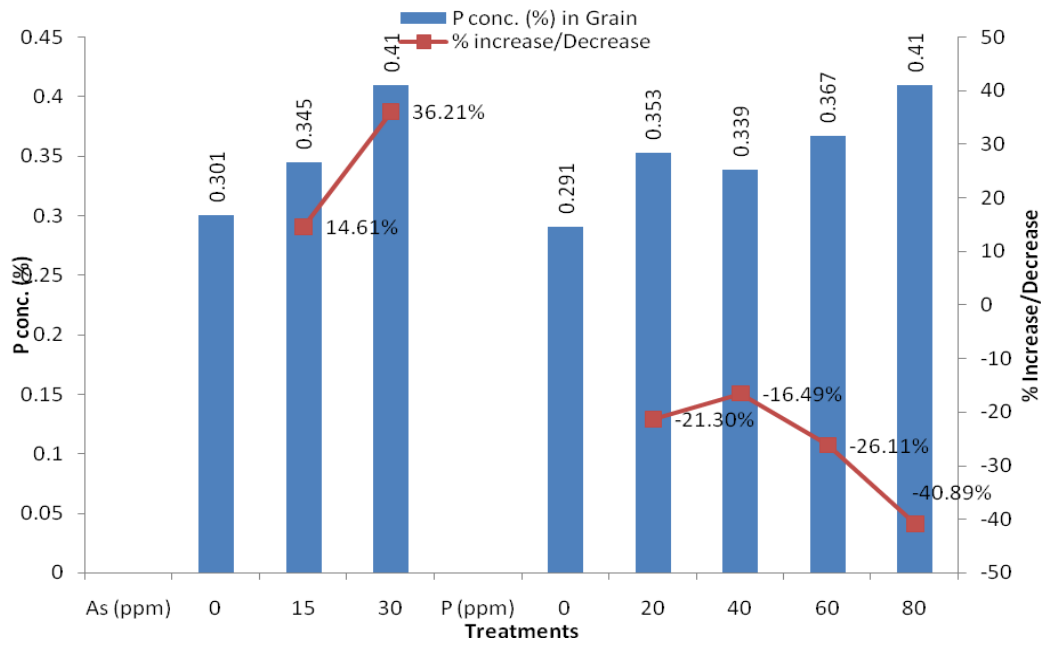


Fig.: 2.13 Effect of arsenic and phosphorus on P conc. and % reduction of BRR1 dhan29

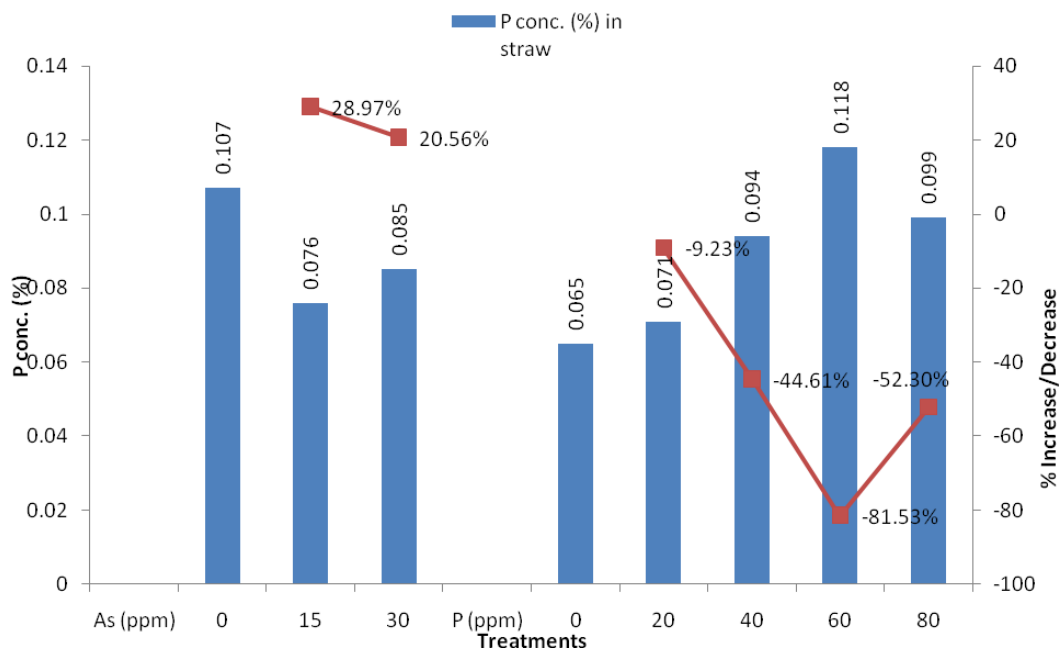


Fig.: 2.14 Effect of arsenic and phosphorus on P conc. and % reduction of BRR1 dhan29

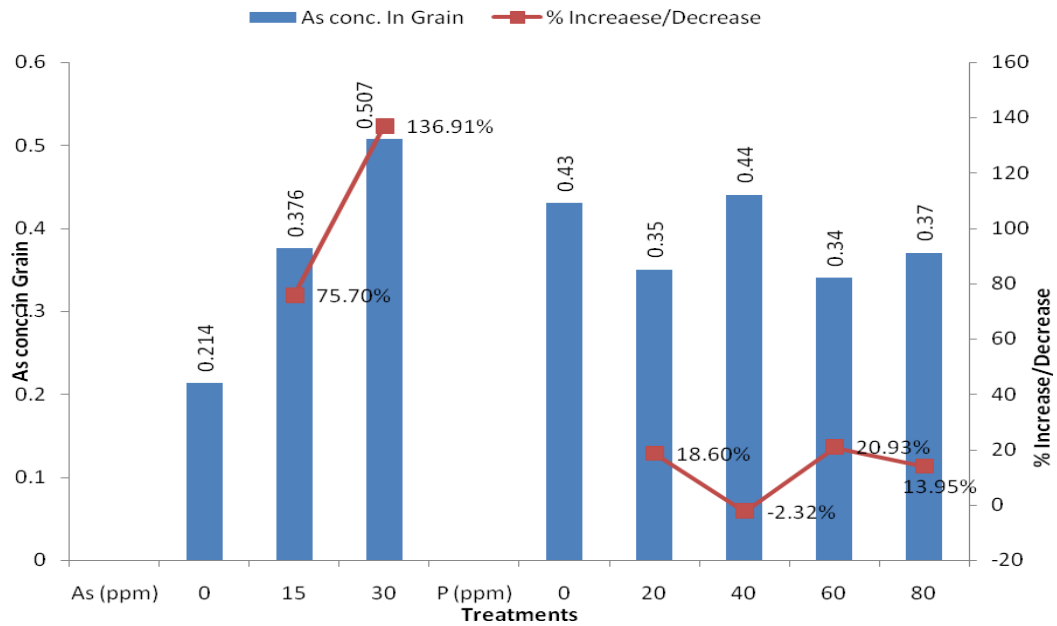


Fig.: 2.15 Effect of arsenic and phosphorus on As conc. in grain and % reduction of BRR1 dhan29

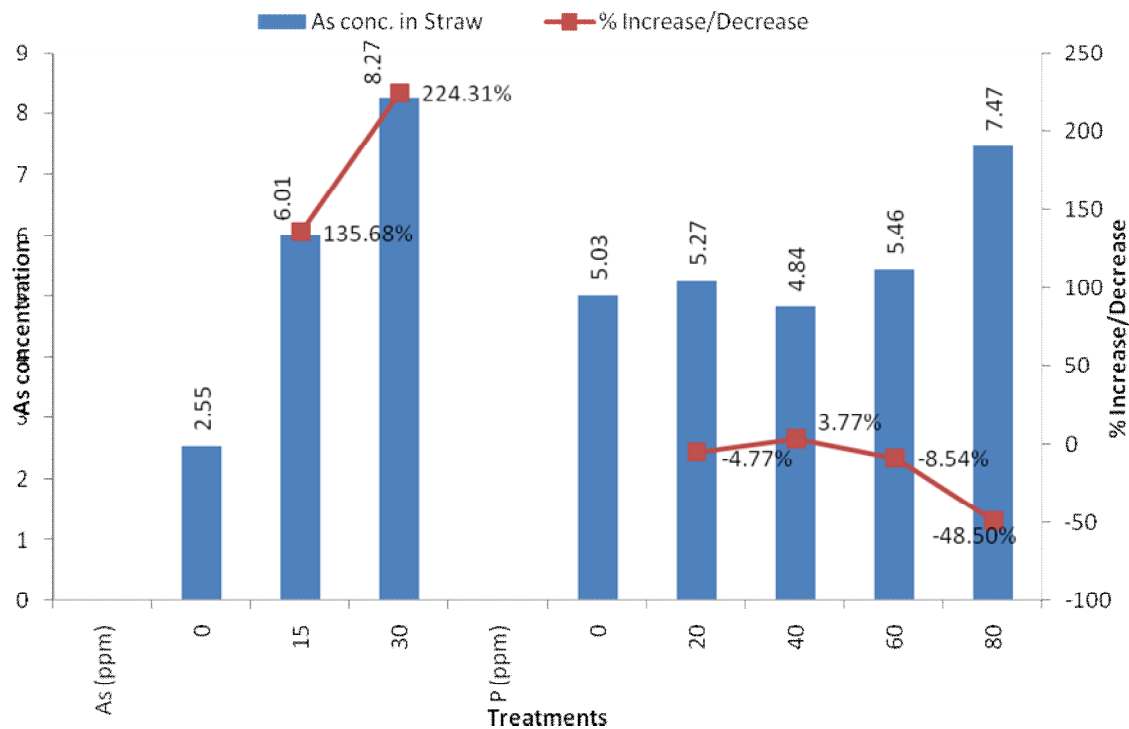


Fig.: 2.16 Effect of arsenic and phosphorus on As conc. in straw and % reduction of BRR1 dhan29

Chapter IV

Summary and Conclusion

A pot experiment was conducted at the Soil Science Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from December 2016 to March 2017 to evaluate the effect of arsenic and phosphorus on the growth and yield of BBRI dhan29. A two factor factorial pot experiment was conducted which comprised with three levels of arsenic doses 0, 15 and 30 ppm and five levels of phosphorus 0, 20, 40, 60 and 80 ppm. The treatment combinations were As0P0, As0P20, As0P40, As0P60, As0P80, As15P0, As15P20, As15P40, As15P60, As15P80, As30P0, As30P20, As30P40, As30P60 and As30P80. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications.

The highest plant height (84.6) was found in As0 treated pot and lowest plant height (54.9) was found in As30 treated pot. On the other hand, the highest plant height (53.4) was found in P0 treated pot and lowest plant height (48.2) was found in P80 treated pot. In interaction effect, highest plant height was observed from As0P80 treatment (88.2) and lowest plant height was recorded from As30P80 treated pot (51.1) (Table 3.2). Furthermore, the reduction of plant height due to application of arsenic and phosphorus were also recorded (35.1% and 9.73%) (Fig. 3.1). The highest effective tillers per pot (33.60) was found in As0 treated pot and lowest effective tillers per pot (23.40) was found in As30 treated pot. On the other hand, the highest effective tillers per pot (32.00) was found in P40 treated pot and lowest effective tillers per pot (27.89) was found in P60 treated pot. In interaction effect, highest effective tillers per pot was observed from As0P0 treatment (37.7) and lowest effective tillers per pot was recorded from As30P80 treated pot (14.8) (Table 3.2). Furthermore, the reduction of effective tillers per pot due to application of arsenic and phosphorus were also recorded (30.35% and 7.85%) (Fig. 3.2).

The highest filled grains per panicle (96.72) were found in As0 treated pot and lowest filled grains per panicle (49.57) were found in As30 treated pot. On the other hand, the highest filled grains per panicle (86.41) were found in P80 treated pot and lowest filled grains per panicle (71.97) were found in P40 treated pot. In interaction effect, highest filled grains per panicle was observed from As0P20 treatment (97.00) and lowest filled grains per panicle was recorded from As30P20 treated pot (24.40) (Table 3.2). Furthermore, the reduction of filled grains per panicle due to application of arsenic and phosphorus were also recorded (48.74% and 12.39%) (Fig. 3.3). The effects of arsenic (As) and phosphorus (P) showed remarkable effect on 1000 grain weight (g) in rice variety BRRI dhan29 (Table 3.1). It was observed that 1000 grain weight (g) of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. 1000 grain weight (g) were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRI dhan29 (Table 1, Fig.3.1). The highest 1000 grain weight (g) (24.94) were found in As15 treated pot and lowest 1000 grain weight (g) (21.11) were found in As30 treated pot. On the other hand, the highest 1000 grain weight (g) (24.49) were found in P60 treated pot and lowest 1000 grain weight (g) (22.58) were found in P0 treated pot. In interaction effect, highest 1000 grain weight (g) was observed from As0P0 treatment (23.8) and lowest 1000 grain weight (g) was recorded from As30P80 treated pot (20.3) (Table 3.2). The highest grain yield (74.17) were found in As0 treated pot and lowest grain yield (23.81) were found in As30 treated pot. On the other hand, the highest grain yield (58.95) were found in P0 treated pot and lowest grain yield (48.37) were found in P40 treated pot. In interaction effect, highest grain yield was observed from As0P80 treatment (80.1) and lowest grain yield was recorded from As30P80 treated pot (8.3) (Table 3.2). Furthermore, the reduction of grain yield due to application of arsenic and phosphorus were also recorded (67.89% and 17.94%) (Fig. 3.5). The highest straw yield (86.00) were found in As0 treated pot and lowest straw yield (29.94) were found in As30 treated pot. On the other hand, the

highest straw yield (66.62) were found in P20 treated pot and lowest straw yield (57.51) were found in P40 treated pot. In interaction effect, highest straw yield was observed from As0P80 treatment (118.07) and lowest straw yield was recorded from As30P80 treated pot (19.5) (Table 3.2). Furthermore, the reduction of straw yield due to application of arsenic and phosphorus were also recorded (65.18% and 11.40%) (Fig. 3.6). The highest nitrogen concentration (N conc.) in straw % (0.839) was found in As30 treated pot and lowest nitrogen concentration (N conc.) in straw % (0.572) was found in As0 treated pot. On the other hand, the highest nitrogen concentration (N conc.) in straw % (0.844) was found in P60 treated pot and lowest nitrogen concentration (N conc.) in straw % (0.588) were found in P20 treated pot. In interaction effect, highest nitrogen concentration (N conc.) in straw % was observed from As30P0 treatment (0.627) and lowest nitrogen concentration (N conc.) in straw % was recorded from As0P60 treated pot (0.423) (Table 3.4). The highest nitrogen concentration (N conc.) in grain % (1.520) were found in As30 treated pot and lowest nitrogen concentration (N conc.) in grain % (1.264) were found in As0 treated pot. On the other hand, the highest nitrogen concentration (N conc.) in grain % (1.644) were found in P80 treated pot and lowest nitrogen concentration (N conc.) in grain % (1.284) were found in P20 treated pot. In interaction effect, highest nitrogen concentration (N conc.) in grain % was observed from As15P40 treatment (1.64) and lowest nitrogen concentration (N conc.) in grain % was recorded from As15P20 treated pot (0.89) (Table 3.4). Furthermore, the reduction of nitrogen concentration (N conc.) in grain % due to application of arsenic and phosphorus were also recorded (20.25% and 27.34%) (Fig. 3.7).

The highest phosphorus concentration (P conc.) in straw % (0.410) were found in As30 treated pot and lowest phosphorus concentration (P conc.) in straw % (0.301) were found in As0 treated pot. On the other hand, the highest phosphorus concentration (P conc.) in straw % (0.410) were found in P80 treated pot and lowest phosphorus concentration (P conc.) in

straw % (0.291) were found in P0 treated pot. In interaction effect, highest phosphorus concentration (P conc.) in straw % was observed from As15P80 treatment (0.137) and lowest phosphorus concentration (P conc.) in straw % was recorded from As30P0 treated pot (0.059) (Table 3.4). Furthermore, the reduction of phosphorus concentration (P conc.) in straw % due to application of arsenic and phosphorus were also recorded (46.67% and 26.13%) (Fig. 3.8). The highest phosphorus concentration (P conc.) in grain % (0.410) were found in As30 treated pot and lowest phosphorus concentration (P conc.) in grain % (0.310) were found in As0 treated pot. On the other hand, the highest phosphorus concentration (P conc.) in grain % (0.410) were found in P80 treated pot and lowest phosphorus concentration (P conc.) in grain % (0.291) were found in P0 treated pot. In interaction effect, highest phosphorus concentration (P conc.) in grain % was observed from As0P60 treatment (0.211) and lowest phosphorus concentration (P conc.) in grain % was recorded from As30P40 treated pot (0.066) (Table 3.4). Furthermore, the reduction of phosphorus concentration (P conc.) in grain % due to application of arsenic and phosphorus were also recorded (36.21% and 40.89%) (Fig. 3.9).

It was observed that arsenic concentration (As conc. in ppm) in straw of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Arsenic concentration (As conc. in ppm) in straw was affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRR1 dhan29 (Table 3.3, Fig.3.10). The highest arsenic concentration (As conc. in ppm) in straw (8.27) were found in As30 treated pot and lowest arsenic concentration (As conc. in ppm) in straw (2.55) were found in As0 treated pot. On the other hand, the highest arsenic concentration (As conc. in ppm) in straw (7.47) were found in P80 treated pot and lowest arsenic concentration (As conc. in ppm) in straw (4.84) were found in P40 treated pot. In interaction effect, highest arsenic concentration (As conc. in ppm) in straw was observed from As30P0 treatment (18.13) and lowest arsenic concentration (As conc. in

ppm) in straw was recorded from As0P20 treated pot (3.19) (Table 3.4). Furthermore, the reduction of arsenic concentration (As conc. in ppm) in straw due to application of arsenic and phosphorus were also recorded (224.31% and 48.50%) (Fig. 3.12).

It was observed that arsenic concentration (As conc. in ppm) in grain of rice plant decreased significantly ($p < 0.05$) with increasing soil As concentration. Arsenic concentration (As conc. in ppm) in grain were affected markedly due to the effects of different arsenic (As) levels and phosphorus in BRRI dhan29 (Table 3.3, Fig.3.11). The highest arsenic concentration (As conc. in ppm) in grain (0.507) were found in As30 treated pot and lowest arsenic concentration (As conc. in ppm) in grain (0.214) were found in As0 treated pot. On the other hand, the highest arsenic concentration (As conc. in ppm) in grain (0.44) were found in P40 treated pot and lowest arsenic concentration (As conc. in ppm) in grain (0.34) were found in P60 treated pot. In interaction effect, highest arsenic concentration (As conc. in ppm) in grain was observed from As30P80 treatment (1.47) and lowest arsenic concentration (As conc. in ppm) in grain was recorded from As0P20 treated pot (0.235) (Table 3.4). Furthermore, the reduction of arsenic concentration (As conc. in ppm) in grain due to application of arsenic and phosphorus were also recorded. Furthermore, the reduction of arsenic concentration (As conc. in ppm) in grain due to application of arsenic and phosphorus were also recorded. The adverse effect of arsenic was further enhanced by P addition. This reaction has an implication to P fertilizer management in rice.

CHAPTER V

RECOMMENDATIONS

The addition of arsenic markedly increased the arsenic concentration of grain and straw, with a concomitant reduction in grain and straw yields of rice. The situation was further deteriorated for P application to soil, probably due to displacement of arsenic from adsorption sites in the bulk soil, with an increased concentration of arsenic in the rhizosphere soil solution for subsequent uptake by rice roots. Rice straw can be a potential exposure of arsenic to cattle. Future research needs to be carried out on arsenic speciation and also to determine the maximum acceptable limit of arsenic in irrigation water, rice grain and straw under Bangladesh situation.

CHAPTER VI

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CHAPTER VIII

APPENDIX

Appendix I. Characteristics of the soil of experimental field

Physical and chemical properties of the initial soil

| Characteristics | Value |
|-------------------------------|------------|
| % Sand | 27 |
| % Silt | 43 |
| % Clay | 30 |
| Textural Class | Silty-clay |
| pH | 6.1 |
| Organic matter (%0 | 1.13 |
| Total N (%) | 0.03 |
| Available P (ppm) | 20.00 |
| Exchangeable K (me/100g soil) | 0.10 |
| Available S (ppm) | 23 |
| As (ppm) | 4.83 |