Mitigation of Arsenic Accumulation in Rice through Organic Amendments and Water Regime Management

Ph.D. THESIS

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Mitigation of Arsenic Accumulation in Rice through Organic Amendments and Water Regime Management

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Declaration

I do hereby declare that the submitted thesis entitled "**Mitigation of Arsenic Accumulation in Rice through Organic Amendments and Water Regime Management"** has been composed by me and all the works presented herein are of my experimental findings. I further declare that this work has not been submitted anywhere for any academic degree, prize, or scholarship and not published anywhere in Bangladesh or abroad.

(**Nazneen Nahar**)

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Dedicated to

My Beloved Father and Mother

Certificate

We have much pleasure to certify that the research work presented in this dissertation entitled "**Mitigation of Arsenic Accumulation in Rice through Organic Amendments and Water Regime Management"** has been performed by **Nazneen Nahar** in the experimental field and laboratory of the Department of Soil, Water and Environment, University of Dhaka, Bangladesh. She accomplished all sorts of research activities under our direct instruction, supervision, and guidance. The part of this dissertation has not been submitted elsewhere for any degree or diploma. It is further certified that the work presented herewith is original and very suitable for submission for the award of the degree of Ph.D.

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Figure 4.72. Correlation analysis of rice grain As and rice grain P concentrations (n = 90). 158

Abstract

A field experiment was carried out with paddy rice (*Oryza Sativa* L.) for two consecutive seasons, namely 2017 *Boro* season and 2018 *Boro* season to observe the effects of two organic amendments (cow dung at 5 and 10 t/ha and poultry manure at 5 and 10 t/ha) and three water regimes (No SW, 3-cm SW, and 5-cm SW) on the accumulation of arsenic in different parts of BRRI dhan 28. The main objective was to see the possibility of mitigating arsenic uptake by water management and the application of organic amendments. The growth parameters such as plant height, fresh weight, dry weight, and yield were recorded. The effects of organic amendments and water regimes were also studied for phosphorus and iron concentrations in plants. The water regime was found to have no significant effects on plant height, fresh and dry weight of biomass, and the yield ($p>0.05$). However, organic amendments were found to have significant effects on the studied growth parameters ($p<0.05$). For both seasons, poultry manure at 10 t/ha was found to be the best treatment in terms of plant height, fresh weight, dry weight, and grain weight. In both seasons, water regime was found to affect the arsenic accumulation in rice root, straw, and husk ($p<0.05$). However, the grain arsenic concentration did not differ significantly among the water regimes ($p>0.05$). No SW regime, i.e. the absence of standing water, was found to be the best treatment in terms of reduced arsenic accumulation. On the other hand, the highest accumulation of arsenic was observed under 5-cm SW regime. Cow dung at 10 t/ha application was found to promote the uptake of arsenic in root, straw, husk, and grain of rice. On the other hand, the lowest uptake of arsenic was observed when plants were dosed with poultry manure at 5 t/ha. Arsenic concentration was significantly different in root from that of straw, husk, and grain ($p<0.05$) and the transfer factor (TF) followed the order: root > straw > husk > grain. The highest root P concentration was observed under 3-cm SW regime (both 2017 and 2018 seasons). For husk and grain P concentrations, the effects of water regime were not consistent. Among the organic amendment treatments, poultry manure at 10 t/ha was found to be the best for root P and straw P concentrations. No significant differences were observed among the organic

amendment treatments for grain P concentrations ($p>0.05$). Rice root and straw iron concentrations did not differ significantly among the water regimes (both 2017 and 2018 seasons). In 2017 and 2018 seasons, the highest grain Fe concentrations were obtained under 3-cm SW regime, which is significantly different from the rest of the treatments. The study demonstrated that No SW water regime along with poultry manure at 5 t/ha was the optimum treatment combination for mitigating arsenic uptake in rice grains. Other water regimes and a higher dose of poultry manure might promote the uptake of arsenic despite having some positive effects in terms of growth parameters.

Chapter One
Introduction

1. INTRODUCTION

Arsenic is a non-threshold class (I) carcinogen with a linear dose-response for chronic low-level exposure (Smith et al., 2003). Over the last few decades, consumption of arsenic-contaminated water caused numerous diseases (or even deaths) to millions of people around the world (Bundschuh et al., 2012). Consumption of rice is an important route through which arsenic finds its way into the human body. Thus, rice consumption is a very important dietary exposure for people who consume rice as their principal food. Consequently, rice consumption constitutes a considerable risk factor for cancer in humans.

Previous studies revealed rice to be an efficient crop in accumulating arsenic in comparison with other cereal crops (Mondal and Polya, 2008). Traditional paddy rice cultivation practices that involve flooding soils from the time of crop establishment to almost harvest time, in an arsenic-contaminated environment may lead to enhanced accumulation of inorganic arsenic in rice grains (Kogel-Knabner et al., 2010). Submerged soil conditions influence the redox chemistry in the paddy environment and thereby enhance the bioavailability of arsenic in the rice rhizosphere for uptake by rice plants (Awad et al., 2018). Other factors that influence the arsenic dynamics in soil (mobility, bioavailability, and speciation of arsenic) include dissolved organic matter, redox-sensitive elements (Fe, Mn, S, and N), the formation of root plaque, competitive ions/compounds (phosphate (PO_4^{3-}) and silicic acid ($Si(OH)_4$)), and the activity of microorganisms (Kumarithilaka et al., 2020). Arsenite (As(III)) and arsenate (As(V)), monomethylarsonic acid (MMA(V)) and dimethylarsinic acid (DMA(V)) are the most commonly found inorganic and organic arsenic species in the paddy agroecosystems, respectively. A number of transporters are associated with the uptake, translocation and grain filling of different arsenic species in rice plants (Tiwari et al., 2014).

In Bangladesh, winter rice or Boro season rice which is dependent on artificial irrigation constitutes 55% of the total rice production. Currently, around 70% of the arable land of the country is irrigated using shallow tubewells. A good number of these shallow tubewells are contaminated with arsenic. Therefore, people residing in the arsenic-affected areas are being exposed to arsenic directly via drinking water and indirectly via the consumption of rice (Panaullah et al., 2009).

Arsenic contaminated soil or water pose risk for food security. Paddy-rice grains contain higher concentrations of arsenic compared to upland rice. The higher concentration of arsenic is attributed to the higher availability of arsenic under puddled conditions. The grain in Bangladesh paddy rice was reported to contain as high as 1.8 mg As/kg (Meharg and Rahman, 2003). Other studies with Bangladesh brown rice reported grain As concentrations ranging from 0.18 to 0.29 mg As/kg (Williams et al., 2006; Zavala and Duxbury, 2008). Assuming that an adult consumes 400 g dry wt rice every day and the grain As concentration is 0.25 mg As/kg, the average daily intake of As by a Bangladeshi adult was computed to be ~100 μg (400 g dry wt × 0.25 mg As/kg) (Panaullah et al., 2009).

To produce safer rice grains with reduced arsenic accumulation, the adoption of mitigation measures is vital in the arsenic-contaminated paddy agroecosystems. Over the last few decades, many researchers have studied the efficacy and feasibility of different mitigation methods to reduce the arsenic content of rice grains. Water management has been reported to influence arsenic content in rice grains. Previous studies revealed that accumulation of arsenic in rice grains can decrease (as much as 10- to 15-fold lower) strikingly under continuous oxic growing conditions than under continuously flooded (CF), or anoxic conditions (Hua et al., 2011). Both DMA and iAs (but to a lesser extent) accumulation can be reduced under oxic conditions compared to anoxic growing conditions (Hu et al., 2015). Inorganic As accounts for a smaller percentage of total As in grain under the anoxic treatment than from the oxic treatment. Nevertheless, the concentration of As in rice under oxic conditions is still less (1.1 to 2.9-fold from different studies) than under anoxic conditions (Li et al., 2009). However, a substantial yield loss has also been observed under sustained oxic conditions (Hu et al., 2015). The loss in yield was ascribed to the build-up of nematodes, soil pathogens, and increased weed pressure (Yamaguchi et al., 2014).

The addition of organic matter is recommended for arable soil. Organic matter plays many important roles in soil. For example, it can improve the soil structure and can act as a reservoir of key elements such as nitrogen, phosphorus, and sulphur (Batey, 1988). Organic matter has a major role in the mobilization of arsenic from paddy fields (Sharma et al., 2011; Williams et al., 2011). Microbes utilizing the organic matter consume oxygen that leads to a decrease in redox potential, which in turn leads to arsenic dissolution from FeOOH (Norton et al., 2013). Organic matter may also affect arsenic availability in soils by desorbing arsenic species from soil micelle (Weng et al., 2009). Dissolved organic matter (DOM) also influences the mobility of arsenic by complexing arsenic species (Liu et al., 2011).

Few studies have investigated the combined effect of water management and the application of organic matter to soil on arsenic concentrations in rice root, straw, husk, and grain. In view of this fact, an investigation was undertaken to observe the effects of different water regimes and different types of organic matter in the form of cow dung and poultry manure. The broader goal is to observe whether water regime and organic amendments could mitigate the accumulation of arsenic by rice crop. The specific objectives were as follows:

1. To determine the main effects of three water regimes on the accumulation of arsenic in the root, straw, husk, and grain of BRRI dhan 28,
2. To determine the main effects of two types of organic matter such as cow dung and poultry manure on the accumulation of arsenic in the root, straw, husk, and grain of BRRI dhan 28,
3. To determine the interaction effects of water regimes and organic matter on the accumulation of arsenic in the root, straw, husk, and grain of BRRI dhan 28,
4. To determine the effects of three water regimes on the accumulation of phosphorus and iron in the root, straw, husk, and grain of BRRI dhan 28, and
5. To determine the effects of two types of organic matter such as cow dung and poultry manure on the accumulation of phosphorus and iron in root, straw, husk, and grain of BRRI dhan 28.

Chapter Two
Literature and Review

2. LITERATURE REVIEW

2.1. Background

Arsenic (As) is a metalloid element that is omnipresent and toxic to plants and animals. Arsenic used to be called a terminator of life. It is naturally present in the earth's crust and the average concentration of arsenic is 1.8 mg/kg (Ravenscroft et al., 2009). Arsenic is the 20th most abundant element in Earth's crust, 14th most abundant in the sea, and the 12th in the human body. (Flora, 2015; Mandal and Suzuki, 2002). Arsenic is mobilized through biogeochemical cycles. A combination of natural and anthropogenic processes drives the mobilization of arsenic in nature. The natural processes include weathering reactions, biological activities, and volcanic activities. On the other hand, anthropogenic activities include gold mining, nonferrous smelting, petroleum refining, combustion of fossil fuel in power plants, and the use of arsenical pesticides and herbicides (Biswas et al., 2008; Violante et al., 2006).

Arsenic has been used as a poison and curative since ancient times. It has also been used in pyrotechnics and metallurgy. Arsenic trioxide was employed in chemical warfare, and copper acetoacetate in wallpapers as a pigment (Nriagu, 2002). Interestingly, many fatal diseases were treated by arsenic in the past and the use of arsenic as a medicinal agent is common in modern times as well. Arsenic trioxide is a potent arsenical that is used as an effective cancer chemotherapeutic agent (Hughes, 2016). Arsenic compounds are very useful for wood preservation. Many arsenic compounds are used as pesticides in agriculture. Despite being toxic to humans at a certain concentration, arsenic is an essential element for human physiology. Humans require 0.00001% arsenic for growth and a healthy nervous system. Pure arsenic is less toxic for humans because the human body cannot readily absorb it. On the other hand, arsenite compounds such as arsine (AsH_3) and arsenic trioxide (As_2O_3) are readily absorbed and can cause cancers at high toxicity.

Consumption of arsenic-contaminated water is the main route through which humans are exposed to arsenic. Other routes included are inhalation and skin absorption. However, these two routes are minor routes compared to the consumption of arsenic-contaminated water (Shi et al., 2004). If arsenic is consumed via drinking water that will lead to carcinogenesis of all vital organs; it also causes skin diseases such as hyperkeratosis. Consequently, different internal cancers and skin cancers ensue. To sum up, arsenic causes irreversible damage to all the vital organs (Bhattacharya, 2017).

Arsenic contaminated groundwater is a hazard for humans all over the world. Bangladesh is one of the countries which is severely affected by the natural source of arsenic. Arsenic contamination of groundwater is regarded as one of the most important disasters in the world. Arsenic from local geologic deposits found its way into groundwater supply and thereby posed a hazard to many populations in Bangladesh and some other countries. Groundwater constitutes the primary source of drinking water in Bangladesh. It is the case for most of the countries of the world. From an estimation, one-third of the world's population consumes groundwater (UNEP, 2000). On the other hand, ~90% population of Bangladesh relies on groundwater for their drinking water purposes. This is because the surface water in the country is unsafe because of the presence of microorganisms. (BGS and DPHE, 2001). The arsenic problem further aggravates because, in the dry season, the arsenic-contaminated groundwater is utilized for irrigation purposes, which brings arsenic to soil and finally to crops. As such, the bulk of the abstracted water (~95%) is utilized for irrigation purposes in Bangladesh. (Heikens, 2006). The arsenic situation in Bangladesh is almost all-pervasive. A vast area of the country's groundwater is contaminated with arsenic, the concentration ranging from less than 1 to 1,500 $\mu g\ L^{-1}$. In some places, the concentration reaches as high as 2000 $\mu g\ L^{-1}$. (BGS and DPHE, 2001; Tondel et al., 1999). From a study in Bangladesh, ~46% of the groundwater was found to exceed the value (0.01 $mg\ L^{-1}$) set by the WHO whereas ~27% exceeded the value (0.05 $mg\ L^{-1}$) set by the Bangladesh drinking water guideline (BGS and DPHE, 2001; Smedley, 2003). As for Bangladesh soils, the arsenic concentrations were found to vary from 0.3 to 49 $mg\ kg^{-1}$, from below detection limit to 56.7 mg kg^{-1}, from <10 to 46 mg kg^{-1}, and from 3.2 to 27.5 mg kg^{-1} in areas with low arsenic concentrations in irrigation water as reported by Islam et al (2005), Alam and Sattar (2000), Meharg and Rahman (2003) and Imamul Huq et al (2006), respectively. On the other hand, the areas having no arsenic in irrigation water were found to contain less soil arsenic, the concentration ranging from 0.10 to 2.75 mg kg^{-1} (Imamul Huq et al., 2006).

Arsenic present in irrigation water may lead to loss of yield in rice and other crops, including vegetables. The food chain is also contaminated with a higher level of arsenic in crops and vegetables. The fact that the food chain is contaminated has implications as human health is associated (Brammer, 2005; Burló et al., 1999; Carbonell-Barrachina et al., 1999a; Duxbury and Zavala, 2005; Imamul Huq and Naidu, 2005; Imamul Huq et al., 2001, 2006; Meharg and Rahman, 2003). The higher arsenic concentration in irrigation water causes an accumulation of arsenic in the receiving soil (Ali, 2003; Heikens, 2006; Meharg and Rahman, 2003).

2.2. Geochemistry of Arsenic

Arsenic is a metalloid that has a chemical symbol of As and an atomic number of 33. It occurs in sedimentary, igneous, and metamorphic rocks. It is naturally found with 200 mineral compounds. The average concentration of arsenic is 1.7 mg kg^{-1} in the earth's crust. (Robinson and Ayotte, 2006). The concentration can be very high (400 mg kg^{-1}) in sedimentary iron ores (ISSI Consulting Group et al., 2000).

Arsenic is present in the soil profile in the form of minerals, and it is virtually nonexistent in all organisms. Arsenic can occur in compounds such as oxygen, chlorine, sulfur, carbon, hydrogen, lead, mercury, gold, and iron. Despite there being as many as 150 arsenic-bearing minerals, only a handful of them are regarded as arsenic ores. Arsenic ores are high in arsenic contents. Some arsenic compounds included are realgar or arsenic disulphide (AsS), orpiment or arsenic trisulphide (As_2S_3), and arsenopyrite or ferrous arsenic sulphide (FeAsS). Arsenopyrite was found to be the main culprit for arsenic pollution in Bangladesh. Figure 2.1 represents the arsenic cycle in soil-water-plant interfaces.

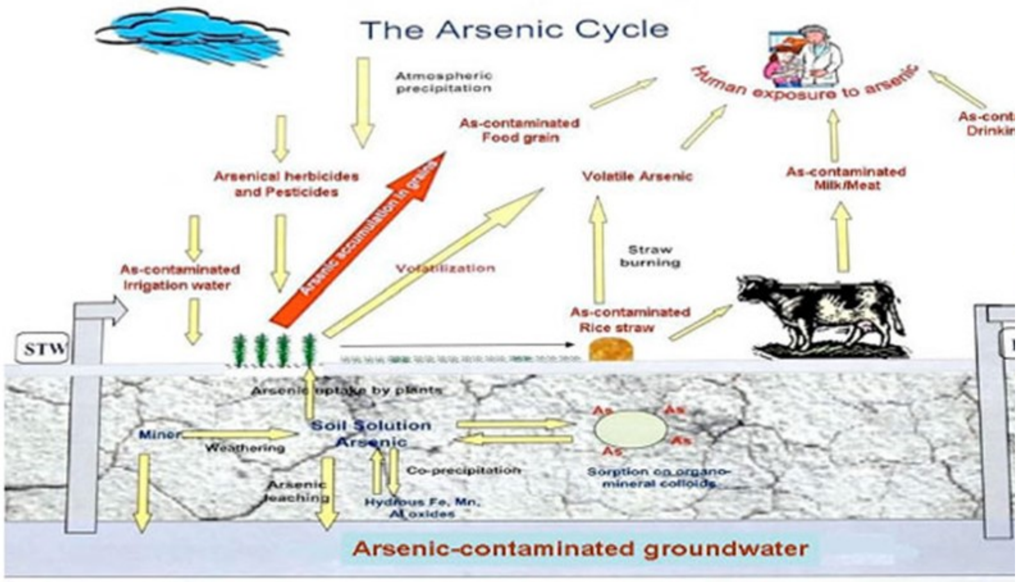

Figure 2.1. The Arsenic cycle in soil-water-plant interfaces (Adopted from DPHE, 2000).

2.2.1. Forms of Arsenic in Soil

Arsenic can occur in the environment in five different valence states, namely arsine (-3), elemental As (0), arsonium metals (+1), arsenites (+3), and arsenates (+5). Arsenite and arsenate are the two common species found in nature whereas elemental arsenic is seldom found. Arsenic occurs in inorganic and organic forms. Arsenic in organic forms contains carbon. Organic arsenic can occur in water, natural gas, and shale oil. It is present in the human body because of enzymatic activities present in the liver (ISSI Consulting Group et al., 2000). Organic arsenic can be present in the following forms: monomethylarsonic acid, $CH_3AsO(OH)_2$; dimethylarsinic acid $(CH_3)_2AsO(OH)$; trimethylarsine oxide, $(CH_3)_3AsO$. Inorganic arsenic is prevalent in almost all rocks and many minerals. The most widespread minerals are arsenopyrite (FeAsS), orpiment (As_2S_3), and realgar (As_4S_4). Arsenopyrite occurs in high-temperature veins, which is mainly found common in tin and tungsten ores. On the other hand, orpiment and realgar are found together in lead and silver deposits (ISSI Consulting Group et al., 2000).

There are several factors that control the forms of arsenic in the soil. The type and amount of sorbing components of the soil, the pH, and the redox potential govern which species will predominate the soil solution. (Nriagu, 1994). The chemical form of arsenic determines the availability and toxicity of arsenic to organisms (Webb, 1966). For example, arsenic in trivalent form is much more toxic, soluble, and mobile than arsenic in pentavalent form.

2.2.2. Adsorption of Arsenic in Soil

Despite being readily soluble, arsenic migration from arsenic minerals and compounds is greatly diminished owing to the strong sorption by clays, hydroxides, and organic matter. The reaction of arsenic is highly governed by its oxidized state. Arsenate ions for example are readily fixed by such soil components as clays, phosphatic gels, humus, and calcium. Hydrated iron and aluminum oxides are the most active player in arsenic retention (Huang, 1975). In soils, arsenic (both natural and added) was found to be strongly associated with iron (mainly goethite) as reported by Norrish (1975).

2.3. Sources of Arsenic

Arsenic is omnipresent in the environment. It is found in the atmosphere, biosphere, hydrosphere, pedosphere, and it moves from one to another sphere by natural processes or anthropogenic activities such as mining, agriculture, industrial processes, etc. (Kannan, 1997).

Large concentrations of arsenic in soils are due to both natural and anthropogenic processes. Both natural phenomena and human activities play roles in the emission of arsenic into the atmosphere. Natural phenomena include weathering, biological activity, and volcanic activity. In Bangladesh, the arsenic problem is unique. Several hypotheses were initially proposed for the As-enrichment problem in the groundwater of Bangladesh. They are as follows: (1) reductive dissolution of iron-oxyhydroxides which frees sorbed As, (2) oxidative dissolution of As-rich pyrite, (3) anion exchange of sorbed As by an augmented concentration of phosphate (PO_4^{3-}) from applied fertilizers. Now it is widely accepted that microbially mediated reductive dissolution of Fe(III) oxyhydroxides (hypothesis 1) under moderate to strong reducing conditions is the chief mechanism to release As in groundwater of the Holocene deltaic aquifers (Ahmed et al. 2004; Bibi, Ahmed, and Ishiga 2008; Maity et al. 2011). The microbially-mediated processes are facilitated by electron donors, e.g. organic matter and/or dissolved organic carbon (DOC). The distribution of arsenic in Bangladesh groundwater is closely related to the major geomorphological units, namely Tertiary hills, Pleistocene uplands, and Holocene plains. The As enrichment is mainly restricted to the Holocene alluvial aquifers as shallow and intermediate depths (Ahmed et al. 2004).

2.3.1. Natural Sources

Arsenic is found in soil and almost all other environmental matrices in detectable concentrations. Arsenic is ranked 20th within the earth's crust and ranked 14th in seawater. The concentration of arsenic in the continental crust of the earth is usually between 1.5 and 2.0 mg/kg. It is a major constituent of more than 245 arsenic-containing minerals and is found in high concentrations in sulfide deposits (Adriano, 1986). Arsenic is distributed rather uniformly in major types of rocks and its common concentrations in most of the rocks range from 0.5 to 2.5 mg/kg. Only in argillaceous sediments, arsenic concentration can go to as high as 13 mg/kg (Kabata-Pendias and Pendias, 1994).

Parent materials are the chief source of arsenic in the soil. The native As content varies significantly within an area and is often governed by the geological history of the area (Wild, 1993). Natural phenomena such as weathering, biological activity together with anthropogenic inputs are the emission sources of arsenic in the atmosphere. From the atmosphere, arsenic is redistributed on the earth's surface by rain and dry fallout.

2.3.2. Anthropogenic Sources

Arsenic can be added to soil, water, and atmosphere due to anthropogenic or human activities. Soil contamination with arsenic has increased as a result of the following factors: 1) Mining and smelting of metals, 2) Coal combustion, 3) Pesticides, 4) Feed additives, 5) Wood preservation, 6) Sewage sludge, and 7) Irrigation (Chilvers and Peterson, 1987). Chilvers and Peterson (1987) pointed out that global emission to the atmosphere from copper smelters is higher than other sources followed by coal combustion. These are the indirect contributions of arsenic to the land and terrestrial waters, whereas a significant amount of arsenic is being added to the soil from the direct dumping of spent sludge, sewage sludge, and industrial wastes. Uses of insecticides, animal dip, feed additives, wood preservatives, etc. cause soil and water contamination.

2.4. Arsenic Contamination in Bangladesh

2.4.1. Arsenic in Soil

Weathering of As-containing rocks leads to the addition of As to soils and sediments. The main source of As in soils is the parent materials from which the soil is derived (Yan-Chu, 1994). The geological history of a region determines the native As content of an area (Wild, 1993). The concentration of As in sediments is largely dependent on source rock. Sediments derived from volcanic rocks generally have higher As concentrations. The As concentration in soil normally varies from 0.1 to 40 mg/kg. Extremely high As concentration of up to 8000 mg/kg can occur in soils associated with sulfidic ores (ISSI Consulting Group et al., 2000). Anthropogenic As compounds used in agriculture, industry, and wood preservation are the other sources of As in soil and sediment. Arsenic in rock, soil, and sediment eventually makes it into the ground and surface water where it can be found in both the arsenite state (As^{+3}) and the arsenate state (As^{+5}) (Deuel and Swoboda, 1972; Walsh and Keeney, 1975). Arsenate is found in oxidizing conditions while arsenite is found in sufficiently reducing conditions (ISSI Consulting Group et al., 2000).

Arsenic is naturally present in soil all over the world, with a concentration that varies depending on the origin of the soil (Matschullat, 2000). The distribution of As in soils may vary with soil type, depending on the nature of the parent material. The background As concentration in soil is approximately 5 mg/kg (Dudas and Pawluk, 1980; Mandal and Suzuki, 2002). Background concentration does not generally exceed 15 mg As/kg (NRCC, 1978), although concentration ranging from 0.2 to 40 mg As/kg soil

(Walsh et al., 1977) and ranged from 8 to 40 mg As/kg (Dudas, 1987) have also been reported.

Soils As concentration ranging between 0.1 to 10 mg/kg are considered as non-contaminated soils (Kabata-Pendias and Pendias, 1992; Walsh and Keeney, 1975). The soil As concentration in Bangladesh is higher than this value and it varies depending on the location. The average As concentration in the soil of Bangladesh is 12.3 mg/kg. Numerous studies documented different As concentration ranges in Bangladesh soil, e.g. from 0.3 to 49 mg/kg (Islam et al., 2005); from below the detection limit to 56.7 mg/kg (Alam and Sattar, 2000); from less than 10 mg/kg to 46 mg/kg in areas with a low concentration of As in the irrigation water (Meharg and Rahman, 2003); from 3.2 to 27.5 mg/kg (Imamul Huq et al., 2006); and from 7.3 to 27.3 mg/kg with an average of 15.7 ± 6.6 mg/kg (Das et al., 2004). In areas where irrigation water did not contain As, the soil As concentration varied from 0.10 to 2.75 mg/kg (Imamul Huq et al., 2006).

2.4.2. Arsenic in Plants

In general, the As content in plants varied considerably with the type of plants, types of soil it was grown in, and the As content of irrigation water. Plants grown in As affected areas had higher concentrations of As than that was grown in unaffected areas. It was reported that the As content in rice and wheat was mostly concentrated in roots and straw. The As content of rice grain samples collected from various districts of Bangladesh varied from below detection limit to >1 mg/kg. The concentration of As in rice roots ranged from less than 1 to 267 mg/kg, while the range was from less than 1 to 30 mg/kg in rice straw. The values of wheat ranged from 0.5 to 1 mg/kg in grain, from 0.2 to 30 mg/kg in straw, and from 1.5 to 3 mg/kg in the root (Imamul Huq et al., 2006).

The mean As level in the grains of Bangladesh rice was 0.13 (range 0.03-0.30) mg/kg (Williams et al., 2005). Other investigators have also reported similar results (Abedin et al., 2002; Alam and Rahman, 2003; Hironaka and Ahmad, 2003; Meharg and Rahman, 2003). The As content in rice grain varied according to the type and the area where it was grown. Abedin and

Meharg (2002) exposed eight Bangladesh rice varieties to As(III) and As(V) and tested for germination and seedling growth in a hydroponic study. Germination was slightly inhibited at 0.5 and 1 mg/L. At 2 mg/L, inhibition was more than 10 percent. As(III) was found to be more toxic than As(V). Root growth was inhibited by ~20 percent at 0.5 mg/L and As(V) was found to be more toxic than As(III). The shoot height was reduced by ~30 percent at 0.5 mg/L, with no significant difference between As species. In a study conducted by Onken and Hossner (1995), a silt loam soil spiked with 25 mg/kg As(III) or As(V) caused a reduced dry matter in rice after 40 days of exposure. The reduction was ~50 percent after 60 days of exposure with no significant difference between As(V) and As(III). In the clayey soil, no toxicity was observed, suggesting that a greater part of the added As was strongly bound to the soil (Onken and Hossner, 1995). Jahiruddin et al. (2004) spiked silt loam soil with As and observed a grain yield reduction of more than 45 percent at 10 mg/kg soil. Abedin et al. (2002) exposed rice cultivar BRRI dhan 11 to As(V) and studied the growth and As uptake. They observed reduced root biomass at 0.2 mg/L. Other effects including a reduction in plant height, spikelet weight, number of spikelets, and grain yield started at 2 mg/L. In an almost similar experiment, reduced root biomass, grain number, and grain weight (g/pot; 26% reduction) was found at ≥1 mg/L (Abedin et al., 2002). Smith et al. (1998) reported that rice, bean, oats can suffer from phytotoxicity at a soil concentration of 20 mg/kg, whereas this value is 100 mg/kg for maize and radish.

Williams et al. (2006) collected a large number of samples (rice: 330, vegetables: 94, pulses and spices: 50) from entire Bangladesh and showed that there was a positive relationship between As levels in rice and As levels in groundwater. *Boro* (dry season) rice contained significantly more As than aman rice (rainy season). The variation was explained by the fact that aman rice was mainly rain-fed, while *Boro* rice was irrigated with groundwater containing As. Of various crops/vegetables analyzed in Bangladesh, As contents were found to range from 8 mg/kg (gourd) to 158 mg/kg (arum) (Imamul Huq et al., 2006). The concentration of As in all plant parts increased with the exposure concentration. This is a common observation for other plants as well (Bleeker et al., 2003; Carbonell-Barrachina et al., 1998; Carbonell-Barrachina et al., 1997, 1998; Hartley-Whitaker et al., 2001; Sneller et al., 1999).

2.4.3. Arsenic in Groundwater

Bangladesh is situated in the Ganges-Brahmaputra delta region. It is a densely populated country with approximately 160 million people and primarily an agricultural economy. Before 1970, surface water was the primary source of drinking water in Bangladesh. However, contamination caused many cases of waterborne diseases with high mortality, notably cholera. To prevent this, in the 1970s tube-wells were installed in Bangladesh to access shallow (10-50 meters) groundwater as an alternate drinking water supply (Mead, 2005; Smith et al., 2000).

In the mid-1980s, patients from Bangladesh were showing the characteristic skin lesions that appear from chronic exposure to high As concentration. In the early 1990s, the groundwater of regions of Bangladesh was tested for As (Smith et al., 2000). A high concentration of As was found, ranging from less than 1 μg/L to greater than 300 μg/L (BGS and DPHE, 2001; Mead, 2005). Out of the over 8 million tube-wells in Bangladesh, more than half were tested for As. It was estimated that about 20% of the tube-wells had unsafe levels of As over 50 μg/L, the national standard for Bangladesh. It was estimated that over 35 million people are exposed to contaminated drinking water with a concentration of 50 μg/L or higher and 57 million people were exposed to drinking water with a concentration greater than 10 μg/L (BGS and DPHE, 2001; Smith et al., 2000).

Various survey and research studies reported different ranges of As concentration in the irrigation water. Imamul Huq et al. (2006) reported that As concentration of the irrigation water varied from 0.14–0.55 mg/L. Another study showed that 87% of irrigation deep tube-wells (DTWs) contained As concentration of more than 0.05 mg/L and the average As concentration in those DTWs was 0.21 mg/L (JICA/AAN, 2004). Ross et al. (2005) estimated that 76% of the *Boro* (dry season) rice is grown in areas where shallow tube-wells (STWs) usually contain less than 0.05 mg/L, 17%

in areas with 0.05–0.10 mg/L, and 7% in areas with more than 0.10 mg/L. The concentration of As exceeding 1.0 mg/L in STWs was also reported from 17 districts in Bangladesh (Ahmad et al., 2006). Besides, over 10,000 people have shown evidence of arsenicosis with this number expected to rise (WHO, 2001).

In the last three decades, the number of STWs has increased abruptly in Bangladesh. The groundwater from these STWs is the main source of drinking water because it is an inexpensive source of drinking water and is mostly free of waterborne diseases. The shallow aquifer is also the main source of irrigation water during the dry season cultivation. These STWs are providing a reliable and inexpensive source of irrigation water, which allows farmers to grow additional crops during the dry season and ensures water security during periods of drought. Approximately, 95% of all groundwater extracted is used for irrigation, mainly for *Boro* rice production in the dry season (Heikens, 2006).

In Bangladesh, alluvial Ganges aquifers used for public water supply are polluted with naturally occurring arsenic, which is posing constant risks to the health of millions of people. As many as a million water wells drilled into Ganges alluvial deposits in Bangladesh are contaminated with arsenic. The arsenic concentration was found to be as high as 1,000 μg/l, which is above the limit set for drinking water in Bangladesh (50 μg/l) or that recommended by the World Health Organization (10 μg/l) (Nickson et al., 1998). Consumption of this contaminated water has resulted in widespread death and disease. Earlier, it was reported that arsenic was derived from the oxidation of arsenic-rich pyrite in the aquifer sediments as atmospheric oxygen invades the aquifer in response to a lowering of the water level by abstraction (Nickson et al., 1998). (Nickson et al., 1998) proposed a different hypothesis with respect to the mobilization of arsenic in the groundwater of Bangladesh. The arsenic derives from the reduction of arsenic-rich iron oxyhydroxides in anoxic groundwater. The arsenic-rich iron oxyhydroxides originated from weathering of base-metal sulphides (Nickson et al., 1998). The present situation of As contamination in the groundwater of Bangladesh can easily be understood from **Figure 2.2.**

The arsenic-rich groundwater is primarily confined to the alluvial aquifers of the Ganges delta. Therefore, it is logical that the source of arsenic-rich iron oxyhydroxides is present along the Ganges source region upstream of Bangladesh. The Ganges basin possesses weathered base-metal deposits. During Late Pleistocene-Recent times, the arsenic-laden base-metal sulphides underwent weathering thereby furnishing arsenic-rich iron oxyhydroxide to downstream Ganges sediments. Now, the arsenic-rich iron oxyhydroxides are being reduced, giving rise to the present problem. Sedimentary organic matter, which concentration is as high as up to 6%, is governing the reduction process (Nickson et al., 1998).

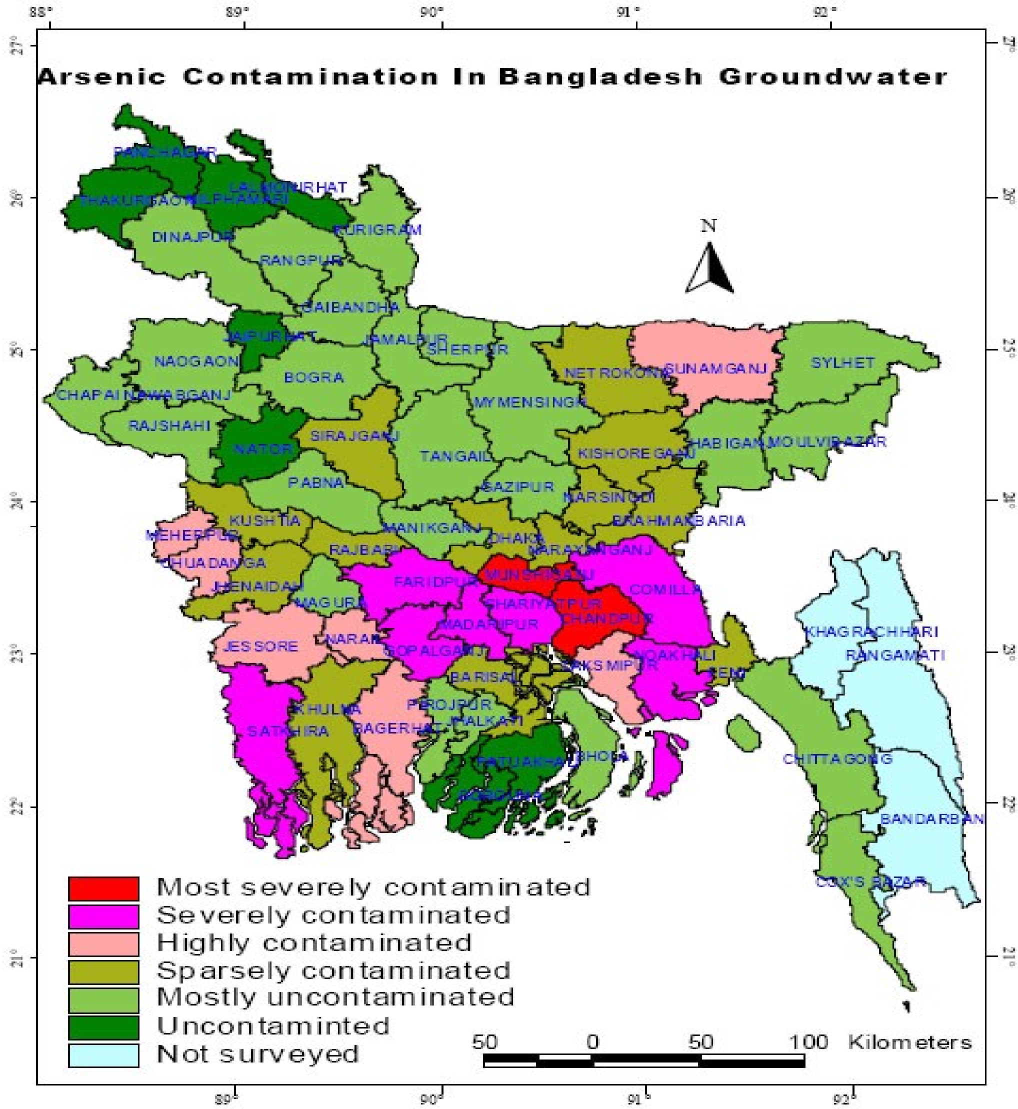

Figure 2.2. Map of Arsenic Contamination in Bangladesh Ground Water (Banglapedia, 2004).

2.5. Extent and Severity of Arsenic Contamination in Bangladesh

Groundwater arsenic contamination in Bangladesh is one of the biggest environmental disasters in the world. Between the 1970s and 1980s, the Bangladesh government with the support of international agencies installed tubewells in the rural areas of the country to provide pathogen-free water to people who had earlier been suffering from water-borne diseases because of the consumption of pathogen-infested surface water (Atkins, Hassan, and Dunn 2007). Approximately, 97% of people are believed to have access to pathogen-free water because of the mass installation of around 12 million tube wells. The arsenic contamination was first confirmed by health professionals when they received chronic arsenicosis patients for the first time in 1995. In 1996, arsenic contamination was confirmed in only 7 districts. By mid-1997, the number of districts increased to 48. Now, the number of districts stands at 61 where arsenic was found at elevated concentrations ($\geq$ 50 µg/L). In a relatively recent survey, 85 million people are drinking water having an arsenic concentration of >50 µg/L (UNICEF, 2008). Therefore, arsenic pollution is a serious health hazard to people all over the country and it is predicted that millions of people may die from consuming arsenic-contaminated water.

2.6. Health Effects of As

Arsenic occurs in inorganic and organic forms. Inorganic As compounds (such as those found in water) are highly toxic while organic arsenic compounds (such as those found in seafood) are less harmful to health.

2.6.1. Acute Effects

The immediate symptoms of acute arsenic poisoning include vomiting, abdominal pain, and diarrhea. These are followed by numbness and tingling of the extremities, muscle cramping, and eventually death, in extreme cases. In survivors, bone marrow depression, haemolysis, hepatomegaly, melanosis, polyneuropathy, and encephalopathy may be observed (WHO, 2001).

2.6.2. Long-term Effects

The first symptoms of long-term exposure to high levels of inorganic arsenic (e.g. through drinking water and food) are usually observed in the skin and include pigmentation changes, skin lesions, and hard patches on the palms and soles of the feet (hyperkeratosis) (**Figure 2.3**). These occur after a minimum exposure of approximately five years and maybe a precursor to skin cancer (WHO, 2001). In addition to skin cancer, long-term exposure to arsenic may also cause cancers of the bladder and lungs. Occupational exposure to arsenic, primarily by inhalation, is causally associated with lung cancer. The International Agency for Research on Cancer (IARC) categorized arsenic and arsenic compounds as carcinogenic to humans and also stated that arsenic in drinking water is carcinogenic to humans.

Figure 2.3. Different skin diseases due to long term exposure: (a) melanosis of the chest, (b) leuco-melanosis, (c) keratosis of the palms, and (d) keratosis of the feet.

There are some other adverse health effects related to long-term ingestion of inorganic arsenic. Health effects include developmental effects, neurotoxicity, diabetes, and cardiovascular disease. Chronic arsenic exposure was also found to cause 'Blackfoot disease' (BFD) in Taiwan. The blackfoot disease is a severe form of peripheral vascular disease (PVD) that results in gangrenous changes. Malnutrition is believed to contribute to its development. Arsenic exposure is likely to cause other forms of PVD (WHO, 2001). The causality of the relationship between arsenic exposure and other health effects are less clear-cut. The evidence is the strongest for hypertension and cardiovascular disease, suggestive for diabetes and reproductive effects, and weak for cerebrovascular disease, long term neurological effects, and cancer at sites other than lung, bladder, kidney, and skin.

2.7. Cyclic Transfer of Arsenic

Most anthropogenic input of arsenic comes from smelting operations and fossil-fuel combustion However, the extent to which anthropogenic activities contribute to the overall Arsenic cycle is yet to be determined (Bhumbla and Keefer, 1994). Arsenic is found in the natural reservoir, such as the ocean, soil, and atmosphere. Soils and water are additionally loaded with arsenic stemming from modern industry, mining operations, agriculture, forestry, and manufacturing and disposal of municipal and industrial wastes. The environmental cycle of Arsenic is shown in **Figure 2.4**.

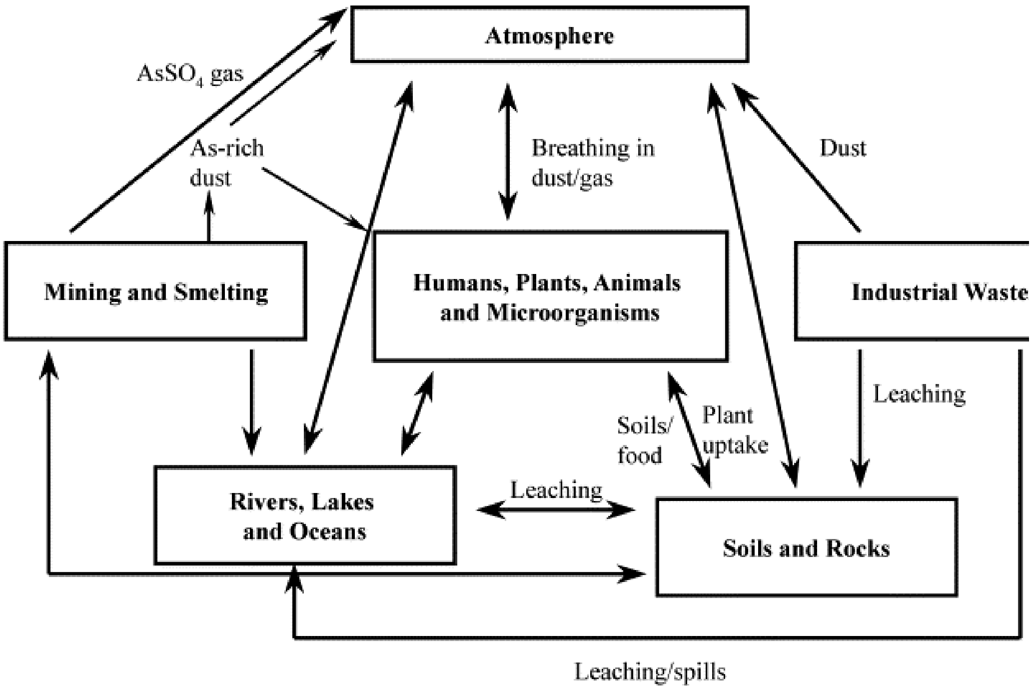

Figure 2.4. The environmental cycle of arsenic (Langdon et al., 2003).

2.7.1. Arsenic Transfer through Food Chain

The elevated concentration of arsenic in irrigation water and topsoil in the root zone increases the likelihood of an increased concentration of arsenic in different parts of plant and food grains. The highest concentration of arsenic was found in the roots of the rice plant, whereas a lower concentration in stem and leaves and the lowest concentration were found in rice grains (Ahmed, 2003). On the other hand, a relatively higher concentration of arsenic was found in leafy vegetables. For instance, the concentration of arsenic in arum *(Colocasia indica)*, which is a popular vegetable in Bangladesh, was found to be ~20.0 mg/kg (Huq et al., 2001). Several other crop plant species (rice, elephant foot yam, green gram, etc.) are also reported to accumulate arsenic in significant amounts. These results suggest that ingestion of food with higher arsenic content could be an important route for human exposure. Organic forms of arsenic in foods are less toxic compared to inorganic forms and most of them are excreted rapidly. In the arsenic-affected areas, it may enter the food chain from water to soil, and from soil to different plant parts such as roots, tubers, leaves, fruits, seeds, etc. In Bangladesh, arsenic is finding its way into rice, Bangladesh's staple crop, through irrigation water pumped from contaminated soils (Meharg and Rahman, 2002). It can be seen from **Figure 2.5** that humans can be exposed to arsenic ingestion in many ways including not only through drinking but also through the food chain.

The arsenic concentration of rice produced in the arsenic-contaminated area of Bangladesh is about 0.3 mg/kg, which is 2 to 3 times higher than that produced in unaffected areas (Hironaka and Ahmed, 2003). The content and range were found higher in the vegetables grown with arsenic-contaminated irrigation water than those grown with arsenic-free water (Farid et al., 2003). The highest concentration of arsenic was always recorded in the plant roots and this may be attributed to contamination from fine colloidal particles adhering to plant roots (Huq et al., 2001). Many crops receiving arsenic-contaminated irrigation water were found to accumulate arsenic at levels that exceed the maximum allowable daily limit (MADL) of 0.2 mg/kg dry weight (dw) by a person (Huq and Naidu, 2005).

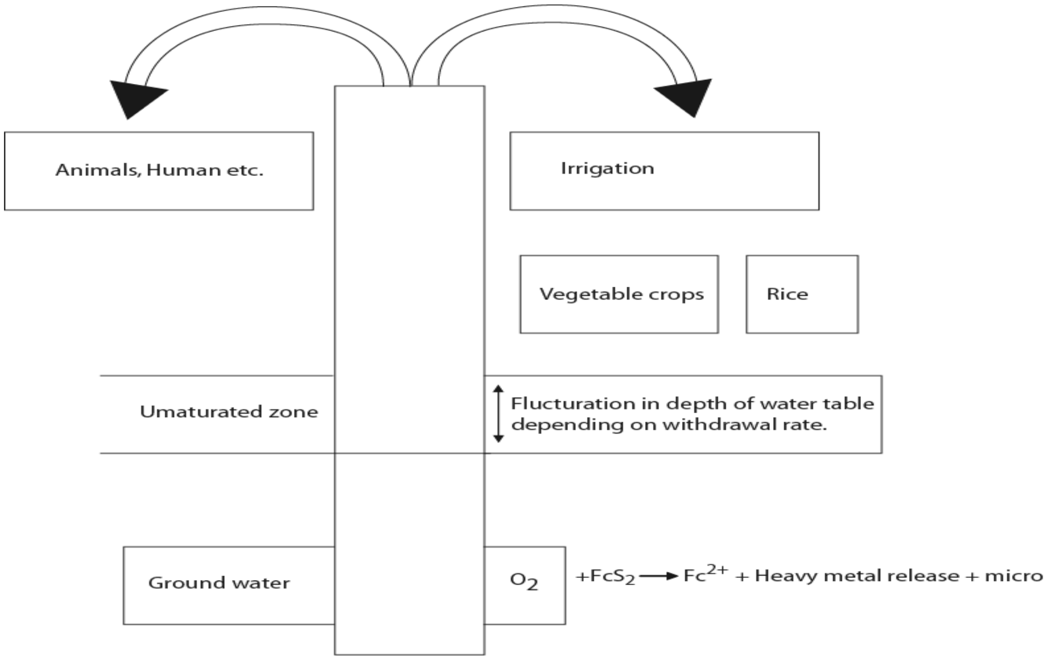

Figure 2.5. Humans can be exposed to arsenic through different pathways in nature (Huq and Naidu, 2003).

2.8. Fate of Arsenic in Soil-Water-Plant Environment

Understanding the fate of arsenic extracted through tube well water in soil-water-plant environment is vital for evaluating its impacts on the food chain and the environment in general. **Figure 2.6** schematically shows the fate of arsenic, extracted via groundwater, in the soil-water-plant environment. The arsenic pumped with tube well water can (i) undergo transformation (e.g., through redox or microbial processes), (ii) volatilize into the atmosphere as a result of the different biological transformation, (iii) undergo adsorption-desorption and thus become retained onto the soil, washed away by surface runoff or leached into the groundwater, and (iv) be taken up by plants and subsequently enter into the food chain (Mclaren et al., 2001).

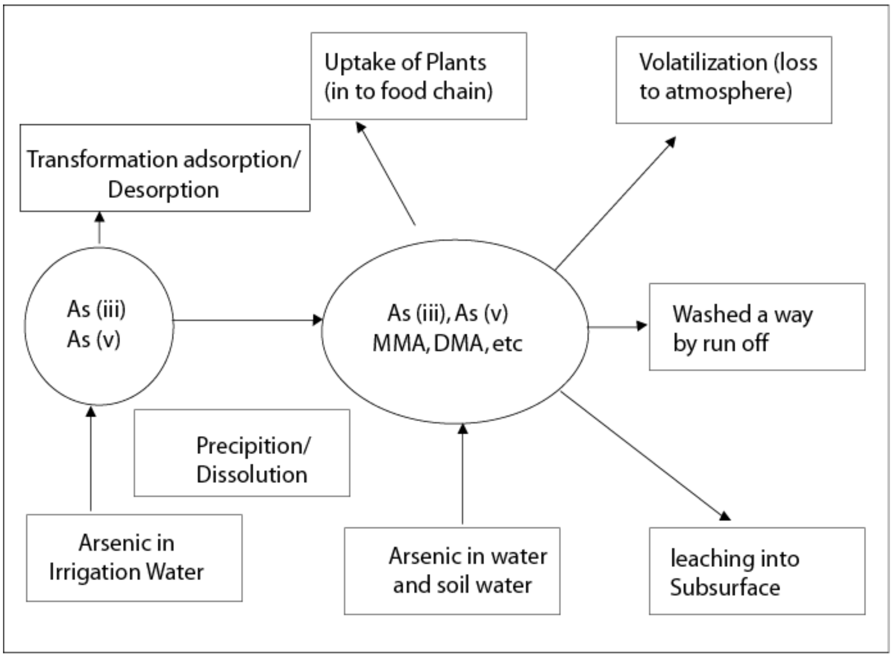

Figure 2.6. Fate of Arsenic in Soil-Water-Plant Environment (Mclaren et al., 2001).

Increased bio-methylation and volatilization of gases such as di-and tri-methyl arsine from soil can reduce arsenic accumulation in agricultural fields. Adsorption-desorption of arsenic into the soil is key to understanding its fate in the environment. Plant uptake is another important pathway that controls the fate of arsenic abstracted via tube well water.

2.9. Effects of Arsenic on Plant Growth

The arsenic problem in Bangladesh is a geological phenomenon. Through the biogeochemical and biochemical pathways, arsenic enters the living biota (Buat-Menard et al., 1987). Accumulation of arsenic by plants depends on plant species (Liebig, 1966; Walsh and Keeney, 1975), the concentration of arsenic present in soil (National Academy of Sciences, 1977), and the presence of other ions (Woolson et al., 1973; Khattak et al., 1991).

Arsenic accumulation in soils reduces soil productivity and is toxic to plants. Trivalent arsenic reacts with cellular sulfhydryl groups and cellular respiration via the tricarboxylic acid cycle. The ability of arsenic to inhibit ATP production suggests that the organ functions will cease rapidly. Adsorption of arsenic by plants and its toxic effects on plants is controlled by many factors, including chemical forms of arsenic (Marin et al., 1992), plant species and genotypes, the concentration of arsenic in soils, soil properties such as pH and clay content (Johnson and Hilbold, 1969), and the presence of other ions (Khattak et al., 1991).

Arsenic phytotoxicity symptoms include leaf wilting (red-brown necrotic spots on old leaves, tips, and margins), violet coloration (as a result of increased anthocyanine), root discoloration (yellowing and browning of roots), cell plasmolysis, and a growth reduction which may lead to death. Kapustka et al. (1995) investigated the toxic effects of arsenic on the growth of alfalfa, lettuce, and wheat. They observed that the concentration of arsenic was found to be positively correlated with phytotoxicity. Zhengmiao and Huang (1994) studied the relationship between arsenic content and rice tillering and found that low concentrations of arsenic in the soils promoted rice tillering, while the high concentration of arsenic was found to inhibit rice tillering. Chino (1981), however, concluded that tillering in rice plants is severely depressed by arsenic as is observed with phosphorous deficiency. The sensitivity of various plants to arsenic is shown in **Table 2.1.**

Table 2.1. Sensitivity of Various Plants to Arsenic (Sheppard, 1992).

Sensitive	Bean, soybean, rice, spinach, green beans, other legumes, onion, cucumber, alfalfa
Moderately sensitive	Apple, cherry, corn, cotton, potato, radish, strawberry, blueberry
Tolerant	Asparagus, tomato, carrot, wheat, oats, corn, cabbage, potato, peanuts, barley, pine

2.10. Rice Production in Bangladesh

Rice constitutes one of the most important staple foods of over half of the world's population. Globally, it ranks third after wheat and maize in terms of production (Bandyopadhay and Roy, 1992). It is a member of genus *Oryza* in the grass family (Gramineae) consisting of 22 species. Only two species are cultivated in this genus: *O. sativa* and *O. glaberrima* (Bounphanousay et al., 2008). The cereal crop is grown in a wide range of climatic zones with over 170 million ha under cultivation globally (Singh et al., 2011). If the global population increases at the current rate, rice requirement will increase dramatically. Many nations will face the huge task of producing more rice at less cost and that also compromising the environment. Therefore, the future of humankind looks bleak considering the fact that there will be a daunting task ahead of them to ensuring food and nutritional security (Tiwari et al., 2011). Future global rice production could be augmented by the dint of increasing rice production area or increasing yield or a combination of both (Mitra et al., 2005).

Bangladesh is ranked among the top ten rice-producing countries globally (Akinbile et al., 2011). In Bangladesh, more than 80% of the districts have arsenic levels exceeding the World Health Organization guideline value for arsenic contamination in drinking water (10 µg/L) (Smith et al., 2006). Long-term use of arsenic-contaminated groundwater to irrigate crops, especially paddy rice (*Oryza sativa,* L.), has resulted in elevated soil arsenic levels in Bangladesh. A number of studies from Bangladesh have reported increased arsenic concentrations in soils and crops because of irrigation with arsenic-contaminated groundwater. Arsenic has been detected in different food items. It was reported by several researchers that rice grown in Bangladesh contains about 80% of inorganic arsenic and people there consume 450 g of rice daily (Potera, 2007). Ahuja (2009) also reported that arsenic in Bangladesh rice contained more As(III) with traces of DMAA and As(V) and that more than 80% of the recovered arsenic was in inorganic form. It was also reported that more than 85% of arsenic in rice was bioavailable compared to only ~28% of arsenic in leafy vegetables such as arum (kochu), gourd leaf, *Amaranthus* and *Ipomea* (kalmi) (Ahuja, 2009). Meharg et al. (2009) reported that ~80% of inorganic arsenic contamination in rice in Bangladesh, which is far more toxic than organic species. This was in sharp contrast to 58% arsenic in U.S. rice, 64% in rice from Europe, and 81% contamination in rice from India. However, basmati rice imported from India and Pakistan and jasmine rice from Thailand were found to contain the least concentration of arsenic (Meharg et al., 2009). Rice is a more efficient arsenic accumulator than any other cereal crop (Stroud et al., 2011). Rice was reported to accumulate up to 2.0 mg/kg which is much above the permissible limit of 1.0 mg/kg, according to the WHO recommendation (Bhattacharya et al., 2009; Delowar et al., 2005; Islam et al., 2005). Data on total and inorganic arsenic in rice from Bangladesh indicate that rice contributes significantly to the daily intake (Williams et al., 2006; Williams et al., 2005). Meharg and Rahman (2002) concluded that the average contribution to total arsenic intake from drinking water was 13% whereas, from cooked rice, it was 56%, thus making it clear that rice contributed most to the daily arsenic intake.

2.11. Toxicity Implications of Rice Produced from Arsenic-Contaminated Irrigation Water

Previous mass balance experiments indicated that the arsenic added via irrigation water was almost quantitatively retained in the soil (Rahman et al., 2008; Panaullah et al., 2009). On the contrary, Dittmar et al. (2007) and Huq et al. (2006) reported results suggesting that soil arsenic concentration increased with groundwater irrigation in the winter (*Boro*) season but declined during the summer monsoon. In a study, Panaullah et al. (2009) found that the long-term use of shallow tube well water (STW), which is contaminated with arsenic, leaves its chemical imprint on the soil by increasing the loading of As and other elements (Fe, Mn, and P) to the soil. This is contrary to the views of Van Geen et al. (2006) who suggested 'modest if any' impact of the arsenic content of irrigation water on rice crop. Lu et al. (2009) reported that further addition of arsenic from irrigation water only leads to a gradual increase in grain arsenic concentration. This view was supported by other scientists who studied the uptake of arsenic by rice (Rahman et al., 2007; Rahman et al., 2009). Rahman et al. (2008)reported that a typical adult can intake 0.20-0.35 mg As/day through rice consumption assuming that a typical adult consumes between 400 and 650 g rice/day in the arsenic-affected areas of Bangladesh. With daily consumption of 4 L drinking water, arsenic intake through drinking water stands at 0.2 mg/day (Rahman et al., 2008).

WHO (2006) opined that the maximum permissible arsenic level in water of 0.01 mg/L was only provisional given the scientific uncertainties; due to the toxic nature, arsenic is likely to cause cancers in 1 person out of 100 people who consumed arsenic-infected rice for a long period. The world body also stated that drinking arsenic-contaminated water exceeding 0.05 mg/L for a similarly long period may result in death. Sengupta et al. (2006) in his study reported varying degrees of arsenic concentrations in raw and cooked rice using different methods. Cooking rice in one of the traditional methods reduced the arsenic load by 57%. However, the remaining arsenic in the rice still poses a considerable health risk to the consumers. Continuous consumption of arsenic-infected rice results in all kinds of skin diseases and cancer in the liver, lung, bladder, kidney, and skin. Consumption of rice straw by cattle could potentially enhance the level of arsenic in meat and/or milk, which in turn further increases the risk of arsenic entering human bodies (Bhattacharya et al., 2009). Chronic arsenic exposure initiates a characteristic pattern of dermal effects that might start with melanosis (pigmentation) to keratosis and hyperkeratosis. On the other hand, consumption of water having more than 300 μg/L arsenic for several years may cause arsenical skin lesions (Ahmed et al., 2006). Panaullah et al. (2009) further pointed out that the animal health and the quality of animal products could also be affected because of the consumption of rice straw produced by arsenic-contaminated irrigation water. In an experiment, rice plants were grown in an arsenic-affected soil (60 mg of As/kg soil) and arsenic concentrations in rice straw were found to be 20.6±0.52 at the panicle initiation stage and 23.7±0.44 at the maturity stage (Rahman et al., 2008). The husk arsenic concentration was 1.6±0.20 mg/kg. Manure which is used as fertilizer and as a kitchen fuel could be an additional route for human exposure to arsenic ingestion (Pal et al., 2007). Increased exposure of children to arsenic-contaminated fields, for example, children playing with sand, has significant impacts on their mental development (Wasserman et al., 2004).

2.12. Risk of Irrigation with Arsenic-Contaminated Water

Irrigation water with elevated levels of arsenic may result in land degradation in terms of crop production (loss of yield) and food safety (food chain contamination). Long-term use of arsenic-contaminated irrigation water in crop production could lead to arsenic accumulation in the soil also. If taken up by the crops, arsenic may add considerably to the intake of dietary arsenic, thereby posing an additional danger to human health (Burló et al., 1999; Carbonell-Barrachina et al., 1999; Imamul Huq and Naidu, 2005; Imamul Huq et al., 2001, 2006; Meharg and Rahman, 2003).

Over time, accumulation of arsenic in soil could render arsenic concentration to reach a toxic level which could be harmful to crops; yield of crops could be reduced as a result. It was estimated that 0.9–1.36 million kg As per year is brought onto the arable land through groundwater extraction for irrigation purposes (Ali, 2003; Heikens, 2006). In Bangladesh, the deposition of arsenic on arable land is high, especially in the south-western and southern parts of Bangladesh where the groundwater arsenic concentration is high (**Figure 2.7**). The north-western part of the country is relatively safer because of the low concentration of arsenic in the shallow aquifer. But the problem lies in the fact that the intensity of extracting water through STWs is very high in that part. Thus, extraction of arsenic from the shallow aquifer is considerable in amount in the north-western part. According to Meharg and Rahman (2003), 150–200 (up to 900) mm water is used for land preparation before planting, and crop growth requires 500–3000 mm. Assuming a land receiving 1000 mm of arsenic-contaminated (0.1 mg As/L) groundwater per year (1000 L/m^2 per year) and the arsenic-contaminated water percolating the first 10 cm of soil (assuming soil density of 1 kg/L), the water input would cause a yearly increase of 1 mg As/kg soil. Wheat, maize, and vegetables are produced on a smaller scale and require much less water. Huq et al. (2003) calculated the arsenic loading in irrigated soils for a *Boro* rice requiring 1000 mm of water per season to be between 1.36 and 5.50 kg As/ha/year. Similarly, for winter wheat requiring 150 mm of irrigation water per season, arsenic loading from irrigation water was calculated to range between 0.12 and 0.82 kg As/ha/year. In a case study in West Bengal (India), data on arsenic in irrigation water and the paddy soil profile suggested a yearly arsenic input of 1.1 mg/kg to the topsoil (Norra et al., 2005). Thus, it is clear that arsenic is entering the soil in different amounts with time through irrigation of crops with arsenic-contaminated groundwater.

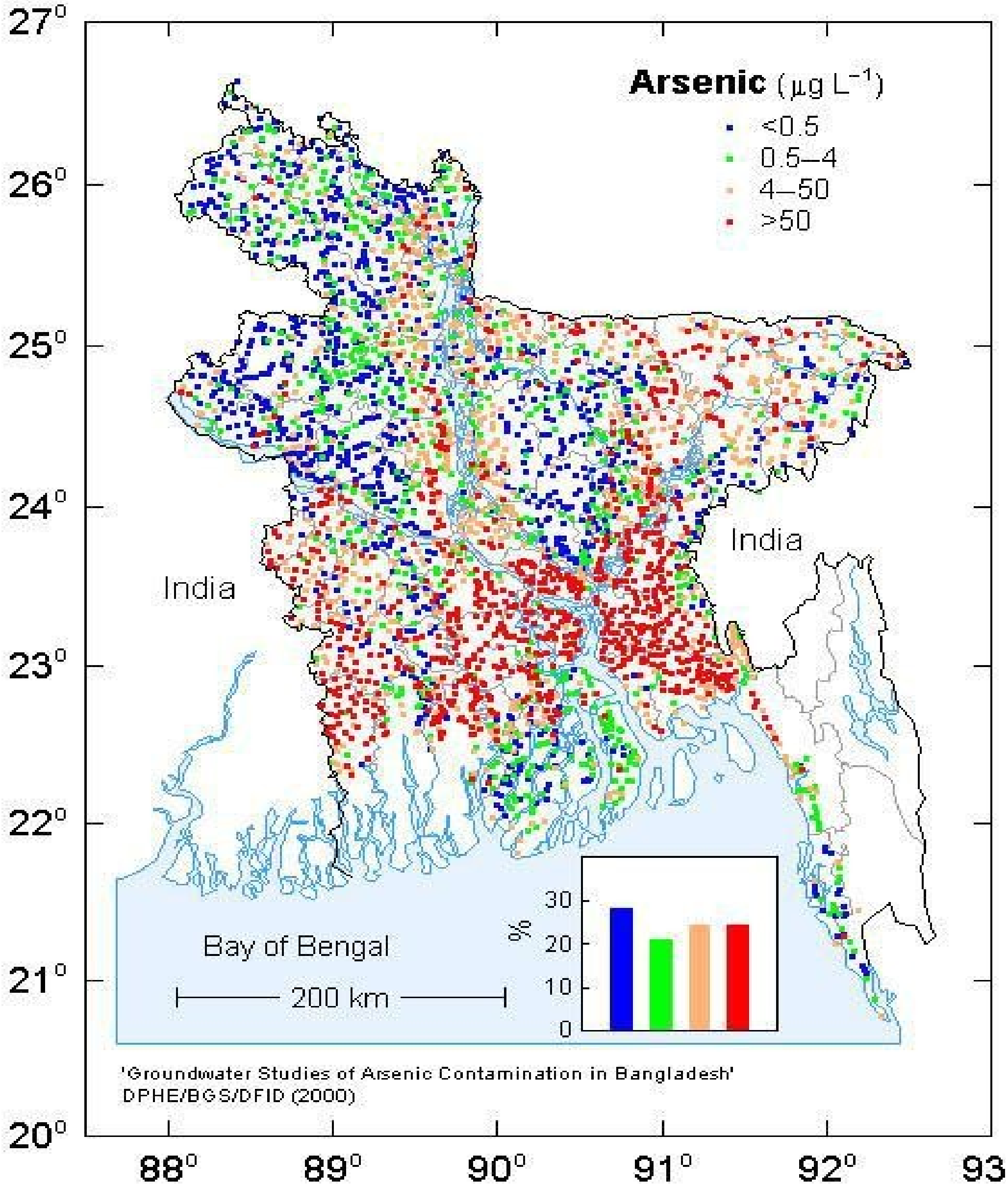

Figure 2.7. Map showing the As distribution in groundwater of Bangladesh (BGS and DPHE, 2001).

2.13. Strategies for Rice Remediation in Arsenic Contaminated Irrigation Water

Arsenic-contaminated water has been found to pose a number of problems towards rice crop in arsenic endemic areas. In Asia, several management strategies have been adopted to minimize the effects of arsenic-contaminated irrigation water on rice production and to have sustained rice production. In recent decades, several practical measures were taken to alleviate the problem of excessive arsenic accumulation in paddy rice. Zhao et al. (2010) recommended a range of mitigation methods, including agronomic measures, plant breeding, and genetic modification to reduce the uptake of arsenic by food crops. In-depth knowledge about the transport of arsenic and key players such as organic carbon is required to deal with the health crisis in South and Southeast Asia (Fendorf et al., 2010). The key role of organic carbon involves triggering the release of arsenic in zones having low groundwater arsenic levels. Carbonell-Barrachina et al. (2009) opined that the processing and cooking of foods could reduce the intake load of arsenic to a great extent. Thus, due considerations should be given to that strategy along with other possible solutions such as breeding rice cultivars having properties of a low arsenic accumulator. One of the recommendations included cooking rice with high volumes of arsenic-free water. This may be a very effective way of reducing arsenic exposure in rural populations of Bangladesh. Bioremediation and phytoremediation were also proposed as strategies in countries with plenty of sunlight (Visoottiviseth and Ahmed, 2008).

Khan et al. (2010) reported that continued long-term irrigation with arsenic-contaminated water poses a potential risk to food security and sustainable rice production in Bangladesh. The grain quality is also compromised as well. The same scenario applies to other countries in central south and south East Asia. Practicing alternate wetting and drying (AWD) was adopted as a mitigation strategy for decreasing arsenic contamination in rice crop (Potera, 2007). Less quantity of irrigation water reduced the uptake of arsenic by rice crops in a significant manner. Li et al. (2009) came up with two potential mitigation methods, namely management of the water regime and silicon fertilization for reducing the accumulation of arsenic in rice. The researchers conducted an elaborate study and found out that silicon fertilization resulted in a decrease in the arsenic concentration in straw and grain by 78 and 16%, respectively. They drew a conclusion saying that water management, Si fertilization, and the proper selection of rice cultivar are effective measures that could be employed to reduce arsenic accumulation in rice. Roberts et al. (2011) suggested that intermittent irrigation or AWD irrigation, which was being popularized in Bangladesh to economize water resources, could be a blessing as it promised toreduce the input of arsenic to paddy soils, which in turn would lessen the arsenic exposure to paddy rice.Garnier et al. (2010) also suggested changes in agricultural practices such as aerobic cultivation (Duxbury and Panaullah, 2007) or breeding (Meharg and Rahman, 2002) of less-arsenic-absorbing rice plants to lower arsenic content of rice grown in the arsenic-affected region. In a separate study, alternate wetting and drying (AWD) with silicon fertilization was found to reduce arsenic levels in pore water and rice grains (Islam et al., 2019). Xu et al. (2008) reported from his study that aerobic conditions led to a decrease in arsenic transfer from soil to the grain of paddy rice. The reduced uptake of arsenic in aerobic condition was attributed to arsenic being adsorbed to oxidized Fe surfaces (Duxbury and Panaullah, 2007). They also opined that arsenic may be present as arsenate, whose uptake is antagonistically suppressed by phosphate. It was also put forward that switching from arsenic-contaminated shallow groundwater to non-contaminated surface or deep groundwater will prevent further buildup of soil arsenic, which will, in turn, reduce the uptake of arsenic in rice plants and vegetables (Farid et al., 2003). However, that will require large irrigation development projects which are economically non-attractive.

2.14. Factors Affecting Arsenic Mobility and Uptake in Rice

There are a number of factors that control the uptake of arsenic by rice plants. The factors are related to the physical, chemical, and biological properties of soil (Delgado and Gomez, 2016). The root rhizosphere also plays an important role in the mobility of arsenic and the uptake by rice. The factors are discussed briefly in the following subsections.

2.14.1. Soil Texture

Soil texture affects the bioavailability and solubility of arsenic in the soil. Fine-textured soil like silt and clayey soils possess higher surface area compared to sandy soils. Consequently, soils dominated by clay particles have more arsenic-adsorbing potential. When clay particles are rich in iron oxides, the arsenic-adsorbing potential of soils accentuates. Therefore, plants growing in clayey soils exhibit fewer arsenic toxicity symptoms. Phytotoxicity of arsenic was found to be five times higher in sandy and loamy soils (Quazi et al., 2011).

2.14.2. Redox Potential

Redox potential is one of the master variables which governs the arsenic speciation and mobility in soil(Williams et al., 2007). In oxidized conditions, arsenic is present as arsenate (As(V)), whereas, in a reducing environment, arsenite (As(III)) is the predominant species. In oxidized conditions, iron oxyhydroxide phases adsorb arsenate thereby limiting its bioavailability. Iron oxides get dissolved in a reducing environment releasing arsenic for plant uptake (Takahashi et al., 2004). Microbial activities aggravate the situation by converting arsenate to arsenite. Some microbes release siderophores which can solubilize the ferric ions at the root-plaque of rice plants exacerbating the situation further (Kraemer, 2004). Arsenic concentration was seen to reach up to 160 mg/kg in the root zone of rice plants (2007).

2.14.3. Soil pH

Soil pH is another master variable that controls arsenic speciation and leaching and consequently its solubility and bioavailability (Quazi et al., 2011). Both low and high pH can influence the uptake and availability of arsenic. Generally, arsenic remains adsorbed onto Fe oxyhydroxide compounds. At low pH (pH <5), Fe oxyhydroxide compounds become more soluble and as a result, arsenic becomes available for plant uptake (Signes-Pastor et al., 2007). On the other hand, at higher soil pH (usually pH 8.5), deprotonation occurs and negative charges such as hydroxyl ions increase, which leads to desorption of arsenic from Fe oxides. Consequently, arsenic mobility increases in the root rhizosphere which in turn facilitates the absorption of arsenic by the standing plant (Ahmed et al., 2011).

2.14.4. Organic Matter

Soil organic matter plays a vital role in limiting the uptake of arsenic. The mechanism lies in its ability to complex arsenic in the form of organo-arsenic complex. The binding of arsenic occurs through phenolic OH, carboxylate, and sulfhydral groups with/without ternary complexes (Suda and Makino, 2016). Therefore, a soil having a high amount of organic matter is likely to reduce the availability of arsenic. The fact that arsenic mobility is reduced by the presence of high organic matter content was demonstrated by an investigation (Paikaray et al., 2005). Some other groups of researchers reported along the same line from their studies (Rahaman et al., 2011; Fu et al., 2011). Diametrically opposite findings were reported by different researchers. A positive relationship between the amount of soil organic matter and arsenic content in the grain of rice was reported. Organic matter was found to promote microbial activities which in turn decreased the redox potential (Turpeinen et al., 1999); lower redox potential is conducive for the reductive dissolution of Fe oxyhydroxides known to complex arsenic (Reza et al., 2010). To sum up, the characteristics of soils and the nature of organic matter should be assessed before using a particular organic matter as an arsenic ameliorator in paddy rice cultivation (Syu et al., 2019).

2.14.5. Nitrogen, Phosphorus and Sulfur Content in Soil

Nitrogen is applied in the form of fertilizers for rice cultivation. The main form of nitrogen is ammonium which is converted to nitrate because microbes can carry out the nitrification process in the oxygenated environment created by paddy rice roots. This transformation may influence iron redox in a paddy rice environment. Nitrate reduction and iron (II) oxidation occurring in tandem could potentially diminish the bioavailability of arsenic in the soil.

Phosphate and arsenate chemistry are the same and the anions may contend for the same sorption sites in the soil. Moreover, paddy rice consumes arsenate {As(V)} via the phosphate transporter across the root plasma membrane. Therefore, when phosphate is applied in the soil in the form of fertilizers two things happen: (a) arsenic leaching is enhanced, and (b) the availability of arsenic in soil solution is increased (Abedi and Mojiri, 2020).

Sulfur plays a vital role in controlling the uptake and translocation of arsenic in plants (Dixit et al. 2015). On sulfur application, the accumulation of arsenic by rice is reduced by three probable mechanisms (Hu et al., 2007): (a) sulfur triggers the formation of Fe plaques thereby reducing the concentration of arsenic in soil, (b) sulfate (SO_4^{2-}) anions may desorb arsenate {As(V)} from

Fe plaques, and (c) at the cell membrane transport site, sulfate could hamper the transport of arsenate into cells.

Sulfur also plays an important role in the arsenic detoxification process. Thus, in the arsenic-affected soil, sulfur metabolism is crucial for plant's survival (Finnegan and Chen, 2012). When plants are exposed to arsenic, it triggers the synthesis of sulfur-rich ligands such as glutathione (GSH) and phytochelatin (PC). Detoxification of arsenic occurs through the conversion of As(V) to As(III), which subsequently binds with sulfhydral groups of GSH and PC; the compound is then transported to vacuoles (Song et al., 2010). Arsenic-thiol complexation may limit arsenic translocation from root to shoot (Dixit et al., 2015). Arsenic mobility is also controlled by the efflux of arsenic from the root to the growing medium. The genes associated with sulfate uptake were found to be upregulated in rice when rice was exposed to arsenate (As(V)) (Srivastava et al., 2016). A higher application of sulfate was seen to enhance glutathione and phytochelatin synthesis, thereby promoting the arsenic complexation in roots, which in turn limiting arsenic translocation from roots to shoots (Dixit et al., 2016). Sulfate application in paddy soils can also precipitate arsenic by forming insoluble arsenic-sulfide (Signes-Pastor et al., 2007).

2.14.6. Iron and Manganese Content in Soil

Arsenic mobility is affected by the presence of iron and manganese-rich compounds such as goethite, ferruginous smectites, nontronite, pyrolusite, and birnessite (Anawar et al., 2018). Arsenate (As(V)) is absorbed by these minerals to a large extent. In paddy soils, iron plaque, which is a coating of iron oxide or hydroxides, is formed on the roots of paddy rice. This iron plaque forms as plants release oxygen into the root rhizosphere (Liu et al., 2005; Ultra et al., 2009). Iron plaque plays an important role in adsorbing or coprecipitating arsenic, thereby reducing the uptake of arsenic by plants (Lee et al., 2013).

2.15. In-practice Strategies for Alleviating Arsenic Uptake in Rice

The strategies which have been in practice for alleviating arsenic uptake in rice include water management and the addition of minerals. Aerating soil by applying less water stops the reduction of arsenic. The aerated condition also helps to create a condition that facilitates the formation and precipitation of insoluble arsenic in the soil. Arsenic uptake may be reduced by the application of mineral nutrients which are known to have an antagonistic relationship with arsenic. In the following subsections, the mitigation strategies are discussed briefly (Bakhat et al., 2017).

2.15.1. Water Management

Water management in the paddy field has been found to govern the bioavailability of arsenic. And, the approach was proposed by a number of researchers in the recent past. Economizing water in the paddy field could be a sustainable solution to limit the uptake of arsenic by rice crop (Mitra et al., 2017). Arsenic mobility is increased when rice is grown under flooding conditions. Dissolution of iron oxyhydroxides occurs in reducing conditions and the associated arsenic becomes available for plant uptake (Takahashi et al., 2004). When less water is used for growing paddy rice, the redox potential of soil tends to decrease and the resulting oxidizing condition inhibits the reduction of As(V) to As(III). As(III) form is the most toxic arsenic species and is significantly more soluble and bioavailable (Takahashi et al., 2004). In oxidized conditions, arsenic's affinity towards soil minerals increases, and Fe plaques formation around root surface is favored (Liu et al., 2004; Roberts et al., 2011). These two factors work in combination to reduce arsenic mobility, which in turn decreases the availability of arsenic for the plants (Xu et al., 2008; Takahashi et al., 2004). This phenomenon was substantiated by the work of Talukdar et al. (2011), who observed less uptake of arsenic (0.23-0.26 mg/kg) by rice under aerobic water regime compared to the uptake of arsenic (0.60–0.67 mg/kg) under the anaerobic regime. Sprinkler irrigation practice was also found to reduce the uptake of arsenic by rice grains (Spanu et al., 2012; Moreno-Jiménez et al., 2014). Under flooding conditions, Fe-reducing bacteria reduce iron oxyhydroxides, thereby increasing the solubility of arsenic in soil (Horneman et al., 2004).

2.15.2. Addition of Mineral Nutrients

Adding elements like Fe, P, S, and Si could considerably decrease the uptake and accumulation of arsenic by plants. Some of the elements could limit translocation within plants as well (Bakhat et al., 2017). Some of the elements are discussed in the following subsections.

2.15.2.1. Role of Fe

Iron (Fe) is a micronutrient element and plays a significant role in alleviating the accumulation of arsenic in rice (Liu et al., 2004; Nath et al., 2014). The external application of iron results in the formation of iron plaque, which reduces the uptake of arsenic. The external application could also enhance coprecipitation of iron and arsenic, thereby decreasing the availability of arsenate(As(V)) (Bakhat et al., 2017). In a study, metallic Fe and Fe-oxide was found to reduce the accumulation of arsenic in rice grains by 51% and 47%, respectively (Matsumoto et al., 2015).

Paddy rice cultivation also promotes the formation of iron plaque on rice roots(Liu et al. 2004). Iron plaque is composed of ferrihydrite (63%), goethite (32%), and siderite (5%). These minerals have a high affinity for arsenate (As(V)). As a result, arsenic is adsorbed onto these minerals which in turn results in reduced translocation of arsenic from roots to shoots(Liu et al. 2004). Arsenic uptake by paddy rice is also reduced due to the elevated concentration of iron oxides in the rhizosphere (Lee et al., 2013; Syu et al., 2019).

2.15.2.2. Role of Phosphorus

As phosphate is an analogue of arsenate, phosphate concentration significantly influences arsenate solubility in soil and its uptake by plants. In the paddy field, phosphate and arsenate compete for the same sorption sites in soils or Fe-plaque via ligand exchange mechanisms (Peryea and Kammereck, 1997). At critical concentration, phosphate was found to inhibit the uptake of arsenate because both use the same transporter during uptake by the plasma membrane

(Abedin et al., 2002c; Meharg and Macnair, 1992). Three important factors are thought to govern the effects of phosphorus on the mobility of arsenic in soil and the uptake of arsenic by rice: (1) the competition between arsenate and phosphate to be sorbed onto soil particles, (2) the antagonistic relationship between arsenate and phosphate to be uptaken by rice roots, and (3) the role of phosphate for the translocation of arsenic from root to shoot (Lee et al., 2016). As such, arsenic toxicity in plants is governed by the As/P ratio in the soil rather than the absolute arsenic concentration. Arsenic accumulation in grains of rice was found to decrease by changes in phosphorus content in shoots of rice (Lu et al., 2010). In arsenic-rich soils, the application of Ca and P combo facilitates the formation of Ca-P-As complex, thereby limiting the mobility of arsenic (Neupane and Donahoe, 2013).

2.15.2.3. Role of Silica

Silicon (Si) is not an essential element for plants. It is rather a beneficial element for tropical grasses such as rice (Tavakkoli et al., 2011). Plants only use the mono silicic acid among the different forms of silicon (Epstein, 2009). Like other elements, silicon solubility is controlled by soil pH, one of the master variables in soil solution. The application of silicon in soil assumes importance owing to silicon's sharing the same transporter with arsenite into plants. Both arsenite and silicon are taken up by plants via nodulin-26 like intrinsic proteins (NIPs)(Ma and Yamaji, 2006). Because of this phenomenon, the availability of high silica reduces the uptake of arsenite (Bogdan and Schenk, 2008). Researchers working with silicon and arsenic found a negative correlation between the concentration of silicon and the uptake of inorganic arsenic (Tripathi et al., 2013; Sanglard et al., 2016). The application of silicon was found to reduce the arsenic concentration in rice straw and grain by 78% and 16%, respectively (Li, et al., 2009a; 2009b). In Southeast Asia, furnace slag and calcium silicate slag, which are iron- and silicon-containing minerals, are used as soil amendments (Bakhat et al., 2017).

2.15.2.4. Role of Sulfur

Sulfur, which is an essential element for plant growth, plays a critical role in limiting arsenic accumulation and translocation in plants (Dixit et al., 2015).

In a study, a higher concentration of sulfur (5 mM) was found to enhance the accumulation of arsenic in roots, thereby inhibiting arsenic translocation from roots to shoots (Dixit et al., 2016). The boosted accumulation was attributed to the enhanced thiolic ligand synthesis (glutathione and phytochelatins) and subsequent arsenic complexation in roots (Dixit et al., 2016). In a genetic study, As(V) was found to upregulate the genes associated with sulfur uptake, transport, and metabolism in rice (Srivastava et al., 2016).

2.15.3. Bioremediation Strategy

2.15.3.1. Role of Soil Microorganisms

Soil microorganisms (SOMs) carry out a number of processes such as mineralization and immobilization which control the concentration of minerals in the soil. The fate and transport of arsenic in the environment are strongly affected by these processes (Huang et al., 2014). Soil microorganisms are capable of detoxifying arsenic species by the sorption process. Their extracellular surface contains uronic acids, proteins, and amino sugars which are able to adsorb arsenic through hydrogen bonding (Bakhat et al., 2017).Various soil bacteria such as *Bacillus* sp., *Rhodococcus* sp., *Halobacterium* sp. were reported to adsorb different species of arsenic (Bakhat et al., 2017; Williams et al., 2013). Arsenic detoxification is also accomplished by the formation of amorphous iron hydroxides on the cell surface; inner-sphere complexes are formed as a result (Yang et al., 2012). Arbuscular mycorrhizal fungi (AMF) limits the translocation of arsenic by suppressing the mRNA expression of OsLsi1 and OsLsi2, which are the mediators of arsenite (As(III)) transport (Chen et al., 2012).

2.15.3.2. Confinement of Arsenic at Non-edible Parts

Genetic intervention showed promising results for reducing build-up of arsenic in rice grain (Grill et al., 2006; Sharma et al., 2014). The overexpression of phytochelatin synthase (PC) will trigger increased synthesis of chelators such as glutathione (GHS) and phytochelatins (PC) in rice plants. Increased synthesis of such chelators means increased levels of arsenite-thiol complexation. The arsenite-thiol complexation phytostabilize the metalloid in roots which is not edible part of the plant (Dhankher et al., 2011).

2.15.3.3. Increase Arsenite (As(III)) Efflux Rate

Transgenic rice plants could be used to reduce the accumulation of arsenic. Transgenic rice plants expressing the *S. cerevisiae* ACR3 gene was found to encode arsenite efflux protein. The arsenite efflux protein enhances arsenite (As (III)) efflux, thereby reducing the accumulation of arsenic in rice grain. Transgenic rice plants showed 30% lower arsenic concentration in root and shoot compared to wild type plants possessing similar arsenic translocation factor (Mitra et al., 2017; Duan et al., 2012).

2.15.3.4. Volatilization of Arsenic

Volatilization of arsenic can be employed to reduce arsenic load of rice. Inorganic arsenic can be converted to methylated organic species like MMA and DMA and finally to the gaseous trimethylarsine (TMA). Here, transgenic plants can be used for the elevated production of gaseous arsenic. Transgenic plant harboring the bacterial gene AsIII-S-adenosyl methioninemethyltransferaseArsM was found to produce gaseous arsenic by a significant extent (Chen et al., 2017; Qin et al., 2006).

2.16. Fertility Status of Bangladesh Soil

2.16.1. Organic Matter

Organic matter is considered the storehouse of all the plant nutrients in the soil. It is the major source of two important mineral elements of P and S, and essentially the sole source of N. Organic matter is vitally important for better soil structural conditions, higher water, and nutrient holding capacity, and higher microbial activities for successful cultivation. The organic matter content of Bangladesh soils is generally low. Bhuiya (1987) studied the organic matter contents of 17 soil series, each from 17 general soil types of Bangladesh. He reported that Peat had the highest (35.37%) organic matter content, followed by Acid basin clays (5.20%) and Acid sulphate soils (3.46%). However, these soils have various constraints and therefore are not agriculturally important in Bangladesh. In his study, only 4 soil series had more than 2% organic matter while the other 10 soil series had below 2% organic matter. Thus, the overall organic matter content is usually low in the agriculturally important soils in Bangladesh. Islam et al. (1992) also did some investigations on the organic matter content in 29 soil series sampled from the different parts of the country. In their investigation, the organic matter content ranged from 0.6 to 1.7%. Soil is suitable for successful crop production if the soil contains at least 2% organic matter (Islam, 1990). From Islam et al. (1992) study, 90% of soils of Bangladesh were found to contain 0.5-1.0% organic matter. Karim et al. (1995) reported that external application of organic residues augmented the organic matter content to the level of 1.1 to 1.3% from the initial level of 0.7% in the Shallow red-brown terrace soil. The researchers added 2.0 Mg/ha of air-dry rice straw, 7.5 Mg/ha of fresh ipil ipil leaves, 25 Mg/ha of compost, and 25 Mg/ha of fresh cow dung per annum under the rice-wheat cropping rotation. As organic matter decomposed at a faster rate, the added residues did not increase the residual organic matter to a great extent; the annual addition of organic residues, however, enhanced the yield of both rice and wheat. Among the different organic residues tested, compost made of rice straw and cow dung was found to be most effective in terms of crop yield.

The organic matter status of Bangladesh soils is becoming worse day by day. Miah (1993) reported that there had been 9 to 46% depletion of soil organic matter in different regions of Bangladesh over a period of 20 years (from 1970 to 1990). There are a number of reasons behind the low organic matter content of Bangladesh soils. The chief reason is the lack of organic matter recycling through addition of crop residues, animal waste, and other organic manures. Because of the fuel scarcity, crop residues including shoots and even roots, and even cow dung are used as fuel in Bangladesh. As Bangladesh is a tropical monsoon climatic country, plants in general grow profusely in the summer season. If plant residues are not removed, soil receives a fair amount of organic matter in the form of biomass. However, the high temperature and rainfall cause the added organic matter to decompose rapidly (Bhuiya, 1987). In Bangladesh, due to the intensive cultivation, the soils are disturbed vigorously. Tillage operations in the form of plowing, puddling, laddering and so on enhance the decomposition of organic matter further aggravating the situation. Use of urea fertilizer, a nitrogenous fertilizer, may also augment microbial activities leading to the decomposition of organic matter (Hoque, 1983). In Bangladesh, low-lying areas of most floodplains possess a good reserve of organic matter and a relatively higher amount compared to high land or medium-high land. Low-lying areas remain under water for a significant period of time of a year. Thus, little decomposition of organic matter can occur in these soils. Moreover, many aquatic weeds grow profusely which add organic matter to these soils (FAO-UNDP, 1988). **Table 2.2** and **2.3** show the nutrient and heavy metal contents of some organic manures used in Bangladesh.

Table 2.2. Nutrient and heavy metal content as well as other characteristics of bio slurry of biogas plants.

Nutrient content	**Cow dung slurry**		**Poultry litter slurry**	
	BARI values	**DU values**	**BARI values**	**DU values**
	%			
Total Nitrogen	1.35	1.23	2.71	2.75
Total Phosphorus	2.89	2.71	3.35	3.24
Total Potassium	0.88	0.62	0.85	0.75
Total Sulphur	0.71	0.67	1.00	0.91
Total Calcium	0.92	0.80	4.50	3.90
Total Magnesium	0.62	0.72	2.60	2.42
Total Iron	0.103	0.800	0.209	0.198
Total Manganese	0.080	0.090	0.067	0.071
Total Boron	0.069	0.060	0.041	0.050
	$mg\ g^{-1}$			
Total Zinc	610	580	717	590
Total Copper	428	450	224	260
Heavy metal				
Total Arsenic	1.47	1.40	1.77	1.32
Total Lead	11.37	12.00	20.09	21.00
Total Cadmium	3.64	4.35	4.28	3.90
Other characteristics				
Moisture (%)	11.25	12.00	11.17	11.79
pH	7.94	8.21	8.31	8.35
Organic matter (%)	26.04	27.78	21.58	30.34
Colour	Brownish		Grayish	
Physical conditions	Powder, free flowing		Powder, free flowing	

Table 2.3. Nutrient concentrations in commonly used organic fertilizers of Bangladesh.

Organic fertilizers	**Nutrient content (%)**		
	N	P	K
Cow dung	0.5-1.5	0.4-0.8	0.5-1.9
Poultry manure	1.6	1.5	0.85
Compost (common)	0.4-0.8	0.3-0.6	0.7-1.0
Farmyard manure	0.5-1.5	0.4-0.8	0.51-1.9
Water hyacinth compost	3.0	2.0	3.0
Bioslurry (cow dung)	1.29	2.80	0.75
Bioslurry (Poultry litter)	2.73	3.30	0.80
Rice straw	0.52	0.25	1.20
Wheat straw	0.63	0.28	0.80
Maize stove	0.45	0.30	0.70
Sugarcane trash	0.35	0.25	0.80
Tobacco stems	0.42	0.25	1.10

2.16.3. Different Types of Organic Manures

Cowdung is a good source of N, P, K and S, which are essential elements for plant growth. Batsai et al. (1997) reported that chemical fertilizers with cowdung produced the highest cabbage yields. Poultry manure, another type of organic manure, is a rich source of macro nutrients and particularly appropriate for acid soils as it has strong liming effect. On application, poultry manure reduces the acidity of soils, thereby protecting crops from aluminum toxicity. Cow dung and poultry manure are suitable for organic farming and can be effectively used for high value crops. On the other hand, using earthworms to decompose and stabilize organic wastes has received attention in the last few decades. When organic wastes are subjected to decompose with the aid of earthworms, the resultant product is called vermicompost and the process is known as vermicomposting. Vermicomposting was reported to be viable, cost-effective and a rapid technique for the efficient management of solid wastes (Payal et al., 2006). It is a technology for degradation of various types of organic wastes into value-added material. Vermicompost is a potential source of readily available nutrients, growth promoting substances and a number of beneficial microorganisms like N-fixing, P-solubilizing and cellulose-decomposing organisms (Suthar, 2012).

2.16.3.1. Cowdung as a Bio-Fertilizer

Cowdung is the main source of bio-fertilizer. However, cow's urine, cow's horn and a dead body of a cow can also be used for preparing bio-fertilizer. The farm animals (cows, bullocks and milk buffaloes) furnish dung and urine to be used as fertilizer. Conversely, crop residues and fodder form most of the feed for these animals. In Bangladesh, farming and agricultural cultivation used to be done with cow as per the traditional age-old system, with cow dung amongst others serving as organic manure.

Cow dung is a good source for sustaining the production capacity of a soil. Cow dung promotes microbial population to a great extent. Increasing population pressure and demand of food resources are forcing humankind to utilize agrochemicals like chemical fertilizer, pesticides and insecticides to the soil, which are degrading the physiochemical properties of soil, including soil texture, porosity, and water holding capacity; agrochemicals are posing problems for the soil microbial population. Therefore, cow dung should be applied in soil along with chemical fertilizers to increase the production capacity of food of a soil (Bargali, 2004). The combined application of cow dung manure and vermicompost improves soil organic matter content, which leads to improved water infiltration and water holding capacity as well as an increased cation exchange capacity. Mandal et al. (2013) reported that a combination of inorganic fertilizer, organic manure and biofertilizers can produce 50-92% more yield in Aonla. C: N ratio in cow dung manure indicates that it could be a good source of protein for the microorganisms which carry out the decomposition of organic matter (Adegunloyeet al., 2007). Vermicomposting of cow manure using earthworm species *E. andrei* (Atiyeh et al., 2000) and *E. foetida* (Hand et al., 1988) was found to favor nitrification, leading to the rapid conversion of ammonium-nitrogen to nitrate-nitrogen. Therefore, vermicomposted cow manure promotes the nutrient cycling and helps in converting unavailable nitrogen into available forms for plants.

The soil biological attributes are important and gives indications about the productivity of a soil. In a study, cattle dung was found to promote microbial biomass. When dung was applied in a grassland soil under controlled conditions, the size of the soil microbial biomass increased compared to the control soil (Lovell and Jarvis, 1996).

2.16.3.2. Poultry Manure

Poultry manure was proved to be a good supplementary source to chemical fertilizers in the rice-rice cropping pattern (BRRI, 1998-1999). Poultry manure contains macro- N, P, K, S, Ca, Mg) and micro-nutrients in different quantities (Egrinya et al., 2001). In a study with poultry litter, it was found that ~75% of the total N and majority (90-100%) of the P and K in poultry litter become accessible for plant use during the year of application (Hammond et al., 1997). Application of 2 tons of poultry manure/ha will obviate the need for P and S from chemical fertilizers and will fulfil the 60% N and K fertilizer requirement for a target yield of 5-6 t/ha rice (Miah et al., 2006).

Poultry litter poses a hazard to human health and the environment because it releases toxic substances and is full of pathogenic microorganisms. In Bangladesh, 1,560,000 metric tons of poultry litter is generated every year (Miah et al., 2016). Poultry litter could be used as fertilizers and soil amendments because of its nutritional properties. Poultry litter could be employed to improve the organic matter content of Bangladesh soil. It is estimated that more than 60% of arable lands of this country possess low organic matter content, usually below 1% (Karim et al., 1994). Under the circumstances, poultry litter can play an important role for maintaining soil fertility. Poultry litter is a good source of nutrient elements for the crop growing in the current season. Poultry litter has lower C:N ratio, which enables microorganisms to mineralize organic matter easily which in turn helps standing crops (Chanyasak et al., 1983). Poultry litter (C:N = 5.3) and composted poultry litter (C:N=8.2) were found to be mineralized easily; as a result, the nutrients became quickly available to plants.

2.17. Organic Matter Depletion & Management

2.17.1. Organic Matter Depletion

Soil organic matter controls long-term soil fertility because it is the pool of all metabolic energy. All soil biological processes associated with nutrient availability are driven by soil organic matter. A soil is considered good in terms of soil fertility when it contains at least 2.5% organic matter. Unfortunately, most of the soils in Bangladesh have less than 1.5% organic matter, and some of them even have less than 1% organic matter (BARC, 2005). Organic matter content in Bangladesh soil declined over the years. During 1967 to 1995, the highest depletion occurred in soils of Meghna River Floodplain (35%) followed by Madhupur Tract (29%), Brahmaputra Floodplain (21%), Old Himalayan Piedmont Plains (18%) and Gangetic Floodplain (15%) (Ali et al., 1997).

2.17.2. Organic Matter Management

As soil organic matter is vitally important for the good physical, chemical and biological properties of a soil, addition of organic materials each year is crucial to maintaining or increasing the soil fertility status of a soil. Adoption of recommended technologies may increase the carbon stock of a soil. The rate of organic carbon sequestration depends on several factors, including soil texture, soil structure, rainfall, temperature, farming system and its management (Lal, 2004). If the organic matter content of a degraded soil is increased by 1 ton, that would enhance the crop yield by 20-40 kg/ha for wheat, 10-20 kg/ha for maize and 0.5-1 kg/ha for cowpea. In addition to maintaining food security, carbon sequestration has the potential to counteract fossil fuel emissions by 0.4-12 gigatons of carbon per year.

Organic matter in soil can be maintained by no-till farming. No-till farming is a complex management system which involves minimum soil disturbance, keeping crop residue cover and diversifying crop rotations and/or cover crops. Cow dung, poultry manure and cow dung-, poultry manure-based bio-slurry could potentially be good source of organic matter in soils. Bio-slurry is produced when cow dung, poultry manure, and buffalo manure etc. are anaerobically digested into combustible gas CH_4. In this process, only 25-30%

of total solid content of organic manure is converted into combustible gas and the rest of the solids (70-75%) come out as sludge (bio-slurry). However, the use of bio-slurry is not without its constraints associated with its management.

Organic matter of a soil may be improved by selecting appropriate crops and cropping pattern. Mung bean, for example, could be used as a summer crop between winter and rainy season crops. After picking pods, mung bean residues could be incorporated into soil as green manure. The growth and yield of T. aman was found to be affected by the green manuring of mung bean residues (Sarkar, 2005). If incorporated, legume crop also benefits the succeeding crop (Ali, 2003). Legume crop obviates the need for inorganic fertilizer to some extent. Ali (2003) compared grain yield obtained from inorganic fertilizer with or without legume residues. The highest grain yield was obtained in treatment having 100% NPKS fertilizer application and the treatment possessing 75% NPKS fertilizers + legume residue incorporation. Thus, to keep agricultural production sustainable, the nutrient elements removed by a crop are to be replenished and appropriate agricultural practices are to be adopted to maintain soil organic matter level.

Chapter Three
Materials and Methods

3. MATERIALS AND METHODS

A field experiment was carried out in a farmer's field of Jashore district, which is a highly arsenic contaminated region in Bangladesh. The experiment was conducted to study the mitigation of arsenic accumulation in rice (BRRI Dhan 28) through water management and organic amendments. The experiment was conducted in two successive *Boro* seasons, namely 2017 *Boro* season and 2018 *Boro* season.

3.1. Description of the Study Area

The study site was selected based on the arsenic contamination in soil. The field experiments were set in a farmer's field situated in Ahsannagar village, Mouza no. 30 under Dogasia thana of Jashore district. The georeference of the sampling spot is 23º13′ N latitude and 89º08′ E longitude (**Figure 3.1**). This region belongs to the western part of the Ganges River floodplain. The soil belongs to Gopalpur soil series under agro-ecological zone (AEZ) 11, known as Ganges River Floodplain. The USDA classification is Aeric Haplaquepts. The general soil type was calcareous dark grey floodplain soils and calcareous brown floodplain soils. The morphological characteristics of the soil of the study area are presented in **Table 3.1.**

Table 3.1. Morphological descriptions of the study area.

AEZ-11	High Ganges River Floodplain
Soil Series	Gopalpur
Land Type	Predominantly high land and medium high land
General Soil Type	Calcareous dark grey floodplain soils and calcareous brown floodplain soils
Flood Level	Moderately well drained
Drainage	Moderate
USDA soil Taxonomy	Aeric Haplaquepts

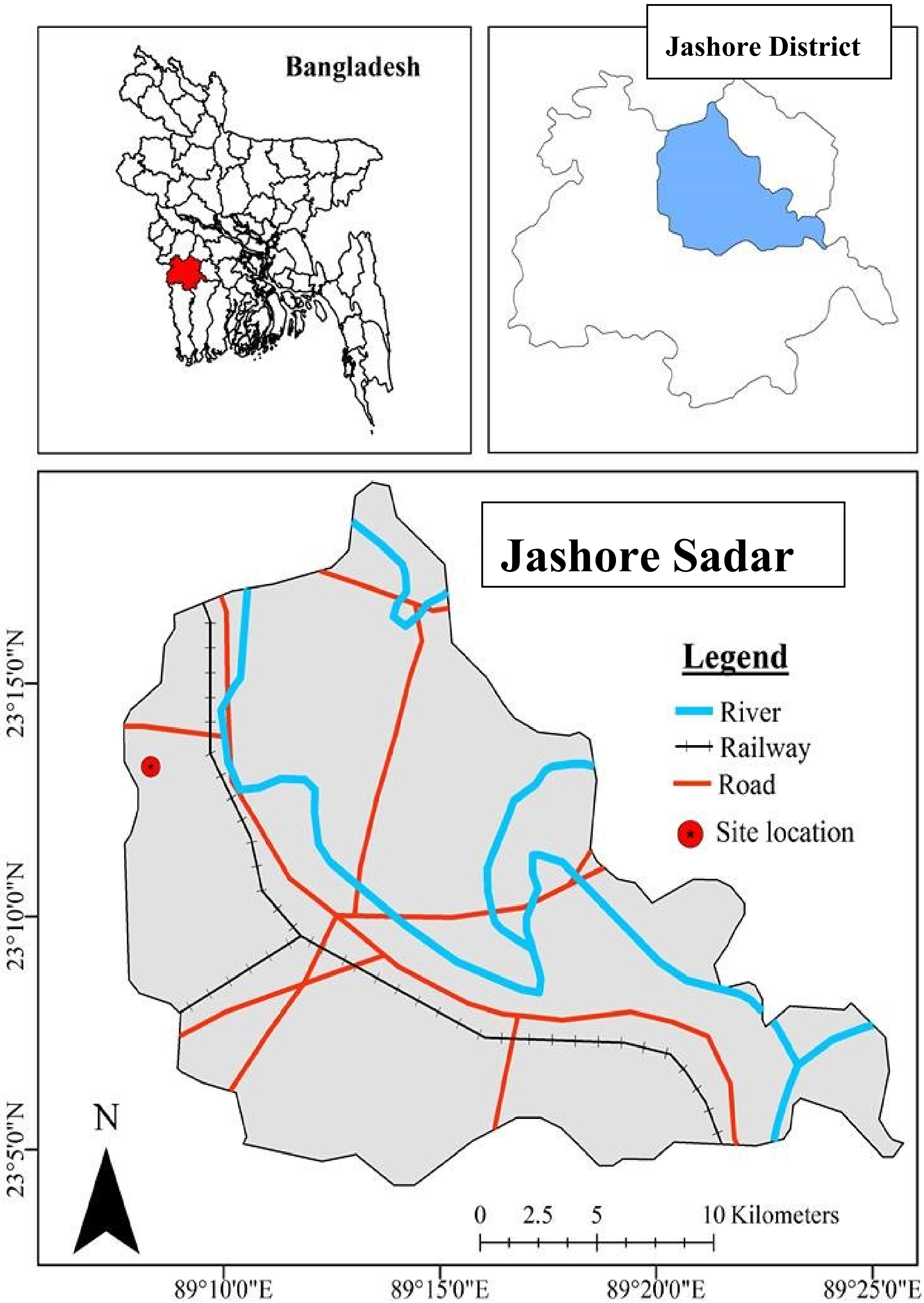

Figure 3.1. Location map showing the geographic position of the sampling site or study area (Source: Personal Communication).

3.2. Sample Collection

3.2.1. Collection of Soil Sample

The soil samples were collected from a paddy field to be used for the field study. The bulk of the soil samples representing 0–15 cm depth from the soil surface were collected by the composite soil sampling method as suggested by the soil survey stuff of the USDA (1951). The collected soil samples were put into polythene bags, tagged with rope, and labeled and were taken into the laboratory for analytical purposes. After harvesting, soil samples in and around the rhizosphere were collected from each plot. The samples were air dried and homogenized and were screened to pass through a 0.5 mm sieve for chemical analysis. The soil samples were preserved in plastic bags and labeled. Irrigation water contaminated with arsenic was also collected for analysis.

3.2.2. Collection of Cow dung and Poultry Manure

Cow dung and poultry manure were procured from a dairy farm and a local poultry, respectively. The manures were put into separate polythene bags, tagged with rope, and labeled. The samples were then taken into the laboratory for analytical purposes.

3.3. Sample Preparation

3.3.1. Preparation of Soil Sample

The collected soil samples were air-dried for 3 days (30 ºC) by spreading in a thin layer on a clean piece of paper. Visible roots and debris were removed from the soil sample and discarded. After air drying, a portion of the larger and massive aggregates were broken by gently crushing them with a wooden hammer. Ground samples were screened to pass through a 2-mm stainless steel sieve. The soil samples were preserved in plastic containers and labeled properly showing sample number, and the date of collection. These soil samples were used for various physical analyses. A portion of soil samples (2-mm sieved) was further ground and screened to pass through a 0.5-mm sieve. The sieved samples were persevered in the same way as above. These soils were used for chemical and physicochemical analyses.

3.3.2. Preparation of Cow dung and Poultry Manure

The collected cow dung sample was also dried in air, ground, and screened to pass through a 0.5-mm sieve and mixed thoroughly. The sample was put into a polythene bag, tagged with rope, and labeled. The poultry manure sample was also prepared and preserved like cow dung. The samples were then taken into the laboratory for chemical and physicochemical analyses.

3.4. Experimental Set-up

The field trial was conducted with rice variety of BRRI dhan 28 for two consecutive years including 2017 *Boro* season and 2018 *Boro* season. The field used for the trial was an arsenic-contaminated region of Bangladesh. In the following subsections, treatments, experimental design, and layout are discussed.

3.4.1. Treatments, Layout and Design of the Experiment

The experiment was laid out in split plot design with three replications (**Appendix 1**). The main plot treatments were three water regimes, and the subplot treatments were different types of organic amendments. The water regimes were field with saturation having no stagnant water (No SW), 3-cm stagnant water (3-cm SW), and 5-cm stagnant water (5-cm SW). The organic amendments included were poultry manure at 5 t/ha, poultry manure at 10 t/ha, cow dung at 5 t/ha, and cow dung at 10 t/ha. The organic amendments were added based on fresh weight basis. A control treatment was included where the plot received no organic amendments. In total, there were 15 treatment combinations. Therefore, with three replications, there were 45 subplots altogether. The dimension of each subplot was 3m x 3m. The space between the plots was 0.5 meters.

3.4.2. Land Preparation

At the beginning of the experiment, the land was prepared with repeated ploughing using a power tiller followed by laddering. After ploughing, the clods and lumps were broken with the help of bamboo stick and spade to make the soil in good tilth. It was ensured that there were no remains of weed and previous crops. A channel was created to store deep tube well water to be utilized to maintain the water regimes properly. Cow dung and poultry manure were incorporated into the soil as per treatment. The amendments were added into the soil before two weeks of transplanting. No chemical fertilizers were applied for this experiment. The tillage was performed very carefully so that the layout remained intact.

3.5. Method of Rice Cultivation

At first, seeds of BRRI dhan 28 were taken into a pot, dipped in water, and allowed to stand overnight. The seeds were then spread onto a seedbed. The seedbed was kept under dark by covering with wet jute bags. It took 2 to 3 days for seeds to germinate. After one month, the seedlings were transplanted in the field. When the seedlings became about 10 cm high, they were sown directly in the plots. Weeds were removed manually. Adequate plant protection measures were taken during the growing period. Agronomic characters like plant height, tiller numbers, panicle numbers, leaf colors, growth, appearance of any symptoms and so forth were noted and recorded during the whole growing period. Intercultural operations like irrigation, soil loosening, weeding, and plant protection were performed as and when necessary. Harvesting, cleaning, drying, and weighing were done following standard protocols.

3.6. Methods of Soil Analysis

The soil samples were analyzed in the laboratory of Soil, Water and Environment Department at the University of Dhaka. Physical, chemical, and physico-chemical properties were analyzed following standard methods. The cow dung and poultry manure were also analyzed for their elemental concentrations. The methods followed for the analysis are described in the following subsections.

3.6.1. Analysis of Physical Properties

- **Particle Size Analysis**

Particle size analysis was done by the Hydrometer method, as described by Day (1965). The textural classification was determined by Marshall's Triangular Coordinates as designed by the USDA (1951).

- **Determination of Moisture Content**

Moisture content of air-dried soil was determined by first oven-drying a known amount of soil in an electric oven at 105°C for 24 hours until constant weight was obtained. The moisture percentage was then calculated from the loss of moisture from the samples as described in Jackson (1962).

3.6.2. Analysis of Physicochemical and Chemical Properties

The physicochemical and chemical parameters were determined following different widely used and some specific methods. The methods used for the determinations are as follows:

- **Measurement of pH**

Soil pH was measured electrochemically using a glass electrode pH meter. The ratio of soil to water was 1:2.5 as described by Jackson (1973).

- **Organic Carbon and Organic Matter**

Organic carbon of the soil samples was determined by wet oxidation method of Walkley and Black as described by Jackson (1973). Organic matter content was determined by multiplying the percentage of organic carbon with conventional Van-Bemmelen's factor of 1.724 (Piper, 1950).

- **Total Phosphorus**

Total phosphorus of soil and manure was determined from the digest (obtained by digesting the soil and manure by conc. HNO_3 and $HClO_4$) spectrophotometrically using the vanadomolybdate yellow colour method on a spectrophotometer at 490 nm wavelength (Jackson, 1962).

- **Available Phosphorus**

Available phosphorus content of the soil was extracted using the Olsen method. The extract was estimated spectrophotometrically following the ascorbic acid blue color method using a spectrophotometer at 880 nm (Murphy and Riley, 1962).

- **Total Sulfur**

Sulfur content in the digest was determined by the turbidimetric method using Tween 80 solution as a surfactant. The digest was analyzed by a spectrophotometer at 420 nm.

- **Total Arsenic**

Arsenic (As) in soil was extracted by digestion with aqua regia. For determination of aqua-regia extractable arsenic, 2.5 gm of soil was digested in about 15 ml of aqua-regia (HCl: HNO_3, 3:1) for approximately 4-5 h using a sand bath as a heating source (~110°C). The sample and acid were placed in 100 ml Pyrex glass beaker. After digestion, samples were diluted up to a volume of 50 ml with distilled water, mixed and filtered prior to analysis (Portman and Riley, 1964). Then arsenic in the extract was estimated by Hydride generation atomic absorption spectrometer (HG-AAS) with the help of potassium iodide, and 10% urea in acid medium following calibration of the equipment. For every 10 sample a certified reference material (CRM) was included to ensure QC.

- **Total Iron**

Total iron was determined by digesting the soil samples with aqua regia (HNO_3:HCl = 1:3) and then measuring by the flame atomic absorption spectroscopy (AAS).

- **Total Manganese**

Total manganese was determined by digesting the soil samples with aqua regia (HNO_3:HCl = 1:3) and then measuring by flame atomic absorption spectroscopy (AAS).

3.7. Collection and Preparation of Plant Samples

Plants of BRRI Dhan 28 were harvested at the age of 135 days after transplantation. The plants were harvested by manual uprooting. The grain samples were collected before two days of harvesting. The harvested roots were first washed with tap water and then copious amounts of deionized water several times to remove solute from ion free space as well as to dislodge any adhering particles on the root surface. The upper parts of the plants were also washed with deionized water. The height of the plant sample was measured from the top leaf blade to the bottom of the plant from where the root started. The plant samples were then wrapped with tissue paper to remove the extra water and dried in the air for half an hour. The fresh weights of the whole plants were then recoded. After taking the weights, the plant samples were separated into four parts, namely root, straw, husk, and grain. The separated plant samples were then air dried before putting into an oven for drying at 70±5 ºC for 48 h. The dry weights of the plant samples were then recorded. The dried plant samples were then ground and were sifted through a 0.2 mm sieve. The ground plant samples of different parts were separately preserved in plastic pots for chemical analysis.

3.8. Methods of Plant Sample Analysis

Approximately 0.5 g of plant sample (root, straw, husk, and grain) was weighed separately into 100 ml Pyrex glass tubes. Five mL of nitric acid (HNO_3) was added, and the tubes were allowed to stand for half an hour. The glass tubes were then placed in a digestion block. Samples were normally predigested at temperatures between 50 and 75 °C before increasing the temperature to 140 °C for the final dissolution of organic material. After dissolution was complete, the samples were diluted to 25 ml, shaken, and filtered. This extract was used for the determination of As, Fe and Mn content of the plant samples (Portman and Riley, 1964).

3.8.1. Total Arsenic

The As content in the extract was estimated by hydride Generation Atomic Absorption Spectrometer with the help of 5% potassium Iodide (KI) and 10% urea in acid medium. Standard solutions were prepared from sodium meta-arsenite. The hydride was generated using 6 N HCl and 1.2% $NaBH_4$ and 1% NaOH in deionized water. Standards ranging from 0 to 40 mg As/L were used.

3.8.2. Total Iron

The iron content of the plant samples was determined from the HNO_3 digest using an atomic absorption spectrophotometer (Portman and Riley, 1964).

3.8.3. Total Manganese

The manganese content of the plant samples was also determined in the HNO_3 digest by using an atomic absorption spectrophotometer (Portman and Riley, 1964).

3.8.4. Total Phosphorus

Total phosphorus in the plant samples was determined by using the Vanadomolybdate yellow colour method (Jackson, 1973). The extract was analyzed by a spectrophotometer at 490 nm.

3.8.5. Total Sulfur

Sulfur content in the digest was determined by the turbidimetric method using Tween 80 as a surfactant. The extract was analyzed by a spectrophotometer at 420 nm.

3.9. Transfer Factor Co-efficient

The Transfer Factor Co-efficient in root, straw, husk, and grain of plants was determined using the following formula:

$$\text{T. F.} = \frac{\frac{mg\ of\ the\ elements}{kg\ dry\ weight\ of\ plants}}{\frac{mg\ of\ the\ elements}{kg\ dry\ weight\ of\ soil}}$$

3.10. Statistical Analysis

Microsoft Excel and MINITAB17 computer program were used for statistical analyses. Data were checked for normality and homoscedasticity (equal variance). A one-way ANOVA followed by Tukey's post-hoc test was used to determine if there were any differences among the water regimes and organic amendments with respect to different growth parameters and different elemental concentrations. Two-way ANOVA was also performed to determine the main effects of water regimes and organic amendments and to determine if there were any interactions between water regimes and organic amendments. Pearson correlation analysis was performed to see if there were any relationships between elemental concentrations of different plant parts. Significance was based on whether p values were <0.05 or not.

Chapter Four
Results and Discussion

4. RESULTS AND DISCUSSION

The present study was carried out to study the effects of water regimes and organic amendments on the uptake of arsenic (As) by rice variety of BRRI dhan 28. The broader goal was to observe whether management of water regimes and addition of organic amendments can reduce the uptake of arsenic by BRRI dhan 28. One of the objectives was to economize the amount of water in a paddy field without compromising the yield. The growth parameters and accumulation of some selected elements were also investigated. Three water regimes, namely complete saturation with no stagnant water (No SW), 3-cm stagnant water (3-cm SW), and 5-cm stagnant water (5-cm SW), and two organic amendments at two levels, namely poultry manure (PM) at 5 t/ha and 10 t/ha and cow dung at 5 t/ha and 10 t/ha were tested with rice variety of BRRI dhan 28. A control was included where no organic amendments were added.

4.1. Initial Characteristics of Soil

Soil sample was collected from the site where the experiment was conducted for two consecutive years including 2017 and 2018. The soil samples were collected before 2017 *Boro* season and 2018 *Boro* season. Composite soil samples were collected from a depth of 0-15 cm. The collected soil samples were analyzed in the laboratory before setting up of the experiment. The analysis was performed to determine the nutrient status of the soil. Background levels of arsenic and some other important elements were also determined. Some important physical, physicochemical, and chemical properties of the studied soil sample are presented in **Table 4.1**. The study site was a clay loam soil with pH ranging from 7.2-7.4. The cropping pattern was rain-fed monsoon rice and *Boro* rice or winter rice, which is irrigated with groundwater contaminated with arsenic. Total soil arsenic concentrations were 10.82 mg/kg and 11.24 mg/kg in 2017 and 2018, respectively. The arsenic concentrations of the irrigation water were tested from time to time. The arsenic concentrations were between 0.10 and 0.17 mg/L.

Table 4.1. Some physical, physico-chemical, and chemical properties of the studied soil sample.

Properties	**2017**	**2018**
pH (Soil:H_2O = 1:2.5)	7.4	7.2
Particle size analysis		
Sand (%)	37%	
Silt (%)	31%	
Clay (%)	32%	
Texture	Clay loam	
Moisture content (%)	7.03	8.12
Organic matter (%)	1.15	1.21
Organic carbon (%)	0.67	0.70
Total nitrogen (%)	0.064	0.071
Total phosphorus (%)	0.085	0.087
Total sulphur (%)	0.245	0.250
Total potassium (%)	0.229	0.234
Total iron (%)	3.088	3.074
Total manganese (%)	0.049	0.046
Available phosphorus (mg/kg)	30	32
Available potassium (mg/kg)	0.12	0.14
Total arsenic (mg/kg)	10.82	11.24

4.2. Initial Characteristics of Organic Amendments

Cow dung and poultry manure were analyzed in the laboratory for their nutrient status and other important elements. Some important properties are presented in **Table 4.2**.

Table 4.2. Some selected chemical properties of cow dung and poultry manure.

Properties	Value	
	Cow dung	**Poultry Manure**
Organic carbon (%)	24.03	27.36
Total nitrogen (%)	1.5	1.6
Total phosphorus (%)	2.71	3.24
Total sulphur (%)	0.67	0.91
Total potassium (%)	0.9	0.85
Available nitrogen (%)	0.056	0.279
Available phosphorus (%)	0.8	1.5
Available potassium (%)	0.62	0.75
Total arsenic (mg/kg)	BDL	BDL
Total iron (%)	0.103	0.198
Total manganese (%)	0.080	0.071

BDL = Below detection limit

4.3. Visual Symptoms

Visual symptoms were observed in the rice plant during the growth period to assess phytotoxicity and deficiency symptoms. Phytotoxicity was monitored because the soil was contaminated with a high arsenic content. Deficiency symptoms were also monitored because the soil was not amended with any chemical fertilizers and the crop was subjected to different water regimes. Plant growth was found to be affected in the cow dung treated soil. There were visible signs of stunting and yellowing in the plants treated with cow dung. On the other hand, rice plants grown in the poultry manure treated soil exhibited deep green colour and profuse growth indicating healthiness. There were no signs of arsenic toxicity in plants. In some previous studies, red brown necrotic spots on old leaves, tips and margins were reported due to arsenic toxicity (Aller et al., 1990 and Martin et al., 1992).

4.4. Plant Growth Parameters

Different plant growth parameters, namely height, fresh weight, dry weight, and yield in the form of grain weight were recorded for *Boro* season in 2017 and 2018 (**Table 4.3**). The parameters were recorded during growth period, at harvest, and after harvest. The biomass sampled for plant growth parameters was from an area of 1 m^2. Plant height was measured after 3 months of transplantation. Fresh weight values were recorded at harvest and the dry weight of biomass was measured after harvest. For dry weight measurement, the harvested plants were first dried in the sun and then transported to the lab. The sundried plant biomass was dried in an oven at 60 ℃ until constant weight. The weight of grain yield was also taken from the plants sampled from an area of 1 m^2. The parameters are discussed in the following sections under water regime and organic amendment.

Height, fresh weight, dry weight, and grain weight values of rice plants grown at different water regimes are presented in **Table 4.3**. The data were subjected to two-way ANOVA and one-way ANOVA. Before parametric analysis, the data were checked for homoscedasticity. The two-way ANOVA was performed to determine the main effects and interaction effects of water regime and organic amendments. The one-way ANOVA followed by Tukey's post-hoc test was performed to determine the best and worst treatments among the water regimes and organic amendments. In the following section, the results from the ANOVA are discussed.

Table 4.3. Main effects and interactions of water regimes and organic amendments on the growth parameters of rice variety of BRRI Dhan 28. The growth parameters recorded were average plant height (cm), fresh weight (t/ha), dry weight (t/ha) and grain weight (t/ha). A one-way ANOVA followed by Tukey's Post-hoc test was performed to determine if there were any significant differences among the treatments ($p<0.05$). Different letters in the same column indicate significant differences between the treatments.

Factor levels/ Interaction	**Growth Parameters**							
Water Regimes	**Height (cm)**		**Fresh weight (t/ha)**		**Dry weight (t/ha)**		**Grain yield (t/ha)**	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
No SW	36.08a	36.30a	20.25a	18.80a	1.47a	1.55a	3.09a	3.04a
3-cm SW	36.45a	36.34a	19.16a	18.75a	1.41a	1.49a	3.03a	3.00a
5-cm SW	36.97a	37.28a	20.97a	20.30a	1.46a	1.51a	3.08a	3.04a
Significance	NS	*	**	**	**	*	NS	NS
Organic Amendments								
Control	34.91c	35.16c	12.66d	12.19d	1.03d	1.09d	2.21d	2.20d
CD 5 t/ha	33.20c	33.69c	14.00cd	13.53cd	1.09d	1.11d	2.74c	2.73c
CD 10 t/ha	33.57c	33.83c	16.10c	15.80c	1.23c	1.29c	2.69c	2.68c
PM 5 t/ha	38.52b	38.39b	22.99b	22.37b	1.71b	1.79b	3.37b	3.30b
PM 10 t/ha	42.30a	42.13a	34.89a	32.53a	2.18a	2.31a	4.32a	4.23a
Significance	***	***	***	***	***	***	***	***
Interactions								
Water regime* Organic Amendments	*	***	***	***	***	***	*	**

NS =Not significant, * = Significant at 5%level, ** =Significant at 1% level, *** = Significant at 0.1% level

Plant Height

Table 4.3 shows the mean height of the plant grown at different water regimes. From the two-way ANOVA, water regime was found to have insignificant effects on the height of rice in 2017 *Boro* season ($F = 1.25$, $p = 0.302$). However, the main effects of organic amendment were found to be highly significant ($F = 55.51$, $p = 0.000$). The interactive effects of water regime and organic amendment were also found to be significant ($F = 2.65$, $p = 0.025$).

In 2018 *Boro* season, water regime had statistically significant effects on rice plant height ($F = 94.54$, $p = 0.019$). Organic amendment also had significant effects on plant height ($F = 115.62$, $p = 0.000$). The interactive effects of water regime and organic amendment was found to be significant as well ($F = 6.06$, $p = 0.000$).

Fresh Weight

In 2017 *Boro* season, significant effects of water regime were observed on the fresh weight of rice of crop ($F = 6.95$, $p = 0.003$). The organic amendment treatment was found to be highly significant ($F = 421.08$, $p = 0.000$). The interactive effects of water regime and organic amendment was also found to be highly significant ($F = 6.58$, $p = 0.000$).

In 2018 *Boro* season, water regime influenced the fresh biomass of rice crop in a statistically significant manner ($F = 8.93$, $p = 0.001$). Organic amendment was also found to be highly significant ($F = 482.62$, $p = 0.000$). Following the previous years' trend, the interaction between those two factors tested was found to be highly significant ($F = 7.20$, $p = 0.000$).

Dry Weight

In 2017 *Boro* season, water regime had significant effects on the dry biomass of rice ($F = 6.06$, $p = 0.006$). Organic amendment was highly significant in influencing the dry weight of rice crop ($F = 742.63$, $p = 0.000$). The interactive effects of water regime and organic amendment was also highly significant ($F = 14.58$, $p = 0.000$).

In 2018 *Boro* season, water regime was found to be highly significant (F = 5.32, p = 0.011). The dry biomass of rice crop was statistically influenced by water regime. The effects of organic amendment on the dry weight of rice was also found to be highly significant (F = 1191.32, p = 0.000). The interactive effects of water regime and organic amendments on the dry weight of rice crop was found to be highly significant (F = 21.45, p = 0.000).

Grain Weight

In 2017 *Boro* season, water regime was found to have non-significant effects on the grain weight of rice (F = 1.52, p = 0.235). However, organic amendment had significant effects on the grain weight of rice (F = 729.91, p = 0.000). The interactive effects of water regime and organic amendment were found to be statistically significant (F = 3.19, p = 0.010).

In 2018 *Boro* season, water regime had no significant effects on the grain weight of rice (F = 1.34, p = 0.278). However, organic amendment had significant effects on the grain weight of rice crop (F = 643.19, p = 0.000). The interactive effects of water regime and organic amendment was found to be significant as well (F = 3.83, p = 0.003).

4.4.1. Plant Growth Parameters as Affected by Water Regimes

Plant Height

A one-way ANOVA followed by Tukey's post-hoc test was performed to determine the best treatment in terms of rice crop height. In 2017 season, the plant height was not found to differ significantly among the water regimes (p = 0.826). However, 5-cm SW water regime produced the tallest plant marginally compared to other water regimes (**Figure 4.1 and Table 4.3**).

In 2018 season, the pattern was the same as was seen in 2017 season. There were no significant differences among the water regimes with respect to plant height (p = 0.705). The tallest plant (37.28 cm) was observed in water regime having 5-cm standing water in the field followed by 3-cm and No SW regimes (**Figure 4.2 and Table 4.3**).

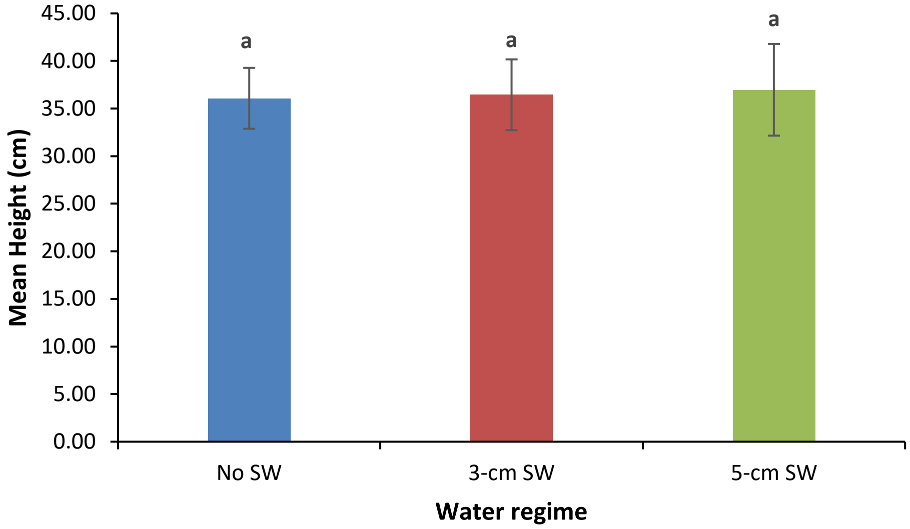

Figure 4.1. Plant height as affected by different water regimes in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post-hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes.

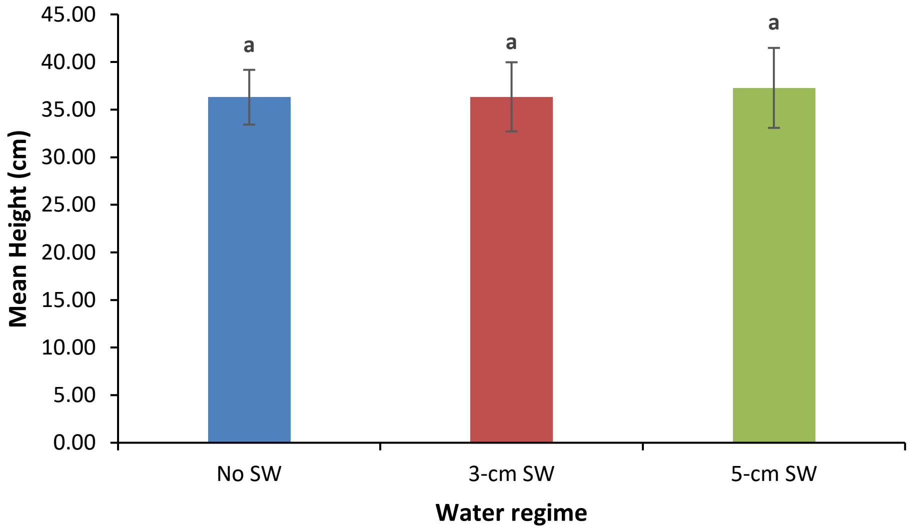

Figure 4.2. Plant height as affected by different water regimes in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post-hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes.

Fresh Weight

In 2017 *Boro* season, fresh weight of rice was not found to be significantly different among the three water regimes as revealed by the one-way ANOVA (F = 0.17, p = 0.848) (**Table 4.3** and **Figure 4.3**). The maximum fresh biomass production was observed under 5-cm SW regime (20.97 t/ha) followed by No SW (20.25 t/ha) and 3-cm SW (19.16 t/ha) regimes.

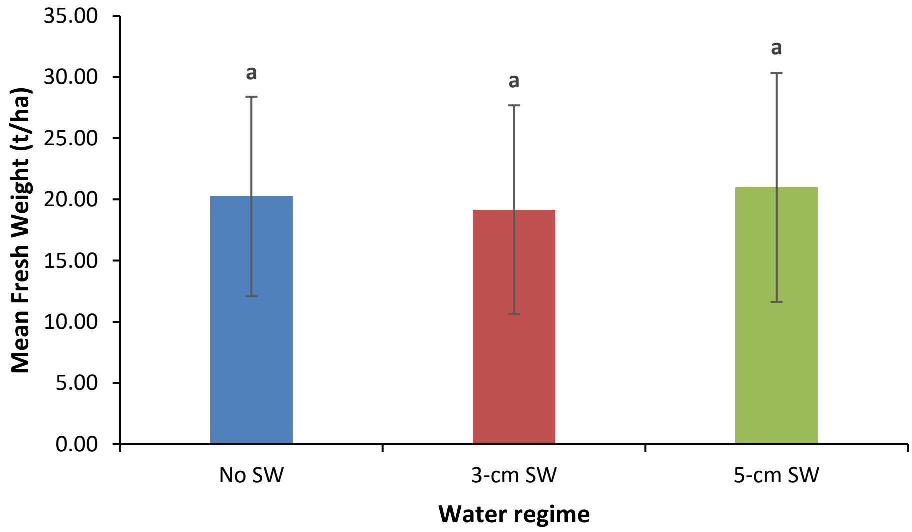

Figure 4.3. Fresh weight as affected by different water regimes in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

In 2018 *Boro* season, fresh weight of rice was not found to be significantly different among the three water regimes as revealed by the one-way ANOVA (F = 0.19, p = 0.831) (**Table 4.3** and **Figure 4.4**). The maximum fresh biomass production was observed under 5-cm SW regime (20.30 t/ha) followed by No SW (18.80 t/ha) and 3-cm SW (18.75 t/ha) regimes.

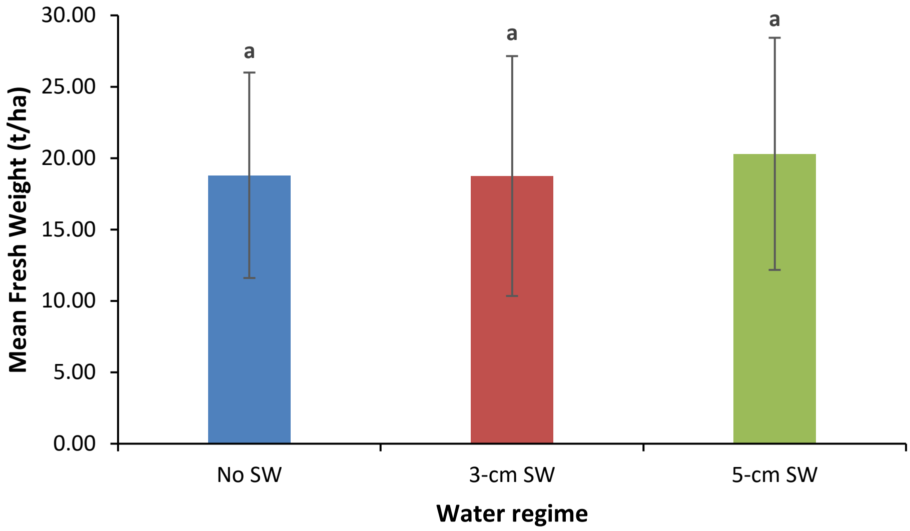

Figure 4.4. Fresh weight as affected by different water regimes in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there are any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

Dry Weight

In 2017 *Boro* season, dry weight of rice was not found to be significantly affected by water regime (one-way ANOVA, p = 0.922) (**Table 4.3** and **Figure 4.5**). The highest amount of dry weight was produced under the water regime of No SW (1.47 t/ha) followed by 5-cm SW (1.46 t/ha) and 3-cm SW (1.41 t/ha) regimes.

In 2018 *Boro* season, water regime did not affect the dry weight of rice significantly (one-way ANOVA, p = 0.956) (**Table 4.3** and **Figure 4.6**). The highest amount of dry weight was produced under the water regime of No SW (1.55 t/ha) followed by 5-cm SW (1.51 t/ha) and 3-cm SW (1.49 t/ha) regimes.

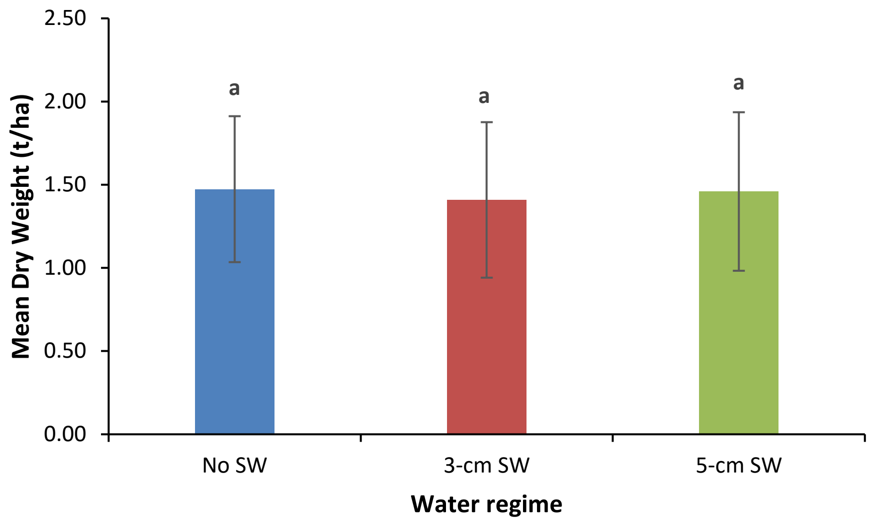

Figure 4.5. Dry weight as affected by different water regimes in 2017 *Boro* season. One-way ANOVA followed by Tukey's post hoc test was done to see if there are any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

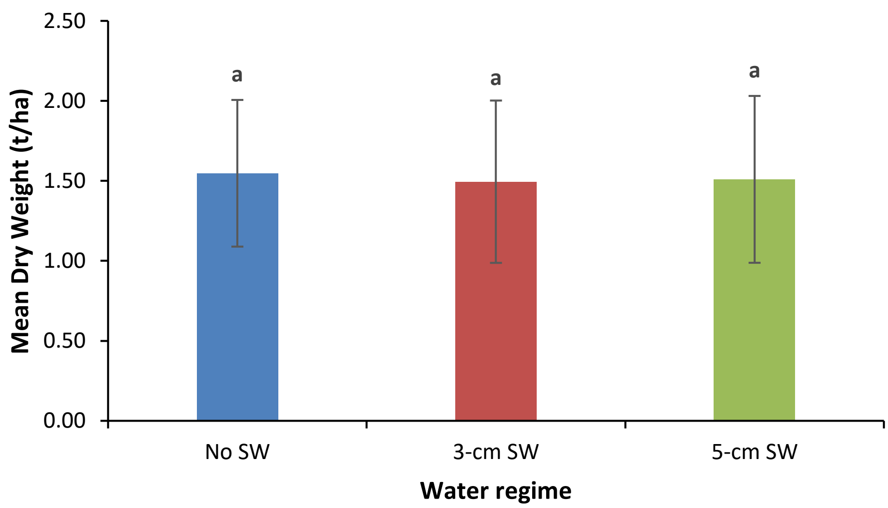

Figure 4.6. Dry weight as affected by different water regimes in 2018 *Boro* season. One-way ANOVA followed by Tukey's post hoc test was done to see if there are any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

Grain Weight

In 2017 *Boro* season, water regimes did not have any significant effects on the grain weight of rice (one-way ANOVA, p = 0.979). However, the highest grain yield was produced under the water regime of No SW (3.09 t/ha) followed by5-cm (3.04 t/ha) and 3-cm SW (3.00 t/ha) water regimes (**Table 4.3** and **Figure 4.7**).

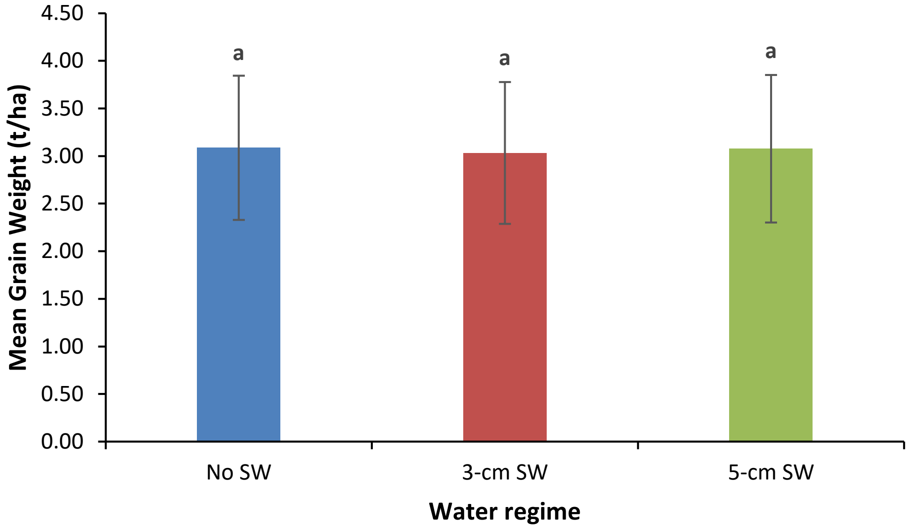

Figure 4.7. Grain weight as affected by different water regimes in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

In 2018 *Boro* season, the mean grain weight was statistically the same among the water regimes (one-way ANOVA, p = 0.979). However, No SW regime produced the highest yield which was on par with the produce of 5-cm SW regime (**Table 4.3** and **Figure 4.8)**.

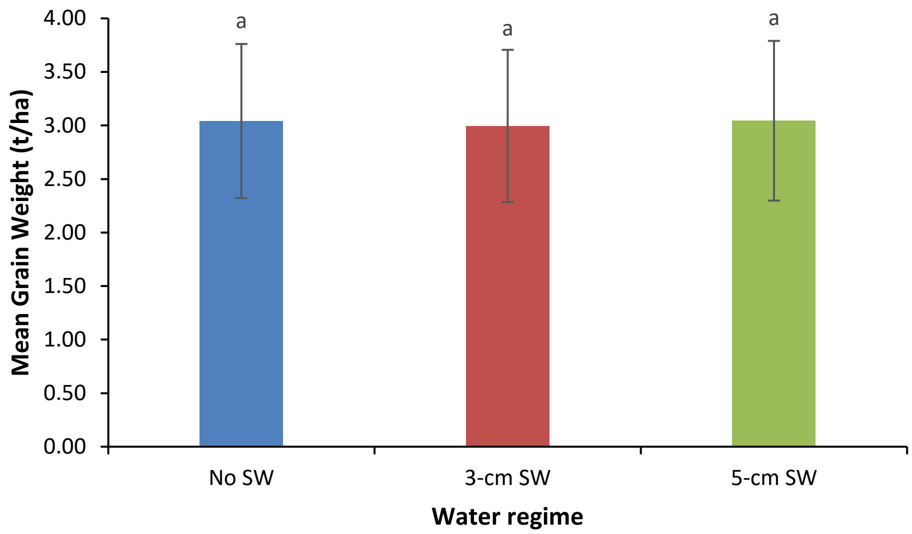

Figure 4.8. Grain weight as affected by different water regimes in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments.

4.4.2. Plant Growth Parameters as Affected by Organic Amendments

A one-way ANOVA followed by Tukey's post-hoc test was performed to determine the best and worst organic amendment in terms of height, fresh weight, dry weight, and grain weight values of rice crop grown in 2017 and 2018 *Boro* seasons (**Table 4.3**).

Plant Height

The one-way ANOVA revealed that organic amendment had significant effects on the plant height of rice crop grown in 2017 *Boro* season (one-way ANOVA, p = 0.000). The post-hoc analysis showed that the application of poultry manure at 10 t/ha was the best treatment in terms of rice plant height, which was significantly different from the rest of the treatments except poultry manure at 5 t/ha (**Figure 4.9**). The second-best treatment was poultry manure at 5 t/ha, which was significantly different from cow dung at 5 t/ha, cow dung at 10 t/ha and the control. On the other hand, cow dung application at 5 and 10 t/ha were found to be inferior to the control.

In 2018 *Boro* season, plant height was significantly affected by the application of organic amendments (one-way ANOVA, p = 0.000). Tukey's post-hoc analysis revealed the best treatment, which was poultry manure at 10 t/ha followed by poultry manure at 5 t/ha. The worst treatment recorded at cow dung at 5 t/ha (**Figure 4.10**).

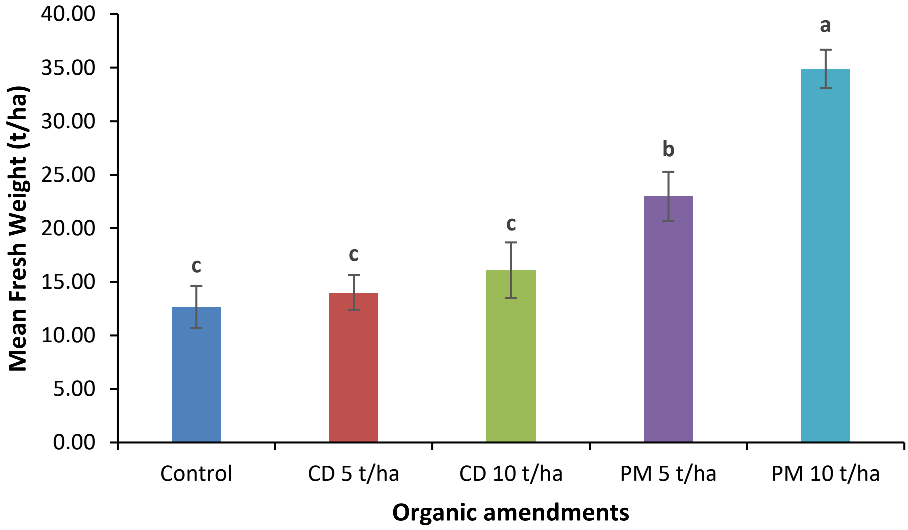

Figure 4.9. Plant height as affected by different organic amendments in 2017 season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

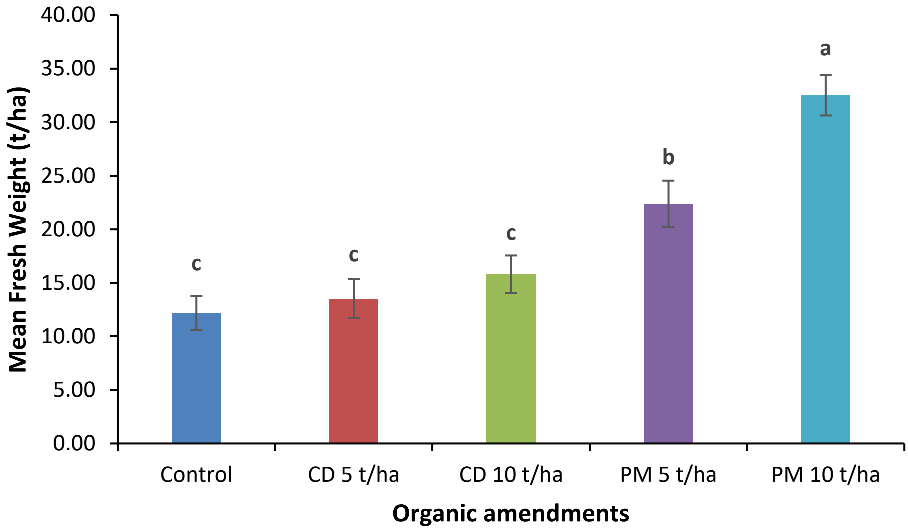

Figure 4.10. Plant height as affected by different organic amendments in 2018 season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

Fresh Weight

As was seen in the plant height data, the mean fresh biomass of rice plant was found to differ significantly among the organic amendments in season 1 (2017 *Boro* season) (one-way ANOVA, p=0.000). The application of poultry manure at 10 t/ha was found to be the best treatment as revealed by the post-hoc test (**Figure 4.11** and **Table 4.3**). The treatment was significantly different from the rest of the treatments including the control. The application of 5 t/ha proved to be the second-best treatment in terms of fresh weight. Cow dung at 10 t/ha was found to be statistically different from the control ($p<0.05$).

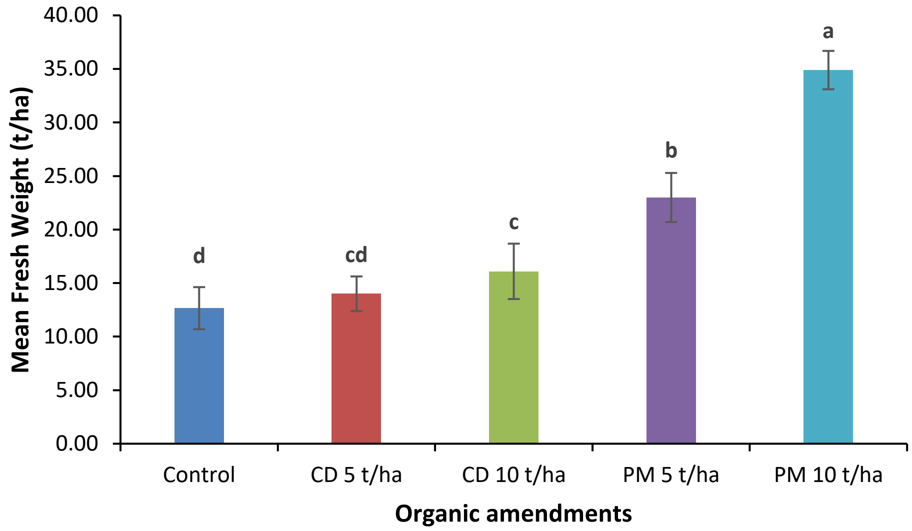

Figure 4.11. Fresh weight as affected by different organic amendments in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments.

The mean fresh biomass of rice plant was found to differ significantly among the organic amendments in 2018 *Boro* season as well (one-way ANOVA, p = 0.000). Following the previous year's trend, poultry manure at 10 t/ha was found to be the best treatment among the organic amendments (**Figure 4.12** and **Table 4.3**). The treatment was statistically different from the rest of the treatments, including the control. The application of 5 t/ha proved to be the second-best treatment vis-à-vis fresh weight. Cow dung at 10 t/ha was found to be statistically different from the control ($p<0.05$).

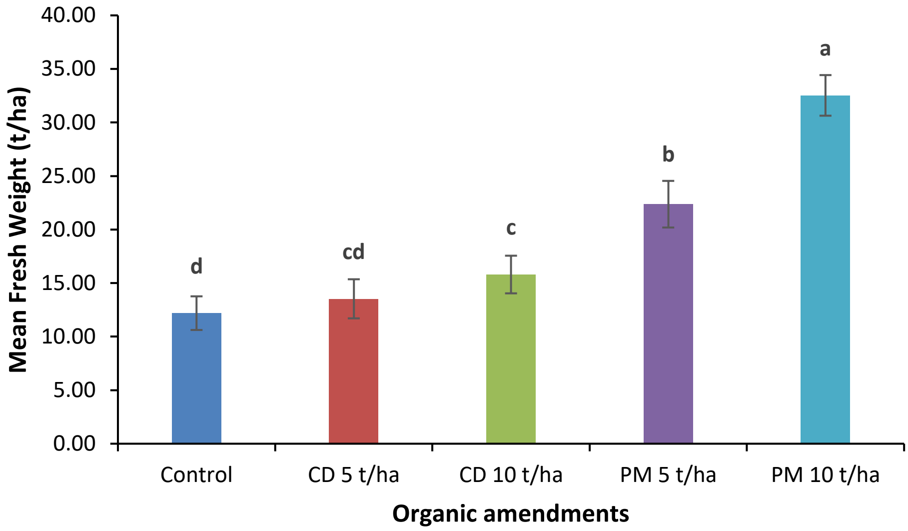

Figure 4.12. Fresh weight as affected by different organic amendments in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments.

Dry Weight

In 2017 *Boro* season, the dry weight of rice plant was found to differ significantly among the treatments (one-way ANOVA, p=0.001). From **Table 4.3** and **Figure 4.13**, it can be seen that the application of poultry manure at 10 t/ha was the best treatment in terms of rice plant dry weight, which was significantly different from the rest of the treatments. The second-best treatment was poultry manure at 5 t/ha, which was significantly different from cow dung at 5 and 10 t/ha and the control. Overall, the application of poultry manure was found to enhance the growth of rice crop.

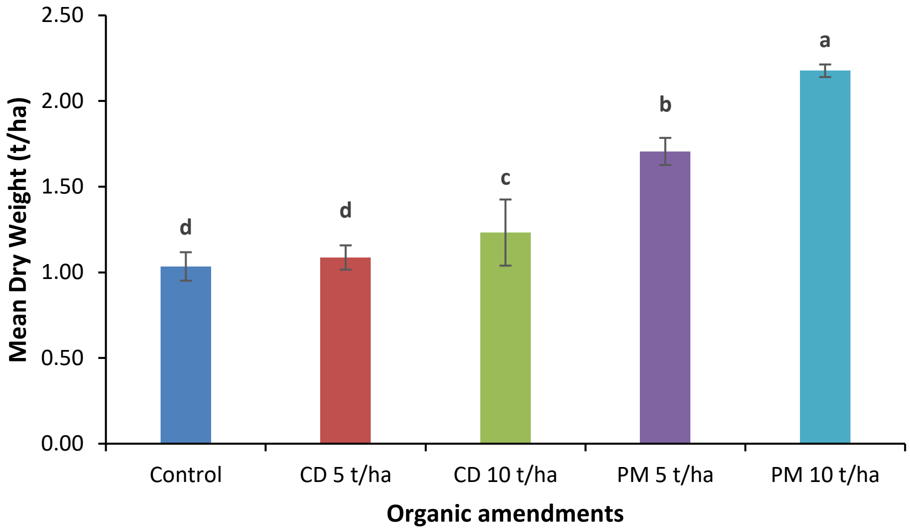

Figure 4.13. Dry weight as affected by different organic amendments in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post-hoc test was performed to determine if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments.

In 2018 *Boro* season, the dry weight of rice plant was also found to differ significantly among the treatments (one-way ANOVA, p=0.001). From **Table 4.3** and **Figure 4.14**, the application of poultry manure at 10 t/ha was the best treatment in terms of rice plant dry weight, which was significantly different from the rest of the treatments. The second-best treatment was poultry manure at 5 t/ha, which was significantly different from cow dung at 5 and 10 t/ha and the control. Overall, the application of poultry manure was found to enhance the growth of rice crop compared to cow dung amendment.

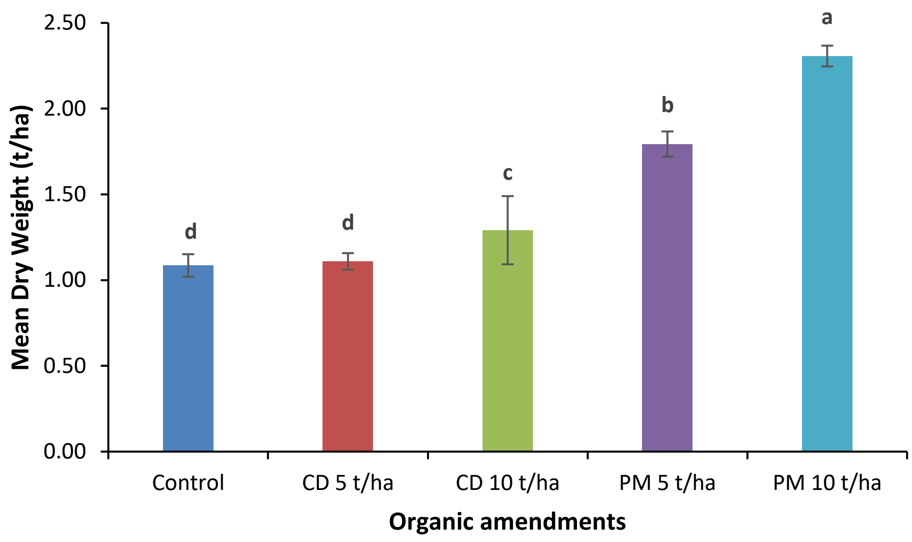

Figure 4.14. Dry weight as affected by different organic amendments in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was performed to determine if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments.

Grain Weight

Like plant height, fresh biomass and dry biomass, the grain weight was found to differ significantly among the treatments in 2017 *Boro* season (one-way ANOVA, $p = 0.000$). The maximum grain yield production was found in the PM 10 t/ha treated soil followed by the PM 5 t/ha treated soil. Poultry manure at 10 t/ha was found to be the best statistically compared to other treatments. On the other hand, the grain yield production in CD 5 t/ha and CD 10 t/ha treated soil were significantly different from the control (**Figure 4.15**).

In 2018 season, the grain weight was found to differ significantly among the treatments (one-way ANOVA, $p = 0.000$). The maximum grain yield production was found in the PM 10 t/ha treated soil followed by the PM 5 t/ha treated soil. Poultry manure at 10 t/ha was found to be the best statistically compared to other treatments. On the other hand, the grain yield production in CD 5 t/ha and CD 10 t/ha treated soil were significantly different from the control (**Figure 4.16**).

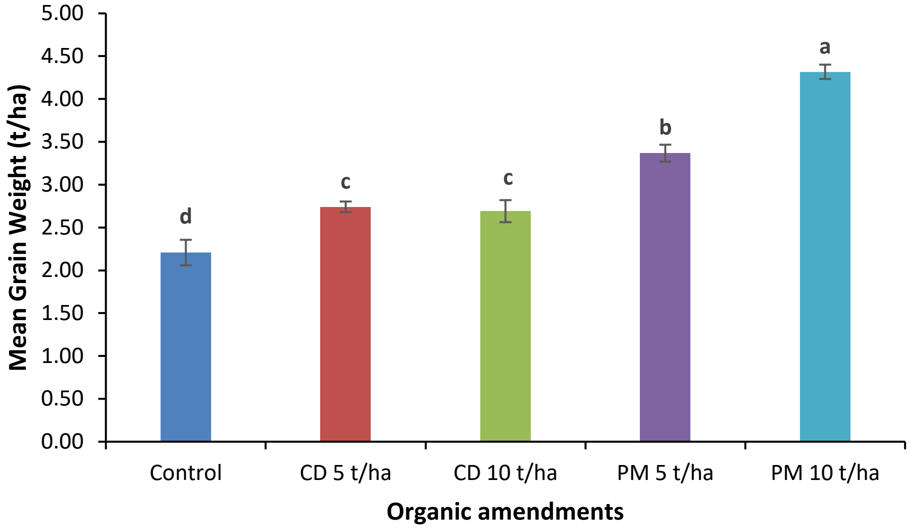

Figure 4.15. Grain weight as affected by different organic amendments in 2017 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was done to see if there are any differences among the treatments. Different letters above bars indicate significant differences between treatments.

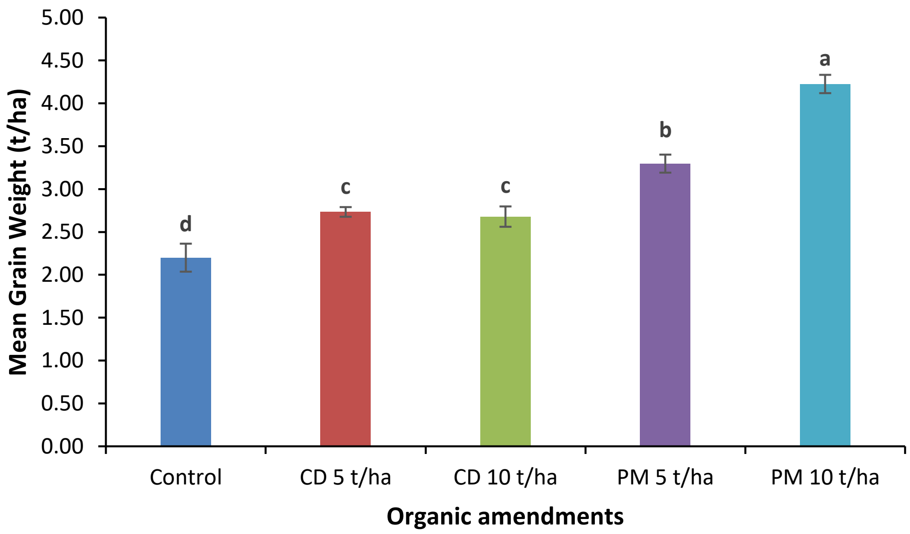

Figure 4.16. Grain weight as affected by different organic amendments in 2018 *Boro* season. A one-way ANOVA followed by Tukey's post hoc test was done to see if there are any differences among the treatments. Different letters above bars indicate significant differences between treatments.

4.5. Concentrations of Arsenic in Rice Plant

The mean arsenic (As) concentrations in rice root, straw, husk, and grain are presented in **Table 4.4**. A two-way ANOVA was performed to determine the main effects and interactive effects of water regimes and organic amendments on the concentrations of arsenic in roots, straw, husk, and grains of rice grown under different water regimes and with different organic amendments. **Table 4.4** contains data for two successive years, namely 2017 *Boro* season and 2018 *Boro* season. The data are discussed in the following subsections.

Table 4.4. Main effects and interaction effects of water regimes and organic amendments on the concentrations of arsenic (mg/kg) in root, straw, husk and grain of rice variety of BRRI Dhan 28 grown in 2017 and 2018. A one-way ANOVA followed by Tukey's Post-hoc test was performed to determine if there were any significant differences among the treatments and water regimes ($p<0.05$). Different letters in the same column indicate significant differences between the treatments. Letters are given alongside average concentrations of As (mg/kg) in root, straw, husk and grain at different water regimes and treatments.

Factor levels/ Interaction	**Arsenic Concentration (mg/kg)**							
	Root As		**Straw As**		**Husk As**		**Grain As**	
Water Regimes	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
No SW	13.93b	16.31a	1.98b	2.66a	1.61b	1.84a	0.55a	0.82a
3-cm SW	17.30ab	19.74a	2.89a	3.00a	2.67ab	1.95a	0.60a	0.90a
5-cm SW	19.54a	20.40a	3.34a	3.11a	3.69a	2.01a	0.61a	0.93a
Significance	***	***	***	***	***	***	*	***
Organic Amendments								
Control	16.23b	17.83bc	2.70bc	3.28b	1.32b	1.19c	0.74a	0.99b
CD 5t/ha	15.20b	15.93bc	2.15bc	2.29c	3.01b	2.46a	0.59b	0.80c
CD 10 t/ha	25.25a	26.78a	3.15ab	3.08b	5.36a	2.54a	0.57bc	1.26a
PM 5 t/ha	12.46b	14.28c	1.87c	2.03c	1.53b	1.67b	0.47c	0.62d
PM 10 t/ha	15.45b	19.25b	3.82a	3.93a	2.06b	1.80b	0.56bc	0.75c
Significance	***	***	***	***	***	***	***	***
Interactions								
Water regime* Organic Amendments	NS	*	***	NS	***	NS	***	NS

NS =Not significant, * = Significant at the 5%level, ** =Significant at the 1% level, *** = Significant at the 0.1% level.

Arsenic in Roots

A two-way ANOVA was performed and the ANOVA test revealed that in season one, both water regime (F = 22.65, p = 0.000) and treatments (F = 40.45, p = 0.000) had significant effects on the concentration of arsenic (As) in the root of rice (**Table 4.4**).In season two, both water regime (F = 20.31, p = 0.000) and organic amendments (F = 58.93, p = 0.000) were found to have significant effects on rice root arsenic concentration. In 2017, no interactive effects of water regime and organic amendment were observed on root arsenic concentration of rice. However, in 2018, water regime and organic amendment was found to have interactions between themselves. The root arsenic concentration was affected by the interaction.

Arsenic in Straw

In 2017, arsenic concentration in rice straw was found to be affected by water regime (F = 45.43, p = 0.000) and organic amendments (F = 34.87, p = 0.000) (**Table 4.4**). There were strong interactive effects of water regimes and organic amendments on the concentration of arsenic. In 2018, water regime (F = 20.36, p = 0.000) and organic amendments (F = 129.28, p = 0.000) had significant effects on straw arsenic concentration of rice as revealed by the two-way ANOVA (**Table 4.4**). However, no interactive effects were found on rice straw concentration owing to water regime and organic amendments.

Arsenic in Husk

In 2017, husk arsenic concentration in rice was found to be affected by water regime (F = 835.31, p = 0.000) and organic amendments (F = 1254.31, p= 0.000) (**Table 4.4**). Husk arsenic concentration was also highly affected by the strong interactions between water regime and organic amendment. Husk arsenic concentration was also affected by water regime (F = 14.76, p = 0.000) and organic amendment (F = 404.77, p = 0.000) in 2018 *Boro* season. However, the interactive effects of water regime and organic amendments on husk arsenic concentration were found to be insignificant.

Arsenic in Grain

In 2017 *Boro* season, water regime (F = 3.97, p = 0.029) and organic amendment (F= 25.79, p = 0.000) had effects on the concentration of arsenic in rice grain at the 5% and 0.1% level of significance (**Table 4.4**). Highly significant interaction effects were seen in rice grain (F = 6.35, p = 0.000). In 2018 *Boro* season, water regime (F = 9.06, p = 0.001) and organic amendment (F = 94.03, p = 0.000) were found to have highly significant effects on the concentration of arsenic in rice grains. However, no significant interactive effects were observed on arsenic concentration in rice grains.

4.5.1. Arsenic Concentration of Rice as Affected by Water Regimes

Table 4.4 also shows the mean arsenic concentrations in root, straw, husk, and grain of rice plants at different water regimes grown in 2017 *Boro* season and 2018 *Boro* season. A one-way ANOVA followed by Tukey's post-hoc test was performed to see if there were any differences among the water regimes in terms of arsenic concentrations in roots, straw, husk, and grains. Arsenic concentrations in roots, straw, husk, and grains under different water regimes are discussed in the following subsections.

Arsenic in Roots

The one-way ANOVA revealed that there were significant differences among the root arsenic concentrations of rice grown in 2017 *Boro* season under different water regimes (F = 4.82; p = 0.013). The highest (19.54 mg/kg) concentration of arsenic was found in plant roots which was under 5-cm SW regime (**Table 4.4 and Figure 4.17**). The plant roots which were under No SW regime had the lowest (13.93 mg/kg) arsenic concentration. There was a statistically significant differences between No SW regime and 5-cm SW regime.

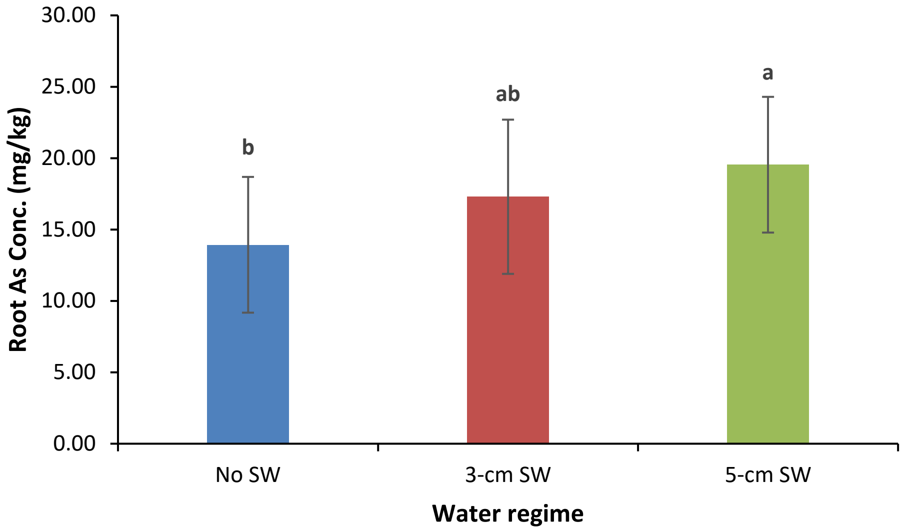

Figure 4.17. Arsenic concentration in rice roots grown in 2017 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes.

In 2018 *Boro* season, no significant differences were observed among the water regimes in terms of rice root arsenic concentrations ($F = 2.99$, $p = 0.061$) (**Table 4.4 and Figure 4.18**). However, following the previous year's trend, the highest (20.40 mg/kg) arsenic concentration was recorded in rice roots grown under 5-cm SW regime and the lowest (16.31 mg/kg) concentration was recorded for rice roots grown in No SW regime.

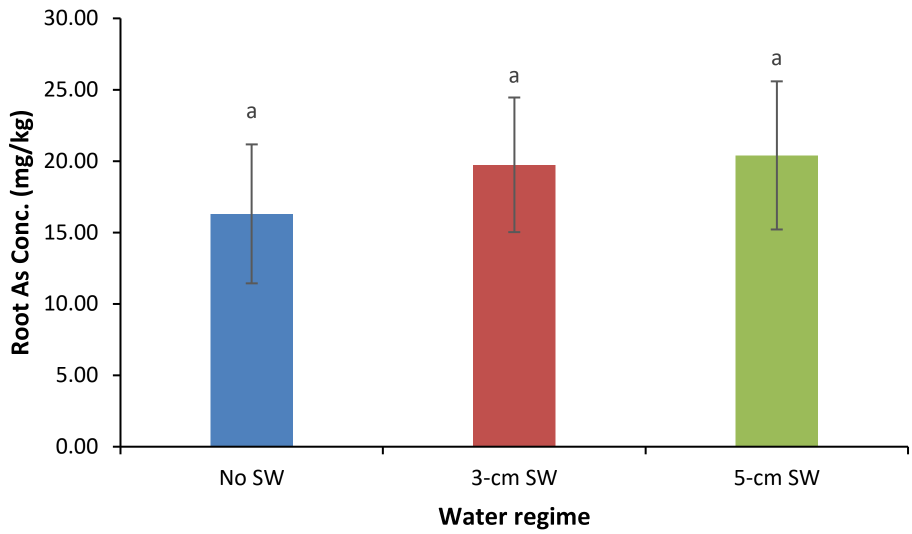

Figure 4.18. Arsenic concentration in rice roots grown in 2018 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes.

It was evident from two years' data that the presence of standing water enhanced the uptake of arsenic by rice roots. The uptake of arsenic was the highest in the plants which was grown under the 5-cm standing water regime followed by the 3-cm standing water regime. The redox potential of the soil could have influenced the bioavailability of arsenic (Honma et al. 2016).

Arsenic in Straw

In 2017 *Boro* season, water regimes were found to have significant effects on the arsenic concentration of rice straw ($F = 9.21$; $p = 0.000$). In the regime, where there was no standing water, rice straw was found to contain the lowest arsenic concentration (1.98 mg/kg). On the other hand, 3- and 5-cm standing water regimes were found to enhance the uptake of arsenic in the straw of rice (**Table 4.4 and Figure 4.19**). Statistically significant differences were found between No SW regime and 3-cm SW regime and between No SW regime and 5-cm SW regime (Figure 4.18). Similar observations were reported in literature.

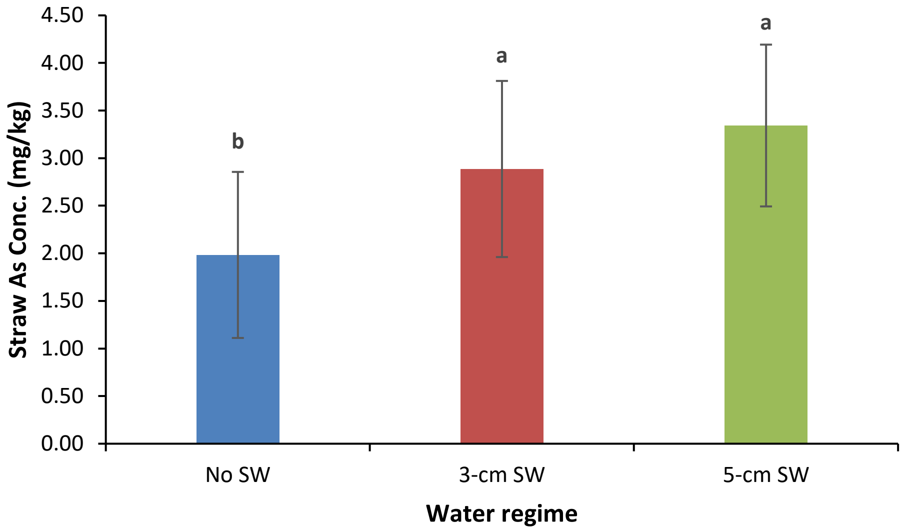

Figure 4.19. Arsenic concentration in rice straw grown in 2017 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post-hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

In 2018 *Boro* season, no significant effects of water regime were registered from the one-way ANOVA ($F = 1.54$, $p = 0.227$) (**Table 4.4 and Figure 4.20**). However, the highest (3.11 mg/kg) straw arsenic concentration was observed under 5-cm SW regime followed by 3-cm SW regime (3.00 mg/kg) and No SW regime (2.66 mg/kg).

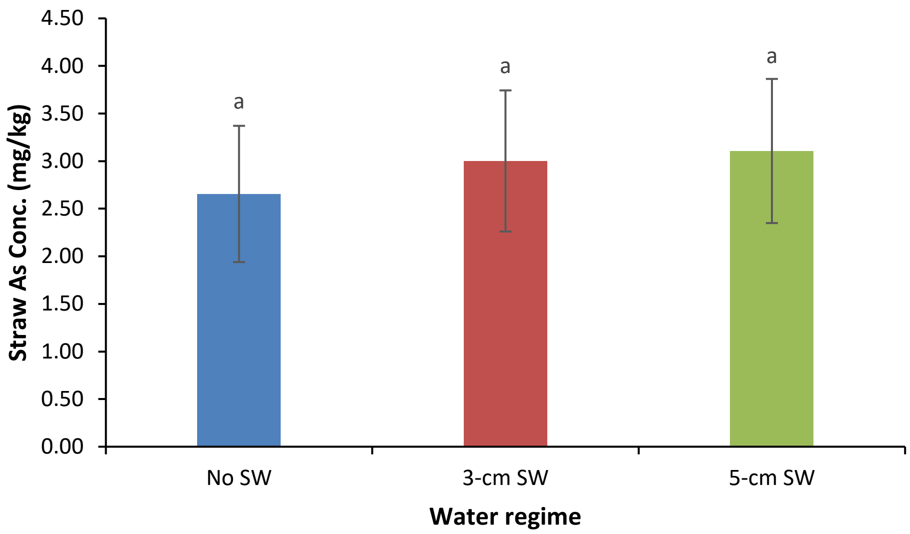

Figure 4.20. Arsenic concentration in rice straw grown in 2018 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post-hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

It was obvious from two *Boro* season's data that the uptake of arsenic was governed by the presence of standing water. The standing water might have reduced the oxidation-reduction potential thereby controlling the mobility of arsenic in soil.

Arsenic in Husk

In 2017 *Boro* season, arsenic concentrations in rice husk were affected by water regimes as was seen for root and straw arsenic concentrations. The trend was also the same as was observed for root and straw. The concentration of arsenic increased with the increase in water in the field during growing period. Standing water apparently triggered the uptake of arsenic by rice husk. The rice plants grown in saturated soil (No SW regime) was found to accumulate less arsenic compared to the plants grown with standing water in the field (**Table 4.4 and Figure 4.21**). Statistically significant differences were found between No SW regime and 5-cm SW regime ($p<0.05$). No differences were found between No SW regime and 3-cm SW regime and between 3-cm SW regime and 5-cm SW regime.

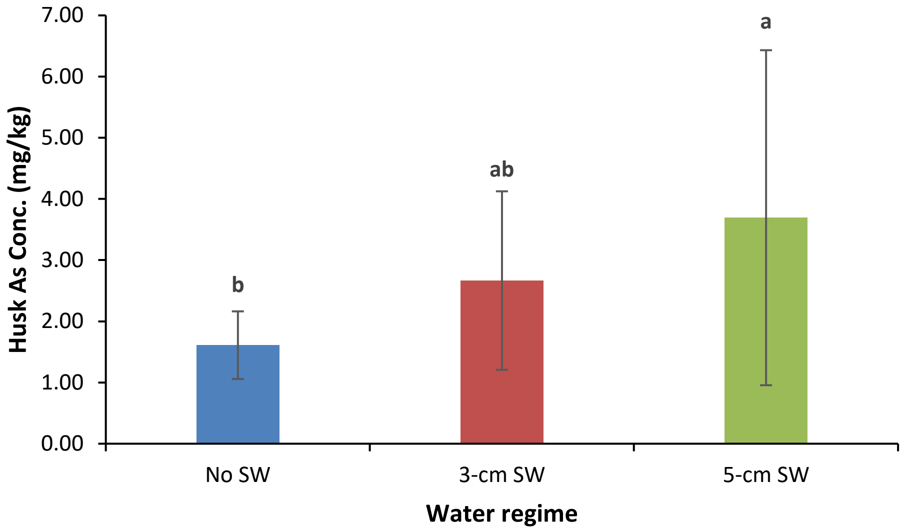

Figure 4.21. Arsenic concentration in rice husk grown in 2017 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

In 2018 *Boro* season, the mean rice husk concentrations did not differ significantly among the water regimes ($F = 0.37$, $p = 0.690$) (**Table 4.4 and Figure 4.22**). However, the highest (2.01 mg/kg) concentration of husk arsenic concentration was found in the water regime having the highest standing water; it means the standing water enhanced the uptake of arsenic by rice crop. No standing water regime, where the soil was only saturated with water, caused the lowest (1.84 mg/kg) accumulation of arsenic in husk of rice crop.

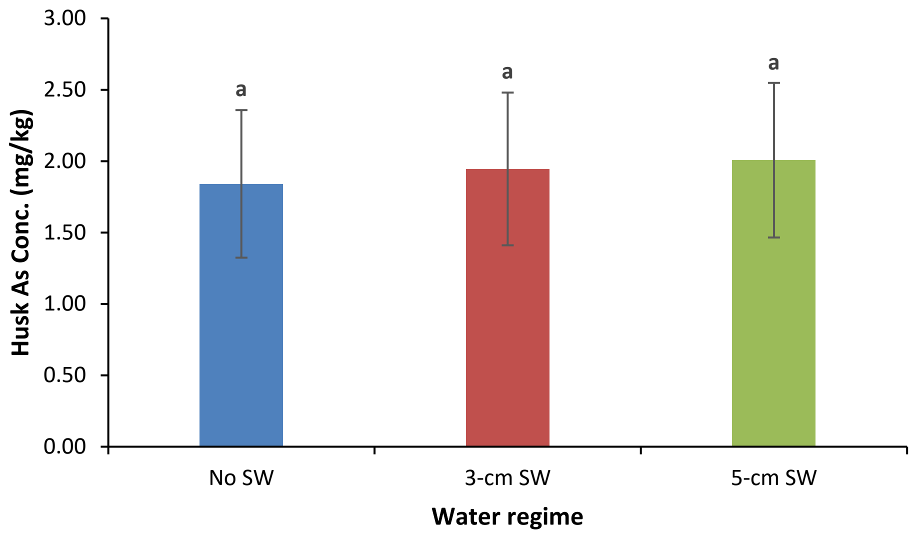

Figure 4.22. Arsenic concentration in rice husk grown in 2018 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was done to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

Arsenic in Grains

A one-way ANOVA followed by Tukey's post-hoc test was performed to determine if there were any differences among the water regimes in terms of grain arsenic concentration. The one-way ANOVA revealed that water regime had no significant effects on the concentration of grain arsenic ($F = 0.91$; $p = 0.411$) in 2017 *Boro* season (**Table 4.4 and Figure 4.23**). However, an increasing trend of arsenic concentration was observed with the increase in water content in the growing medium. The post-hoc test also revealed that there were no significant differences among the different water regimes with respect to grain arsenic concentration.

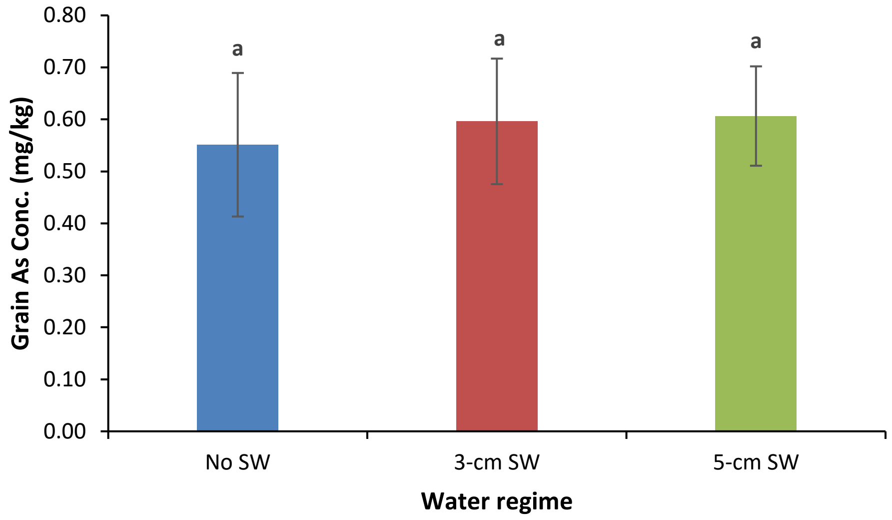

Figure 4.23. Arsenic concentration in rice grains grown in 2017 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

In 2018 *Boro* season, the highest (0.93 mg/kg) rice grain arsenic concentration was observed under 5-cm standing water regime followed by 3-cm standing water regime (0.90 mg/kg) and No SW regime (0.82 mg/kg) (**Table 4.4 and Figure 4.24**). However, the one-way ANOVA revealed that there were no statistically significant differences among the water regimes with respect to grain arsenic concentration ($F = 0.92$, $p = 0.405$).

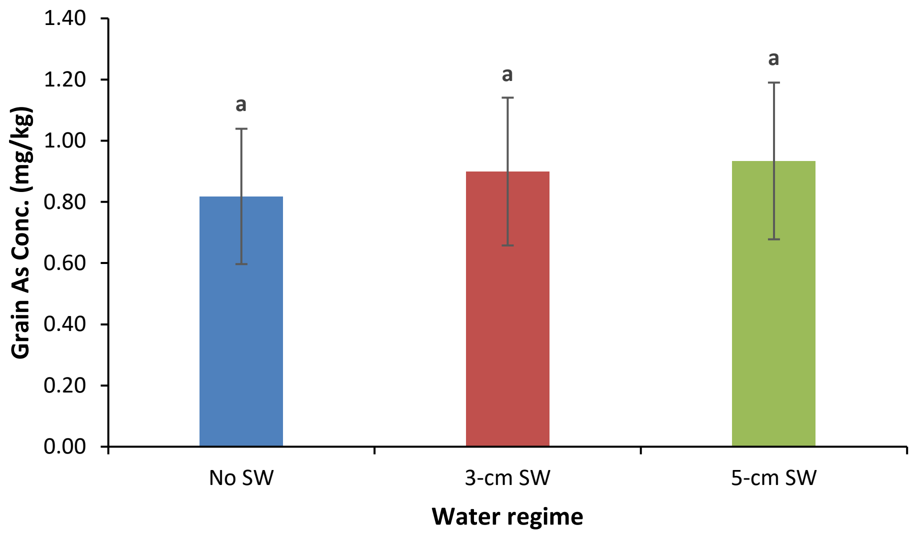

Figure 4.24. Arsenic concentration in rice grains grown in 2018 *Boro* season under different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the water regimes. Different letters above bars indicate significant differences between water regimes at $p<0.05$.

In previous studies, arsenic concentrations were found to be higher in rice roots. It was revealed from previous studies that most of the accumulated arsenic stays in root. In a study with BRRI dhan 28, root arsenic concentration was found to be 28 and 75 times higher than that of straw and raw rice (Rahman et al., 2007). Abedin et al (2002) also reported that the bulk of arsenic is retained by rice roots compared to other parts of the plant. Other scientists reported findings which substantiate the findings of the present study (Duxbury et al., 2002; Rahman et al., 2007). The higher accumulation of arsenic in rice roots could be ascribed to the presence of iron oxides or iron plaques which is formed around the roots of a rice crop. These iron plaques bind the arsenic, thereby preventing the upward translocation of rice to other tissues of the rice plant (Liu et al., 2004). In the present study, arsenic concentrations in the rice crop were in the following descending order: root>straw>husk>grain. The same order was reported by other groups of scientists (Odanaka et al., 1987; Marin et al., 1992; Abedin et al., 2002).

In the present study, water management was administered to reduce the uptake of arsenic. The hypothesis was that the absence of standing water will prevent the lowering of oxidation-reduction potential in soils, which is a master variable in soil and govern the bioavailability of arsenic. In the present study, water management was found to lower the uptake and accumulation of arsenic. In the first season, a significantly lower accumulation of arsenic was observed with the water regime where no standing water was allowed. Water regimes with standing water promoted the uptake of arsenic. However, in the second season, the accumulation of arsenic was not significantly different among the water regimes. The lower accumulation of arsenic in the first season could be attributed to the higher redox potential in the soil having no standing water. Under oxidized condition, the solubility of arsenic is low (Talukdar et al., 2011) because most of the arsenic remains sequestered with iron oxides; plants have no access to bound arsenic (Lauren and Duxbury, 2005). On the other hand, under moderately reduced conditions (Eh = 0 to + 100 mV), the solubility of arsenic increases owing to dissolution of iron oxyhydroxides. Li et al. (2009) conducted an experiment with rice where he administered different water regimes, including an aerobic one. They found out that rice crop takes up less arsenic if grown aerobically throughout the entire season. Islam et al. (2019) studied the impact of water and silicon fertilizer management on the bioaccumulation of arsenic. Alternate wetting and drying (AWD) irrigation practice along with silicon fertilization was found to significantly decrease the amount of arsenic in pore water compared to continuous flooding (CF) irrigation practice.

4.5.2. Arsenic Concentration of Rice as Affected by Organic Amendments

Arsenic concentrations in different parts of rice were also influenced by the application of organic amendments at different levels. One-way ANOVA was performed to determine if there were any significant differences among mean arsenic concentrations in different plant parts (root, straw, husk, and grain) dosed with cow dung and poultry manure at different levels. Tukey's post-hoc test was performed to make a pairwise comparisons among the concentrations. The mean values of arsenic concentrations in roots, straw, husk, and grains of rice plants at different treatments are presented in **Table 4.4**. In the following sub-sections, arsenic concentrations in roots, straw, husk, and grains are discussed as a function of organic amendments.

Arsenic in Roots

Table 4.4 shows the mean arsenic content in the roots of rice plants treated with different organic amendments at different levels. The one-way ANOVA revealed that organic amendments had significant effects on the arsenic concentration of rice roots ($F = 20.02$; $p = 0.000$) in 2017 *Boro* season. The root arsenic concentration was the highest in rice plants grown with cow dung at 10 t/ha (**Figure 4.25**). The concentration was significantly different from the rest of the treatments including the control ($p<0.05$). The lowest root arsenic concentration was observed in plants treated with poultry manure at 5 t/ha. However, the concentration was not statistically different from the control ($p>0.05$) **(Figure 4.25)**.

In 2018 *Boro* season, organic amendments had significant effects on the arsenic concentration of rice roots ($F = 26.05$; $p = 0.000$) as indicated by the one-way ANOVA (**Table 4.4**). Following the previous year's trend, the root arsenic concentration was the highest (26.78 mg/kg) in rice plants grown with cow dung at 10 t/ha (**Figure 4.26**). The concentration was significantly different from the rest of the treatments including the control ($p<0.05$). The lowest (14.28 mg/kg) rice root arsenic concentration was observed in plants treated with poultry manure at 5 t/ha. The lowest concentration was not statistically significantly different from the control ($p>0.05$) **(Figure 4.26)**.

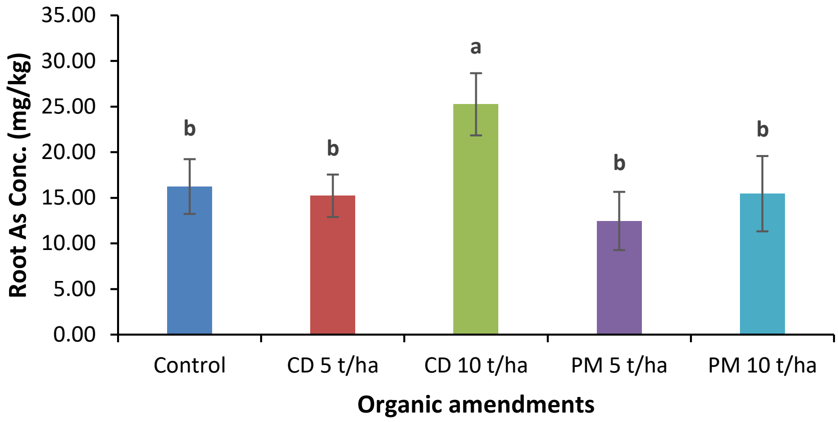

Figure 4.25. Arsenic concentration in rice roots grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

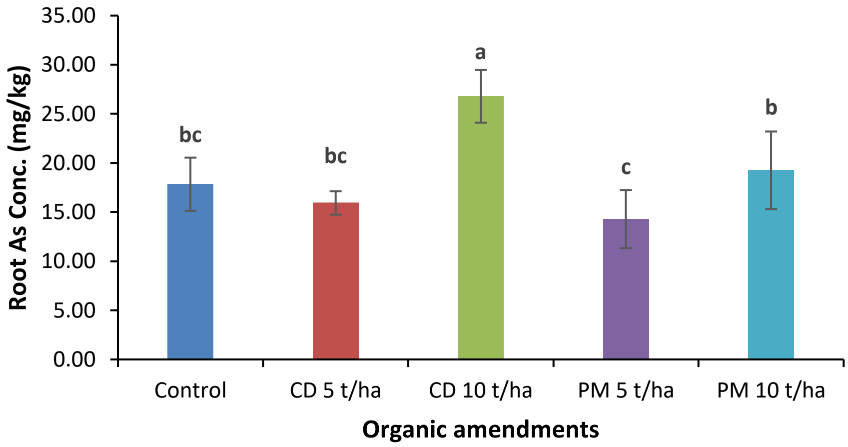

Figure 4.26. Arsenic concentration in rice roots grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

Arsenic in Straw

In 2017 *Boro* season, like root arsenic concentration, the mean straw arsenic concentrations were found to differ significantly among the treatments as revealed from the one-way ANOVA ($F = 8.80$; $p=0.000$). The highest straw arsenic concentration was observed in rice plants treated with poultry manure at 10 t/ha (3.824 mg/kg) followed by cow dung at 10 t/ha (3.147 mg/kg) (**Table 4.4 and Figure 4.27**). The lowest straw arsenic concentration was observed in rice plants amended with poultry manure at 5 t/ha (1.871 mg/kg). There was a statistically significant difference between poultry manure at 10 t/ha and 5 t/ha levels ($p<0.05$).However, the lowest concentration of arsenic was not significantly different from the control ($p>0.05$).

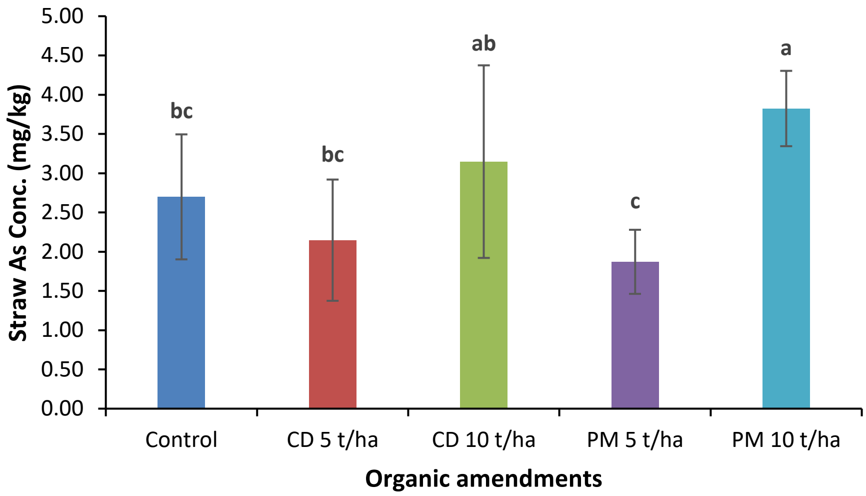

Figure 4.27. Arsenic concentration in rice straw grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post-hoc test was performed to determine if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

In 2018 *Boro* season, the straw arsenic concentrations in rice were significantly affected by the application of organic amendments ($F = 64.00$, $p = 0.000$) (**Table 4.4 and Figure 4.28**). The highest (3.93 mg/kg) straw arsenic concentration was observed in plants dosed with PM at 10 t/ha. The highest concentration reported for PM at 10 t/ha was statistically significant from the rest of the treatments ($p<0.05$). On the other hand, the lowest (2.03 mg/kg) straw arsenic concentration was reported for plants amended with PM at 5 t/ha; the treatment was significantly different from the control ($p<0.05$).

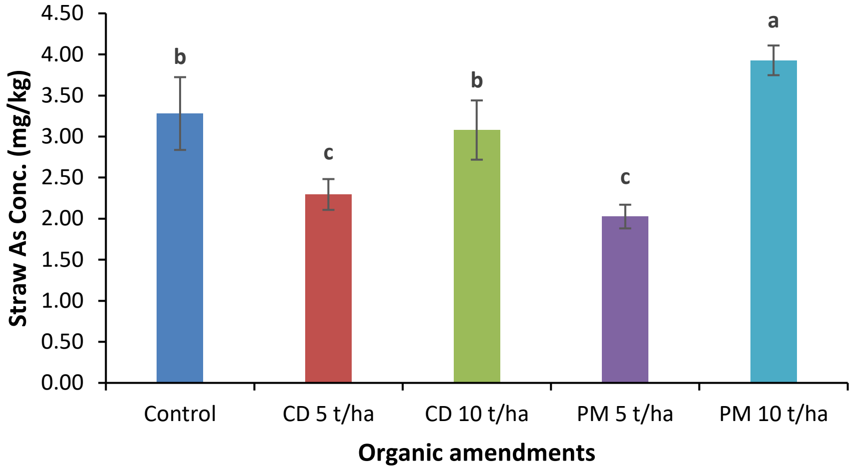

Figure 4.28. Arsenic concentration in rice straw grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post-hoc test was performed to determine if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

Arsenic in Husk

In 2017 *Boro* season, the mean husk arsenic concentrations were found to be affected by the application of organic amendments as indicated by the one-way ANOVA ($F = 13.25$; $p = 0.000$). The highest arsenic concentration (5.363 mg/kg) was obtained in plant husk dosed with cow dung at 10 t/ha (**Table 4.4 and Figure 4.29**). The treatment differed significantly from the rest of the treatments. The other organic amendment treatments and the control were statistically the same ($p>0.05$). It was noticeable that cow dung treatments promoted the uptake of arsenic compared to poultry manure treatments.

In 2018 *Boro* season, the mean husk arsenic concentrations were found to be significantly affected by the application of organic amendments as indicated by the one-way ANOVA ($F = 251.38$; $p = 0.000$). The highest (2.54 mg/kg) arsenic concentration was obtained in plant husk dosed with cow dung at 10 t/ha (**Table 4.4 and Figure 4.30**). The treatment differed significantly from the rest of the treatments except CD at 5 t/ha. The lowest (1.19 mg/kg) husk arsenic concentration was observed in plants grown in the control. In both seasons, cow dung amendment was found to enhance the uptake of arsenic compared to poultry manure treatments.

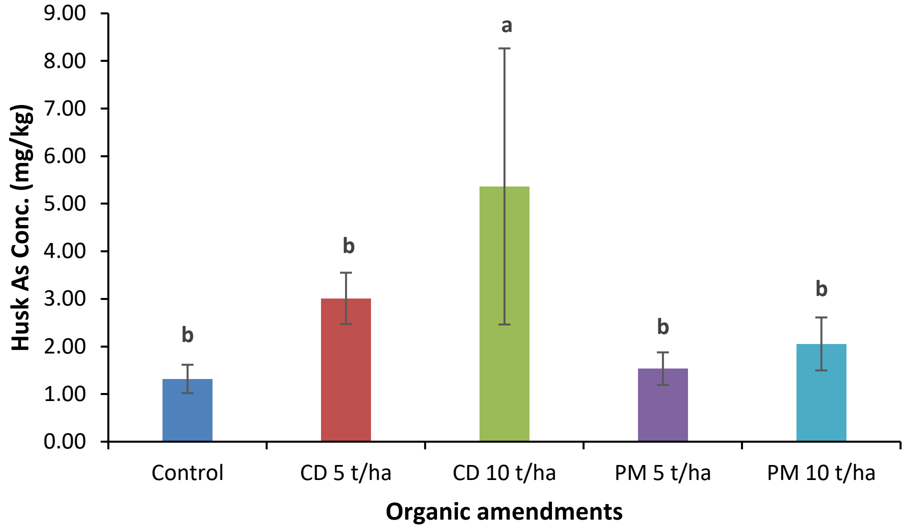

Figure 4.29. Arsenic concentration in rice husk grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

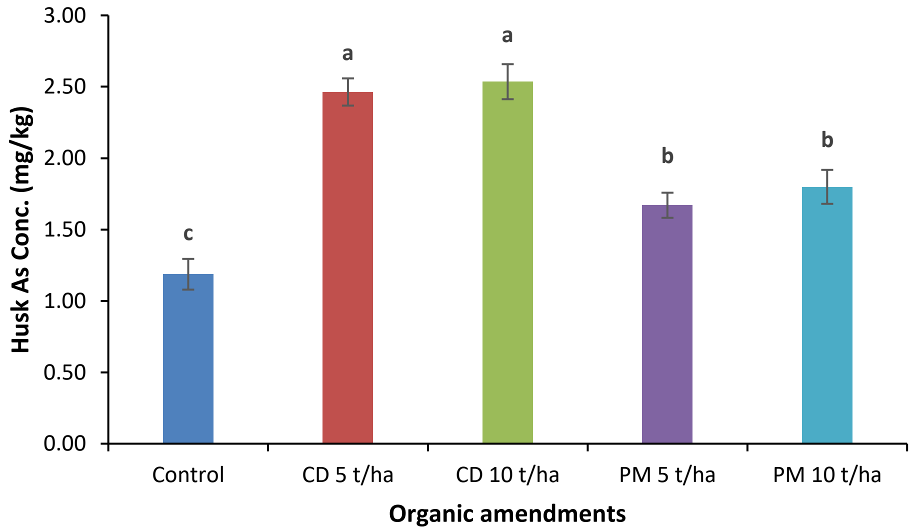

Figure 4.30. Arsenic concentration in rice husk grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to see if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments.

Arsenic in Grains

In 2017 *Boro* season, the mean arsenic concentrations of grains of rice plant were found to differ significantly among the treatments as was revealed from the one-way ANOVA (F = 11.63; p= 0.000).The highest grain arsenic concentration was found in plants grown in the control (**Table 4.4 and Figure 4.31**). The control was significantly different from the rest of the treatments (p<0.05). The lowest grain arsenic concentration was recorded in plants dosed with poultry manure at 5 t/ha. The cow dung amendment enhanced the uptake of arsenic more compared to poultry manure treatment.

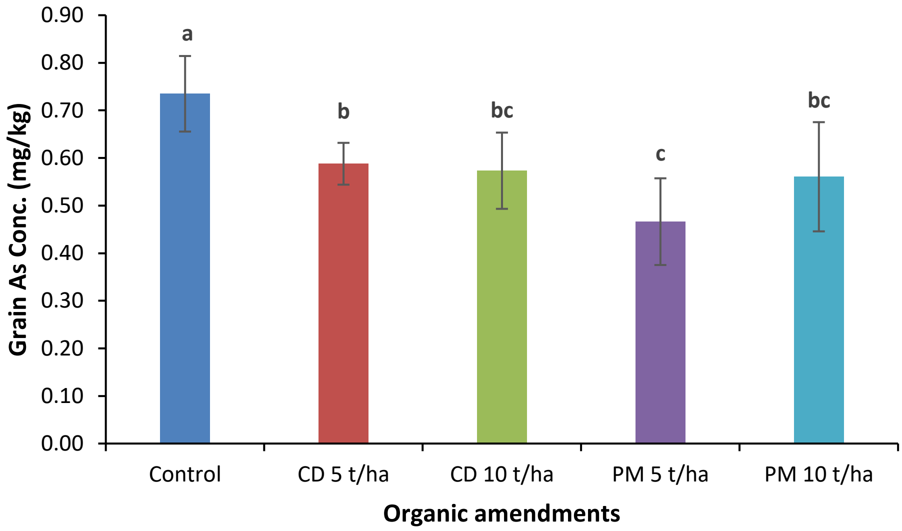

Figure 4.31. Arsenic concentration in rice grains grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments at p <0.05 level.

In 2018 *Boro* season, the mean arsenic concentrations of grains of rice plant were found to differ significantly among the treatments as revealed from the one-way ANOVA (F = 69.21, p= 0.000). The highest (1.26 mg/kg) grain arsenic concentration was found in plants amended with CD at 10 t/ha (**Table 4.4 and Figure 4.32**). The treatment i.e., CD 10 t/ha was significantly different from the rest of the treatments (p<0.05). The lowest (0.62 mg/kg) grain arsenic concentration was recorded in plants dosed with poultry manure at 5 t/ha. The cow dung amendment was found to promote the uptake of arsenic compared to poultry manure treatment.

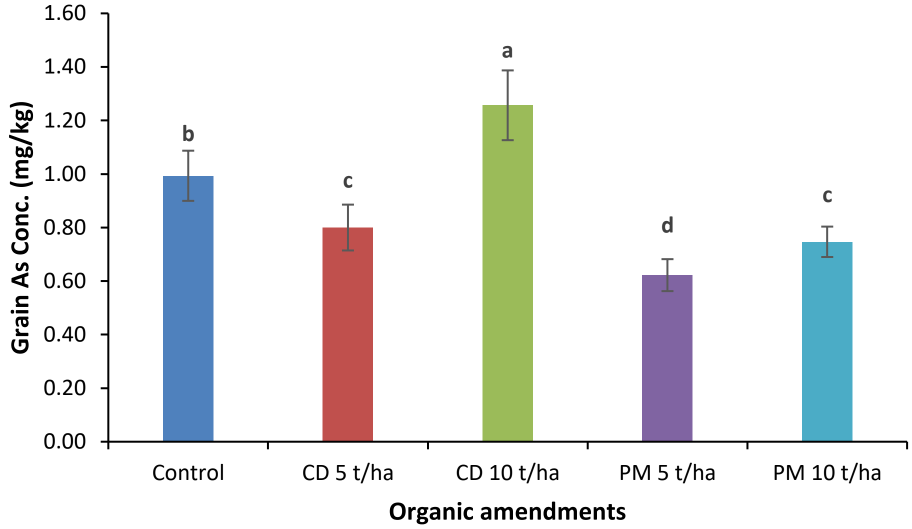

Figure 4.32. Arsenic concentration in rice grains grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the treatments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Arsenic concentrations in different plant tissues were found to be affected by the application of organic amendments. In roots, the mean arsenic concentrations ranged from 12.46 mg/kg to 26.78 mg/kg. On the other hand, the mean arsenic concentrations in rice straw were between 1.87 and 3.93 mg/kg. The mean husk arsenic concentrations ranged from 1.19 to 5.36 mg/kg. The grain arsenic concentrations ranged from 0.47 to 1.26 mg/kg. In general, the application of poultry manure at 5 t/ha was found to reduce the uptake of arsenic by the different parts of rice crop. At higher dose (10 t/ha), the application of poultry manure increased the uptake compared to the control. On the other hand, cow dung manure at 5 t/ha was found to reduce the uptake of arsenic by root, straw, and grain tissues. However, the treatment was found to enhance the uptake of arsenic by husk tissues. Cow dung manure at 10 t/ha was the least effective among the organic amendments for the treatment caused an enhanced uptake of arsenic by all the parts of rice crop. Norton et al. (2013) investigated the combined effects of organic matter amendment, arsenic amendment and water management on the concentrations of arsenic species in rice grains. They found that farmyard manure (FYM) at higher dosage (10%) enhanced the mobilization of arsenic. In the present study, the same phenomenon was observed for both the organic amendments. At higher dosage, both cow dung and poultry manure enhanced the accumulation of arsenic in root, straw, husk and grains of rice. Organic matter enhancing the availability of arsenic is attributed to the activities of microbes. Microbes utilizes organic matter and for utilizing organic matter they require oxygen. Consumption of oxygen by microbes result in a decrease in redox potential, which triggers the dissolution of arsenic from FeOOH (Nickson et al., 2000; Norton et al., 2013). The present study indicates that application of organic matter in reasonable amount is beneficial for crop in terms of nutrition and arsenic mitigation strategy.

4.5.3. Transfer Factor of As in Different Plant Parts

Transfer factors of arsenic (As) were calculated for soil to root, soil to straw, soil to husk and soil to grain from arsenic analysis data of rice crop grown in 2017 and 2018 seasons. The transfer factors were compared among the different parts of rice plant grown in 2017 and 2018. This was done to find the predisposition of rice plant to accumulate arsenic in different parts of its body. A one-way ANOVA followed by Tukey's post-hoc test was performed to see whether or not the transfer factors calculated for different parts of the rice plant differed significantly among themselves. For 2017 data, the one-way ANOVA indicates that the accumulation in root was significantly different from the rest of the parts ($p<0.05$). For 2018 data, the accumulation of arsenic was significantly different in root from the rest of plant parts ($p<0.05$). There was no significant variation between straw and husk ($p>0.05$) in terms of arsenic accumulation. No significant difference was observed between husk and grain of rice vis-à-vis arsenic accumulation. In previous studies, arsenic content of rice plant parts was found to follow the trend: root > straw > husk> grain (Wang et al., 2006; Smith et al., 2008). Findings from the present study are in agreement with the previous studies.

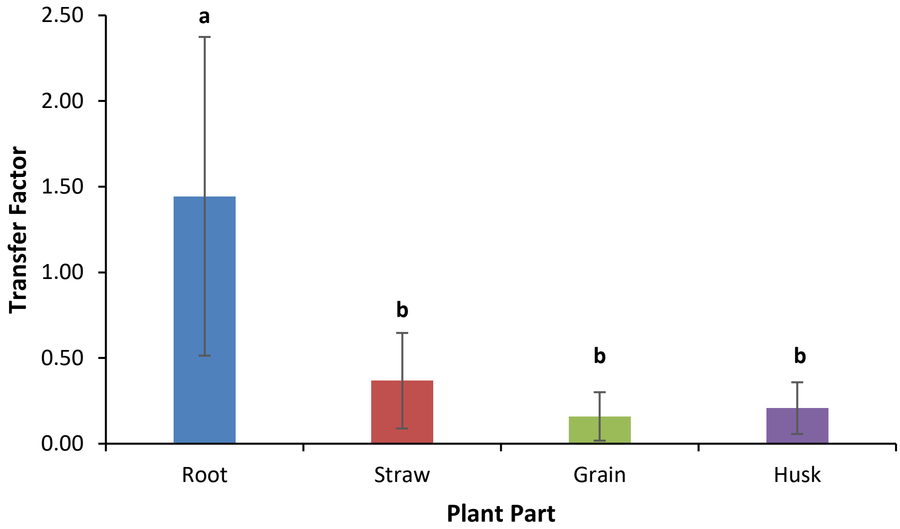

Figure 4.33. Transfer factors of arsenic in different parts of the rice plant grown in 2017. A one-way ANOVA followed by Tukey's post-hoc test was performed to find if there are differences among the different parts of the plant. Different letters above bars indicate significant differences between the treatments ($p<0.05$).

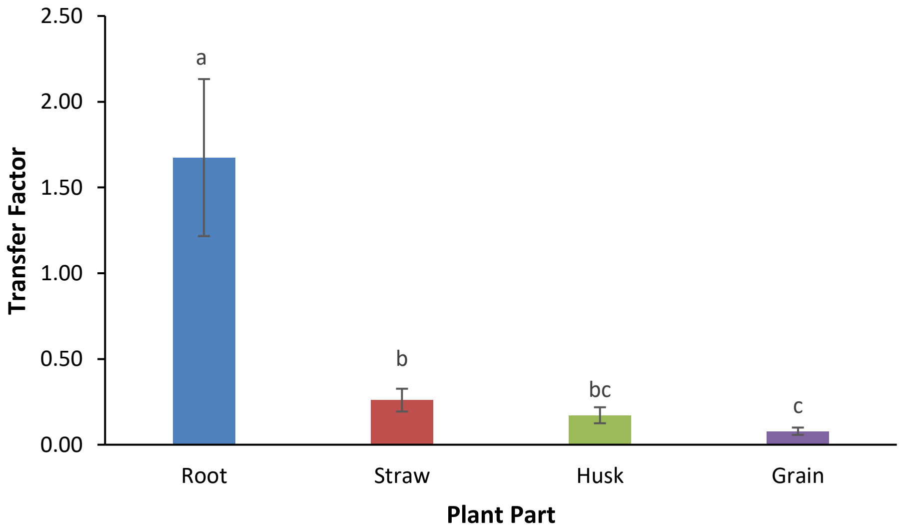

Figure 4.34. Transfer factors of arsenic in different parts of the rice plant grown in 2018. A one-way ANOVA followed by Tukey's post-hoc test was performed to find if there are differences among the different parts of the plant. Different letters above bars indicate significant differences between the treatments ($p<0.05$).

4.5.4. Relationship between Different Parts of Rice

Pearson correlation analysis was performed to assess if there are any linear relationship between different parts of rice crop in terms of arsenic concentrations. Significant correlations with r>0.5 (therefore explaining 25% of total variation) were only considered for further discussion. Significance was determined based on whether p-values are <0.05 or not (Hossain, 2016). For this analysis, 2017 and 2018 data were combined. A significant relationship was found between root As concentrations and straw As concentrations (r = 0.504, p = 0.000). A significant relationship was found between root As concentrations and grain As concentrations as well (r = 0.544, p = 0.000). No significant linear relationship was found between straw As concentrations and grain As concentrations (r = 0.250, p = 0.018). However, Talukder et al. (2012) found significant positive relationship (r = 0.88; **p ≤ 0.001) between As concentration in the straw and grain. They also grew rice under different water management regimes.

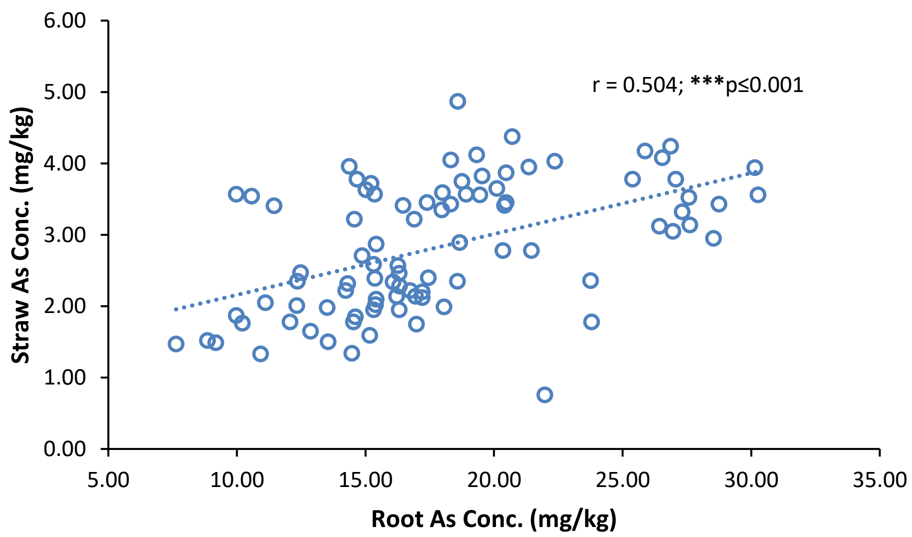

Figure 4.35. Correlation analysis of rice root As and rice straw As concentrations (n = 90).

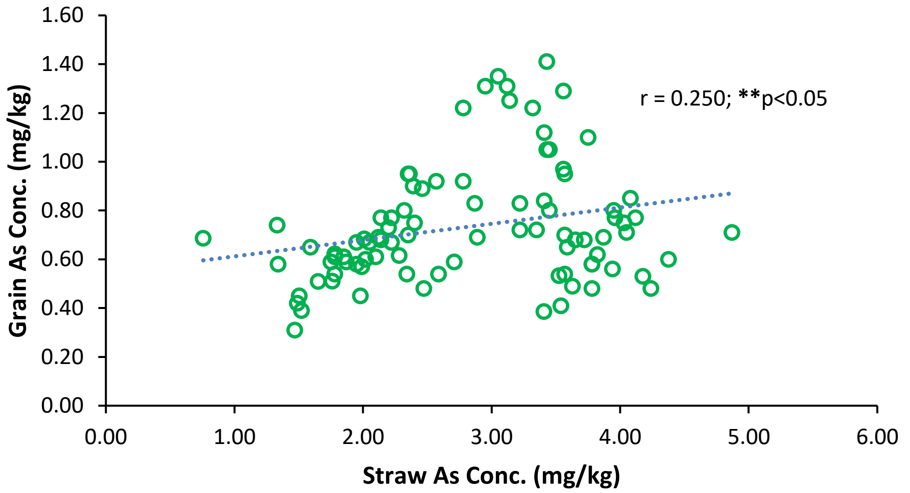

Figure 4.36. Correlation analysis of rice straw As and rice grain As concentrations (n = 90).

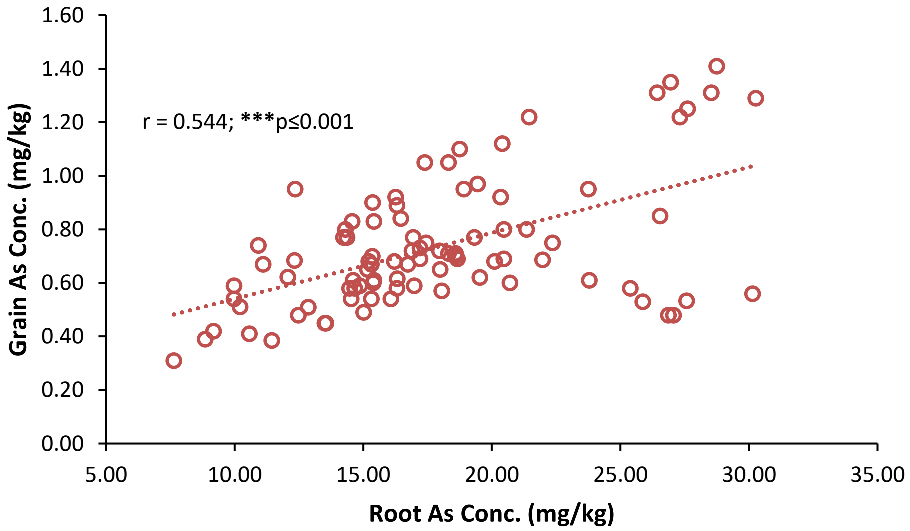

Figure 4.37. Correlation analysis of rice root As and rice grain As concentrations (n = 90).

4.6. Availability of Some Other Elements as Affected by Water Regimes and Organic Amendments

The availability of arsenic is affected by some mineral nutrients like P, S, Fe and Si. In the paddy field, phosphorus is thought to play a vital role for arsenic solubility and its uptake by plants. Phosphorus competes with arsenate at the sorption sites through ligand exchange mechanisms. Moreover, arsenic toxicity in plants is governed by the As/P ratio in the soil. In the following section, the accumulation of phosphorus element is discussed in great detail.

4.6.1. Concentration of Phosphorous in Rice Plant

The concentration of phosphorus in different parts of rice crop was analyzed. **Table 4.5** represents the mean values of phosphorus (P) in root, straw, husk and grain at different water regimes and treatments. A two-way ANOVA was performed to determine if there were any effects of water regime and organic amendments on the concentration of phosphorus in different parts of rice crop. Interactive effects of water regime and organic amendments were also revealed. A one-way ANOVA followed by Tukey's post-hoc test was also performed to determine the best and worst water regime and organic amendment treatment.

Table 4.5. Main effects and interaction effects of water regimes and organic amendments on the concentrations of phosphorus (%) in root, straw, husk, and grain of rice variety of BRRI Dhan 28 grown in 2017 and 2018.A one-way ANOVA followed by Tukey's Post-hoc test was performed to determine if there were any significant differences among the treatments and water regimes ($p<0.05$). Different letters in the same column indicate significant differences between the treatments. Letters are given alongside average concentrations of phosphorus (mg/kg) in root, straw, husk and grain at different water regimes and treatments.

Factor levels/ Interaction	**Phosphorus Concentration (%)**							
Water Regimes	**Root P**		**Straw P**		**Husk P**		**Grain P**	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
No SW	0.6255a	0.6500a	0.7279a	0.7460a	0.2843a	0.3047a	0.1493a	0.1553a
3-cm SW	0.7180a	0.6827a	0.5316b	0.4933b	0.2822a	0.2433b	0.1748a	0.1420a
5-cm SW	0.6727a	0.6160a	0.5200b	0.5027b	0.2917a	0.2587ab	0.1079b	0.0965b
Significance	***	***	***	***	NS	***	***	***
Organic Amendments								
Control	0.6625bc	0.6178b	0.5597bc	0.5456b	0.2669b	0.2456bc	0.1513a	0.1353a
CD 5 t/ha	0.5789cd	0.5589b	0.4717c	0.4578b	0.2543b	0.2278c	0.1252a	0.1044a
CD 10 t/ha	0.7480ab	0.7278a	0.5124bc	0.5011b	0.3336a	0.3078ab	0.1711a	0.1556a
PM 5 t/ha	0.5531d	0.5511b	0.6612ab	0.6544ab	0.3349a	0.3256a	0.1327a	0.1311a
PM 10 t/ha	0.8177a	0.7922a	0.7609a	0.7444a	0.2406b	0.2378c	0.1397a	0.1300a
Significance	***	***	***	***	***	***	***	***
Interactions								
Water regime* Organic Amendments	*	*	***	***	***	***	***	***

NS =Not significant, * = Significant at the 5% level, ** = Significant at the 1% level, *** = Significant at the 0.1% level.

Phosphorus in Roots

The two-way ANOVA test indicates that both water regimes (F = 13.51, p = 0.000) and organic amendments (F = 47.24, p = 0.000)significantly affected the concentration of P in rice roots grown in 2017 *Boro* season (**Table 4.5**).Interaction effects were also found to be significant(F= 2.72, p= 0.022).

In 2018 season, water regime (F = 11.79, p = 0.000) and organic amendment (F = 72.27, p = 0.000) were found to be significant in terms of having effects on the concentration of phosphorus in rice roots (**Table 4.5**). Water regime and organic amendment were found to have significant interactive effects on the phosphorus concentration of roots (F = 2.59, p = 0.028).

Phosphorus in Straw

In 2017 season, both water regime (F = 133.50, p = 0.000) and organic amendment (F = 80.88, p = 0.000) had highly significant effects on the concentration of P in straw as revealed by the two-way ANOVA test (**Table 4.5**). Interaction effects were also found to be highly significant (F= 17.92, p= 0.000).

In 2018 *Boro* season, water regime (F = 309.22, p = 0.000) and organic amendment (F = 124.14, p = 0.000) were found to have highly significant effects on P concentration in straw (**Table 4.5**).Interactive effects of water regime and organic amendment were found to be highly significant.

Phosphorus in Husk

The two-way ANOVA test indicates that water regime (F = 0.34, p = 0.712) had no significant effects on P concentration in rice husk (**Table 4.5**) grown in 2017 *Boro* season. However, organic amendment (F = 16.47, p = 0.000) had highly significant effects on P concentration in rice husk.The ANOVA test also suggested that there were no interactions between water regime and organic amendment (F= 6.00, p= 0.000).

In 2018 *Boro* season, both water regime (F = 29.77, p = 0.000) and organic amendment (F = 34.74, p = 0.000) had significant effects on P concentration in rice husk (**Table 4.5**). Interactive effects were also found to be highly significant (F = 11.33, p = 0.000).

Phosphorus in Grains

The two-way ANOVA indicates that both water regime (F = 36.19, p = 0.000) and organic amendment (F = 6.11, p = 0.001) had highly significant effects on P concentration in rice grains grown in 2017 *Boro* season (**Table 4.5**). Interactive effects of water regime and organic amendment were found to be highly significant (F = 14.14, p = 0.000).

In 2018 *Boro* season, both water regime (F = 74.78, p = 0.000) and organic amendment (F = 15.57, p = 0.000) were found to have significant effects on P concentration in rice grains (**Table 4.5**). Water regime and organic amendment had highly significant (F = 30.51, p = 0.000) interactive effects on P concentration in rice grains.

4.6.1.1. Phosphorus Concentration of Rice as Affected by Water Regimes

A one-way ANOVA followed by Tukey's post-hoc test was performed to see if there were any significant differences among the water regimes. Fifteen values were combined to calculate the mean and the standard deviation and to do the analysis of variance. The data was checked for the assumption of normality before doing the parametric test.

Phosphorus in Roots

In 2017 *Boro* season, water regimes were found to have no significant effects on the concentration of rice root P (F = 2.36, p = 0.107). The maximum concentration of P in roots was observed under 3-cm SW regime followed by 5-cm SW and No SW regimes (**Table 4.5** and **Figure 4.38**).

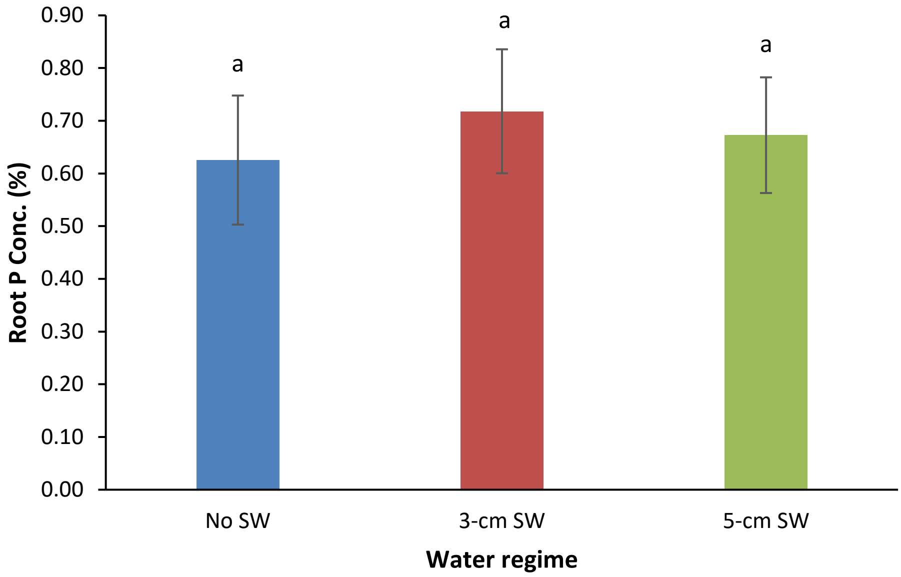

Figure 4.38. Phosphorus concentration in rice roots grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018*Boro* season, water regimes had no significant effects on the concentration of rice root phosphorus concentration ($F = 1.46$, $p = 0.244$). The maximum P concentration was found in plant roots under 3-cm SW regime followed by No SW and 5-cm SW regimes (**Table 4.5** and **Figure 4.39**).

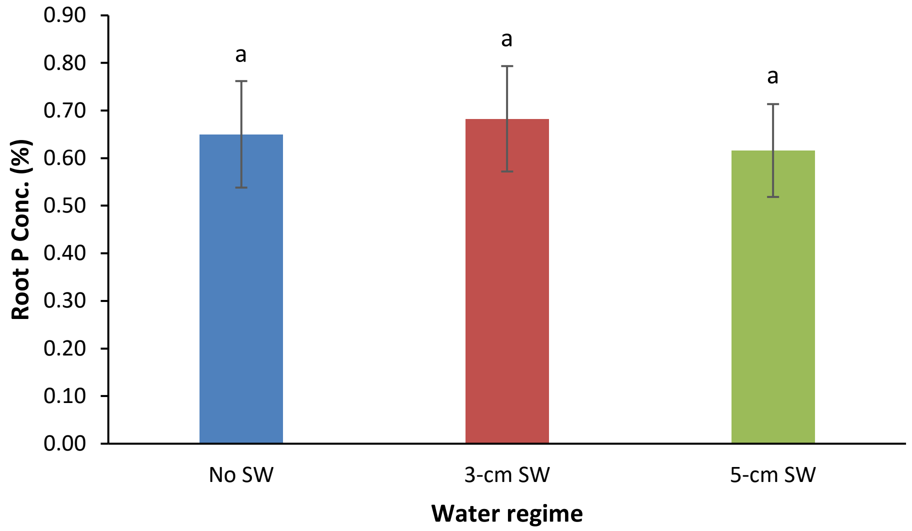

Figure 4.39. Phosphorus concentration in rice roots grown in 2018 Boro season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Straw

Table 4.5 and **Figure 4.40** show that there is a decreasing trend in the rice straw P concentration with increasing water levels in 2017 *Boro* season. Rice straw P concentration under No SW regime was significantly different than the concentrations under 3-cm and 5-cm SW regimes ($p<0.05$). There were no significant differences between 3-cm and 5-cm SW regimes with respect to rice straw phosphorus concentration ($p>0.05$). The one-way ANOVA revealed that water regime had significant effects on the concentration of rice straw P ($F = 11.28$, $p = 0.000$).

In 2018 *Boro* season, phosphorus concentration in rice straw was found to be affected by the administration of water regime ($F = 17.51$, $p = 0.000$). Following the trend of the previous year, rice straw phosphorus concentration under No SW regime was found to be significantly different from 3-cm and 5-cm SW regimes ($p<0.05$) **Table 4.5** and **Figure 4.41**). 3-cm and 5-cm SW regimes were statistically the same in terms of straw P concentration.

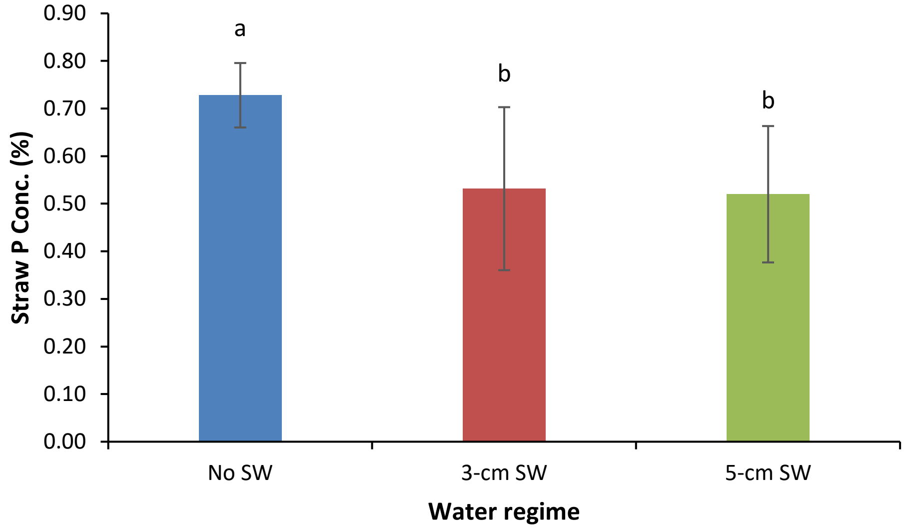

Figure 4.40. Phosphorus concentration in rice straw grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

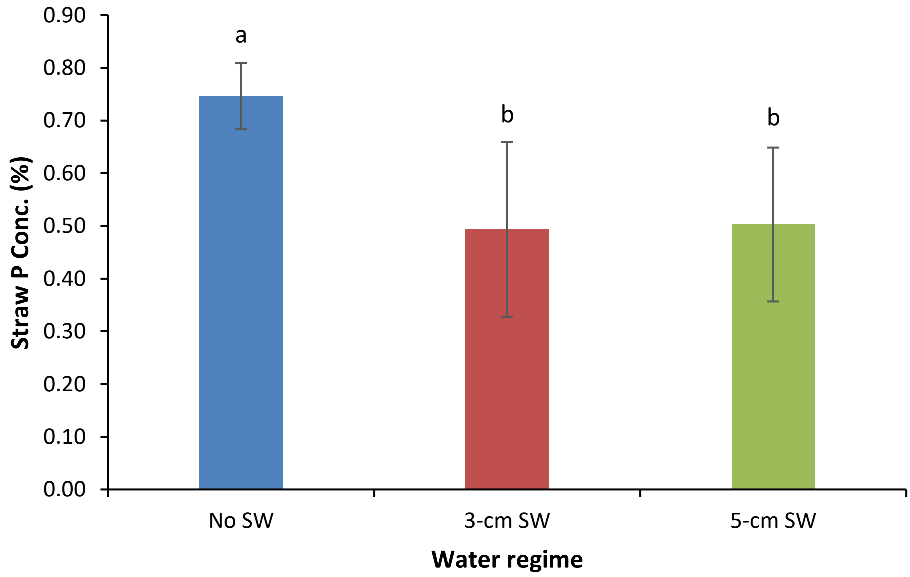

Figure 4.41. Phosphorus concentration in rice straw grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Husk

In 2017 *Boro* season, there were no significant differences among the rice husk phosphorus concentration grown under three water regimes (No SW, 3-cm SW, and 5-cm SW) as revealed by the Tukey's post-hoc test (**Table 4.5** and **Figure 4.42**). However, the highest husk P concentration (0.2917 mg/kg) was observed under 5-cm SW regime followed by No SW and 3-cm SW regimes. The one-way ANOVA also suggested that there were no differences among the three water regimes vis-à-vis husk P concentration ($F = 0.10$, $p = 0.905$).

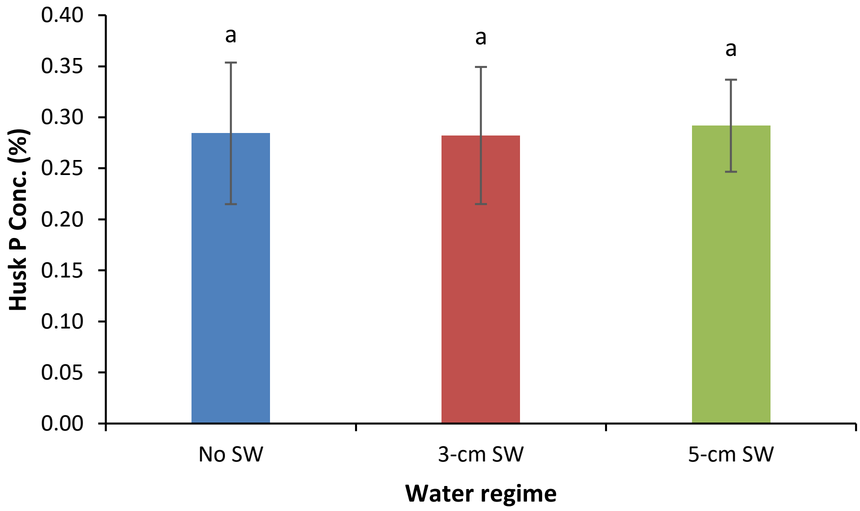

Figure 4.42. Phosphorus concentration in rice husk grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 *Boro* season, significant differences among the rice husk phosphorus concentration grown under three water regimes (No SW, 3-cm SW, and 5-cm SW) were observed as revealed by the Tukey's post-hoc test (**Table 4.5** and **Figure 4.43**). The highest husk P concentration (0.3047 mg/kg) was observed under No SW regime followed by 5-cm SW and 3-cm SW regimes. The husk P concentration under No SW regime was significantly different that 3-cm SW regime. The one-way ANOVA also indicated that there were differences among the three water regimes vis-à-vis husk P concentration ($F = 4.82$, $p = 0.013$).

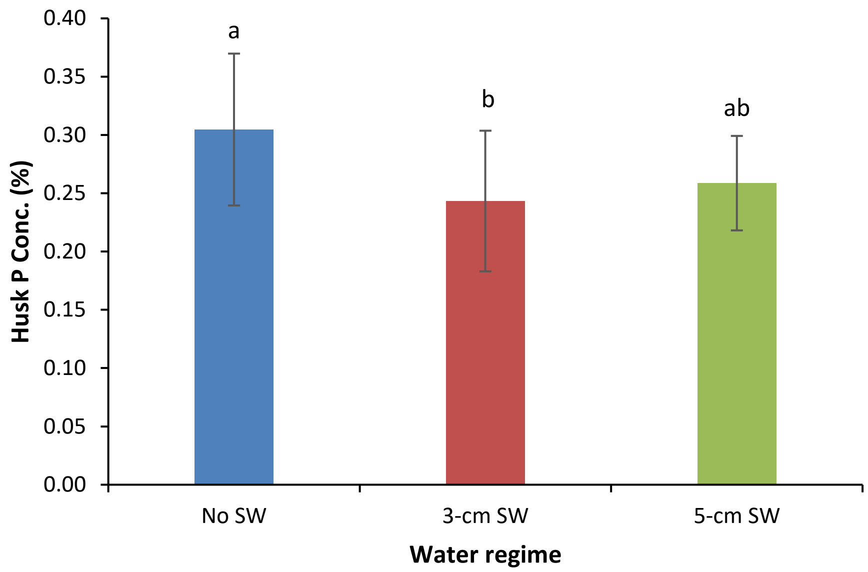

Figure 4.43. Phosphorus concentration in rice husk grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Grains

In 2017 *Boro* season, water regime was found to have significant effects on the concentration of rice grain P as revealed by the one-way ANOVA ($F = 9.07$, $p = 0.001$). The grain phosphorus concentration was found in rice plants grown under 3-cm SW regime followed by No SW and 5-cm SW regimes (**Table 4.5** and **Figure 4.44**). The grain P concentrations under No SW and 3-cm SW regimes were significantly different from 5-cm SW regime.

In 2018 *Boro* season, water regime was found to have highly significant effects on rice grains P concentration as indicated by the one-way ANOVA ($F = 9.30$, $p = 0.000$). Tukey's post-hoc test revealed that the highest concentration of phosphorus was observed under No SW regime followed by 3-cm and 5-cm SW regimes (**Table 4.5** and **Figure 4.45**). Phosphorus concentrations under No SW regime and 3-cm regime were statistically significantly different from that under 5-cm SW regime ($p<0.05$).

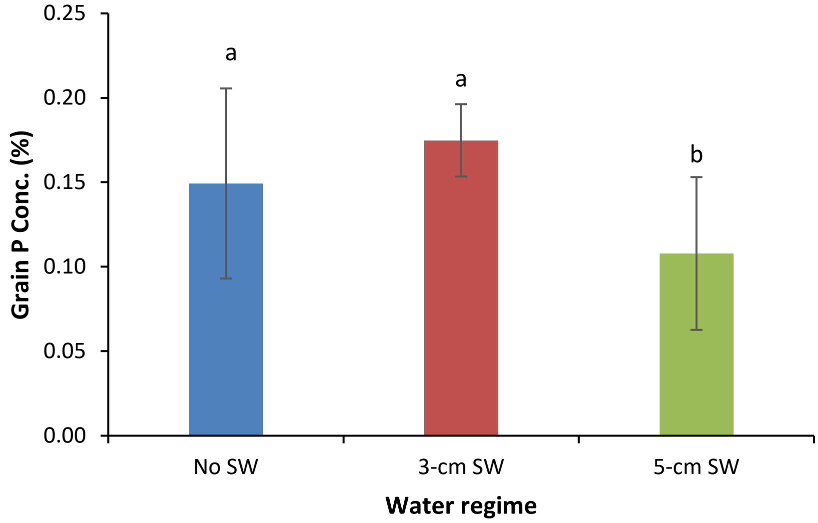

Figure 4.44. Phosphorus concentration in rice grains grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at p <0.05 level.

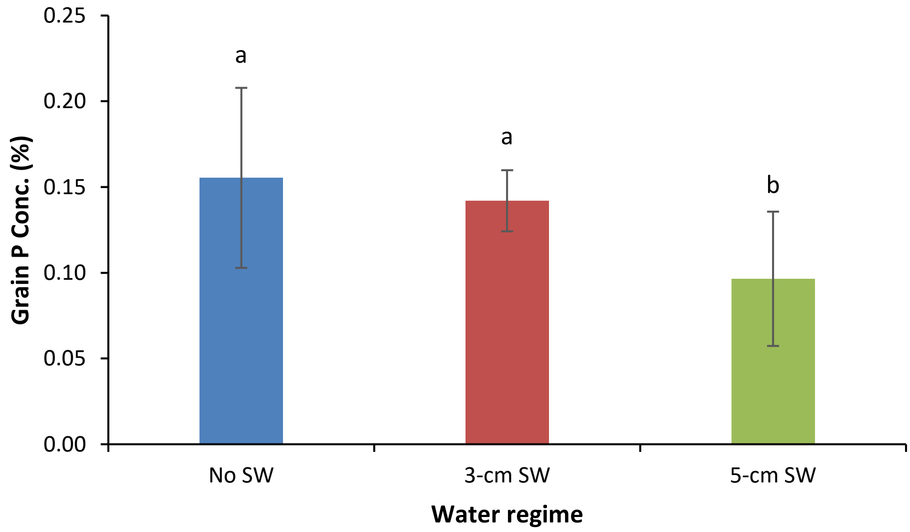

Figure 4.45. Phosphorus concentration in rice grains grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at p <0.05 level.

4.6.1.2. Phosphorus Concentration of Rice as Affected by Organic Amendments

A one-way ANOVA followed by Tukey's post-hoc test was performed to find if there were any statistically significant differences among the organic amendments in terms of phosphorus concentrations in root, straw, husk and grains. The data was checked for the assumption of normality. The distribution of data was found to be normal. Therefore, the parametric test was carried out using the original data. **Table 4.5** represents the average values of phosphorus concentration in roots, straw, husk and grains of rice plants at different organic amendment treatments.

Phosphorus in Roots

In 2017 *Boro* season, root phosphorus concentration in rice crop was found to be affected by the application of organic amendments as indicated by the one-way ANOVA ($F = 23.97$, $p = 0.000$). The highest concentration (0.8177 mg/kg) was observed at the application of 10 t/ha of poultry manure. The concentration was significantly different from the concentrations observed with the rest of the treatments excluding CD 10 t/ha (**Table 4.5** and **Figure 4.46**). The phosphorus concentration in the control was significantly better than that of PM 5 t/ha.

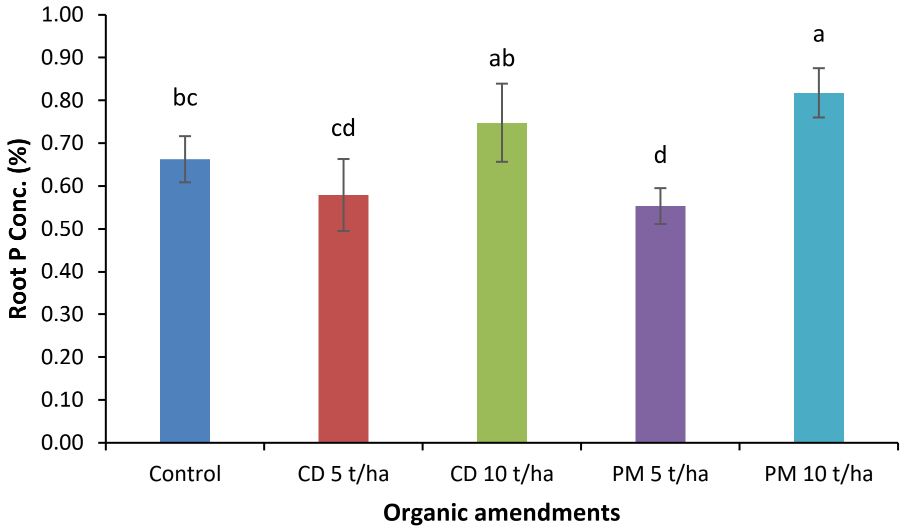

Figure 4.46. Phosphorus concentration in rice roots grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 *Boro* season, the application of organic amendment was found to affect the root phosphorus concentration in rice crop significantly as indicated by the one-way ANOVA ($F = 38.89$, $p = 0.000$). The highest concentration (0.7922 mg/kg) was observed in rice plants treated with 10 t/ha of poultry manure. 10 t/ha dose was significantly different from the rest of the treatments excluding CD 10 t/ha (**Table 4.5** and **Figure 4.47**).

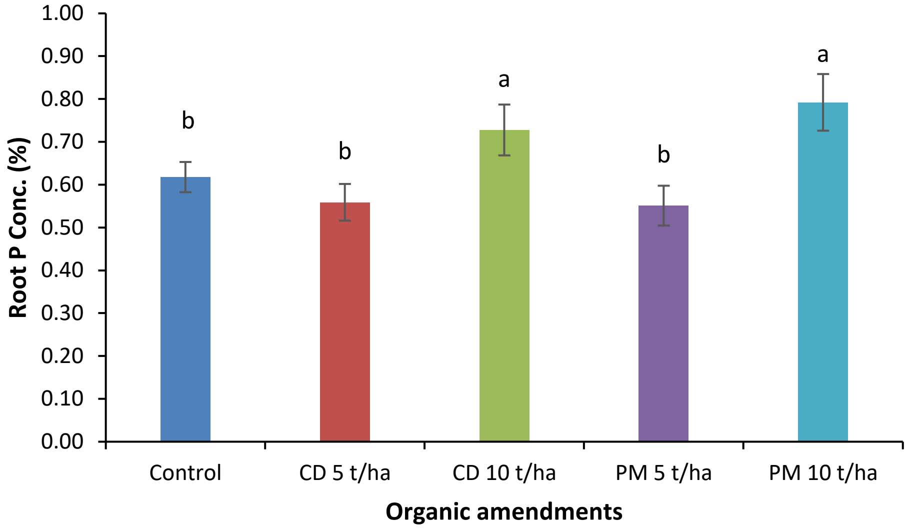

Figure 4.47. Phosphorus concentration in rice roots grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Straw

In 2017 season, the effects of organic amendment treatment were found to be significant on rice phosphorus straw concentrations as indicated by the one-way ANOVA ($F = 7.35$, $p = 0.943$). The highest straw phosphorus concentration (0.7609 mg/kg) was observed in the PM treatment at 10 t/ha (**Table 4.5** and **Figure 4.48**). The treatment was significantly different from the rest of the treatments excluding PM at 5 t/ha. The control was statistically the same with all the treatments barring PM at 10 t/ha.

In 2018 *Boro* season, the phosphorus concentration in straw followed the same trend as was observed in season 2017. The one-way ANOVA suggested that the organic amendment treatment significantly affected the phosphorus content of rice straw ($F = 5.75$, $p = 0.001$). From the post-hoc test, poultry manure at 10 t/ha was found to be the best in terms of straw phosphorus concentration. The second-best treatment was poultry manure at 5 t/ha followed by the control, CD at 10 t/ha and CD 5 t/ha (**Table 4.5** and **Figure 4.49**).

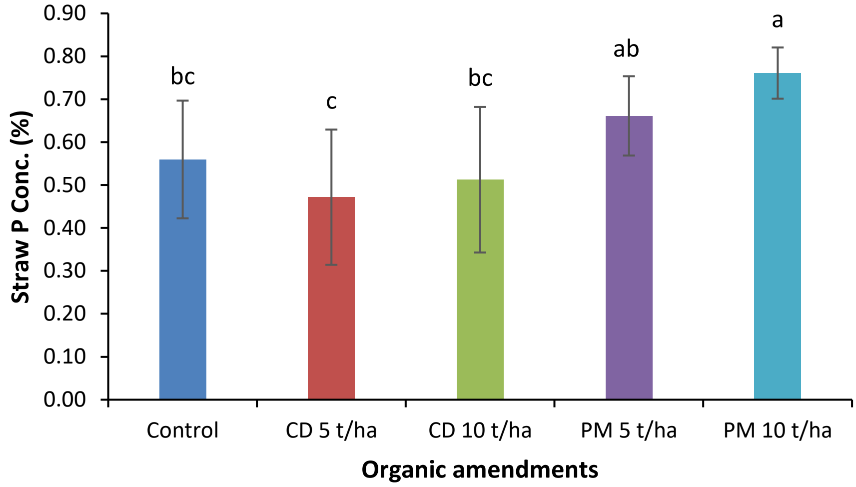

Figure 4.48. Phosphorus concentration in rice straw grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

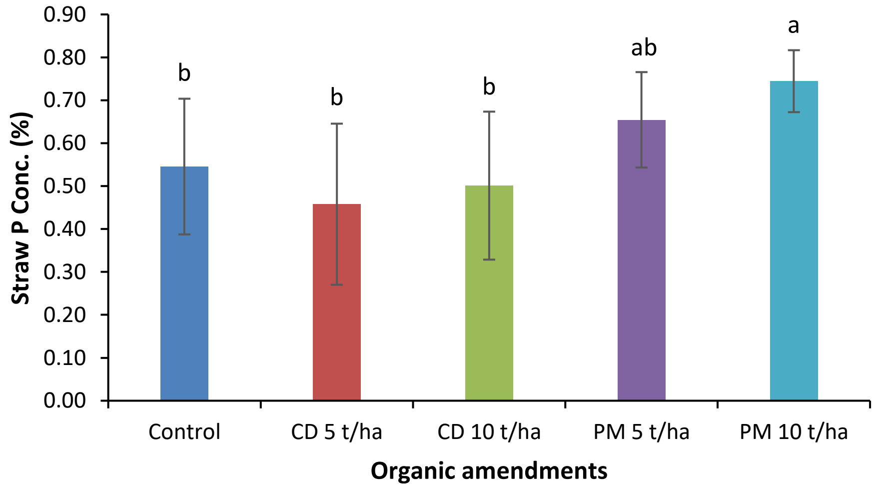

Figure 4.49. Phosphorus concentration in rice straw grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Husk

In 2017 season, phosphorus concentrations in the rice husk differed significantly among the different treatments as revealed by the one-way

ANOVA (F = 8.37, p = 0.000). The post-hoc test indicates that the poultry manure treatment at 5 t/ha and cow dung treatment at 10 t/ha were the best in terms of rice straw phosphorus content (**Table 4.5** and **Figure 4.50**). The rest of the treatments including the control were statistically the same.

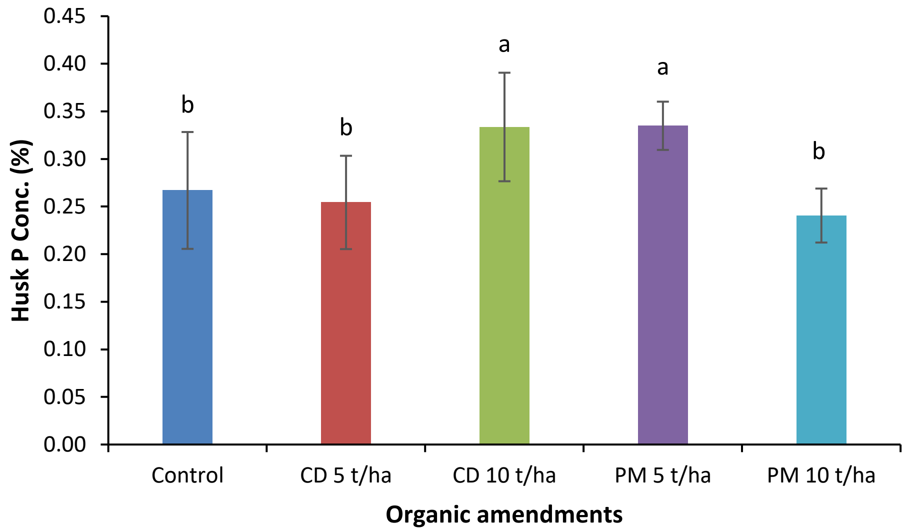

Figure 4.50. Phosphorus concentration in rice husk grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 season, following the previous year's trend, the phosphorus concentrations in the rice husk differed significantly among the different treatments as shown by the one-way ANOVA (F = 7.71, p = 0.000). The post-hoc test revealed that the poultry manure treatment at 5 t/ha and cow dung treatment at 10 t/ha were the best in terms of rice straw phosphorus content (**Table 4.5** and **Figure 4.51**). Poultry manure at 5 t/ha was significantly different from PM at 10 t/ha, CD at 5 t/ha and the control ($p<0.05$).

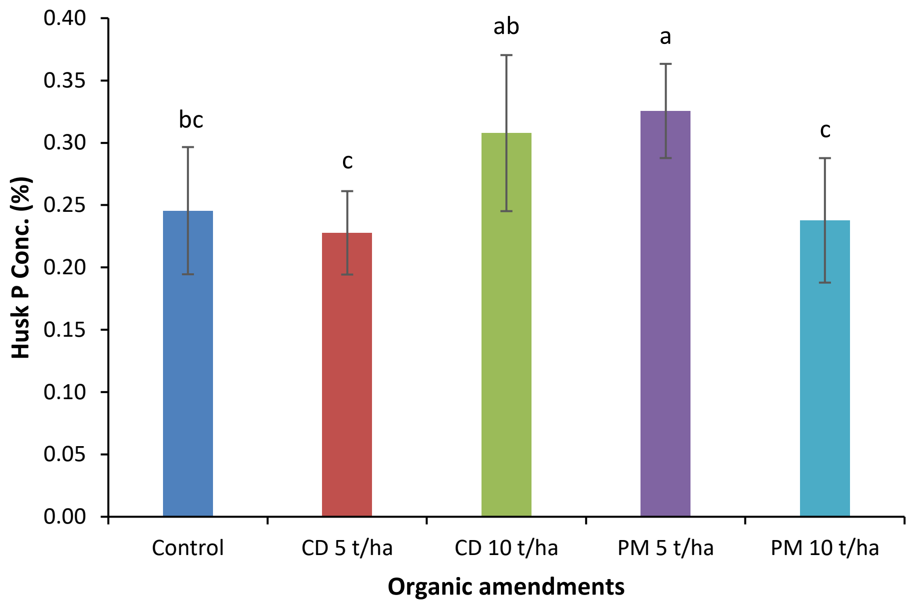

Figure 4.51. Phosphorus concentration in rice husk grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Phosphorus in Grains

In 2017 season, the mean rice grain phosphorus concentrations were not different statistically as indicated by the one-way ANOVA ($F = 1.13$, $p = 0.354$). The post-hoc test produced the same result suggesting that the treatments are statistically the same. However, the highest grain P concentration was found in rice plants treated with cow dung at 10 t/ha (**Table 4.5** and **Figure 4.52**). On the other hand, the lowest phosphorus concentration was observed at CD5 t/ha.

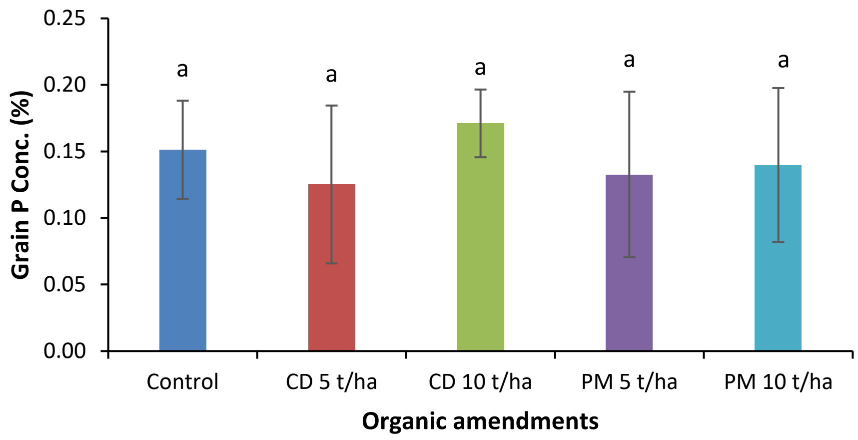

Figure 4.52. Phosphorus concentration in rice grains grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at p <0.05 level.

In 2018 season, the mean rice grain phosphorus concentrations were not different statistically either as revealed by the one-way ANOVA ($F = 1.47$, $p = 0.229$). The post-hoc test produced the same result indicating that the treatments are statistically the same. However, the highest grain P concentration was observed in rice plants treated with cow dung at 10 t/ha (**Table 4.5** and **Figure 4.53**). On the other hand, the lowest phosphorus concentration was observed at CD 5 t/ha.

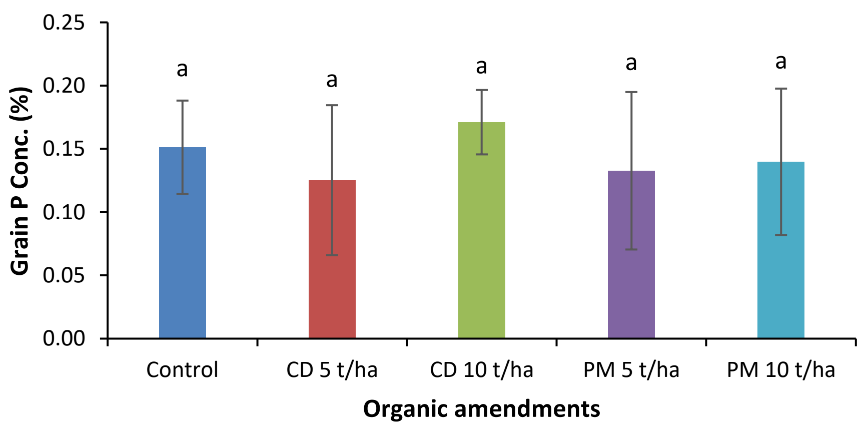

Figure 4.53. Phosphorus concentration in rice grains grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at p <0.05 level.

4.6.2. Concentration of Iron in Rice Plant

A two-way ANOVA was performed to determine the overall effects of water regimes and organic amendments on the concentration of iron in different parts of rice. A one-way ANOVA followed by Tukey's post-hoc test was also performed to see if there were any significant differences among the water regimes and among the organic amendments. Table **4.6** represents the mean values of iron (Fe) in root, straw, husk and grain at different water regimes and treatments.

Table 4.6. Main effects and interaction effects of water regimes and organic amendments on the concentrations of iron (mg/kg) in root, straw, husk, and grain of rice variety of BRRI Dhan 28 grown in 2017 and 2018. A one-way ANOVA followed by Tukey's Post-hoc test was performed to determine if there were any significant differences among the treatments and water regimes ($p<0.05$). Different letters in the same column indicate significant differences between the treatments. Letters are given alongside average concentrations of iron (mg/kg) in root, straw, husk and grain at different water regimes and treatments.

Factor levels/ Interaction	**Iron Concentration (mg/kg)**							
Water Regimes	**Root Fe**		**Straw Fe**		**Husk Fe**		**Grain Fe**	
	2017	**2018**	**2017**	**2018**	**2017**	**2018**	**2017**	**2018**
No SW	5418a	5315a	273.47a	278.73a	39.33b	42.20a	47.00b	46.33b
3-cm SW	5499a	5509a	277.40a	287.00a	43.20b	45.07a	81.67a	79.93a
5-cm SW	5467a	5494a	277.53a	288.47a	53.13a	53.27a	53.80b	55.80b
Significance	NS	NS	*	**	***	***	***	***
Organic Amendments								
Control	5176c	5216bc	258.33d	264.22c	34.78b	35.78b	52.33b	53.11b
CD 5 t/ha	5331b	5131c	275.67bc	284.67ab	42.44b	45.00b	60.11ab	58.33ab
CD 10 t/ha	5614a	5621ab	283.67ab	294.22ab	67.00a	68.33a	80.33a	80.67a
PM 5 t/ha	5520a	5559abc	273.56c	283.33b	38.67b	38.56b	50.67b	50.44b
PM 10 t/ha	5665a	5669a	289.44a	297.22a	43.22b	46.56b	60.67ab	60.89ab
Significance	***	**	***	***	***	***	***	***
Interactions								
Water regime* Organic Amendments	NS	NS	***	**	***	***	***	***

NS =Not significant, * = Significant at the 5% level, ** = Significant at the 1% level, *** = Significant at the 0.1% level.

Iron in Roots

A two-way ANOVA was performed to observe if water regime and organic amendment treatments had any effects on iron concentration in roots. The ANOVA was also performed to find if these two factors interacted and thereby influenced the iron concentration in roots. For 2017 *Boro* season data, the two-way ANOVA test indicates that water regimes had no significant (F= 2.45, p=0.104) effects on root iron concentration in rice crop (**Table 4.6**). On the other hand, treatments were found to have significant (F= 36.92, p = 0.000) effects on the concentration of Fe in roots. The interactive effects of water regime and organic amendments were found to be non-significant (F= 1.83, p=0.111).

In 2018 *Boro* season, the main effects of water regime were found to be non-significant (F = 1.74, p = 0.192) as indicated by the two-way ANOVA (**Table 4.6**). The main effects of organic amendments were found to be significant (F = 5.48, p = 0.002), though. The interaction effects of water regime and organic amendments were non-significant (F = 0.88, p = 0.544).

Iron in Straw

A two-way ANOVA was also performed for straw iron concentration in rice grown in 2017 and 2018 *Boro* season. For 2017 *Boro* season data, the two-way ANOVA indicates that the water regimes had significant (F = 3.97, p = 0.030) effects on straw iron concentration in rice (**Table 4.6**). The main effects of organic amendments were found to be significant (F = 62.18, p = 0.000) also. The interaction effects of water regime and organic amendments on straw iron concentration of rice were found to be significant (F= 6.60, p=0.000).

In 2018 *Boro* season, the main effects of water regime were found to be significant (F = 9.00, p = 0.001) (**Table 4.6**). The main effects of organic amendments were found to be significant (F = 32.77, p = 0.000), also. The interaction effects of water regime and organic amendments on straw iron concentration of rice were found to be significant (F = 3.88, p = 0.003), as well.

Iron in Husk

A two-way ANOVA was conducted to evaluate the main effects of water regime and organic amendments on husk iron concentration in rice. The two-way

ANOVA was also indicative of interaction effects of water regime and organic amendments. For 2017 *Boro* season data, the ANOVA test suggests that both water regimes ($F = 43.47$, $p = 0.000$) and treatments ($F= 82.09$, $p = 0.000$) had highly significant effects on the concentration of Fe in husk of rice (**Table 4.6**). The interaction effects of water regime and organic amendments were also found to be highly significant ($F= 21.07$ and $p= 0.000$).

For 2018 season data, the ANOVA suggested that the main effects of both water regime ($F = 18.81$, $p = 0.000$) and organic amendments ($F = 56.14$, $p = 0.000$) on husk iron concentration of rice were highly significant. The interaction effects of these two factors were highly significant as well.

Iron in Grains

A two-way ANOVA was performed to evaluate the main effects of water regime and organic amendments on grain iron concentration of rice grown in 2017 and 2018 *Boro* season. The two-way ANOVA also revealed if water regime and organic amendments interacted to affect the concentration of iron in rice husk. For 2017 *Boro* season, the two-way ANOVA suggests that both water regimes ($F = 209.53$, $p = 0.000$) and treatments ($F= 51.83$, $p = 0.000$) had highly significant effects on the Fe concentration of rice grains (**Table 4.6**). The interaction effects were found to be highly significant ($F = 15.02$, $p = 0.000$) as well.

For 2018 *Boro* season data, the two-way ANOVA reveals that the main effects of both water regime ($F = 294.92$, $p = 0.000$) and organic amendments ($F = 83.57$, $p = 0.000$) had highly significant effects on grain arsenic concentration of rice grown in this study (**Table 4.6**). The interaction effects of water regime and organic amendments were found to be highly significant ($F = 25.29$, $p = 0.000$).

4.6.2.1. Iron Concentration of Rice as Affected by Water Regimes

A one-way ANOVA followed by Tukey's post-hoc test was performed to assess if there are any significant differences among the water regimes with respect to iron concentration. The one-way ANOVA was performed among the three water regimes tested in this study. In order to do so, all the organic amendments treatments under a water regime were combined. Therefore, 15 (fifteen) values were combined to calculate the mean and the standard deviation of iron (Fe) concentration under a water regime and to do the analysis of variance. The data was checked for the assumption of normality. As the data was normal, the data was not subjected to any transformation before doing the parametric test. The average values and standard deviation of Fe concentrations in root, straw, husk and grain of rice plants at different water regimes are presented in **Table 4.6**.

Iron in Roots

In 2017 *Boro* season, the mean root iron concentration of rice under different water regimes did not differ significantly from one another as indicated by the one-way ANOVA ($F = 0.53$, $p=0.590$) and Tukey's test (**Table 4.6**). However, the highest root Fe concentration of rice was observed under 3-cm SW regime, followed by 5-cm and No SW regime (**Figure 4.54**).

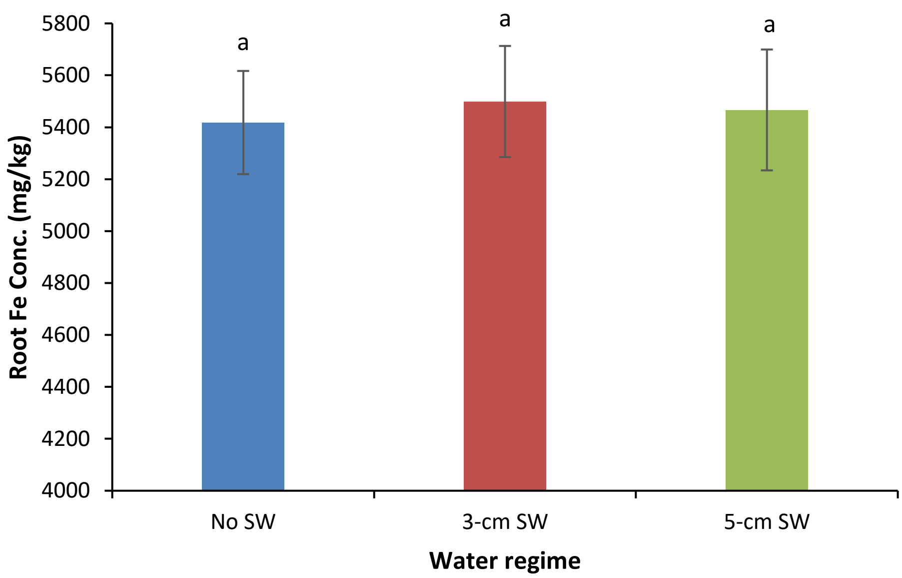

Figure 4.54. Iron concentration in rice roots grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 *Boro* season, the mean root iron concentration of rice under different water regimes did not differ significantly from one another as indicated by the one-way ANOVA ($F = 1.24$, $p=0.299$) and Tukey's test (**Table 4.6**). However, the highest root Fe concentration of rice was observed under 3-cm SW regime, followed by 5-cm and No SW regime (**Figure 4.55**).

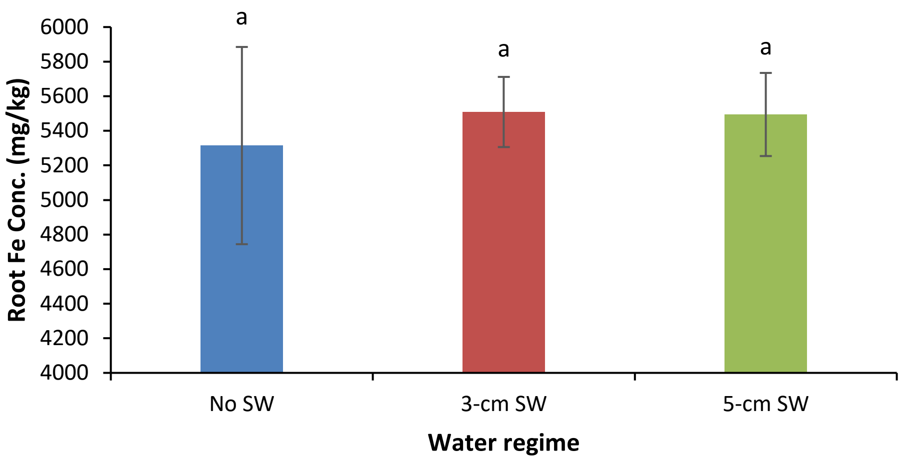

Figure 4.55. Iron concentration in rice roots grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Straw

In 2017 *Boro* season, the mean straw iron concentration of rice was not affected significantly by the different water regimes administered in this study (one-way ANOVA, p= 0.608) (**Table 4.6**). However, the highest straw iron concentration in rice was observed in plants grown in 5-cm SW regime, followed by 3-cm and No SW regime (**Figure 4.56**).

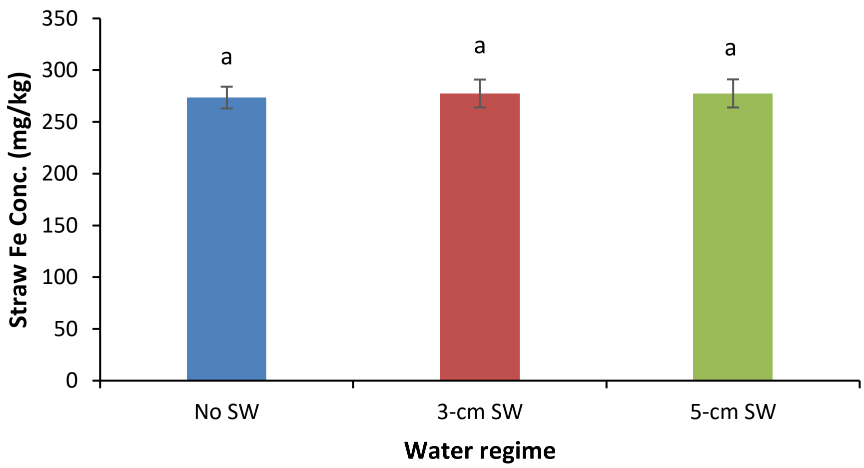

Figure 4.56. Iron concentration in rice straw grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 *Boro* season, the mean straw iron concentration of rice was not affected significantly by the different water regimes administered in this study (one-way ANOVA, p= 0.153) (**Table 4.6**). However, the highest straw iron concentration in rice was observed in plants grown in 5-cm SW regime, followed by 3-cm and No SW regime (**Figure 4.57**).

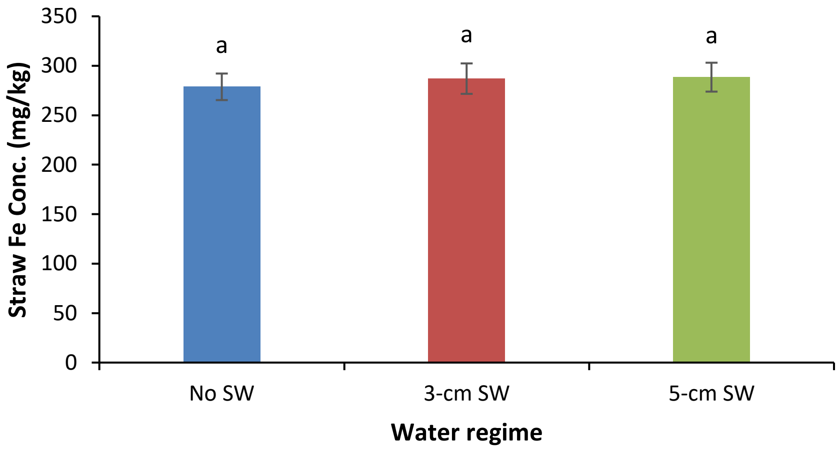

Figure 4.57. Iron concentration in rice straw grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Husk

For 2017 *Boro* season data, a one-way ANOVA was conducted, and it was found that the mean husk iron concentrations differ significantly among the different water regimes (F = 3.46, p = 0.040) (**Table 4.6**). The highest husk iron concentration was observed in rice plants grown under 5-cm SW regime (**Figure 4.58**). 5-cm SW regime was significantly different from No SW regime with respect to husk iron concentration. No significant difference was observed between 5-cm and 3-cm SW regimes.

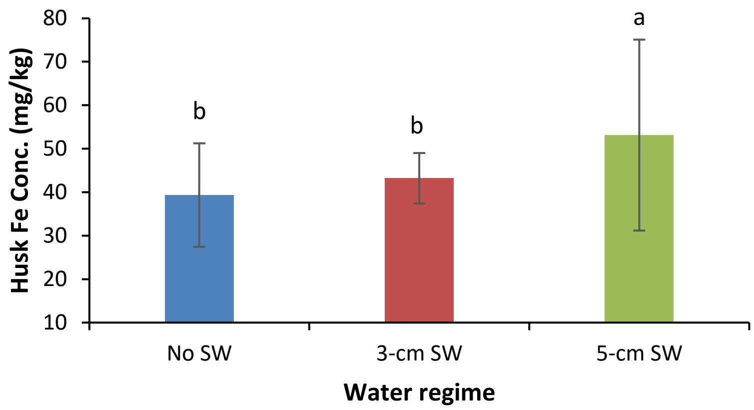

Figure 4.58. Iron concentration in rice husk grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

For 2018 *Boro* season data, a one-way ANOVA was also conducted, and the mean husk iron concentrations were found to be the same under different water regimes in terms of statistical significance ($F = 2.25$, $p = 0.118$) (**Table 4.6**). The highest husk iron concentration was observed in rice plants grown under 5-cm SW regime followed by 3-cm SW regime and No SW regime (**Figure 4.59**).

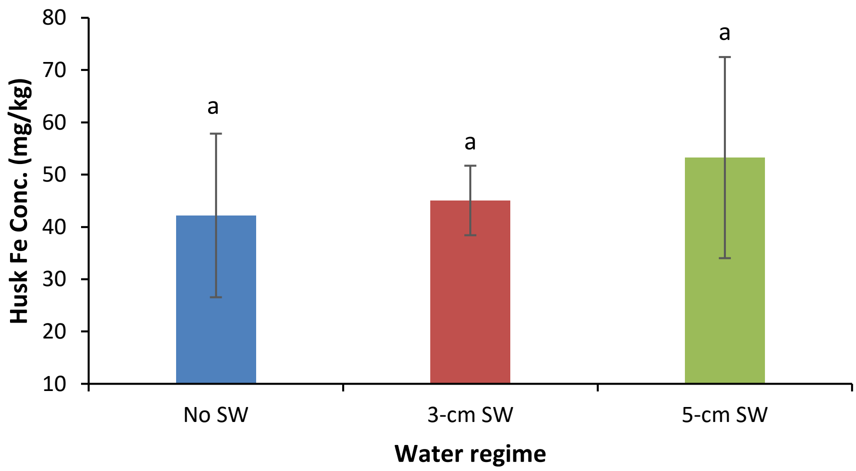

Figure 4.59. Iron concentration in rice husk grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Grains

In 2017 *Boro* season, the mean grain iron concentrations in rice plants grown under different water regimes differ significantly from each other as indicated by the one-way ANOVA ($F = 24.62$, $p = 0.000$) (**Table 4.6**). The highest grain iron concentration was observed in plants grown under 3-cm SW regime followed by 5-cm SW regime and No SW regime. The highest concentration under 3-cm SW regime was significantly different from that of 5-cm and No SW regimes (**Figure 4.60**).

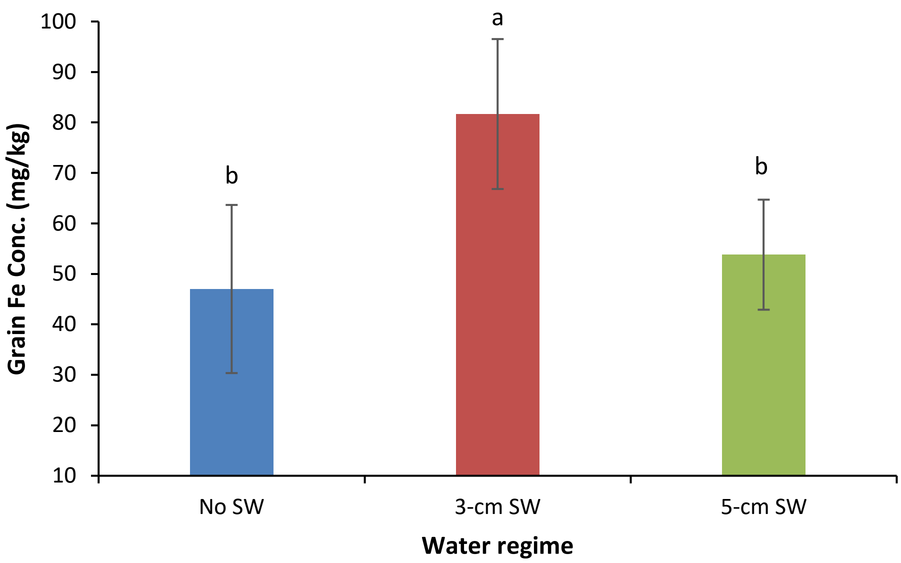

Figure 4.60. Iron concentration in rice grains grown in 2017 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

In 2018 *Boro* season, the mean grain iron concentrations in rice plants grown under different water regimes differ significantly from each other as indicated by the one-way ANOVA ($F = 21.86$, $p = 0.000$) (**Table 4.6**). The highest grain iron concentration was observed in plants grown under 3-cm SW regime followed by 5-cm SW regime and No SW regime. The highest concentration under 3-cm SW regime was significantly different from that of 5-cm and No SW regimes (**Figure 4.61**).

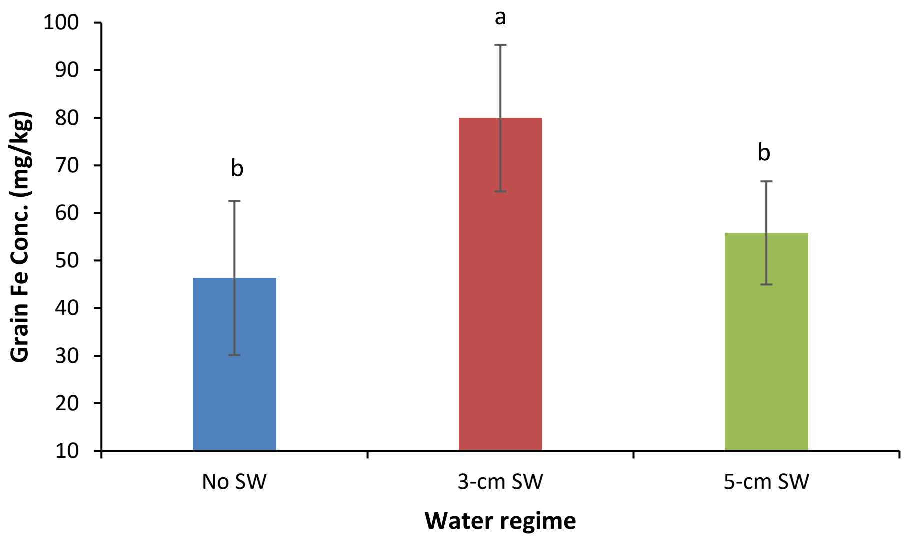

Figure 4.61. Iron concentration in rice grains grown in 2018 *Boro* season as affected by different water regimes. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the water regimes. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

4.6.2.2. Iron Concentration of Rice as Affected by Organic Amendments

A one-way ANOVA followed by Tukey's post-hoc test was performed with the data of iron concentrations recorded for roots, straw, husk and grains of rice to find if there are any statistically significant differences among the organic amendments. To do the ANOVA, iron concentrations under an organic amendment treatment were lumped together regardless of their water regimes. Therefore, 9 (nine) iron concentration values were combined for each treatment to compute their mean and standard deviation and to do the analysis of variance. The data was checked for the assumption of normality. The distribution of data was found to be normal. Therefore, parametric test was then carried out using the original data. **Table 4.6** represents the average values and standard deviation of iron concentration in roots, straw, husk and grains of rice plants at different organic amendment treatments.

Iron in Roots

For 2017 *Boro* season data, the one-way ANOVA revealed that the mean root iron concentrations differ significantly among the organic amendment treatments ($F = 29.83$, $p = 0.000$) (**Table 4.6**). The highest iron concentration (5664.9 mg/kg) was recorded in PM at 10 t/ha, which was significantly different from that of CD at 5 t/ha and the control. The highest root iron concentration in PM at 10 t/ha was not statistically different from that of PM at 5 t/ha and CD at 10 t/ha (**Figure 4.62**).

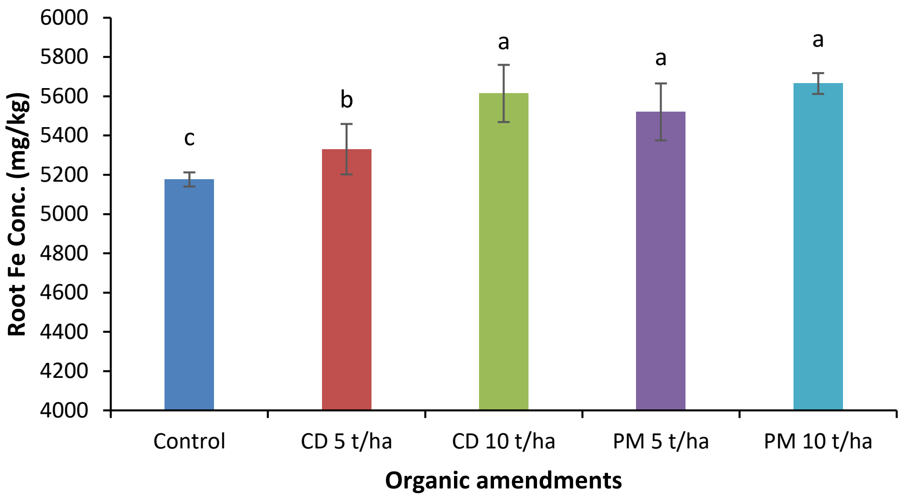

Figure 4.62. Iron concentration in rice roots grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

For 2018 *Boro* season data, the one-way ANOVA revealed that the mean root iron concentrations differ significantly among the organic amendment treatments ($F = 5.40$, $p = 0.001$) (**Table 4.6**). The highest iron concentration (5669.3 mg/kg) was recorded in PM at 10 t/ha, which was significantly different from that of CD at 5 t/ha and the control. The highest root iron concentration in PM at 10 t/ha was not statistically different from that of PM at 5 t/ha and CD at 10 t/ha (**Figure 4.63**).

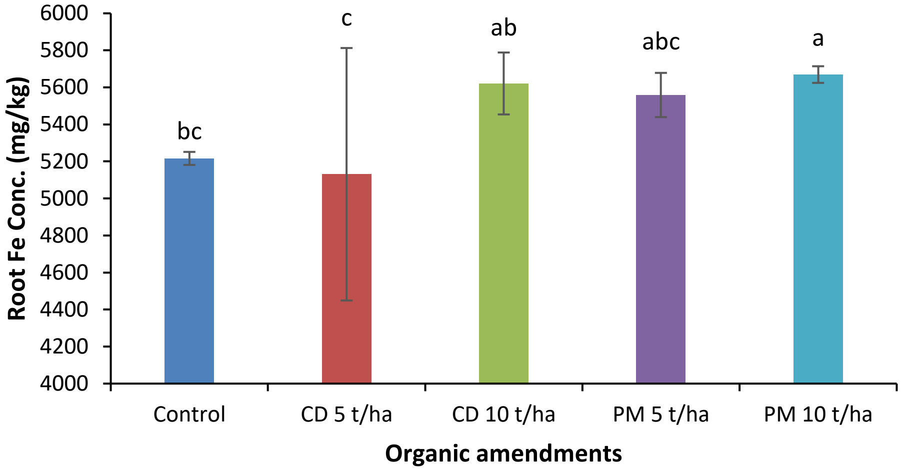

Figure 4.63. Iron concentration in rice roots grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Straw

For 2017 data, the one-way ANOVA suggests that the mean iron concentrations differed significantly among the organic amendments ($F = 27.42$, $p=0.000$) (**Table 4.6**). The highest iron concentration in rice straw was obtained in PM 10 t/ha treated soil followed by CD 10 t/ha, CD 5 t/ha, PM 5 t/ha and the control treatments (**Figure 4.64**). PM 10 t/ha treatment was significantly different from CD 5 t/ha, PM 5 t/ha and the control vis-à-vis straw iron concentration in rice.

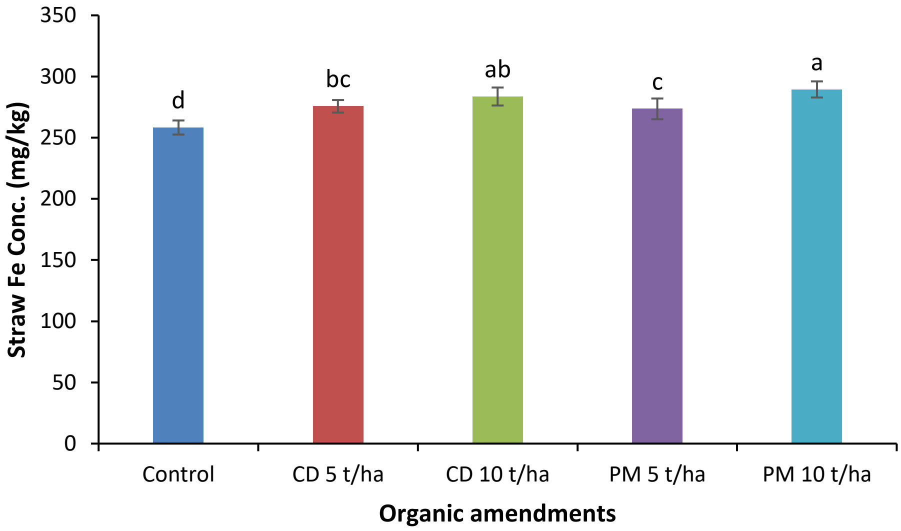

Figure 4.64. Iron concentration in rice straw grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

For 2018 data, the one-way ANOVA reveals that the mean iron concentrations differed significantly among the organic amendments ($F = 16.58$, $p = 0.000$) (**Table 4.6**). Following the previous year's trend, the highest iron concentration (297.22 mg/kg) in rice straw was obtained in PM 10 t/ha treated soil. The highest concentration was followed by CD 10 t/ha, CD 5 t/ha, PM 5 t/ha and the control treatments (**Figure 4.65**). PM 10 t/ha treatment was significantly different from PM 5 t/ha and the control vis-à-vis straw iron concentration in rice.

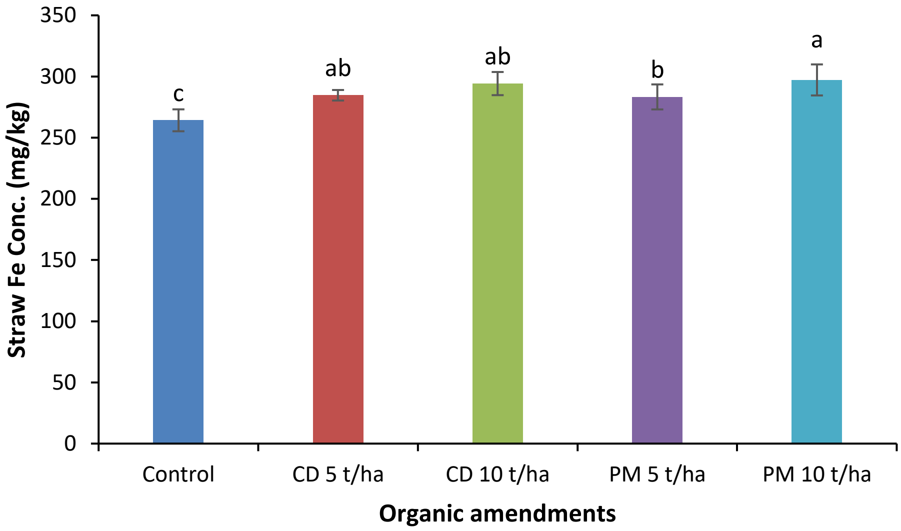

Figure 4.65. Iron concentration in rice straw grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Husk

For 2017 *Boro* season data, the mean rice husk iron concentrations differed significantly among the organic amendments as indicated by the one-way ANOVA ($F = 11.50$, $p = 0.000$) (**Table 4.6**). The highest husk iron concentration was observed in CD 10 t/ha treatment followed by PM 10 t/ha, CD 5 t/ha, PM 5 t/ha and the control treatments (**Figure 4.66**). The highest iron concentration at CD 10 t/ha was significantly different from the rest of the treatments including the control.

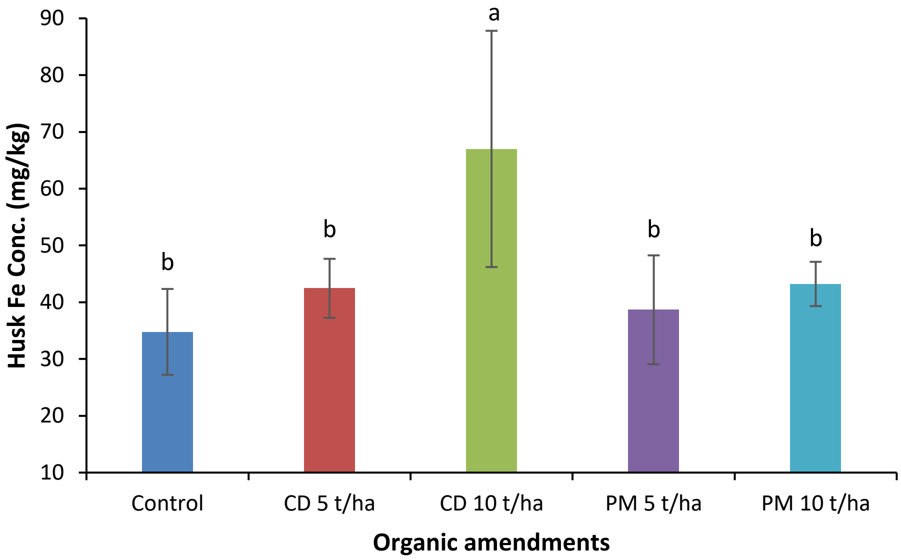

Figure 4.66. Iron concentration in rice husk grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

For 2018 *Boro* season data, the mean rice husk iron concentrations differed significantly among the organic amendments as indicated by the one-way ANOVA ($F = 13.72$, $p = 0.000$) (**Table 4.6**). The highest husk iron concentration was observed in CD 10 t/ha treatment followed by PM 10 t/ha, CD 5 t/ha, PM 5 t/ha and the control treatments (**Figure 4.67**). The highest iron concentration at CD 10 t/ha was significantly different from the rest of the treatments including the control.

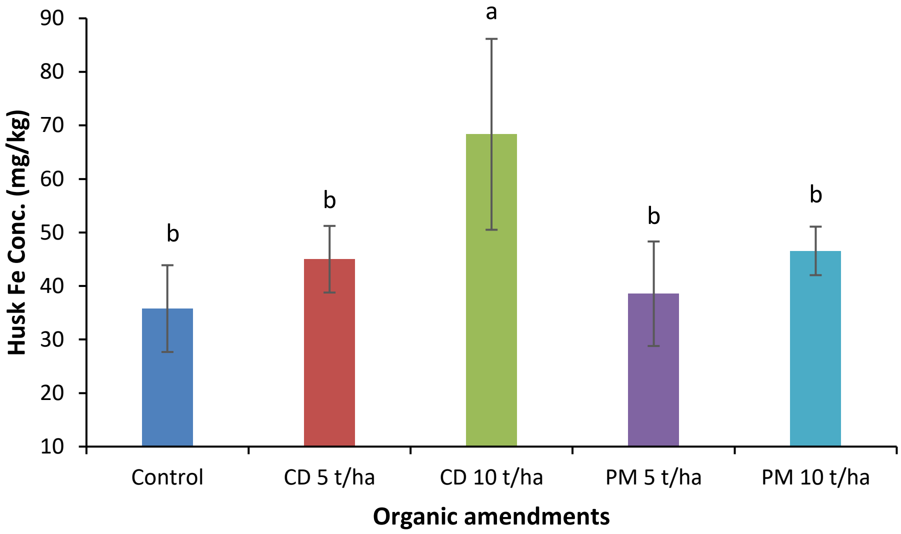

Figure 4.67. Iron concentration in rice husk grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

Iron in Grains

For 2017 data, the mean rice grain iron concentrations differed statistically significantly among the organic amendments as revealed by the one-way ANOVA ($F = 3.64$, $p = 0.013$) (**Table 4.6**). The highest iron concentration was observed in CD 10 t/ha treatment followed by PM 10 t/ha, CD 5 t/ha, the control and PM 5 t/ha treatments (**Figure 4.68**). The highest concentration at CD 10 t/ha was significantly different from that of PM 5 and the control.

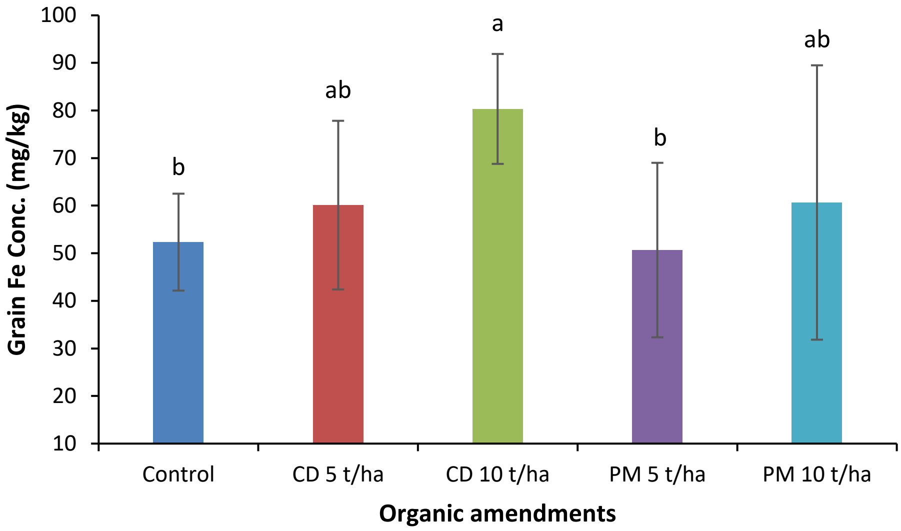

Figure 4.68. Iron concentration in rice grains grown in 2017 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

For 2018 data, the mean rice grain iron concentrations differed statistically significantly among the organic amendments as revealed by the one-way ANOVA ($F = 4.07$, $p = 0.007$) (**Table 4.6**). The highest iron concentration was observed in CD 10 t/ha treatment followed by PM 10 t/ha, CD 5 t/ha, the control and PM 5 t/ha treatments (**Figure 4.69**). The highest concentration at CD 10 t/ha was significantly different from that of PM 5 and the control.

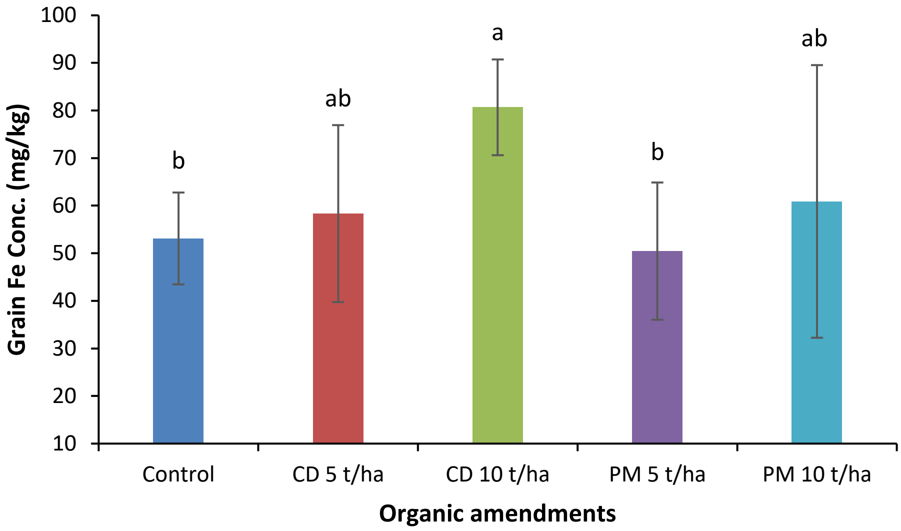

Figure 4.69. Iron concentration in rice grains grown in 2018 *Boro* season as affected by different organic amendments. A one-way ANOVA followed by Tukey's post hoc test was performed to observe if there were any differences among the organic amendments. Different letters above bars indicate significant differences between treatments at $p < 0.05$ level.

4.6.3. Relationship between Different Elements within the Plant

Pearson correlation analysis was performed to assess if there are any linear relationship between different elemental concentrations within the rice crop. Significant correlations with $r > 0.5$ (therefore explaining 25% of total variation) were only considered for further discussion. Significance was determined based on whether p-values are <0.05 or not (Hossain, 2016; Hossain et al., 2018). For this analysis, 2017 and 2018 data were combined. A significant relationship was found between grain P concentration and grain Fe concentration ($r = 0.345$, $p = 0.001$) (**Figure 4.70**). However, the r value is lower than the cut-off value. Therefore, the relationship was not considered and discussed. Likewise, a significant relationship was found between grain As concentration and grain iron concentration (**Figure 4.71**). However, the r value was low to be considered for further discussion ($r = 0.241$; $p = 0.022$). No significant relationship was found between grain As concentrations and grain P concentrations ($r = -0.048$, $p = 0.655$) (**Figure 4.72**).

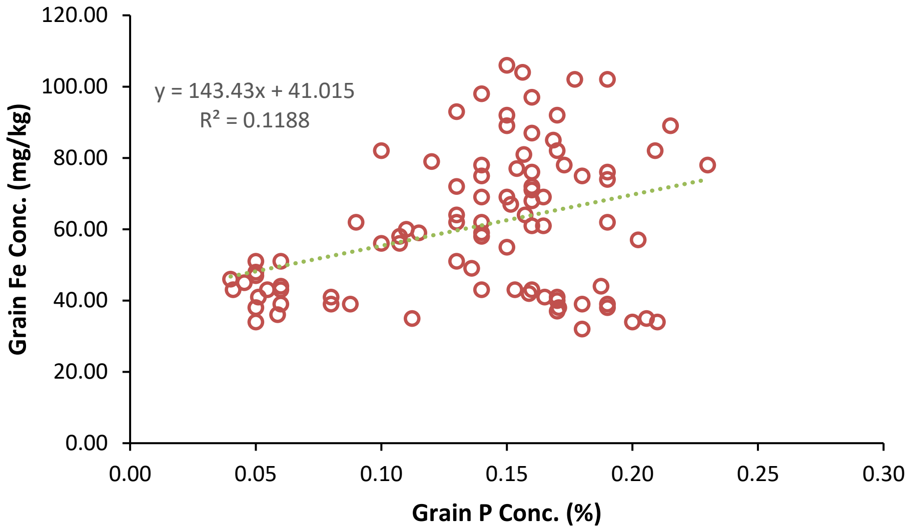

Figure 4.70. Correlation analysis of rice grain P and rice grain Fe concentrations (n = 90).

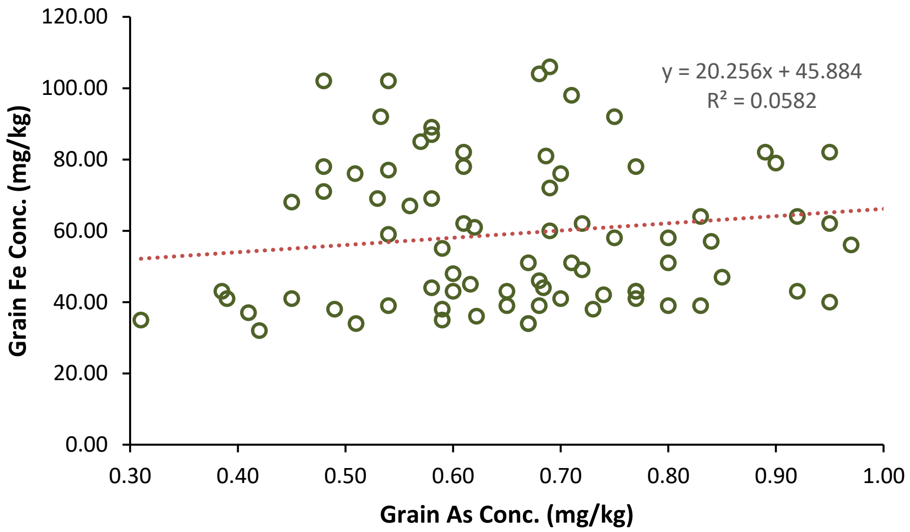

Figure 4.71. Correlation analysis of rice grain As and rice grain Fe concentrations (n = 90).

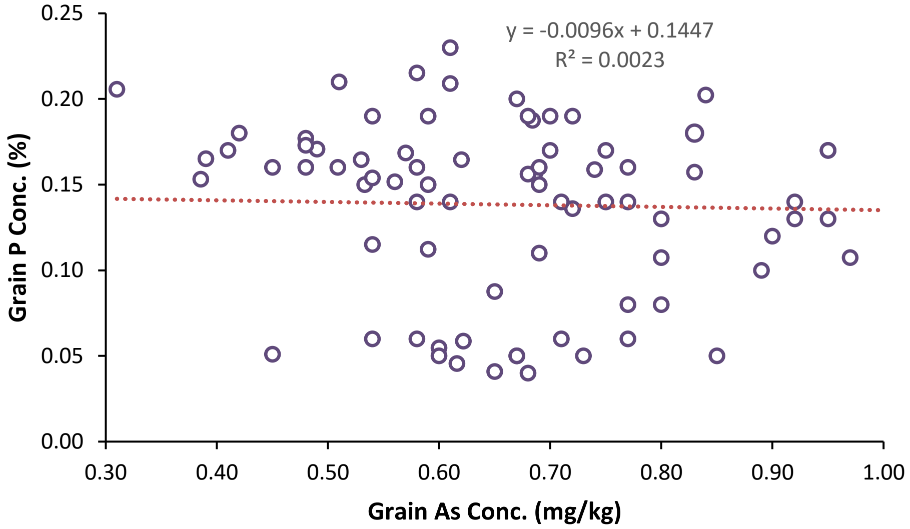

Figure 4.72. Correlation analysis of rice grain As and rice grain P concentrations (n = 90).

Chapter Five
Conclusions and Recommendations

5. CONCLUSIONS AND RECOMMENDATIONS

In the present study, three water regimes and five organic amendment treatments were tested in factorial combination for mitigation of arsenic accumulation in BRRI dhan 28 grown in two Boro seasons in 2017 and 2018. Different growth parameters of rice were also recorded to evaluate the effects of arsenic contaminated irrigation water and soil. Some important elements were also analyzed for their possible association with arsenic accumulation in rice. The water management was found to have no significant effects on plant height, fresh and dry weight of biomass and the yield in the form of grain weight. However, organic amendments were found to have significant effects on the studied growth parameters. Poultry manure at 10 t/ha was seen to be the best treatment in terms of plant height, fresh weight, dry weight, and grain weight. Cow dung at 5 t/ha was statistically the same with the control with respect to plant height (both 2017 and 2018 seasons), fresh weight (both 2017 and 2018 seasons) and dry weight (both 2017 and 2018 seasons). However, the yield was significantly different from the control for cow dung application at 5 t/ha. With few exceptions, cow dung at 10 t/ha was significantly different than the control. Poultry manure at 5 t/ha was significantly different from cow dung and the control for all the growth parameters.

Water regime was found to affect the arsenic accumulation in rice root, straw, and husk in 2017 and 2018 seasons. The grain arsenic concentration did not differ significantly among the water regimes. No SW regime was found to be the best treatment in terms of less arsenic accumulation. The highest accumulation of arsenic was observed under 5-cm SW regime. Organic amendments were found to behave interestingly with respect to arsenic accumulation. Cow dung at 10 t/ha application was found to promote the uptake of arsenic in root, straw, husk, and grain of rice. On the other hand, the least uptake of arsenic was observed when plants were dosed with poultry manure at 5 t/ha. Transfer factors of arsenic was calculated for soil to different parts of plants. Transfer factor was found to follow the order: root > straw > husk > grain. Thus, arsenic accumulates mainly in the roots of rice crop.

Phosphorus concentration was also affected by water regime and organic amendments. However, there was no definite pattern. The highest root P concentration was observed under 3-cm SW regime (both 2017 and 2018 seasons). On the other hand, the highest straw P concentration was seen in No SW regime (both 2017 and 2018 seasons). For husk and grain P concentrations, the effects of water regime were not consistent. Among the organic amendment treatments, poultry manure at 10 t/ha was found to be the best for root P and straw P concentrations. For husk P concentration, poultry manure at 5 t/ha was found to be the best. No significant differences were observed among the organic amendment treatments for grain P concentrations.

Rice root and straw iron concentrations did not differ significantly among the water regimes (both 2017 and 2018 seasons). The highest husk iron concentration was observed in rice crops grown under 5-cm SW regime in 2017. In 2018, the concentrations were not statistically different among the water regimes. In 2017 and 2018 seasons, the highest grain P concentrations were obtained under 3-cm SW regime, which is significantly different from the rest of the treatments.

The study demonstrated that No SW water regime along with poultry manure at 5 t/ha was the optimum treatment combination for mitigating arsenic uptake in rice grains. Other water regimes and higher dose of poultry manure might promote the uptake of arsenic despite having some positive effects in terms of growth parameters.

Some recommendations are made from the present study. It would be interesting to see the effects of alternate wetting and drying in the future. The researchers can also look into the interactive effects of water regime and each of the organic amendments. The interactive effect will help us to determine the optimum dose of organic amendment and water regime.

Chapter Six
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6. REFERENCES

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Appendix 1. Layout of the experiment.

No SW	3-cm SW	5-cm SW	No SW	3-cm SW	5-cm SW	No SW	3-cm SW	5-cm SW
PM 5 t/ha	Control	PM 10 t/ha	PM 5 t/ha	Control	PM 10 t/ha	PM 5 t/ha	Control	PM 10 t/ha
Control	PM 5 t/ha	PM 5 t/ha	Control	PM 5 t/ha	PM 5 t/ha	Control	PM 5 t/ha	PM 5 t/ha
CD 5 t/ha	CD 10 t/ha	Control	CD 5 t/ha	CD 10 t/ha	Control	CD 5 t/ha	CD 10 t/ha	Control
PM 10 t/ha	CD 5 t/ha	CD 5 t/ha	PM 10 t/ha	CD 5 t/ha	CD 5 t/ha	PM 10 t/ha	CD 5 t/ha	CD 5 t/ha
CD 10 t/ha	PM 10 t/ha	CD 10 t/ha	CD 10 t/ha	PM 10 t/ha	CD 10 t/ha	CD 10 t/ha	PM 10 t/ha	CD 10 t/ha

*No SW, 3-cm SW and 5-cm SW constitute the main plot treatments

**PM (poultry manure) and CD (cow dung) constitute the subplot treatments