ACCUMULATION OF ARSENIC IN RICE PLANT FROM ARSENIC CONTAMINATED IRRIGATION WATER AND ITS EFFECT ON NUTRIENT CONTENT

A THESIS SUBMITTED TO THE

DEPARTMENT OF CIVIL ENGINEERING

BANGLADESH UNIVERSITY OF ENGINEERING AND TECHNOLOGY

DHAKA, BANGLADESH

BY

MD. ZAHANGIR ALAM

IN PARTIAL FULFULLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL ENGINEERING

APRIL, 2005

The thesis titled "**Accumulation of arsenic in rice plant from arsenic contaminated irrigation water and its effect on nutrient content**" by **Md. Zahangir Alam,** Roll No. 040004104P, Session April, 2000 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of M. Sc. Engineering (Environmental) on 12 of April, 2005.

BOARD OF EXAMINERS

Dr. Md. Mujibur Rahman Professor Department of Civil Engineering BUET, Dhaka-1000	:	Chairman (Supervisor)
Dr. Sk. Sekender Ali Professor and Head Department of Civil Engineering BUET, Dhaka-1000	:	Member
Dr. M. Ashraf Ali Associate Professor Department of Civil Engineering BUET, Dhaka-1000	:	Member
Dr. Md. Mafizur Rahman Associate Professor Department of Civil Engineering BUET, Dhaka-1000	:	Member
Rezaur Rahman Dr. Md. Rezaur Rahman Associate Professor Institute of Water and Flood Management (IWFM), BUET, Dhaka-1000	:	Member (External)

DECLARATION

I hereby declare that this thesis or any part of it has not been submitted elsewhere for the award of any degree or diploma.

April, 2005

Md. Zahangir Alam

Student No. 040004104P

ACKNOWLEDGEMENT

At the beginning the author would like to express his heartiest gratitude to the thesis supervisor, Dr. Md. Mujibur Rahman, Professor, Department of Civil Engineering, BUET for his continuous support and guidance during the course of this research work starting from selection of thesis proposal to the completion of thesis writing. His careful scrutinising of the draft, effective comments and constructive criticism has made the thesis more comprehensive. The author is also grateful to the Civil Engineering Department, BUET, for providing laboratory facilities and equipments, which enabled him to carry out a part of laboratory works.

The author expresses his profound gratitude to Dr. Khandaker Aminul Kabir, Head and other research officers of Grain Quality & Nutrition Division of Bangladesh Rice Research Institute (BRRI), Gazipur for their advice and co-operation for the tests of nutrient contents in rice grain and also for collection of literature regarding nutritional aspects of rice. The author is also grateful to the Librarian including staffs working in the library of BRRI for their intimate and sincere help in studying and collecting some related books, reports and journals.

The author wishes to thank the Chief Chemist, Dr. Musharraf Ashraf and the Chemist, Mr. Md. Shariful Islam, Ministry of Shipping for co-operation in conducting the tests for arsenic in rice the Environmental Monitoring and Control Laboratory (EMCL).

The author also expresses his sincere thanks and indebtedness to Dr. Ashraf Ali, Associate professor, Department of Civil Engineering, BUET, Dhaka for his imperative suggestions, guidance and active help throughout the research work.

The author is also grateful to his colleagues and friends for their co-operation during this study. Finally, the author likes to offer sincere thanks to the Ministry of Food for allowing him to pursue M. Sc. in Environmental Engineering Degree in BUET.

Abstract

To achieve self-sufficiency in food grain production, the HYV rice varieties are widely produced in Bangladesh, particularly in the dry season, and this requires a large volume of groundwater for irrigation. Groundwater of many areas of our country is severely contaminated with arsenic. So, there is a possibility of arsenic accumulation in rice plants from arsenic contaminated irrigation water. Generally, consumption of protein is not adequate for our people due to insufficient production of major protein sources like fish, meat, milk, eggs, etc. and also due to economic constraints. Any adverse effect on protein content in rice due to use of arsenic contaminated irrigation water may, therefore, aggravate the malnutrition problem of our people. Similarly any adverse effect on amylose content, which is about one forth portion of rice grain by weight, would cause deficiencies in calorie supply. This study aims at assessing the accumulation of arsenic in rice plants and its possible effect on nutrient (protein and amylose) content of rice grains. Arsenic contents in different parts of rice plants were tested using hydride generation Atomic Absorption Spectrophotometry (Shimadzu, AA6800). For assessing nutrient contents (protein and amylose), rice samples were tested at the Laboratory of Grain Quality and Nutrition Division of Bangladesh Rice Research Institute (BRRI), Gazipur. Results from this study show that arsenic accumulates in different parts of rice plants, though the accumulation varies from variety to variety. Highest arsenic concentration was found in root hair with a mean 16.7 mg/kg and maximum 38.5 mg/kg, and least in grain with a mean 0.32 mg/kg and maximum 1.1 mg/kg. Mean arsenic concentrations in husk, leaf and stem were found to be 1.06 mg/kg, 3.98 mg/kg and 3.32 mg/kg, respectively. Presence of arsenic in straw (leaf and stem) at moderately high concentration poses risks for cattle and human health. Arsenic concentration in top soil samples of study fields were found to be up to 12 mg/kg. From linear regression analysis only a moderate correlation (R^2 = 0.45) was found between arsenic in water and arsenic in root hair. Measured nutrient contents were compared with the standard values. Test results for protein content show rice grain contain less protein than standard values. Similarly, for most samples, amylose content was found to be less than standard value. More studies are needed to better understand these phenomena.

Contents

List of Tables

List of Figures

Abbreviations

ANHMRC	Australian National Health and Medical Research Council
BADC	Bangladesh Agricultural Development Corporation
BAU	Bangladesh Agricultural University
BGS	British Geological Survey
BAMWSP	Bangladesh Arsenic Mitigation and Water Supply Project
BRRI	Bangladesh Rice Research Institute
BUET	Bangladesh University of Engineering and Technology
DAF	Day after flowering
DMAA	Dimethylarsinic acid
DPHE	Department of Public health Engineering
EMCL	Environmental Monitoring and Control Laboratory
FAO	Food and Agricultural Organization
GLV	Green leafy vegetable
HYV	High yield variety
IRRI	International Rice Research Institute
MMAA	Monomethylarsonic acid
NAMIC	National Arsenic Mitigation Information Centre
NRCC	National Research Council of Canada
PEM	Protein-energy-malnutrition
PETRRA	Poverty Elimination Through Rice Research Project
STW	Shallow Tube Well
TVZ	Taupo Volcanic Zone
UNDP	United Nations Development Program
UNICEF	United Nations International Children Education Fund
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 General

Bangladesh and the adjacent areas of West Bengal, India are currently facing the challenge of high arsenic concentration in groundwater. In Bangladesh, ground water is heavily contaminated with arsenic and in some areas it reaches ≥ 2 mg/L (Tonden et al., 1999; BGS, 2000; Dainichi, 2001). A large number of tube wells in at least 61 districts out of 64 have been identified to have arsenic concentration above the national recommended limit of 0.05 mg/L. Conservative estimates indicate that about 29 million people are exposed to the risk of being affected by arsenic contaminated drinking water (Ahmed, 2001).

The country is heavily dependent on its agricultural sector for its gross domestic product. National growth is reliant on the growth of agricultural output. The population growth rate is still quite high and therefore the demand of food grain production specially rice production has always been a challenging issue for our country. To ensure food security Bangladesh Government has emphasized on increasing rice production. To become self sufficient in food grain, Bangladesh Government has insisted on our people to produce huge quantity of HYV rice which require a large volume of irrigation water. The use of ground water for irrigation has increased abruptly over the last couple of decades. About 86% of total groundwater withdrawn is utilized in agricultural sector (WRI, 2000). It is therefore, apparent that the use of arsenic contaminated irrigation waters in Bangladesh may cause accumulation of arsenic in rice and rice plants, which needs to be examined.

As a poverty stricken country, many people are living under the absolute poverty level measured on the basis of calorie intake, which is 2122 Kcal/capita/day (BBS, 1996). Recommended allowances for protein requirements for the people of Bangladesh are 1.85 g/day per kg of body weight for infant, 23-41 g/d for children, 53-79 g/d for adolescents, 60 g/d for adult male, 50 g/d for adult female, with additional 15 g/d for pregnant mother and 25 g/d for lactating mother (Kabir Y, 2002). The major sources of protein are meat, fish, eggs and milk. However, due to lack of availability and economic reasons, peoples' access to these protein sources is inadequate. Many people especially children suffer from malnutrition in Bangladesh. Since, rice is the staple food in this country, any adverse effects on nutrient content of rice due to arsenic contaminated irrigation water would only enhance the malnutrition problem.

The use of this arsenic contaminated water for irrigation of agricultural crop may lead to accumulation of arsenic in crops and plant body. Rice is the main crop in this country and a vast quantity of water is needed for its cultivation especially during the dry season. Presently, 75% of the total cropped area and 83% of the total irrigated area are used for rice cultivation (Dey et al., 1996). Varieties of rice produced in Bangladesh are mainly in two seasonal categories namely Boro and Aman rice. Boro variety, cultivated in dry season is completely dependent on irrigation water but Aman variety requires the minimum quantity of irrigation water, and in most cases no irrigation is required. Arsenic uptake and accumulation in rice plant from irrigation water may differ depending on cultivars used (Xie and Huang, 1998). Abedin (2002) found considerable differences in uptake of arsenic among different varieties of rice grains and he has also found that the affinity to absorbing arsenic of Aman variety is more than Boro varieties cultivated in Bangladesh. The present study was aimed at examining whether or not rice and rice plants absorb arsenic from arsenic contaminated irrigation water and subsequently to see if there is any adverse effect on the nutritional (protein and amylose) level of rice.

1.2 Objectives

The specific objectives of the study are

(i) to find out the level of arsenic absorbed by rice and rice plant produced on underground irrigation water contaminated with arsenic;

(ii) to determine the relationship between the amounts of arsenic absorbed by rice and rice plant and arsenic concentration in irrigation water;

(iii) to find out the level of arsenic content in the soil samples of the study fields and

(iv) to examine the possible changes in nutrient content (protein & amylose) in rice.

1.3 Scope of Research

HYV rice's cultivated in Boro seasons (dry season) are dependent on irrigation water, which comes mainly from underground water. Due to contamination of ground water with arsenic soil of agricultural fields as well as rice plants may be contaminated with arsenic. Many researchers found arsenic is accumulated by rice plant. Abedin et al. (2002) conducted a greenhouse experiment on accumulation of arsenic by Bangladesh HYV rice and he observed that arsenic was accumulated by rice plant. Some researchers (Hiromi Hironaka, 2003) collected rice samples from different markets of Bangladesh in basket survey experiment and identified accumulated arsenic in rice grain. Accumulation of arsenic in rice grain may cause health risk for human being if accumulation occurs at an elevated concentration. These reports on arsenic accumulation in rice plant leads to work on arsenic accumulation in rice plant during practical cultivation in the rural areas where arsenic groundwater is severely contaminated with arsenic and it is also used for irrigation purpose.

1.4 Methodology

In order to achieve the above objectives, the following program of works were undertaken to select the study fields:

(1) selection of 23 work fields in Comilla district from analyzing the data of arsenic contaminated ground water;

(2) collection of water samples from the primarily selected fields and testing of water samples for arsenic using Atomic Absorption Spectrophotometer (Shimadzu, AA6800) attached with a graphite furnace at environmental engineering laboratory in BUET. On the basis of arsenic content found in the water samples above national safe limit (>0.05 ppm) 13 work fields were finalized (Append.-A).

Out of these finalized study fields one is located in North Dhamti Mauza of Debidwar Upazila and the rests are in Galimpur, Deora and Jhalam Mauza of Barura Upazila. Rice plant samples and also soil samples surrounding the plant hills were collected from the study fields during harvesting of rice in April' 2002. Collected samples were brought to the laboratory and tested for arsenic and nutrient (protein and amylose).

1.5 Limitations of the Study

The limitations of the study were as follows-

- study for speciation of accumulated arsenic in rice plant could not be determined due to technical limitations
- rice plant samples were collected from the selected areas where variety selection for cultivation was dependent only on the willingness of the farmer; therefore, there was no scope for selection of particular one or two target variety
- soil quality does not remain uniform in the agricultural field in rural Bangladesh
- rain fall and stagnation of rain water were not similar for all study locations.

1.6 Organization of the Thesis

The thesis consists of five chapters. Chapter one is introduction, which includes background, objectives, scope of research, methodologies and also limitations of the study. Chapter two is used literature review. It includes discussion on (i) different research works on arsenic contamination and literature on arsenic such as health risk assessment, fate of arsenic in soil, cause of arsenic contamination, arsenic in rice and other plants, phytotoxicity of arsenic, etc., (ii) rice chemistry which includes structure of rice, brief discussion on protein and amylose, development of protein and amylose in rice, varieties of rice cultivated in Bangladesh and (iii) nutritional aspect in Bangladesh. Chapter three includes experimental methodologies for arsenic in rice plant and soil, results and discussion and also analysis for arsenic content. Similarly, chapter four includes laboratory analysis for protein and amylose content in rice grain, experimental test results, comparison of test results with standard value for protein and amylose and discussion. Finally, chapter five includes conclusions drawn from the study and proposed recommendations for future works.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Ground water in Bangladesh is contaminated with arsenic. To produce HYV rice ground water is used for irrigation which may cause accumulation of arsenic in rice plant. On the other hand this irrigation water continuously added arsenic to the soil during irrigation season. Inorganic arsenic is the predominant from of Arsenic in soil (Jonson and Hiltbold, 1969) and in ground water (Samanta et at., 1999). Under aerobic soil conditions arsenate dominates, whereas in submerged soil condition the predominant species is arsenite (Masscheleyn et al., 1991; Marin et at., 1993; Onken and Hossner, 1995; Onken and Hossner, 1996). There is an evidence of arsenic methylation in paddy soil systems, where inorganic species were converted to organic form by micro-organisms (Takamatsu et al., 1982). Takamatsu et al. (1982) found arsenate as the major component in paddy soils, with lower levels of arsenite, MMAA (monomethylarsonic acid [$CH_3AsO\text{-}(OH)_2$]) and DMAA (dimethylarsinic acid [$(CH_3)_2AsO\text{-}(OH)$]).

Arsenic accumulated by agricultural plants produced in the contaminated area. There are a number of studies investigating the mechanism of arsenic uptake by different plant species (Asher and Reay, 1979; Meharg and Macnair, 1992a; Meharg and Macnair, 1992b; Meharg and Macnair, 1994), but little work has been done on the uptake mechanism of rice plant. Arsenic uptake by a range of plants is via the high affinity phosphate transporter, as arsenate and phosphate are analogous (Jung and Rothstein, 1965; Asher and Reay, 1979; Beever and Burns, 1980; Silver and Misra, 1988; Ullrich-Eberius et al., Meharg and Macnair, 1990; Meharg and Macnair, 1992a).

Rice fruit, a caryopsis, consists of nitrogen free material (about 77%), protein (about 7.5%), fat (1.9%), fibre (0.9), ash (1.2) etc. Many researchers reported about reduction in shoot biomass and/or growth in rice grown in arsenic contaminated soil or water (Tsutsumi, 1980; Milam et al., 1988; Tang and Miller, 1991; Marin et al., 1992 and 1993;). So, there is a possibility of adverse effect on nutrient content of rice grain. Reduction in protein development in rice is also reported by Jahiruddin et al., (2004).

This chapter briefly describes the sources of arsenic, its distribution in groundwater of Bangladesh and its health effects. It also provides a review of available information on arsenic contents in irrigation water, soil and plants and effect of arsenic bearing water on plants. This chapter also briefly describes rice chemistry, including its structure, composition and nutrient content.

2.2 Source of Arsenic

2.2.1 Natural Sources of Arsenic

Major sources of arsenic (As) contamination in the environment through out the world are smelting of metals, burning of coal, the use of arsenical pesticides and geogenic abnormality of As. The high concentration of As in lead-zinc and copper ores and its presence in most pyrites causes As pollution of the environment in the vicinity of smelters treating such ores. The mine dumps and smelter slag tips containing high concentrations of As can be found through the world. Arsenic is a component of more than 245 minerals (Fredrick et al., 1994). These are mostly ores containing sulfide, along with copper, nickel, lead, cobalt or other metals. The most important ores are arsenopyrites or mispickel (FeS), realger (As_4S_4), orpiment (As_4S_6), cludite, Iolligngite ($FeAs_2$), nicoliotite (NiAs), cobalt glance (CoAsS), Gersdroffite (NiAsS) and smaltite ($CoAs_2$). Within these minerals, arsenopyrite is probably the most common mineral. Table 2.1 shows some naturally occurring minerals containing arsenic. Arsenic and its compounds are mobile in the environment. Weathering of rocks converts arsenic sulfides to arsenic trioxide, which enters the arsenic as dust or by dissolution in rain, rivers or ground water (Clifford and Zhang, 1993). Volatile forms of arsenic [$(CH_3)_3As$] enter the atmosphere from land and water ate returned by rain and atmosphere fall out. The oxidized forms of arsenic are converted back to sulfides by anaerobic processes occurring on land and water sediments (Tamaki, 1992). The concentration of arsenic in natural reservoir with respect to soils have been shown in Table 2.2. The rate of transfer of fluxes among the individual reservoirs are shown in Table 2.3. Soils and oceans are the remaining major reservoirs that have much more inherent arsenic than do biota (plants, animals, man and microbes) and the atmosphere. The average concentration of arsenic in soils of the world is 7.2 ppm (Annonymous, 1978). Arsenic in the natural environment occurs in soils at an average concentration of about 5 to 6 mg/kg, but this varies among geographic region (Peterson et al., 1981).

Table 2.1 Naturally occurring minerals containing arsenic (NRCC, 1978).

Endogenous	Formula	Supergeneous	Formula
Arsenite	As	Arsenolite	As_2O_3
Antimony arsenite	AsSb	Mutite	$Pb_5(PO_4, AsO_4)_3Cl$
Realger	AsS	Adamite	$Zn_2AsO_4(OH)$
Orpiment	As_2S_3	Erythrite	$Co_3AsO_4.8H_2O$
Arsenopyrite	FeAsS	Annarbergite	$Ni_3(AsO_4)_2.8H_2O$
Nicolite	NiAsS	Scorodite	$(Fe.Al)AsO_4.2H_2O$
Gersdorffite	CoAsS	Pharmacosiderite	$Fe_3(AsO_4)_2(OH)_3$
Cobaltite	CoAsS	Olivenite	$Cu_2(AsO_4)OH$
Smaltite	$(Co, Ni)As_x$	Beandanite	$PbFe_3(AsO_4)SO_4$
Skutteridite	$(Co, Ni)As_x$		
Loallingite	$(FeAs_2)$		
Tennantite	$(Cu_{12}As_4S_{13})$		
Jordanite	$(Pb,Ti)_{13}As_7S_{23}$		
Pearcite	$Ag_{16}As_2S_{12}$		
Prunsifite	$Ag_2As_2S_3$		
Energite	Cu_3AsS_4		
Rathine	$Pb_3As_5S_{10}$		

Table 2.2: Calculated Ratios of Arsenic Concentrations in Natural Reservoirs with Respect to Soil (Mackenzie et al., 1979).

Reservoir	Approximate ratios with respect to soil
Rocks	25,000
Oceans	4
Soil	1
Biota	0.0005
Atmosphere	0.000001

Table 2.3: Calculated Arsenic Rates of Transfer.

From:	To:	Approximate Amount ($x\ 10^8$ g/yr)
Land	Oceans	3,000
	Atmosphere	1,000
	Biota	300
Atmosphere	Oceans	2,000
	Land	1,000
Oceans	Sediment	2,500
	Biota	1,300
	Dissolved	1,000
Sediments	Land	2,400
Minning, Smelting Terrestrial biota	Land	300
Volcanoes	Land	54
	Sediment	40
	Atmosphere	3

2.2.2 Anthropogenic Sources

Recent estimates have placed the ratio of natural to anthropogenic inputs of arsenic at 60:40 (Chilvers et al., 1985). The global production rates of arsenic compound, determined in a recent survey are shown in Table 2.4.

Table 2.4: Production rates of the main arsenical compounds (Alloway, 1990).

Compound	Production (tons As/year)
Herbicides	8,000
Cotton desiccant	12,000
Wood preservative	16,000

The anthropogenic influence on the level of arsenic in soils depends on the human activity, the distance from the pollution sources, and the pollution dispersion pattern

(Yan-chu, 1994). Arsenic may accumulate in soil through use of arsenical pasticides, application of fertilizers, irrigation, dust from the burning fuels, and disposal of industrial and animal wastes (Sandber and Allen, 1975). It is natural contaminant in lead, zinc, gold and copper ores and can be released during the smelting process (Crecelcious et al., 1974; Ragaini et al., 1977). The stack dust and flue gases from smelters often contaminate soil with arsenic downwind from the operation (Crecelcious et al., 1974; Ragaini et al., 1977). Arsenic is also coming associated with phosphate minerals in an average concentration of 7.7 μg/L (Alloway, 1990).

2.3 Distribution of Arsenic in Groundwater of Bangladesh

In case of Bangladesh and West Bengal, India, the cause of arsenic contamination is different from that of other countries. The source of arsenic in this area is ground water. It is now generally agreed that the source of arsenic contamination in groundwater of Bangladesh is geological (BGS, 2000). The distribution of arsenic in ground water is related to the geology of the country rather than depth (Ministry of LGRD, 2002). The arsenic derives from the geological strata underlying Bangladesh. The primary sources for arsenic the sedimentology of Bangladesh are likely to have originated from Eastern India, the Shillong Plateau and possibly from Himalayas (National Water Management Plan, Working Paper, 1999).

The British Geological Survey (BGS, 2001) conducted a comprehensive study to assess the extent of the Arsenic contamination of groundwater in Bangladesh. The work included their own measurements in UK laboratories, in addition to field measurement in Bangladesh. The UK results were at some variance with local measurements. Both field kit and laboratory methods were used for analysis of water. Arsenic concentration data for 3534 shallow tube wells (depth < 150 meters) are shown in Table 2.5. About 27% of the wells were found to have Arsenic concentrations in excess of the national standard of $50 \mu g L^{-1}$. These wells are distributed in 61 districts out of 64 districts in the country which corresponds to 433 upazilas out of a total of 496. The data for the As concentration in 570 shallow wells in the 12 worst affected districts are shown separately. The highest concentrations in contaminated wells are found in these districts situated in the south and east of

Bangladesh and where some 76% of shallow wells exceed the Bangladesh standard for arsenic.

Table 2.5: BGS Screenning survey results for 61 districts out of 64 and 12 worst affected districts (Kinniburgh et al., 2001).

Area Type	**Districts (Upazillas)**	**Tube wells**		**Comments**
		Total	**Contaminated (%)**	
All	61 (433)	3,534	27	61 out of 64 districts and 433 out of 496 Upazilas.
Worst affected	12	570	76	

World Bank financed Bangladesh Arsenic Mitigation and Water Supply Project (BAMWSP) the LGED, Local Government Division, GOB, is responsible for As screening the As contamination level in shallow tube well water in 188 Upazilas out of 496 through out the country in another survey. The data obtained by BAMWSP are archived by National Arsenic Mitigation Information Centre (NAMIC), which are shown in Table 2.6.

Table 2.6: Status of the NAMIC screening survey-Phase I and II. (NAMIC, 2003).

Phase	**Upazilas**	**Tube wells**		**Population**	**Patients**
		Total	**Contaminated(%)**		
I	6	80,390	48.19	1,698,410	1,139
II	35	544,651	46.49	8,674,202	9,188
Total	41	625,041	46.71	10,372,612	10,327

The percentage of contaminated tube wells is higher in this studies compared to BGS survey. This is not necessarily a disagreement as hot spots were chosen for screening on the basis of prior knowledge.

2.4 Health Effect of Arsenic

The routes of Human exposure to Arsenic can be through food, water and air. The general values of exposure through air, water and food are shown in Table-2.7 (Vahter, M., 1994).

Table 2.7: Intake of inorganic and organic Arsenic compounds in the general population.

Source	Inorganic Arsenic (μg/d,p)	Organic Arsenic compounds (μg/d,p)
Air	0.05	-
Food	5-20	5-1000
Water	<1-10	-
Smoking	1-20	-

Normally the concentrations of Arsenic in air are in the range of 0.4 - 30 x 10^{-3} $\mu g/m^3$ (Wu et al, 1989). 30-85% of the inhaled arsenic can be absorbed and USEPA (United States Environmental Protection Agency) has estimated that the general public's intake of Arsenic would be in the range of 0.04-0.09 μg/d (Pontius F.W. et al., 1994). People living near smelters, power plants using coal and oil with high arsenic concentrations, and households using coal with high concentrations of arsenic can get exposed to arsenic concentrations as high as 1 μg/m3 resulting in a real intake of 6-17 μg/d (assuming that an adult inhales 20 m^3 of air per day). Liu et al. (2002) reported an incidence of indoor air arsenic concentrations in the range of 20-400 $\mu g/m^3$ in China, where people used coal with high arsenic concentration for preparation of food.

The amount of Arsenic intake through food is widely varied depending on the type of food consumption. The importance of the various routes of exposure depends on the actual concentration. Usually in places where the arsenic concentration in the groundwater is less (1μg/l) and there is no pollution of air due to industries, the major route of exposure to arsenic would be through food. Based on market basket survey of total As the US Food and Drug Administration has estimated that adults ingest on average about 53 μg/d from the diet (Pontius F.W. et al., 1994). A Japanese study showed a daily intake of Arsenic at 182 μg. In a Dutch study the daily intake of total Arsenic range between <5 to 950 μg/d. According to WHO the mean daily intake of Arsenic through food by adults is in the range of 17-129 μg (Wu et al, 1989). Generally it is only the inorganic Arsenic that is considered toxic to human, while the organic forms appear virtually non-toxic. Ali et al. (2003) found average arsenic concentration in rice grain produced in different parts of Bangladesh is 0.48 mg/kg. Considering average consumption of rice grain 454gm/capita/day average arsenic intake by a Bangladeshi people through only rice grain is 218 μg/day.

Arsenic concentration in most natural waters generally varies between 1-2μg/l resulting in an arsenic intake of 2-4 μg/d assuming that an adult consumes 2L of water per day (Wu et al, 1989). This value is very low compared to the exposure through food. But as mentioned before elevated concentrations of Arsenic occur in some parts of the world especially in Bangladesh & West Bengal, India and the arsenic concentration varies widely, resulting in very high exposure to Arsenic concentrations. In another study, Raychowdhury et al. (2002) estimated that the average intake of drinking water per day for adult male is 4 L and for females 3 L. With a mean concentration of 133 μg/l in one of the studied blocks they found the average daily intake of arsenic from drinking water by an adult is 532 μg and the average intake of inorganic arsenic through food it is 86 μg/d assuming that 50% of total arsenic in food is inorganic.

The toxicity of arsenic is a complex subject. The most common toxic mode of an element is the inactivation of enzyme systems which serve as biological catalyst. The acute toxicity of arsenic compounds is predominantly a function of their rate of removal from the body. Chronic exposure to low concentrations of arsenic is of primary interest when the health significance is of arsenic in drinking water is elevated, because of general population is normally exposed to low concentration. Chronic oral exposure of As generally affects the same organs and systems as those affected by acute exposure. For chronic toxicity of arsenic, arsenite [inorg. As(III)] and arsenates [inorg. As(V)] are equally toxic. The inorganic arsenic compounds are about 100 times more toxic than organic arsenic compounds (DMAA and MMAA) (Nagy, G. et al., 1983). The methylation of inorganic arsenic in the body is a detoxification process which reduces the affinity of the compound for the tissue (Vahter, M. and Marafante, E., 1988). Man, cow and dog can methylate both trivalent (As^{+3}) and pentavalent (As^{+5}) arsenic to form monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) and the steps of methylation reactions are-

As(V) → As(III) → MMAA(V) → MMAA(III) → DMAA(Thompson, D.J., 1993)

Arsenic is a known carcinogen and incidences of population suffering from Blackfoot disease due to long term exposure to Arsenic concentrations at 0.1 - 0.9 mg/l (Tseng W. P. et al., 1968). Acute dosages of 1.5 mg Arsenic (di-arsenic trioxide)/kg of body weight can lead to death. The most common symptoms of long-term low-level arsenic exposure are variations in skin pigments, hyperkeratosis, and ulcerations. There is documentation that Arsenic can cause kidney, bladder, skin, lung and liver cancer. In the majority of cases in which the internal cancer is ascribed to As exposure, some dermatological hallmark of As poisoning is identified (Tsai et al., 1999). However, the total quantity of arsenic consumed per day and the duration of exposure are important factors (WHO, 1981; Chakrabarti and Saha, 1987; Niu et al. 1999). In Taiwan, Chen and Wu (1962) reported a high incidence of hyperkeratosis and skin cancer after consumption of water containing more than 0.3 mg L^{-1} arsenic. Tseng et al. (1961) also observed an association of skin cancer with drinking well water containing 0.5 mg arsenic. It is reported (WHO, 1981) that approximately 1 mg of arsenic per day may give rise to skin effects within a few years of exposure.

Gastrointestinal manifestations have also been reported due to chronic As exposure and includes noncirrhotic portal hypertension (NCPH) (Nevens et al., 1990) hepatic or splenic enlargement, hepatocellular carcinoma (Nevense et al., 1990) and liver angiosarcoma (Neshiwat et al., 1992). Due to increasing severity of risk from arsenic contamination ANHMRC (1996), Australian National Health and Medical Research Council reduces the guidelines for arsenic in water from 0.01 mg/L to 0.007 mg/L because of the concerns for health/cancer risk. Sharma et al. (2003) found the risk of getting skin and internal cancer can be reduced by 77% if the water containing high concentrations of Iron and Arsenic are allowed to stand for 24 hours and then filtered properly before drinking.

There were many studies, which showed that at lower exposure levels there was no excess risk of getting cancer. These studies are from the Oregon, Utah Study (Kartinen et al., 1995) Belgium, and Finland. The study from Utah showed that there was no observed excess incidence of death due to cancer and high Arsenic concentrations in drinking water. The Arsenic concentrations were in the range between 14-166 μg/L.

Long time intake of inorganic arsenic is known to induce a build-up in the ectodermic tissues like hair and nails (Eisler, 1994). The health effects of Arsenic vary for each individual depending on genetic conditions, nutrition level, amount of exposure, duration of exposure etc (Brown K. G. et al., 1995; Pontius F. W. et al., 1994; Viraraghavan et al., 1994).

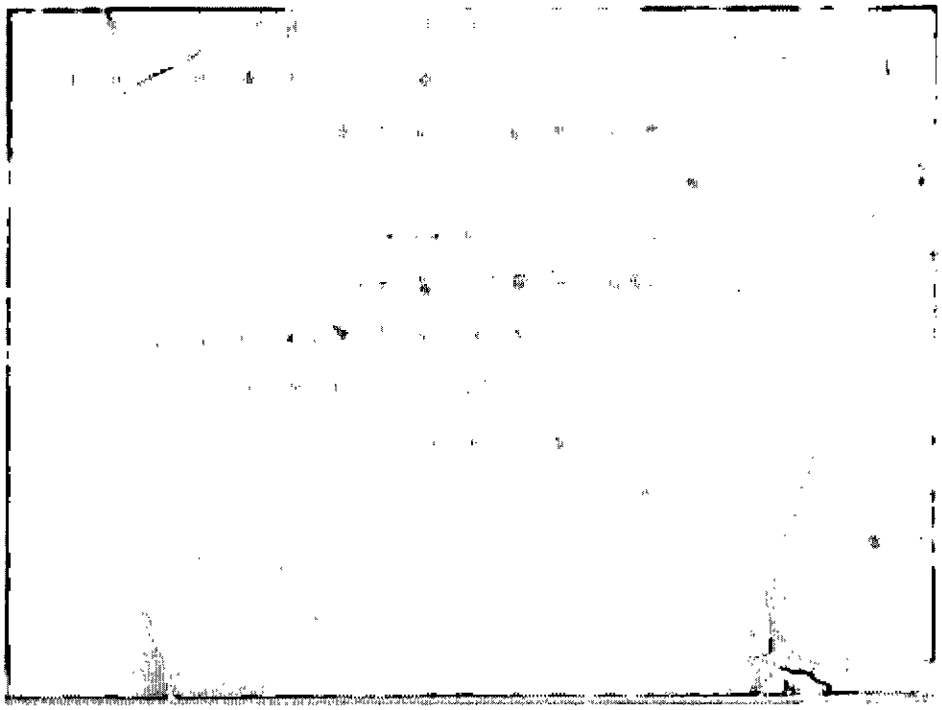
Generally, a dose of 120 to 200 milligrams of As would prove lethal in an adult. Levels between 0.1 and 0.5 mg/kg on a hair sample indicate chronic poisoning whilst 1.0 to 3.0 mg/kg indicates acute poisoning. In acute poisoning the clinical presentation includes nausea, vomiting, diarrhea, abdominal pain, excessive salivation and seizures. Increased capillary permeability along with diarrhea, vomiting and excessive salivation leads to reduced blood volume and eventual death due to circulatory failure, usually within twenty-four hours to four days following ingestion. Chronic As poisoning has a more insidious onset. The symptoms are non-specific such as abdominal pain, diarrhea and sore throat. There are numerous skin changes associated with long-term exposure.

The ground water contamination causes serious health hazards as reported from many countries of the world. In Bangladesh, shallow groundwater is heavily contaminated with arsenic and in some areas it reaches ≥2 mg/L (Tonden et al., 1999; BGS, 2000; Dainichi, 2001). The source of this contamination is geogenic (DPHE-BGS, 2000). Chronic arsenic poisoning or arsenicosis occurs as a result of prolonged exposure (Ministry of Health and Family Welfare, 1990). The symptoms include melanosis (blackening of skin), keratosis (thickening of the hands and soles), skin lesions, muscular weakness and paralysis of lower limbs (National Water Management Plan, Working Paper, 1999). Studies indicate the incidences of skin cancer and cancer of specific internal organs increases with increased exposure to inorganic arsenic (USEPA, 1998). It is reported that about 7500 arsenicosis patients have been identified in 37 districts (Ahmad, 2000). In another study it is also reported that about 2 lac people in Bangladesh are suffering from arsenicosis ranging from melanosis to skin cancer (Das, 2000). Yet in another report (Ahmed, 2001) estimates that 29 million people are exposed to high arsenic contents in drinking water exceeding the Bangladesh standard of 50 μgL^{-1} while 49 million people are exposed to the some risk for exceeding the WHO guideline value of 10 μgL^{-1}. It is extremely toxic on long-term exposure to very low concentrations. The WHO acceptable skin cancer risk is calculated to be 0.17μg arsenic/L of water (WHO, 1996). The estimated number of people under the risk of excess skin cancer due to arsenic contamination of drinking water in Bangladesh is 375,000 (Ahmed, 2003)

A recent paper on cancer risk estimation in Bangladesh, on the basis of Taiwan data, showed that the lifetime risk of deaths from internal cancers among Bangladesh population would be more than double due to arsenic ingestion (Chen and Ahsan, 2002).

Das et al. (2003) studied for As in scalp hair collected from Daudkandhi and Begumgonj Upazila of Bangladesh found arsenic concentration 8-13 ppm in human scalp hair that is more than 4 times higher than the proposed NRCC (National Research Council of Canada, 1978) critical concentration of 2 ppm. Photographs of some arsenicosis patients of Bangladesh are shown in figure 2.1.

Conversely, As has been found to have an anti-carcinogenic effect and a potential role in the treatment of acute promyelocytic leukaemia. It must be remember that As has been extensively used in the past as a healing agent and an example is Fowler's solution, which was used for leukaemia. Arsenic was also used for the primary treatment for syphilis until World War II.



(a) Melanosis patients

(b) Keratosis with cancer patients

Fig. 2.1. Photographs of arsenicosis patients (Source: NGO Forum, 2002).

2.5 Irrigation Practices in Bangladesh

Agricultural activity is of critical importance to both the household and national income of Bangladesh. The country itself is heavily dependent on its agricultural sector for domestic employment, foreign exchange earnings, and its gross domestic product. It is the most dominant sector of national economy, accounting for nearly 80% of trade. More than 80% of the population depends on agriculture for its livelihood. The agricultural sector employs 90% of rural males as well as 80% of rural females (World Development Indicators, 1998); for rural populations of Bangladesh, activities in agricultural operations are virtually the only means of earning income and increasing welfare. This emphasis on agricultural production has prompted the government of Bangladesh to adopt policies for the continued and increased development of water resources. In 1983 the government of Bangladesh, along with the World Bank and the United Nations Development Program collaborated on the formulation of a National Water Plan to take into account the needs of all water-use sub sectors. The specific objectives of the National Water Plan are to: 1) maximize value added in agriculture and fisheries, 2) provide water in a timely manner and in sufficient amounts to users 3) manage and control of flooding, and 4) achieve growth in equity. The underlying goal of this plan was to ultimately achieve food grain self-sufficiency for the country, a goal that the country is close to attain in the case of rice.

In Bangladesh, due to absence of adequate surface water in the dry season, irrigation is heavily dependent on ground water. During the last fifteen years, the area under irrigation has been increased significantly to raise food production. Much of this increase has been accomplished through installation of shallow tube well. Irrigation season is divided into two seasons in this country, namely Rabi season and Kharif season. According to WARPO report (WARPO, 1999) in the 1996/1997 Rabi season, the total irrigated area was 3.8 million hectares, among which 2.63 million hectares irrigated using groundwater. In the Kharif season a total of 0.2 million hectares was irrigated, among which 0.158 million hectares was irrigated using ground water. In the Rabi (1996/1997) season, STW irrigated 2.15 million hectares (63% of the total area under minor irrigation), among which boro rice field accounted for 1.68 million hectares.

According to BADC survey (BADC, 2002) a total of 865213 shallow tube wells and 23182 deep tube wells were used for irrigation during the 2001 boro season. Shallow tube wells irrigated about 2.295 million hectares (about 61% of total area under irrigation) and deep tube wells accounted for about 0.538 hectare (about 14% of irrigated area). The contribution of ground water to total irrigated area has increased from 41% in 1982/83 to over 75% in 2001.

Thus, for Bangladesh, attainment of self-sufficiency in food production is strongly related to its ability to produce food during the dry season with the help of irrigation. Since we are increasingly depending on shallow aquifer (a large part of which is arsenic contaminated) for irrigation water, a huge quantity of arsenic is being added in the agricultural fields each year with irrigation water. If concentration of arsenic in ground water is 100 ppb and in an average 1000 mm of water is applied for irrigation of boro rice then 1 kg/ha arsenic is added to the rice field each year. According the estimation of BADC (BADC, 2002) the districts with high deposition of arsenic in agricultural land include Chandpur, Munshigonj, Comilla, Gopalgonj, Faridpur, Madaripur, Jessore, Jhenaidah, Bagerhat and Kustia.

2.6 Arsenic Content in Soil and Plants

2.6.1 Arsenic Content in Soil

2.2.6 Fate of Arsenic in Soil

Arsenic contaminated soil causes potential risk to human and environmental health. Arsenic contamination of soil can occur as a result of both natural and anthropogenic activity. This type of contamination associated with use of arsenical pesticides and herbicides, atmospheric deposition, mining activity, waste disposal and many other sources of As. Currently, the use of As contaminated groundwater for irrigation in Bangladesh, India and Vietnam is raising concerns.

The fate of As once it enters the soil environment can be complex. The fate of arsenic in the soil can be summarised as shown in Fig. 2.2. Essentially, As can (i) react with, and become retained by the solid phase of the soil, (ii) be volatilised into the atmosphere as a result of various biological transformations, (iii) be taken up by plants, (iv) be leached out of the soil in groundwater and (v) be washed out by rain water and flood.

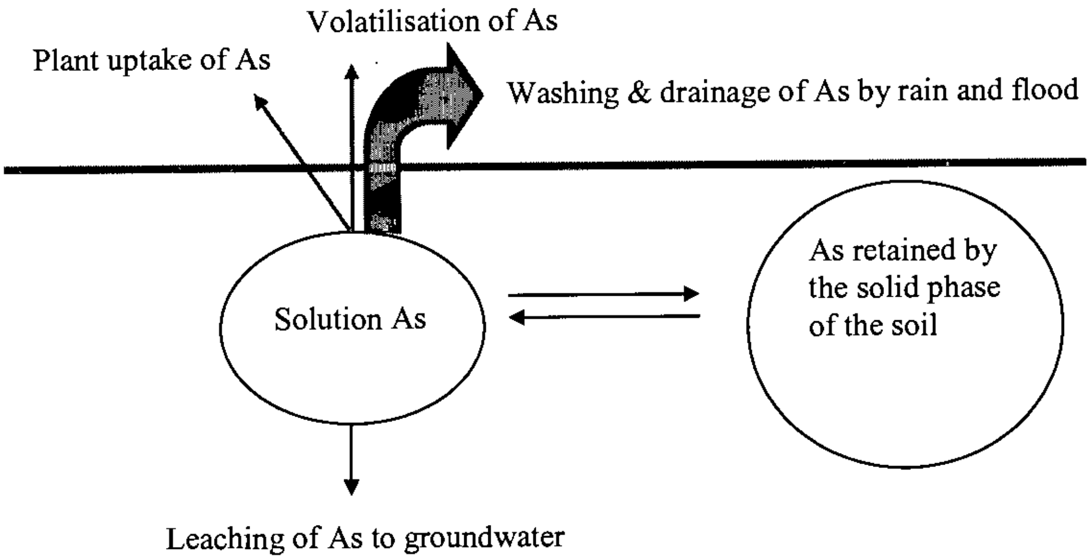


Fig. –2.2: Simplified fate of As in the soil environment.

Sorption of As on solid phase of soil affected by many factors such as soil pH, clay minerals, iron and alluminium oxides and competing inorganic and organic ligands. Various biotransformation in particular microbial oxidation and reduction process complicate the chemical form of As in solution and solid phase. Certainly, microbial influences on As sorption and desorption process. Microbial methylation process also produces volatile gases such as di- and trimethylarsine from soil. Depending on conditions, 17 to 60% of the total arsenic present in soils may be volatilized (NCCR, 1978). The plant uptake pathway is another potential fate of As in contaminated soil. However, in most plants, As concentrations are relatively low. But the fern *pteris vittata* (brake fern) is extremely efficient in extracting As from soils and translocating it into its aboveground biomass. The redox process may be involved in acquisition of As by the hyperaccumulating fern *pteris vittata* (Fitz et al., 2001).

Probably of great concern in relation to As-contaminated soil is the potential for arsenic to be leached through the soil into groundwater. The potential for As to leach has indeed been recognized for many years. For example, there are many reports of As movement down through the soil profile following the use of As containing herbicides and pesticides. Substantial leaching has also been observed at sites contaminated with copper-chromium-arsenic timber preservative, and at sites containing As rich mining and processing wastes (McLaren et al., 2001). However, estimates of half-life of arsenic in soil vary from 6.5 years for arsenic trioxide to 16 years for lead arsenate.

In aerobic soils, the dominant arsenic species is As^{+5} and small quantities of arsenate and monomethylasonic acid are present in mineralised areas; in anaerobic soils As^{+3} is the major soluble species (Haswell et al., 1985). Inorganic arsenic is more mobile than organic arsenic and thus poses greater problems by leaching into surface waters and ground water (NCCR, 1978). The trivalent arsenic species are generally considered to be more toxic, more soluble and more mobile than As^{+5} species (Thanabalasingam and Pickering, 1986). Arsenic from solid phase of soil and soil solution are dissolved in water logged over soil surface. Water logging due to rain and flood dilute As from soil and soil solution and drainage it out to river and other places.

As is coming to the soil surface from the arsenic contaminated ground water during irrigation because 86% of total ground water withdrawn uses for irrigation (WRI, 2000) that is mostly inorganic arsenic. The experiment used to study the sorption and transformation of arsenic [As(III)] and arsenic [As(IV)] in the soil system by Xie et al. (2001) showed that the chemistry of soil-water system as follows:

The order of As(III) and As(IV) sorption by soil components tested was synthetic Al_2O_3 > synthetic Fe_2O_3 > synthetic MnO_2 > synthetic $CaCO_3$ > montmorillonite > kaolinite > verviculite. Of the above mentioned soil components, the birnessite can oxidize As(III) to As(V). It was suggested that amorphous Fe_2O_3 and/or MnO_2 in the soil-water systems could reduce As toxicity. On average, As(III) added to the soil was transformed into water-soluble As (H_2O-As) 4.0%, As-Al 6.04%, As-Fe 30.05%, As-Ca 4.74% and As-O (occluded As) 55.19%. Increased soil pH decreased the As sorption on the soil sharply. Anions in the soil increased H_2O-As released from the soil, the order being $H_2PO_4^-$ > SO_4^- > NO_3^- > Cl^-. Cations are opposite, the decreasing order being Mg^{2+} > Ca^{2+} >> K+ ~ Na^+.

Despite repeated application of As contaminated irrigation water soil may adsorb certain amount of arsenic and rice and other agricultural plant uptake arsenic from soil solution. There is no direct relationship with As in ground water and corresponding

As in soil (Huq, 2003). However, there is a positive relationship of As adsorption in soils with its clay content and soil having clay more than 84% adsorb more As (Huq, 2003).

Since arsenic in ground water was recognized as a potential threat to human life, much effort has been directed towards ensuring safe drinking water either through mitigation techniques or through finding alternative water sources. Even when alternative sources of water are used for potable purposes, continued cropping of long-term irrigated soils subjected to As contaminated ground water will pose significant risk to animal and human health through soil-crop transfer of As. Food crops such as vegetables and cereals can become a path by which As may enter the food chain, because they can reflect the levels of As that exist in the environment in which they are cultivated (soil and irrigation water). Recent studies by Meharg et al. (2002) and Huq et al. (2001) demonstrate significant uptake of As by rice and a range of vegetable crop commonly grown in Bangladesh. Numerous greenhouse studies by a number of researchers have revealed that an increase in As in cultivate soils leads to an increase in the levels of As in edible vegetables (Burlo et al., 1999; Carbonell Barrachina et al., 1999, Helgensen and Larsen, 1999) with many complex factors affecting bioavailability, uptake and phytotoxicity of As (Carbonell-Barrachina et al., 1999). Although, there are many studies on As in Bangladesh few have focused on soil As. Similar greenhouse study by Abedin et al. (2002) revealed that the most predominant forms of arsenic in soil solution were inorganic and constitute about 56-82% and in the paddy soil environment the applied arsenate was readily converted to arsenic. The presence of arsenic in the soil solution at a larger proportion corroborates the results of a number of investigations (Marin et al., 1993a; Onken and Hosner, 1995; Onken and Hosner, 1996), as arsenite is the predominant species in the soil solution. Microbial transformation of inorganic species to the organic form occurs in the paddy soil (Takamatsu et al., 1982, Abedin et al. 2002) that may be considered beneficial because of the lower toxicity of organic species (Fowler, 1977; NRCC, 1978). According to another study by EPA (1980) inorganic arsenic can be converted to organic alkyl arsenic acid and methylated arsine under anaerobic conditions by fungi, yeast and bacteria although biomethylation may also occur under aerobic conditions. A recent study to see the residual effect of arsenic in pot experiment conducted by Shah et al. (2004) reported that when arsenic application exposed for longer time, the phytotoxiciy effect of arsenic became less compared to immediate application of arsenic.

Arsenic found in soil, where contamination occurs, all over the world at an average concentration of 5 to 6 mg/L. The average concentration of arsenic in alluvial sand and mud/clay has been reported to be 2.9 mg/kg and 6.5 mg/kg respectively in Bangladesh (DPHE and BGS, 2001). Arsenic concentrations in soils as high as 51 mg/kg in Faridpur and 83 mg/kg in Comilla have also been reported (Ullah, 1998). A concentration varying from 1.5 to 19 mg/kg showing higher concentration in the top layers of soil has been found in Samta village in Jessore (Kubota, 1998). In a recent study conducted by Bangladesh University of Engineering & Technology and United Nations University (Ahmed et al., 2003) on Bangladesh shows that:

- Arsenic content of sediment of the Jamuna-Padma-Meghna rivers system varies from 1.0 to 6.5 mg/kg depending on particle size of the sediment.
- Arsenic builds up in the top soil when irrigated with arsenic contaminated water and maintain a decline profile of depth.
- Arsenic content of agricultural soils increases during irrigation with contaminated water but it is reduced during post irrigation period probably by leaching with flood/rain water and/or methylation process to the level of background concentration.
- Arsenic content of soil does not build up to a critical level that affects growth of crops.

2.6.2 Arsenic in Rice Plant

2.6.2.1 Accumulation of arsenic in rice

Accumulation of arsenic is affected by arsenic concentration in soil or nutrient media and increased greatly with increasing arsenic levels (Marin et al., 1992, Xie and Huang 1998). Arsenic concentration pattern in rice plant parts generally follow the pattern: root > straw > husk > whole grain >husked rice (Xie and Huang, 1998). Odanka et al., (1987) and Marin et al. (1992) also reported higher accumulation of arsenic in roots than any other plant parts. Rice grain generally has lower arsenic concentration (Schoof et al., 1999, Heitkemper et al., 2001) and the concentration remain much below maximum permissible limit of 1 mg As kg^{-1}. However, a study conducted by Chakraborti et al. (2001) showed 95% inorganic and 5% organic As species in rice. Irrespective of arsenic chemical forms root arsenic concentration was 10.5 mg/kg in the 0.05 mg As/L^{-1} treatment, which increased to 212.7 mg/kg in the 0.8 mg As L^{-1} treatment (Marin et al., 1992). Bennett et al., (2000) tested wild rice collected from Seely, Willow Flowage, Crandon and Port Edwards area of Northern Wisconsin, USA and found arsenic concentrations (ppm) 12.00, 0.34, 0.81 and 0.11 in root (36), stem (27), leaf (30) and seed (4) respectively and average concentration 4.82 ppm. The mean for As is almost double that of Nriagu and Lin's value of 0.066 ppm for store-bought wild rice (Nriagu and Lin, 1995). Yan Chu (1994) has posed a relationship between arsenic concentration in soil solution and rice also tested to quantify the effect of arsenic level uptake into rice. The regression equation found between the amount of arsenic present in the rice plant, Y, and the amount of arsenic in aqueous solution, X, to be: $Y = 0.042X - 0.0413$.

Xie et al. (2001) determined As contents of roots, flag leaf, grain and husked rice of 11 new cultivars of rarely rice variety cultivated in paddy soils polluted from tailing at an abandoned lead-zinc-silver mine at Xhoxing, Zhejang, China. They found As concentrations in husked rice exceeded the hygienic standard (0.7 mg As/kg) when they grew in the soil having total As content 70-100 mg/kg.

After confirmation of ground water contamination with arsenic in Bangladesh many researchers including government, non-government and foreign organizations works to find out the extent and severity and also to mitigate the problem but a few number of researchers are trying to find out the presence of arsenic in rice as rice is the staple food and about 70% of produced rice cultivated using ground water irrigation.

Recent studies by Meharg et al. (2002) demonstrate significant uptake of As by rice grown in Bangladesh. Huq et al. (2001) reported 0.11 to 0.35 mg/kg As found in cooked rice collected from Munshigonj, Bangladesh. Hiromi Hironaka and Sk. Akhtar Ahmad (2003) collected rice samples from different part of the country and conducted test for arsenic and found arsenic contamination range from 0.1 to 0.3 ppm which was similar to that of Japan's rice. But the limitations of the study were samples collected from farmer's house and market so there was no perfect indication of arsenic in soil and water.

Das et al. (2003) conducted test for arsenic in rice grown on the soils adjacent to arsenic contaminated water source. They found roots of paddy accumulated the highest arsenic concentration followed by shoot and rice grain (≈ 0.23 ppm). The ratio of As concentration in root, shoot and grain is 1 : 6 : 10. But they didn't show the level of As in soil.

Abedin et al. (2002) found arsenic, arsenate, DMAA and MMAA in soil solution where rice (8 Bangladesh Varieties) was irrigated with arsenic (arsenate, arsenite, DMAA, MMAA) contaminated water having arsenic concentration 0 to 8 mg/L^{-1} in greenhouse experiment. Uptake of both arsenite and arsenate by Boro varieties was less than that of Aman varieties. Arsenite uptake was carrier-mediated, and was taken up at approximately the same rate as arsenate. In presence of phosphate arsenate uptake strongly suppressed while arsenite transport was not affected. However, at a slow rate, there was a hyperbolic uptake of MMAA and limited uptake of DMAA. They found accumulation of arsenic in rice grain was limited and was less than the maximum permissible limit of 1 mg/kg (National Food Authority, Australia, 1993). The presence of very high concentrations of arsenic in rice straw could pose a potential health hazard to the cattle population as rice straw is used as cattle feed in Bangladesh and in other countries.

In another study conducted by Ali et al., (2003), the highest concentration of arsenic was found in the roots (2.81 – 16.8 mg/kg) of rice plant, and the comparatively a lower concentration in stem and leaves and the lowest concentrations were found in rice grain (0.05 – 1.52 mg/kg with a mean of 0.48mg/kg).

Duxbury et al., (2002), collected 150 samples of paddy grains from Barisal, Comilla, Dinajpur, Rajshahi and Rangpur districts in Bangladesh and determined the arsenic concentration in the grain. He found arsenic concentration in the range from 0.01 to 0.415 mg/kg dry weight. As expected grain arsenic concentrations were higher for Boro rice (mean value 183 μg/kg dry wt.) compared to Aman rice (mean value 117 μg/kg dry weight).

In case of rice, Ghani et al (2004) also observed higher accumulation of As in rice roots (121.56 ±117.03 ppm) followed by straw (13.01 ± 3.57 ppm) and least in grain (0.12 ± 0.05).

In a different type of study Huq et al. (2005) found green algae accumulate arsenic at a significantly high concentration while grown in boro rice field in Bangladesh. This accumulation of arsenic by green algae from irrigation water may cause lower accumulation of arsenic by rice plant, which would be beneficial to the people of Bangladesh.

2.6.2.2 Rice growth and yield

Presence of As in soil or soil solution or water cause adverse effect on growth and yield. Tsutsumi (1980) and Marin et al. (1992) reported about reduced plant height, Tang and Miller (1991) and Marin et al. (1993) reported about reduced root and Tsutsumi (1980), Milam et al. (1988), Tang and Miller (1991), Marin et al. (1992, 1993) reported reduced shoot biomass and/or growth in rice grown either in soil or in solution culture with arsenic. However, improved tillering (Xie and Huang 1994) and no reduction in dry matter production (Marin et al. 1993) have also been reported while arsenic concentrations in the growing media were low. Significant reduction in dry biomass (root and shoot), leaf area and net photosynthesis was observed with increasing concentration of DMAA (o - 1.6 mg As L^{-1}) by Marin et al. (1993). A physiological disease, straighthead has been reported to be associated with arsenic (Wells and Gilmour, 1977; Gilmour and Wells, 1980). It was also found that 7 ppm of arsenic causes injury to rice plant and would affect farm output productivity (Yan-Chu, 1994).

There are reports in literature of grain yield reduction due to arsenic application for rice (Gilmour and Wells, 1980; Liu and Gao, 1987; Milam et al., 1988; Frans et al., 1988). The arsenic concentration at which rice yield is decreased by 10% is judged to be the maximum allowable limit or critical content of arsenic in soil (Yan-chu, 1994). Nriagu (1994) reported rice yield decreases by 10% at 25 ppm of arsenic in soil. While reviewing soil arsenic concentration effect on rice yield Yan-chu (1994) reported a reduction of yield by 10% at 12.5-29 mg As kg^{-1}; 50% at 47-52 mg As kg^{-1}; no yield leading to death of plants occurred at 109-157 mg As kg^{-1}. Onken and

Hossner (1995) also reported rice yield reduction by 66% when mean soil solution arsenic concentration reached 1.5 mg/L^{-1}. However, Shah et al., (2004) has reported the level of arsenic in soil having concentration above 20 ppm may affect rice yield of Bangladesh variety.

2.6.3 Arsenic in other Plants

2.6.3.1 Direct effect of arsenic on plants

Arsenic (a toxic element) and phosphate (a macronutrient) are taken up by the same plasma membrane transport system in plants. In well aerate soils, the chemical behavior of arsenate is similar to that of phosphate. Both elements adsorb strongly to certain soil components (Fe-and Al-oxides and clays) and are therefore not readily available for plants. The effect of As on plant growth is often different in hydrophonics compared to soil systems (Cox et al., 1996). In hydrophonic uptake studies, phosphate decreased the As uptake in both arsenic tolerant and non-tolerant populations of Holcus lanatus L. (Macnair, 1991). In soils however, phosphate is able to displace arsenate into the soil solution (Alam et al., 1991). The presence of phosphate could therefore result in an increase in the availability of arsenate and enhance its uptake.

Carbonell et al. (1999) tested arsenic uptake by turnip growing under soil less culture conditions conducted with four As species [arsenite, arsenate, monomethylarsonic acid (MMAA), dimethylarsinic acid DMAA] and there As concentrations (1.0, 2.0 and 5.0 mg L^{-1}). They found both organic arsenicals showed a higher upward translocation than their inorganic counterparts, contributing to the greater phytotoxicity and lower dry matter productions of these organic treatments. Both inner root and outer root skin As concentrations were above the maximum limit set for As content in food crops (1.0 mg/kg^{-1}). Smith et al. (2001) also found yield of vegetable crops (Silver beet, Tomatoes, Peas and Capsicum) were severely affected. With increased As concentration in agricultural soils. They found yields of silver beet and tomatoes at higher rate arsenic (100 and 200 mg/kg) were only 5-10% of the yield at control soil and the variation also depends on plant species and soil p^{H}.

The accumulation of arsenic by plants grown on As contaminated soils varied between plant type and plant part with elevated levels of As being accumulated in all the plants studied. Smith et al. (2001) found different high arsenic concentration (>1 mg/kg, Australian food standard) in Rye grass, Silver-beet and Radish produced in long-term contaminated in South Australia. Similarly, study of Bolan et al. (2001) showed that arsenic concentration in watercress (*Lipidium sativum*) and other tropical water plants (Table 2.8) grown in Taupo Volcanic Zone (TVZ), New Zealand was less than the maximum value permitted in food stuffs (2 mg/kg) by the WHO except watercress (2.42 mg/kg).

Table 2.8: As concentration in different plant species grown in TVZ, New Zealand.

Sl. No.	Species	Common name	As concentration mg/kg^{-1}
1.	*Hygrophila difformis*	Hygrophilla	0.07
2.	*Hygrophila cosata*	Hygrophilla	0.18
3.	*Saurus cernus*		0.19
4.	*Hygrophila lacustris*	Hygrophilla	0.42
5.	*Echinodorus*	Amazon Sward	0.72
6.	*Bacopa monniera*	Baby's tears	0.75
7.	*Ludwigia repens*		0.95
8.	*Limnophila heterophylla*	Ambuli	1.41
9.	*Leidium sativum*	Watercress	2.42

Source: Bolan et al., 2001.

There is significant variation in the As content of plant tissues of commonly grown vegetables in Bangladesh (Huq et al., 2001) that is shown in the Table-2.9. Recent studies by Farid et al. (2003) reported that As content in vegetables (tomato, potato, brinjal, lalshak, better gourd, amaranth, china shak, katuadata, cabbage, Indian spinach, cauliflower, chilli, okra) produced in different part of Bangladesh using both As contaminated and uncontaminated irrigation water varied from species to species and also from uncontaminated to contaminated water. They found the trend of arsenic accumulation was higher in leafy vegetables and lower in fruit vegetables. Similarly, in other study Huq et al. (2001) reported that Arum accumulated excessively high arsenic than other vegetables tested.

Table-2.9: As concentration in different plant species grown in Bangladesh.

Sl. No.	Vegetables	Plant tissue arsenic mg/kg^{-1} dry weight
1	Gourd (leaves)	0.48
2	Gourd	0.66
3	Arum	19.80
4	Papaya	0.83-1.13
5	Data Shak (leaf + stem)	0.87-2.78
6	Data Shak (root)	1.92-4.54
7	Cauliflower	1.77-2.66
8	Cauliflower (leaves)	1.28-1.86
9	Cucumber (flower)	0.05-7.23
10	Cucumber (leaves)	0.38-3.25
11	Potato (leaves)	0.26-0.57
12	Potato (tuber)	0.33-0.41

Conversely, in other study Shah et al., (2004) found none of the 8 vegetables under study had arsenic level above maximum permissible limit when grown in different arsenic concentration in irrigation water (Table-2.10).

In a separate study, Shah et al., (2004) also found high concentration of As (0.03 – 0.96 ppm) in roots than leaves (0.03 – 0.1 ppm) and stems (0.03 – 0.6 ppm) in early growth stages of vegetables of age 4-5 weeks (Table-2.11). Another field survey by Shah et al., (2004) on 24 vegetable crops in Chapai Nawabgonj showed that arsenic content varied from 0.33 – 0.88 ppm (Table-2.12).

Table-2.10: Effect of irrigation water with different arsenic levels on the yield and arsenic contents in different vegetables, 2004.

Vegetables	High water arsenic (614 ppb)			Low water arsenic (38 ppb)		
	Yield (t/ha)	As load (mg/ha)	As in edible parts (ppb)	Yield (t/ha)	As load (mg/ha)	As in edible parts (ppb)
Amaranth	17.32	421.61	211.3	17.23	36.73	83.33
Red Amaranth	23.06	442.1	146.7	19.92	36.23	57.66
Spinach	25.59	468.69	68.35	19.94	36.73	50.00
Onion	13.25	1369	48.14	13.13	65.61	45.45
Onion flower	-	-	148.0	-	-	52.19
Radish	47.45	751.1	33.98	34.32	73.21	29.70
Potato	26.59	1448	27.97	17.38	110.8	42.67
Cabbage	46.07	751.1	25.26	37.15	50.41	43.33
Cauliflower	34.36	751.1	21.03	27.90	53.20	32.02

From the above test results conclusion were drawn that if irrigation water contain higher amount of As, the concentration of As will become more in the plants though it does not influence the yield of the vegetable.

Table-2.11: Distribution of arsenic content (ppm) in different parts of vegetable crops during early stage.

Crop	Root	Stem	Leaf
Red Amaranthus	0.10	0.06	0.05
Amaranthus	0.08	0.06	0.08
Tomato	0.96	0.06	0.07
Cauliflower	0.03	-	0.03
Cabbage	0.04	-	0.03
Radish	0.10	-	0.07
Potato	0.17	0.03	0.10
Onion	0.39	-	0.09

Source: Shah et al., 2004.

Table-2.12: Arsenic content of different types of vegetables grown in arsenic contaminated soil at Chapai Nawabgonj sadar upazila, 2003.

Fruit type	As content (ppm)	Leafy vegetable	As content (ppm)
Tomato	0.73	Red Amaranthus	0.05
Papaya	0.61	Coriander leaf	0.58
Bringal	0.66	Indian spinach	0.44
Ladies finger	0.54	Spinach	0.68
Chilli	0.60	Potato	0.47
Bean	0.59	Radish	0.77
Cauliflower	0.59	Onion	0.46
Bitter gourd	0.88	Garlic	0.33

Source: Shah et al., 2004.

In a study of Huq et al., (2001) arsenic concentration in aurum found excessively high, 19.80 mg/kg dry weight, which indicates the alarming situation in Bangladesh.

2.6.3.2 Residual effect of arsenic on plants

To see the residual effect of arsenic two sets of pot experiments were conducted by Shah et al., (2004) in a green house experiment for arsenic accumulation in different parts of Indian spinach as moderate sensitive crops to arsenic. In first port experiment was conducted by adding fresh As at a concentration of 0, 5, 10, 15 and 20 ppm. Results showed that the growth and dry matter yield decreased with increasing As rates (Table-2.13). Growth of Indian spinach drastically suppressed with 10 ppm As/pot. As accumulation in different plant parts was the highest in roots, lesser in leaves and least in stems.

In the 2nd pot experiment in the first year, rice was grown with high rates of As (0.0 to 140 kg/ha) and in the following year, residual effect of As on the growth and yield was observed where soil As level was 5.4 – 85.3 ppm. The higher As content in soil had no growth suppression effect on spinach and had no effect on green dry matter yield (Table-2.14). Results indicated that when As application exposed for longer time, the phytotoxicity effect of As became less compared to immediate application of As.

Table-2.13: Effect of arsenic rates on Indian spinach dry matter yield and its As content in different parts.

As level	Dry matter (g/pot)	As content (ppm)		
		Root	Stem	Leaf
As_0	50.7	1.66	0.22	0.52
As_5	39.02	3.12	0.31	0.21
As_{10}	37.60	8.26	0.70	1.05
As_{15}	22.50	9.48	1.64	1.71
As_{20}	16.46	14.03	1.72	1.70

Source: Shah et al. (2004).

Table 2.14: Residual effect on fresh dry matter weight of Indian spinach and its influences on the arsenic content in different plant parts.

Treatment	Weight of plant parts (g/pot)				As content (ppm)		
	Root	Stem	Leaf	Total	Root	Stem	Leaf
As_0 (5.4)	11.18	76.92	127.96	191.17	0.29	0.04	0.08
As_5(8.2)	11.46	91.38	121.48	212.86	0.44	0.02	0.09
As_{10}(14.4)	11.36	87.17	119.01	206.18	0.63	0.03	0.12
As_{20}(24.8)	12.00	92.30	123.61	215.92	0.82	0.04	0.11
As_{40}(37.0)	12.49	87.90	110.79	198.69	1.13	0.04	0.13
As_{60}(43.9)	12.05	95.11	130.80	225.91	1.49	0.07	0.17
As_{80}(53.5)	12.59	101.30	124.53	225.83	2.22	0.06	0.15
As_{100}(68.3)	10.98	105.38	121.44	227.32	2.37	0.06	0.13
As_{120}(74.2)	10.01	97.97	115.74	213.76	2.96	0.14	0.13
As_{140}(85.3)	10.96	105.89	106.37	212.26	2.65	0.18	0.18

Figure in parenthesis indicates pot soil As in ppm. Source: Shah et al. (2004).

2.7 Fluctuation of Arsenic in Groundwater and Soil

To see the fluctuation of arsenic concentration in groundwater in Bangladesh a study was conducted by Rashid et al, (2004) under the areas of PETRRA subprojects of IRRI. Monthly monitoring of As concentration in groundwater in the selected locations in Charghat upazila of Rajshahi district and Nawabgonj upazila of Chapainawabgonj district showed that the concentration was not constant through out the year. It varied from time to time. As concentration started to increase with the beginning of dry season and continued up to May/June. Then the As level started to minimum level after the monsoon. Variable arsenic concentrations over time have been given in table 2.15. Fig. 2.3 and 2.4 present the monthly average arsenic concentration in selected locations.

A study conducted by Ali et al., (2003) in Srinagar upazila of Munshigonj district also shows that arsenic concentration varies over time during irrigation of HYV rice varieties in Boro season.

Another study on irrigated agricultural soil over time for a rice field site in Srinagar, Munshigonj, by Ali (2003) showed that arsenic concentration in soil was significantly higher at the end of the irrigation season in June, compared to concentrations at the beginning of the season in February. However, after the flood (i.e., in Nov.) during which the rice field at the Srinagar site was under flood water for prolonged periods of time, arsenic concentration in soil came down to levels comparable to those at the beginning of the (2001-2002) irrigation season. The reduction in arsenic concentration in soil after the flood may result from bio-geochemical process (e.g., bio-methylation, dissolution/desorption) occurring in the rice fields.

Table 2.15: Monthly arsenic content (ppm) in irrigation water in the selected locations of Rajshahi and Nawabgonj district during, 2003.

Name of Upazila	Location (village name)	Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Charghat	Talbaria north	0.190	0.212	0.215	0.207	0.200	0.163	0.134	0.130	0.142	0.163	0.180	0.174
	Talbaria south	0.075	0.072	0.077	0.074	0.076	0.060	0.056	0.054	0.051	0.051	0.074	0.104
	Bamnidah	0.105	0.109	0.114	0.147	0.174	0.170	0.168	0.171	0.164	0.170	0.155	0.162
	Bhatpara	0.052	0.063	0.067	0.079	0.102	0.117	0.112	0.110	0.112	0.106	0.099	0.081
	Bhatpara (DTW)	0.021	0.026	0.031	0.015	0.015	0.008	0.003	0.003	0.004	0.004	0.002	0.004
	Shibpur	0.017	0.021	0.021	0.028	0.038	0.035	0.030	0.035	0.033	0.028	0.031	0.027
Nawabgonj Sadar	Laxshmipur (STW)	0.107	0.173	0.224	0.235	0.251	0.340	0.458	0.379	0.206	0.014	0.192	0.237
	Laxshmipur	0.105	0.148	0.160	0.205	0.247	0.275	0.257	0.227	0.201	0.184	0.136	0.215
	Vadail	0.078	0.107	0.138	0.186	0.211	0.233	0.218	0.216	0.177	0.068	0.053	0.113
	Hatathpara	0.554	0.595	0.852	1.108	1.536	1.275	1.107	0.814	0.747	0.747	0.842	1.117
	Rajarampur	0.082	0.130	0.159	0.125	0.125	0.119	0.131	0.133	0.131	0.060	0.065	0.103
	Laxshmipur (DTW)	0.052	0.090	0.140	0.166	0.179	0.161	0.146	0.124	0.082	0.073	0.062	0.052

Source: Rashid et al., 2004.

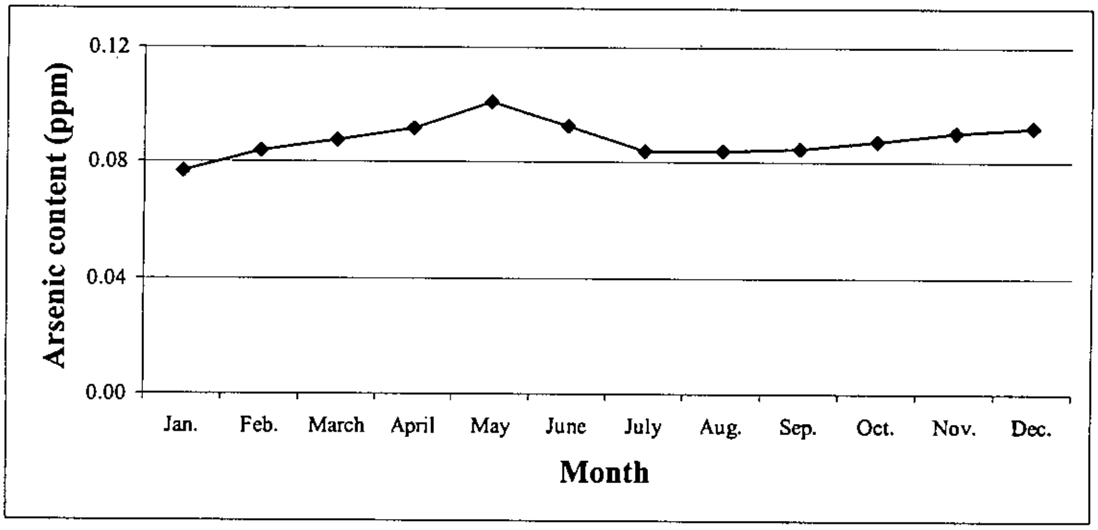


Fig. 2.3: Fluctuation of As content in irrigation water in Charghat.

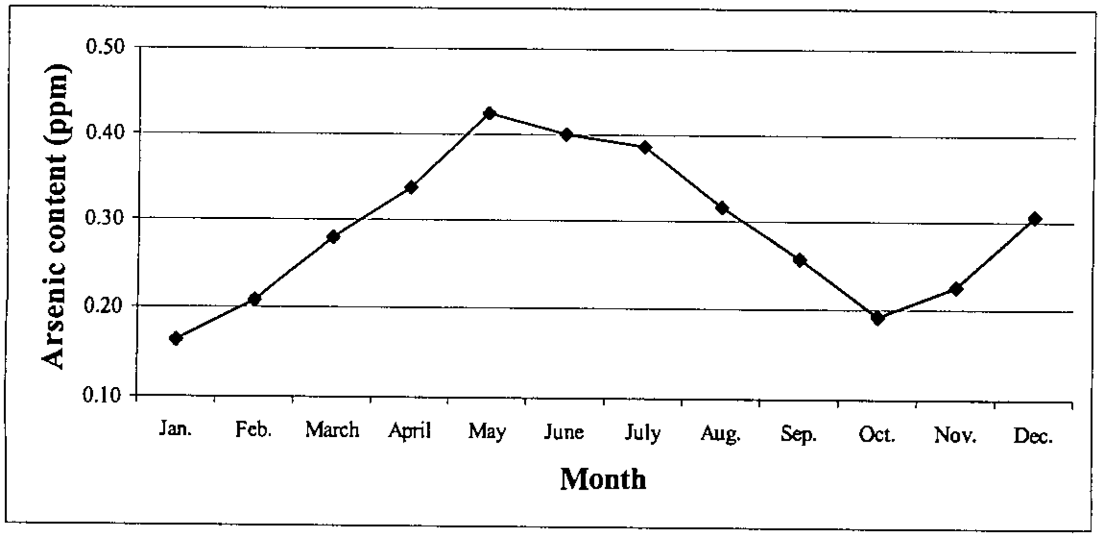


Fig. 2.4: Fluctuation of As content in irrigation water in Chapai Nawabgonj.

2.8 Arsenic contamination due to carried over effect

Studies were conducted by Rashid M. A. et al. (2003) under PETRRA sub-projects for three treatments with As contaminated groundwater in each of Charghat and Chapai Nawabgonj areas. The treatments were as follows-

T_1 = Continuous standing water (1-7 cm),

T_2 = Alternate wetting and dry (irrigation after three days of disappearing standing water) and

T_3 = Saturation to field capacity.

Three plots were selected under all the tube wells for detail monitoring. The experiments were conducted in the Boro seasons of 2003 and 2004 for the variety of BRRI dhan 28. Control plots were irrigated with permissible As concentration (<0.05 ppm). Arsenic load was higher in continuous standing water condition than in other two treatments. The highest load was 2.26 kg/ha in continuous standing water condition and lowest was 1.46 kg/ha under alternate wet and dry condition (Table-2.16). In this studies arsenic concentrations in straws were found in the ranges from 3.84 to 5.20 ppm. Arsenic concentrations in grain were found in the range from 0.428 to 0.56 ppm. Highest arsenic concentration found in straw for the treatment of continuous standing water. But more arsenic concentration found in grain for the treatment of saturation to field capacity and least concentration found for the treatment of continuous standing water.

Table 2.16: Arsenic accumulations by rice plant in different treatment conditions.

Location (Upazila)	Treatment	Yield (Kg/ha)	Water depth (cm)	As load (kg/ha)	Straw As in (ppm)	Grain As in (ppm)
Charghat	T_1	5074	107.0	2.26	5.20	0.465
	T_2	5174	78.1	1.65	4.38	0.540
	T_3	5527	74.2	1.57	4.05	0.560
Chapai Nawabgonj	T_1	4697	101.6	2.14	4.72	0.428
	T_2	4494	72.6	1.53	3.91	0.476
	T_3	4794	69.0	1.46	3.84	0.492

Source: Rashid et al., 2004.

2.9 Influences of Phosphorus and other soil parameters

A pot experiments were carried out by Jahiruddin, et al. (2004) at BAU to evaluate the effect of As in presence of P (phosphorus) on Boro rice (cv. BRRI dhan 29). These two element were added to soil at 0, 10, 25 and 50 ppm and 0, 25, 50 and 100 ppm from sodium arsenate and potassium di-hydrogen phosphate, respectively. Addition of As adversely affected the growth, grain and straw yields of rice. The application of As to soil at or above 10 ppm markedly affected the grain and straw yields of rice and such adverse effect of As was further aggravated due to P addition to soil. The application of P enhanced the As accumulation in grain and straw while the P content in grain and straw remained unaffected by As addition to soil. Therefore, it can be concluded that application of high levels of phosphorus may increase the arsenic toxicity in soils.

In another study by Abedin et al., (2002) in a greenhouse experiment it was found that presence of phosphate strongly suppressed the uptake of arsenate while arsenite transport was not affected.

In another analysis, Shah et al., (2004) collected soil and water samples from selected fields of nine districts under PETRRA subprojects. Based on test results of arsenic content they established a correlation matrix. The correlation matrix showed that water extracted As had -

i) positive but insignificant relationship of total soil As, and
ii) similar relationship with As in straw and grain of rice plant.

In contrast total As in soil showed-

i) positive but insignificant relationship with As in straw and grain of rice plant;
ii) significant positive relationship with soil p^H and
iii) negative relationship with available iron content in soil.

2.10 Residual effects of soil arsenic

Jahiruddin et al. (2004) were studied the effects of As contamination on crop yield and As accumulation under control conditions. Two sets of pot experiments, one with soil added arsenic and the other with irrigation water arsenic, were conducted in the net-house of BAU, Mymenshingh. The levels of soil added arsenic were 0, 5, 10, 15, 20, 30, 40 and 50 ppm, and that of irrigation water As were 0, 0.1, 0.25, 0.5, 0.75, 1.0, 1.5 and 2 ppm. The effect of added As (plus 2.6 ppm soil As) was tested directly on Boro rice (cv. BRRI dhan 29) and its residual effect on T. Aman rice (cv. BRRI dhan 33). The pots for both crops received an equal amount of fertilizers. They found that the grain protein was adversely affected due to arsenic contamination.

For water added arsenic grain yield decreased with higher arsenic doses at 1.5 ppm in irrigation water for the first crop and doses at 0.75 ppm in irrigation water for the 2nd crop (Table 2.17). For soil added arsenic it was observed that there was a significant yield reduction at and above 10 ppm As. The arsenic concentration in rice grain increased with arsenic applications either added through irrigation water or added directly to the soil, still it did not exceed 1 ppm. The straw-arsenic concentrations in all cases were well above 1 ppm. There was a residual effect of both soil and water added arsenic on the second crop (T. Aman rice).

Table 2.17: Direct and residual effects of added arsenic through irrigation water on growth, yield and arsenic contents of rice.

Concentration of irrigation water arsenic (ppm)	Direct effect on first crop (Boro rice, BRRI dhan 29)				Residual effect on second crop (T. Aman, BRRI dhan 33)			
	grain yield (g/pot)	Straw yield (g/pot)	Grain arsenic content (ppm)	Straw arsenic content (ppm)	grain yield (g/pot)	Straw yield (g/pot)	Grain arsenic content (ppm)	Straw arsenic content (ppm)
0.0	99.87	119.10	0.239	2.292	56.33	61.26	0.094	1.480
0.10	103.73	122.50	0.330	4.836	58.60	66.68	0.107	2.646
0.25	108.90	148.00	0.348	6.536	71.50	83.52	0.133	2.741
0.50	106.60	148.83	0.390	7.068	56.54	67.39	0.137	3.083
0.75	101.97	133.57	0.447	7.491	53.10	62.16	0.156	3.269
1.00	99.02	115.77	0.485	7.869	50.28	61.38	0.171	3.841
1.50	74.01	99.07	0.579	9.008	45.19	59.91	0.206	4.601
2.00	49.77	87.90	0.622	10.830	42.64	57.42	0.294	5.024

Source: Jahiruddin et al., 2004.

Based on the study by Jahiruddin et al., (2004) it can be concluded that-

i) the level of arsenic in irrigation water bellow 1 ppm may not affect growth and yield of rice;

ii) the level of arsenic in soil having above 15 ppm may affect rice yield.

2.11 Arsenic tolerance and phytotoxicity

Arsenic is not an essential nutrient element for plant and not involved in specific metabolic functions when supplied at low concentrations (Leibig, 1996; Lepp, 1981; Marin et al., 1993b). At higher concentrations, arsenic may interfere with the metabolic process and could hamper normal growth and development of plants through arsenic induced phytotoxicity (Marin et al., 1993b). Plants can develop toxicity symptoms while they are exposed to excess arsenic either in soil or solution culture such as inhibition of seed germination (Abedin et al., 2002; Liebig, 1966); decrease in plant height (Tsusuni, 1980; Marin et al., 1992); depressed tillering (Labata-pendias and Pendias, 1984); reduction in root growth (Carbonell-Barrachina et al., 1987); reduction in leaf area and photosynthesis (Marin et al., 1993b); decrease in shoot growth (Carbonell-Barrachina et al., 1998; Cox et al., 1996); lower fruit and grain yield (Tsutsumi, 1980; Carbonell-Barrachina et al., 1995); sometimes leading to death (Marin et al., 1993b). Decreased dry matter production was recorded due to application of both MMAA and DMAA in Spartina sp. (Carbonell-Barrachina et al., 1998); MMAA in turnip (Carbonell-Barrachina et al., 1999); DMAA in rice (Marine et al. 1993b); arsenate in garden pea (Paivoke, 1993); and rice (Tang and Miller, 1991). However, a number of species have been reported to show arsenate tolerance (Paliouris and Hutchinson, 1991, Macnair, 1993). The tolerance of plants to arsenic have been described to be heritable, often as a result of multiple or single gene differences, and genetic modifiers (Macnair, 1993).

Abedin et al. (2002) conducted tests in greenhouse experiment to cheek the tolerance to germination of some Bangladesh HYV rice varieties to arsenic and found that most of the varieties showed tolerance upto 2 mg As L^{-1}. Percentage germination decreased considerably at 4 mg As L^{-1} and at the highest concentration of 8 gm As L^{-1} treatment germination was completely halted for all the varieties except purbachi. Purbachi was the only variety that showed tolerance up to 4 mg As L^{-1} treatment for arsenite and up to 8 mg As L^{-1} treatment for arsenate. Because of the tolerance of rice seeds for germination up to 2 mg As L^{-1} which is equivalent to highest reported concentration in Bangladesh groundwater (BGS, 2000), the use of contaminated groundwater might have little impact on germination.

Resistance to high concentrations of arsenic could also be achieved through some detoxification mechanisms such as, i) complexation of arsenite and arsenate (production of thiol rice peptides phytochelatins [PCs]) (Hartley-Whitaker et al., 2001a) ii) reduction of arsenate to less toxic methylated arsenic compounds and non-toxic organo-phospho-lipids (Nissen and Benson, 1982) iii) enhanced production of antioxidants that detoxify free reactive oxygen species (ROS) produced in response to arsenite and arsenate (Hartley-Whitaker et al., 2001b) and iv) efflux of arsenic species from the root to the rhizosphere (Sharples et al., 2000).

2.12 Rice Varieties in Bangladesh

About 12487 traditional varieties of rice were cultivated in through out the country except Kishorgonj district of Bangladesh (Talukder, M. H. R., 1982). Currently, in parallel to HYV rice varieties some of those traditional varieties are cultivated in moderate scale in different parts of Bangladesh. Moreover, a few numbers of foreign varieties and IRRI developed HYV varieties are also cultivated. **Purbachi** is such an important China variety whose original name is Cheng-chu-ai.

From more than last 2 decades Modern high-yielding varieties (HYV) become more popular to our farmer. Modern HYV contribute more than 74% of the rice production in Bangladesh. These rice are classified in three group namely, Boro, Aus and Aman. The physiochemical characteristics of HYV rice are different from those of the traditional varieties. A list of HYV rice cultivated in our country has given in **Appendix -B** which are developed by Bangladesh Rice Research Institute (BRRI). Due to difference in soil quality all varieties are not cultivated in all over the country. Table- 2.17 shows a list of more popular HYV rice varieties (Boro) jointly developed by BRRI, UNDP and FAO with respective cultivated areas in Bangladesh (Jameel et al., 1999). A map of Bangladesh presented in figure-2.18, shows the district wise indication of some important HYV rice developed by BRRI, which are most commonly cultivated in Boro and Aman seasons.

Table-2.18: List of HYV rice (Boro) produced in different divisions of Bangladesh.

Division	Early Variety (Life 140-145days)	Mid Life Variety (Life 150-155days)	Late Variety (Life 160-170days)
Dhaka	BR-1, BR-26 BRRI Dhan28 BRRI Dhan28	BR-14 BR-17	BR-3, BR-12, BR-16 BR-18, BR-19 BRRI Dhan29 BRRI Dhan35
Chittagong	BR-1, BR-26 BRRI Dhan28 BRRI Dhan28	BR-14	BR-3, BR-12, BR-16 BRRI Dhan29 BRRI Dhan35
Khulna	BR-1, BR-26 BRRI Dhan28 BRRI Dhan28	BR-14	BR-3, BR-16 BRRI Dhan29 BRRI Dhan35
Rajshahi	BR-1, BR-26 BRRI Dhan28 BRRI Dhan28	BR-9 BR-14	BR-3, BR-16 BRRI Dhan29 BRRI Dhan35
Barisal	BRRI Dhan28 BRRI Dhan28	BR-14	BR-3, BR-16 BRRI Dhan29 BRRI Dhan35
Sylhet	BR-26 BRRI Dhan28 BRRI Dhan28	BR-14 BR-17	BR-3, BR-18, BR-19 BRRI Dhan29 BRRI Dhan35

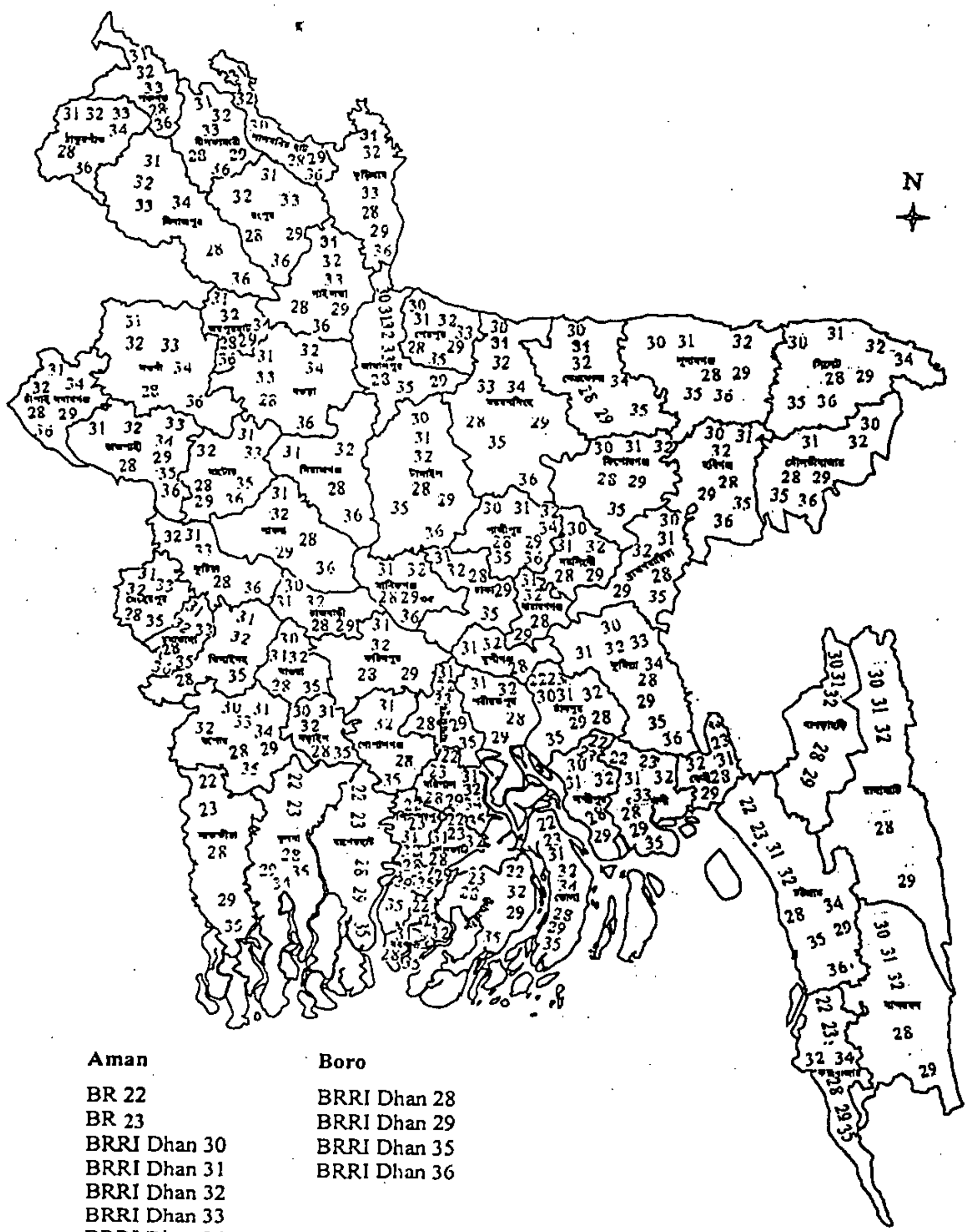


Figure 2.5: Map of Bangladesh showing district wise distribution for cultivation of some HYV Rice developed by BRRI.

2.13 Rice Chemistry

2.13.1 Structure of Rice

Rice is the principal cereal food in Asia and in some countries in Africa and Latin America, and is used as a food in all countries in the world. It is generally considered to be a tropical crop. Detailed studies have been made on the gross structure of brown rice by Santos (1933), Juliano and Aldama (1937), Hinton (1948). The rice fruit is caryopsis in which the single seed is fused with the wall of the ripened ovary (pericarp), forming a seed-like grain (Figure-2.6).

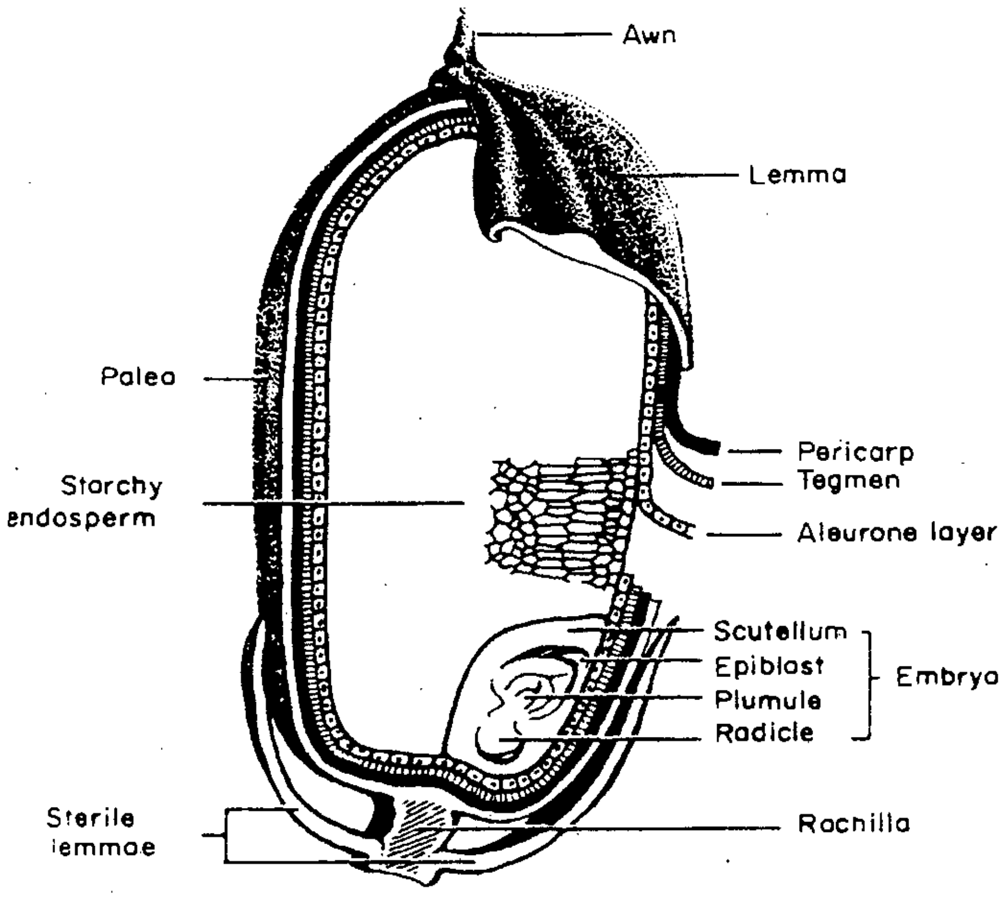


Figure 2.6: Structure of the rice grain (Juliano and Aldama, 1937).

The caryopsis is called brown rice because of its brownish pericarp. The weight distribution of the various parts of brown rice is: pericarp, 1-2%; aleurone plus testa, 4-6%, germ, 2-3%; and endosperm, 89-94%. The cell walls of the pericarp are 2μ thick and absorb stains for protein, hemicellulose, and cellulose. Both the endosperm and the embryo are enclosed by the aleurone, which lies beneath the tegmen. The aleurone may have from 1 to 7 cell layers (Fig. 2.7) and the aleurone layer is composed of quadrangular or rectangular parenchyma cells with 2μ thin walls. These cells are filled with small, protein-rich aleurone grains enclosed in a sheath of fat-staining material. The cell-walls of the aleurone layer react positively to stains for protein, hemicellulsoe, and cellulose.

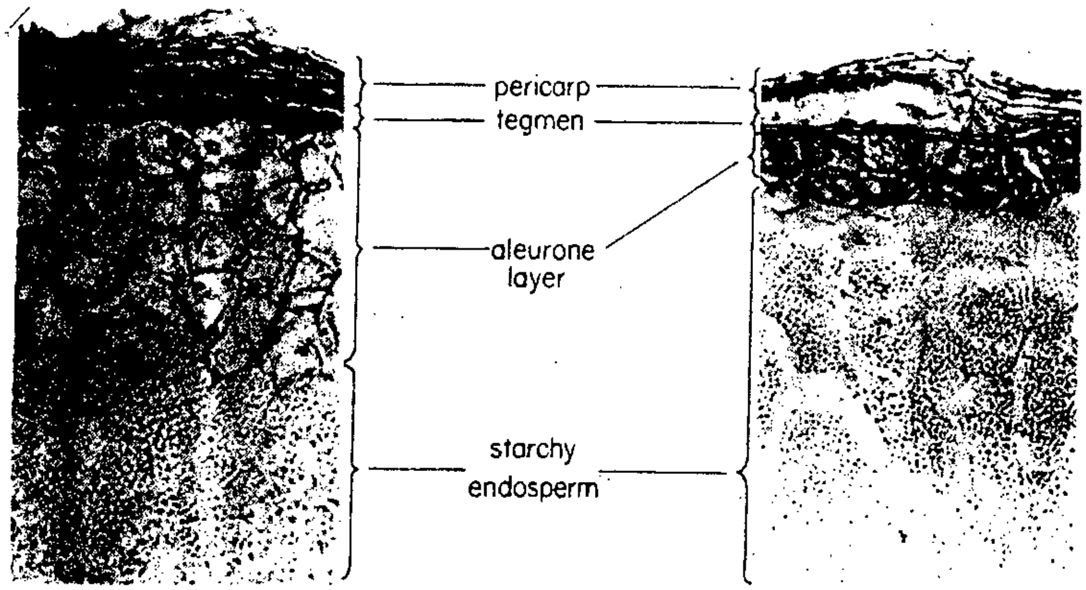


Figure 2.7: Longitudinal section of the dorsal (left) and ventral (right) portions of the mature caryopsis (Juliano, B. O., 1978).

The starchy endosperm consists of thin-wall parenchyma cells, usually radially elongated and heavily loaded with compound starch granules and some protein bodies [Figure-2.8 (Juliano, B. O., 1978)]. The number of endosperm cells is highly correlated to the size of the starchy endosperm but not to its shape. Endosperm cell-walls react positively to stains for protein, hemicelluloses, and cellulose. Rice starch granules are polygonal and compound. The granules have the structure of a pentagonal dodecahedron, and ranges in size from 2 to 10μ. Starch granules are small (2 to 4μ) in the peripheral cells of the starchy endosperm; they are fewest in the lateral and ventral peripheral cells, forming tiny clusters well separated by the surrounding dense protein material. Starch granules of the major, central portion of the kernel are larger (5 to 9μ) and occur in closely packed compound groups, several groups in each cell. Extremely small granules are found in cells near the embryo; they are usually considered devoid of starch. However, the embryo has been reported to contain minute starch granules, the largest ones being in the scutellum.

Up to 95% of endosperm protein has been observed to be in the form of discrete particles called protein bodies. These range in size from 1 to 4 μ. Protein bodies are most concentrated in peripheral-lateral and peripheral-dorsal cells (whereas starch granules are fewest and least concentrated in peripheral-dorsal cells) and are found more along he periphery than in the center of each cell. Fat seems to occur in combination with protein bodies. Differences among cultivars in their distribution in the starchy endosperm have been reported. The protein bodies are about 1μ in size at peripheral cells and 2 to 3μ in size in the inner cells.

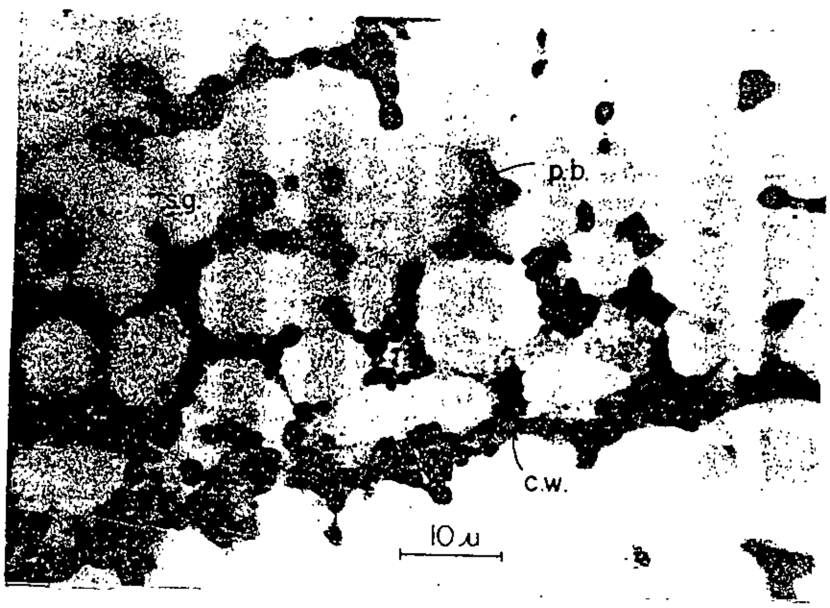
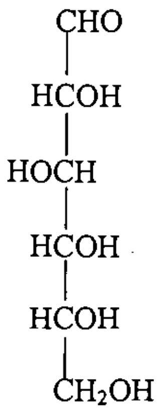


Figure 2.7: Cross-section of 14% protein BPI-76 endosperm stained with mercuric chloride-bromophenol blue, showing protein bodies (p.b.), compound starch granules (s.g.), and cell-walls (c.w.).

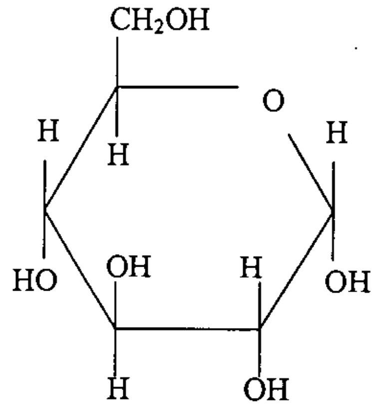
2.13.2 Starch and Protein

Starch is the reserve carbohydrate of plants and occurs as granules in the cell in plastids, separated from the cytoplasm. Most starch granules contain both amylose and amylopectin molecules. Waxy or glutinous starch from corn and other cereals contains little or no amylose, while a sugary mutant corn and some of the legumes contain amylose in greater abundance than amylopectin. Amylopectin is usually the more plentiful type of starch.

Amylose molecules are straight chain polysaccharides in α-D-glucose units are joined 1-4. Chain lengths vary from 250 to about 350 glucose units, and the long molecules appear to be coiled in an α helix. The formula for α-D-glucose is given below:



or



D-glucose α

Every living cell contains protein. Indeed, the name "protein" comes from the Greek root which means "to be first". In man synthesis of the many proteins that make up body tissue requires the presence of amino acids. Although man's need for protein and its amino acids is rather stringent, his method of fulfilling his need varies considerably in different parts of the world. Primarily the difference has depended on the availability of foods. In the tropics many peoples have developed dietary patterns based primarily on plant foods with the cereals most abundant. In most of these diets animal protein in the form of meat, eggs, fish and milk is added when available. Most of the proteins required for body synthesis are available in rice that has been shown in table-2.13.

2.13.3 Composition of Rice

In many Asian countries, the average individual gets half or more of his total caloric intake from rice. The composition of rice is influenced by variety and environment. The composition of brown, milled raw and parboiled rice and other cereal grain is shown in Table-2.19 (Houston, 1978).

Table 2.19: Composition of rice and other cereals.

Composition	Rice			Wheat	Maize
	Brown	Milled Raw	Milled Parboiled		
Moisture (%)	12.0	12.0	10.3	12.5	13.8
Calories/100gm	360	363	369	330	348
Protein, %	7.5	6.7	7.4	12.3	8.9
Fat, %	1.9	0.4	0.3	1.8	3.9
N-free extract, %	77.4	80.4	81.3	71.7	72.2
Fiber, %	0.9	0.3	0.2	2.3	2.0
Ash, %	1.2	0.5	0.7	1.7	1.2
Thiamine, mg/100gm	0.34	0.07	0.44	0.52	0.37
Riboflavin, mg/100gm	0.05	0.03	...	0.12	0.12
Niacin, mg/100gm	4.7	1.6	3.5	4.3	2.2

The Principal difference between brown rice and milled raw rice is that the protein, fibre, thiamine, and niacin are higher in brown rice. Parboiled milled rice is similar to brown rice in protein, thiamin and niacin content. The nutritional level of rice is comparatively high among cereals and other grain and root crops. Among cereals rice has a comparatively high content of essential amino acids and fairly low content of glutamic acid and some other nonessential amino acids. High-protein rice has better nutritional value because it has higher level of essential amino acids. The amino acid composition of the protein in cereals is shown in Table- 2.2 (Houston, 1978).

Table 2.20: Amino acid content of cereals, % amino acid in protein

Amino acid	Rice (brown)	Wheat(RHS)	Maize(field)
Tryptophan[b]	1.08	1.24	0.61
Threonine[b]	3.92	2.88	3.98
Isoleucine[b]	4.69	4.34	4.62
Leucine[b]	8.61	6.71	12.96
Lysine[b]	3.95	2.82	2.88
Methionine[b]	1.80	1.29	1.86
Cystine	1.36	2.19	1.30
Phenylalanine[b]	5.03	4.94	4.54
Tyrosine	4.57	3.74	6.11
Valine[b]	6.99	4.63	5.10
Arginine[b]	5.76	4.79	3.52
Histidine[b]	1.68	2.04	2.06
Alanine	3.56	3.50	9.95
Aspartic acid	4.72	5.46	12.42
Glutamic acid	13.69	31.25	17.65
Glycine	6.84	6.11	3.39
Proline	4.84	10.44	8.35
Serine	5.08	4.61	5.65

Note: [b] Nutritionally essential

Bangladesh Rice Research Institute developed released 45 varieties of HYV rice which are cultivated in the country. The standard protein and amylose content of these rice varieties are given in Appendix–B.

2.13.4 Change in Dry Matter content in developing rice grain

The protein in matured rice grain is consistently high in some varieties compared to some other varieties. The difference in grain protein content may be due to difference in rates of protein accumulation in grain rather than difference in soil N absorption. Table 2.21 shows the changes in dry matter content of paddy and brown rice in BR-3 and Hbj IV varieties. At 4 DAF, BR3 and Hbj IV had 4.4 and 5.4 mg/paddy dry matter respectively but at 20 DAF, dry matter content was 29.3 and 22.5 mg/paddy respectively.

Table 2.21. Weight (mg) of single paddy and brown rice of Hbj IV and BR-3 at different stages of development.

DAF	Hbj IV		BR-3	
	Paddy	Brown rice (Kernel)	Paddy	Brown rice (Kernel)
4th	5.4	3.1	4.4	1.3
8th	14.6	9.0	14.7	8.5
12th	20.6	15.0	25.7	19.0
16th	21.8	16.2	28.5	21.7
20th	22.5	16.7	29.3	22.7

Table 2.22 shows the protein accumulation in paddy and brown rice. The protein content increased most rapidly from four DAF to 16 DAF in Hbj IV and to 12 DAF in BR-3. Then it almost leveled off. BR3 brown rice had 16.5% at four DAF and rapidly decreased to 7.8% at 12 DAF. Dry matter was found to accumulate at the faster rate from four DAF to 12 DAF in BR-3. On the other hand, actual percentage of protein in Hbj IV brown rice decreased from 12.3% at four DAF to 9.6% at 20 DAF. Actual percentage of protein in brown rice decreased, but protein per brown rice increased between four DAF to 16 DAF in Hbj IV.

Amylose content in brown rice was found to be 15.1% in Hbj IV and only 3.1% in BR-3 brown rice at four DAF. The content increased slowly to 24.5% at 20 DAF in Hbj IV .On the other hand, in BR-3 brown rice, amylose content increased to 26.6%

at 20 DAF. The % of amylose accumulation was observed higher in BR-3. The variations of amylose content in rice at different stages are given in the Table-2.23.

Table 2.22. Protein accumulation in paddy and brown rice at different stages of development

DAF	Hbj IV			BR-3		
	Paddy	Brown rice(Kernel)		Paddy	Brown rice(Kernel)	
	mg/paddy	%	Mg/brown rice	mg/paddy	%	mg/brown rice
4th	0.47	12.30	0.66	0.30	16.50	0.19
8th	1.15	10.20	1.08	1.02	8.50	0.76
12th	1.41	9.00	1.60	1.73	7.80	1.70
16th	1.48	9.60	1.77	1.74	7.80	1.73
20th	1.89	9.60	1.79	1.76	7.60	1.75

Table 2.23: Amylose content in brown rice at different stages of development.

DAF	Hbj IV		BR-3	
	%	mg/Brown rice (Kernel)	%	mg/Brown rice (Kernel)
4th	15.1	1.0	3.1	0.0
8th	15.8	1.8	6.7	0.6
12th	17.1	3.1	17.1	3.6
16th	24.2	4.2	22.5	4.6
20th	24.5	4.2	26.6	5.8

CHAPTER 3

ARSENIC IN RICE PLANT AND SOIL

3.1 Introduction

In Bangladesh ground water is severely contaminated with arsenic. Due to application of this contaminated water in agricultural field for irrigation purpose soil could be contaminated with arsenic. Rice plants as well as other agricultural plants may accumulate arsenic from this irrigation water and also from contaminated soil. In this chapter results of analysis of arsenic contents in rice plant and soil samples collected from the study locations have been presented.

3.2 Methodology

3.2.1 Selection of Study Area

Study areas were selected on the basis of arsenic concentration in irrigation water and distance from BUET considering communication feasibility. Firstly, analyzing the data of arsenic concentration in ground water 23 study areas were selected in different locations in Barura and Debidwar upazila of Comilla district. Then water sample were collected from irrigation pumps of these locations and tested for arsenic using atomic absorption spectrophotometry attached with graphite furnace. Finally, on the basis of arsenic concentration > 0.05 mg/L, the national acceptable limit 13 study locations were selected. The locations of the selected study areas were shown in figure 3.1- 3.2.

3.2.2 Collection of water, soil and rice plant samples

Water samples were collected from irrigation water pumps of the selected study fields at the beginning of the irrigation season. Before collection of water bottles were rinsed out 3-4 times with the sampling water. Samples were collected at least 30 minutes after starting the pump. The bottles were filled with 90 ml of sampling water mixed with 10 ml 2N HCl acid and the bottles were marked properly. During harvesting full rice plants (including root) and soil samples were collected from 13 selected study fields. Samples were packed in separate poly bags and brought to the laboratory. Initially the plant samples were washed with distilled water, air-dried and

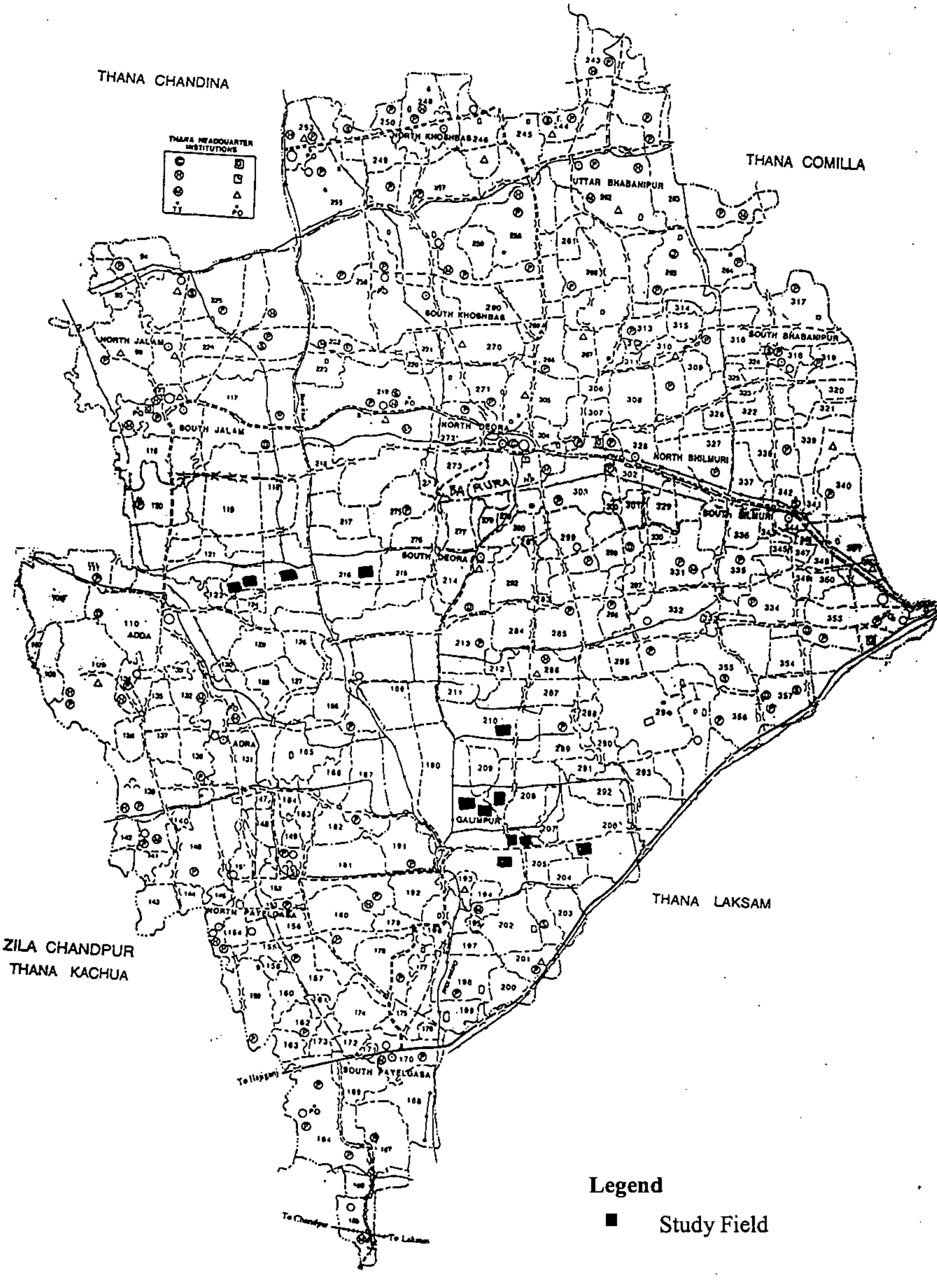
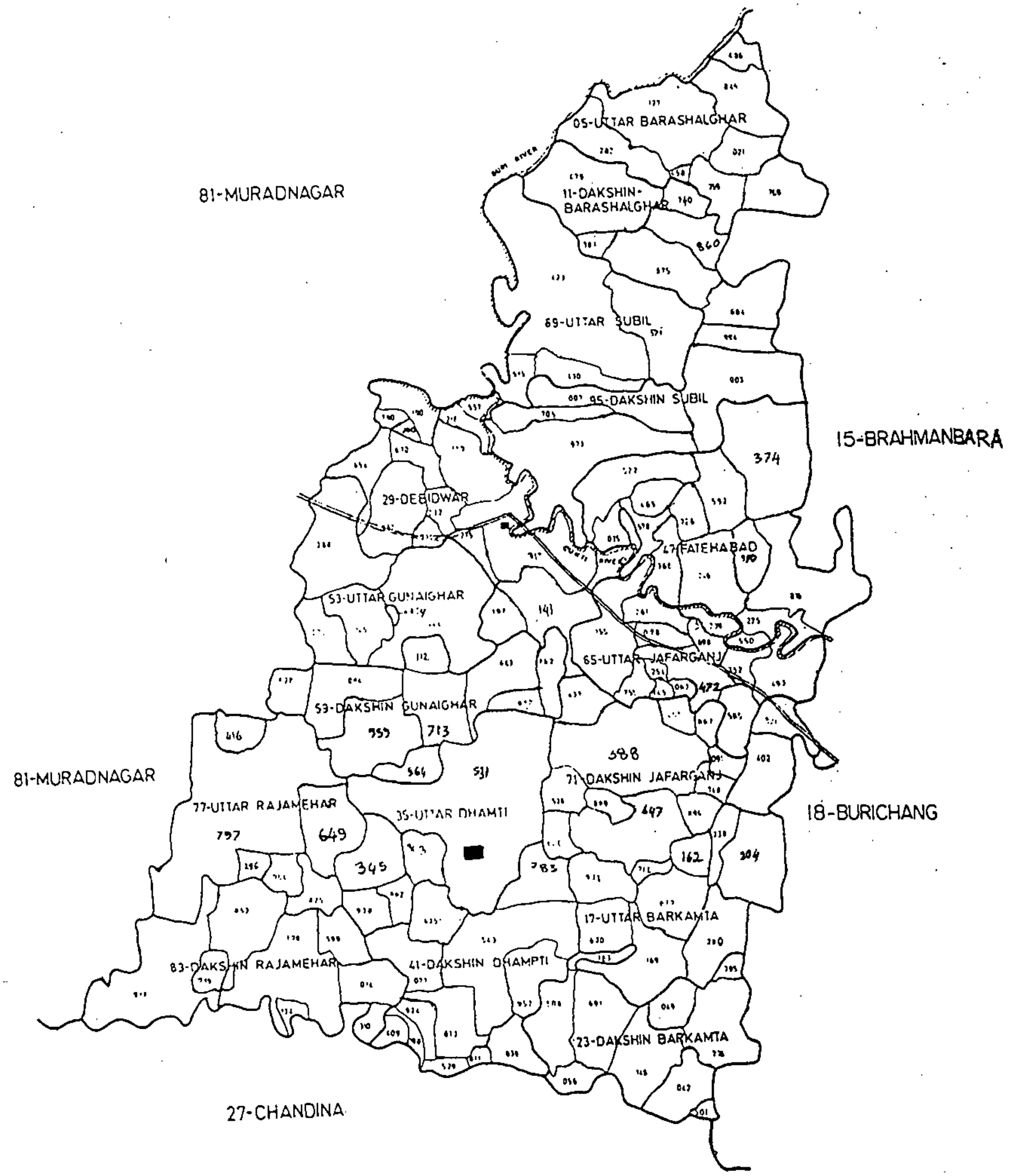


Figure 3.1: Map of Barura Upazila of Comilla District showing 12 Study Fields.



Legend

■ Study Field

Figure 3.2: Map of Debidwar Upazila of Comilla District showing 1(one) Study Field.

segmented into rice, stem, leaf and root hair and packed in different poly bags. During sample collection varieties name of rice were recorded and that were re-confirmed from Seed Division of BRRI, Gazipur. Collected samples were of 5 varieties, these were BR-14, BRRI dhan-28, BRRI dhan-29, Purbachi and IR-50.

3.2.3 Analysis of Water Samples

Water samples were analyzed for arsenic using an atomic absorption spectrophotometer attached with graphite furnace (Shimadzu, AA6800)

3.2.4 Analysis of soil samples

Soil samples were also collected from the selected field during rice plant sample collection. Soil samples were collected only from the top layer surrounding the hill and root of rice plant and packed in poly-bags. Soil samples were initially air dried and grounded into powder and cleaned from root hair and other foreign materials. Then soil samples were digested as (i) oven-dry at 110°C for 24 hours, (ii) take 5 gram oven-dried sample in a volumetric flask, add 2.5 ml concentrated nitric acid and 7.5 ml of concentrated hydrochloric acid and keep it overnight, (iii) heat the sample for 2 to 3 hours to boiling, then allow to cool and adjust the volume to 500 ml by adding de-ionized water. Then the digested sample was filtered and stored in plastic bottle for analysis. Test for total arsenic was carried out using an AAS (Shimadzu, AA6800) attached with a graphite furnace.

3.2.5 Analysis of Rice Plant Samples

Before experimental analysis rice samples are divided into brown grains and husks. The samples of grain, husk, leaf, stem and root hair were digested following the procedure described in section 6 of Cookbook of Shimadzu AAS. The digestion procedures were as (i) oven-dry the sample at 65°C for 24 hours, (ii) take 2 gm of grounded samples into volumetric flask of 500 ml capacity and make it moist by adding a few ml of deionized water, then add 25 ml concentrated nitric acid to the flask and keep it overnight, (iii) heat the flask for two hours to boiling, then after cooling add 10 ml of concentrated perchloric acid to the flask and heat again (to boiling) for one hour, (iv) if color of the sample turns yellow, digestion is assumed to be complete, (v) if color of the sample turns dark, add 2 to 3 ml of nitric acid to the flask and apply heat, repeat the process until the color turns yellow, (vi) then allow to cool and adjust the volume to 100 ml by adding de-ionized water. Digested samples were filtered and preserved in plastic bottles for testing. Total arsenic concentration in digested sample was tested with hydride generation atomic absorption spectrophotometry using an AAS (Shimadzu, AA6800).

3.3 Results and Discussions

The results of arsenic concentration found in different parts of rice plant samples tested as the procedure of article 3.2.3 and in soil samples tested as the procedure of article 3.2.4 are given in table 3.1. Corresponding groundwater arsenic concentration and varieties of rice are also given in this result sheet. Test results shows that arsenic is accumulated in different parts of rice plant. To analyse the level of arsenic contents in rice grain, husks, leaves, stems and root hairs, arsenic concentration found in laboratory tests for all varieties are shown graphically in figures 3.3 - 3.8. Arsenic concentration in different parts of the rice plants of different varieties: BR-14, BRRI dhan-28, BRRI dhan-29 and *Purbachi* (Cheng-chu-ai) have also been presented separately in figures 3.9 - 3.12. Figure 3.13 also represents the trend of accumulation of arsenic (considering average value) in different parts of the rice plants of different varieties.

3.3.1 Arsenic Content of Soil Samples

Arsenic found in soil samples tested with in 12mg/kg but no similarity was found with the concentration of irrigation water arsenic. In some cases it is found comparatively al low concentration (2 - 4 ppm). It may be due to over flow of rainwater or flood-water and stagnation of rainwater over soil surface for long time (more than 15 days). Because soil arsenic could be released into the water. Soil can contain certain amount of arsenic from irrigation water or other sources though the supply of arsenic contaminated irrigation water applied repeatedly. Accumulation of arsenic in soil and also in plants depends on arsenic concentration of groundwater but it is affected by many other factors discussed in literature review. Many researchers found that soil arsenic concentration depends on many factors like pH, EC , P, Fe, soil texture etc

(Shah et al. 2004). It also depends on groundwater arsenic (Shah et al., 2004; Huq et al., 2003; and Xie et al., 2001). Adsorption of arsenic in soils positively dependent on clay content (Huq et al., 2003). Moreover, the release of soil As has no bearing on total As contents of soil.

3.3.2 Arsenic Content of Rice Plnat Samples

As per test results, presence of arsenic in different parts of rice plants such as rice, husk, leaf, stem & root hairs and soil samples are found. Arsenic concentration found in rice grain 0.0 – 1.1 mg/kg, in husk 0.4 – 2.0 mg/kg, in leaf 2.0 - 7.6 mg/kg, in stem up to 5.7 mg/kg and in root hair up to 38.5 mg/kg. This results shows similarity to the results found by Ali et al. (2003) while testing for rice plant grown with arsenic contaminated irrigation water in Srinagar during boro season. However, in a recent study Ghani et al. (2004) observed higher accumulation of arsenic in roots (121.56 ± 111.0.03) followed by straw (13.01 ± 3.75 ppm) and least in grain (0.12 ± 0.05 **ppm**). Test results show rice grain absorb less amount of arsenic that also shows the similarity to the result found in greenhouse experiment conducted by Abedin et al. (2002). Root hair found having the highest arsenic concentration that is similar to the findings of Odanka et al., (1987) and Marin et al., (1992), Das et al. (2003), Bennett et al. (2002). The trend of arsenic absorbing capabilities of different parts of rice plants found are as **root hair > leaf > stem > husk > rice grain** that is similar to the trend found by Xie and Huang (1998) which is **root > straw > husk > whole grain > husked grain**.

Test result also indicates that arsenic absorption capability of rice varies from variety to variety. In green house experiment Abedin et al. (2002) also found that arsenic absorption capability of some Bangladesh varieties varies from one variety to other varieties. The affinity of arsenic absorption in rice found for ***Purbachi*** variety is more than other varieties such as BRRI dhan-28, BRRI dhan-29, BR14 & IR 50 tested though the variety wise sample size was low. Most of them absorb arsenic in an amount less than 1 mg/kg of Australian maximum permissible limit (National Food Authority, 1993) except sample FGB-22 of Purbachi variety that contain 1.1 mg/kg of arsenic. But all samples contain arsenic less than 2 mg/kg of Bangladesh Pure Food Limit (The Bangladesh Pure Food Rules, 1967).

Table 3.1: Arsenic concentration in different parts of rice plants and corresponding water and soil samples.

Sl. No	Sample ID	As in water (ppb)	Arsenic in soil (mg/kg)	Variety of rice	Rice grain (mg/kg)	Husk (mg/kg)	Leaf (mg/kg)	Stem (mg/kg)	Root Hair (mg/kg)
1	FDD-6	199	3.764	BRRI dhan-28	0.56	2.07	7.62	4.94	18.04
2	FGB-12	105	3.363	Purbachi	0.54	0.47	2.97	3.01	11.91
3	FDB-13	131	3.976	BRRI dhan-28	0.31	0.83	4.79	3.47	13.17
4	FGB-14	188	3.295	BRRI dhan-28	0.12	1.31	2.89	4.24	0.32
5	FGB-15	156	7.518	BR-14	0.17	0.83	3.73	1.56	16.66
6	FGB-16	62	2.008	BRRI dhan-29	0.00	0.49	2.33	0.15	4.92
7	FGB-17	364	2.069	BR-14	0.26	2.05	4.23	4.02	38.46
8	FJB-18	208	3.628	BRRI dhan-29	0.20	1.26	2.09	3.28	24.26
9	FJB-19	277	12.03	BR-14	0.04	1.89	3.59	5.66	38.51
10	FJB-20	222	2.243	Purbachi	0.22	1.12	4.99	3.97	19.41
11	FGB-21	255	2.417	BRRI dhan-28	0.17	0.43	5.04	5.13	6.83
12	FGB-22	177	3.022	Purbachi	1.10	0.66	3.70	2.33	5.68
13	FGB-23	278	9.926	IR-50	0.44	0.38	3.72	1.46	18.40

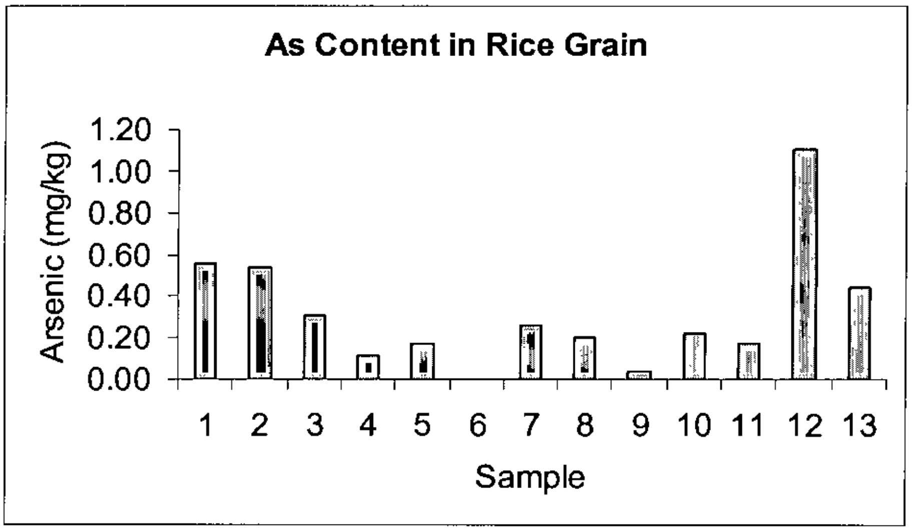


Figure 3.3: Arsenic content of brown rice grain (mg/kg).

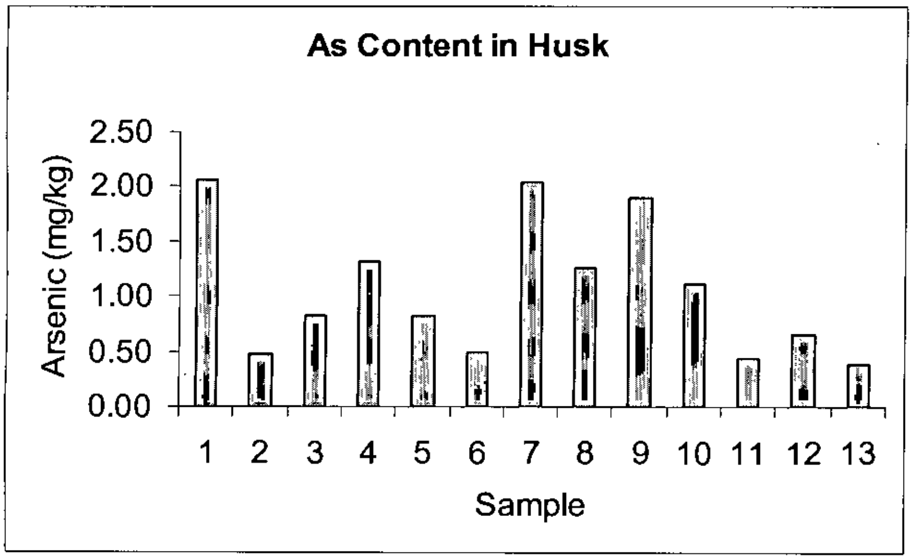


Figure 3.4: Arsenic content of husk (mg/kg).

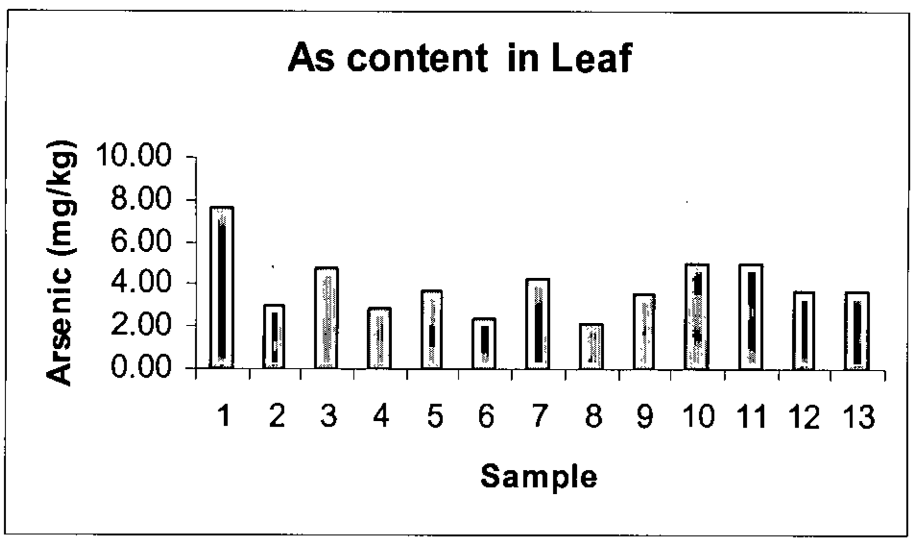


Figure 3.5: Arsenic content of leaf (mg/kg).

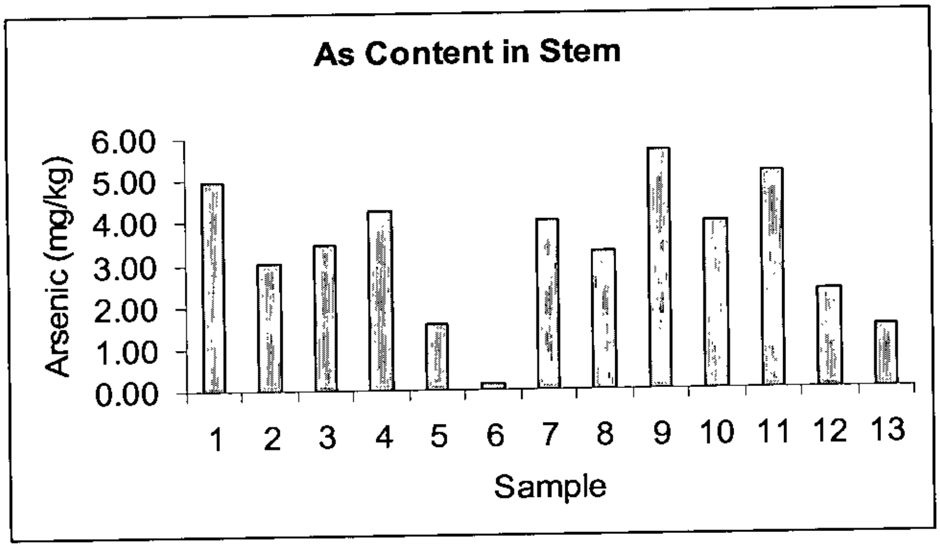


Figure 3.6: Arsenic content of stem (mg/kg).

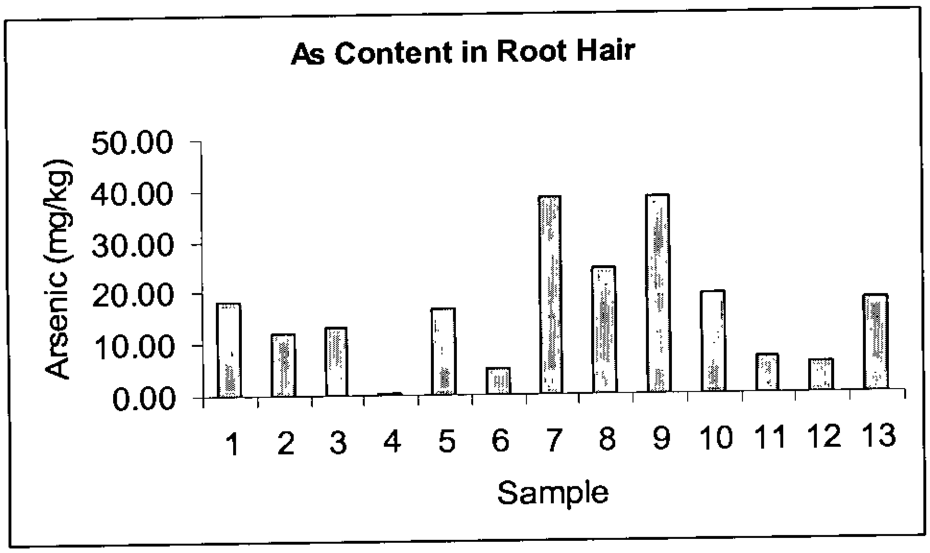


Figure 3.7: Arsenic content of root hair (mg/kg).

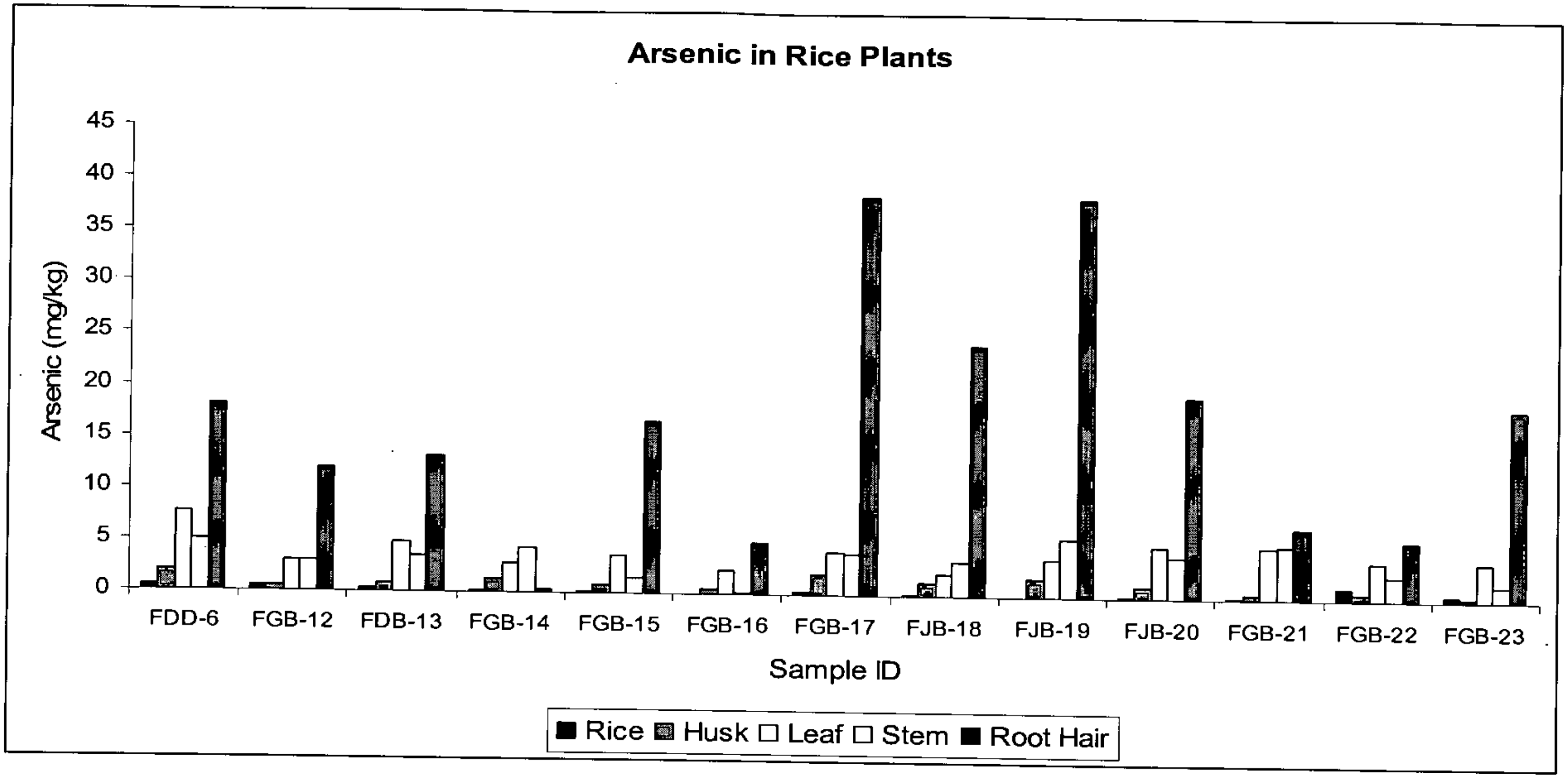


Figure 3.8: Arsenic accumulation in different parts of rice plants of different samples (mg/kg).

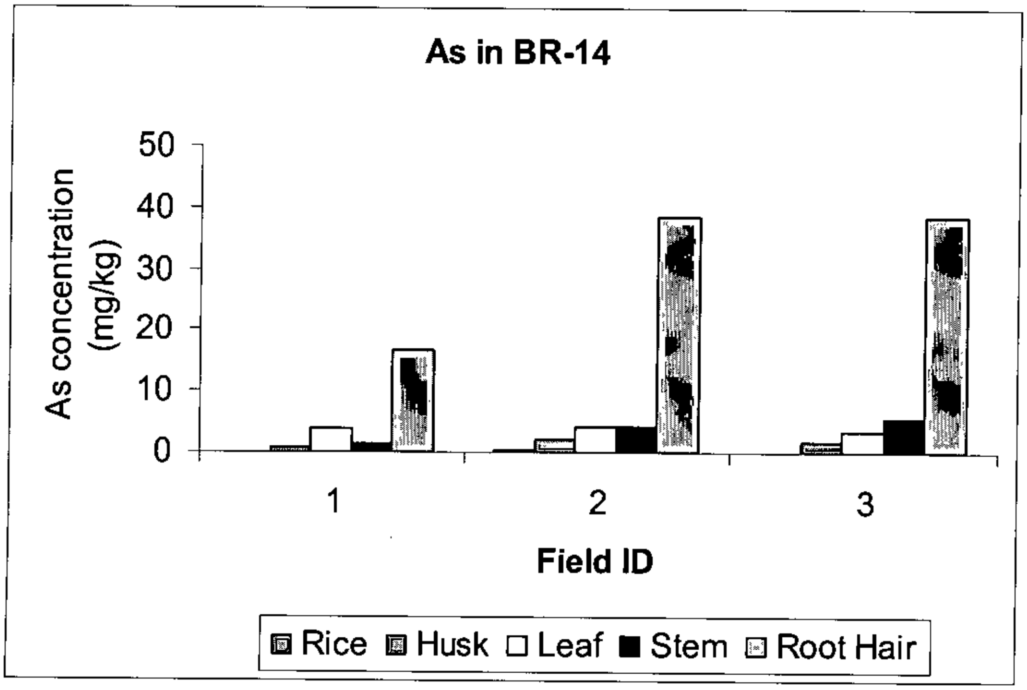


Figure 3.9: Arsenic accumulation in different parts of BR-14 rice plants.

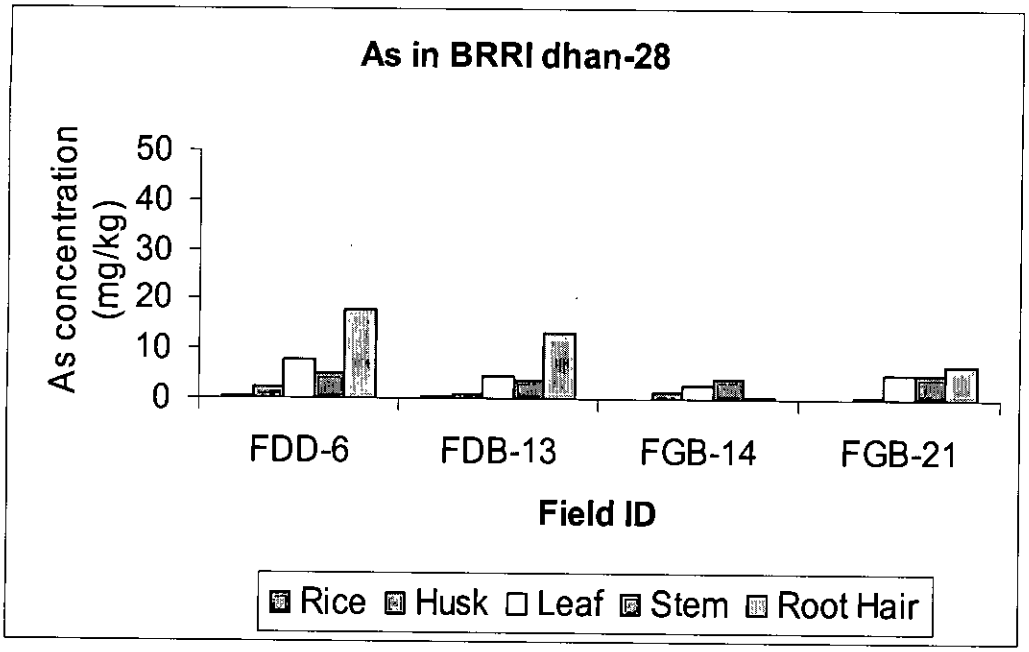


Figure 3.10: Arsenic accumulation in different parts of BR-28 rice plants.

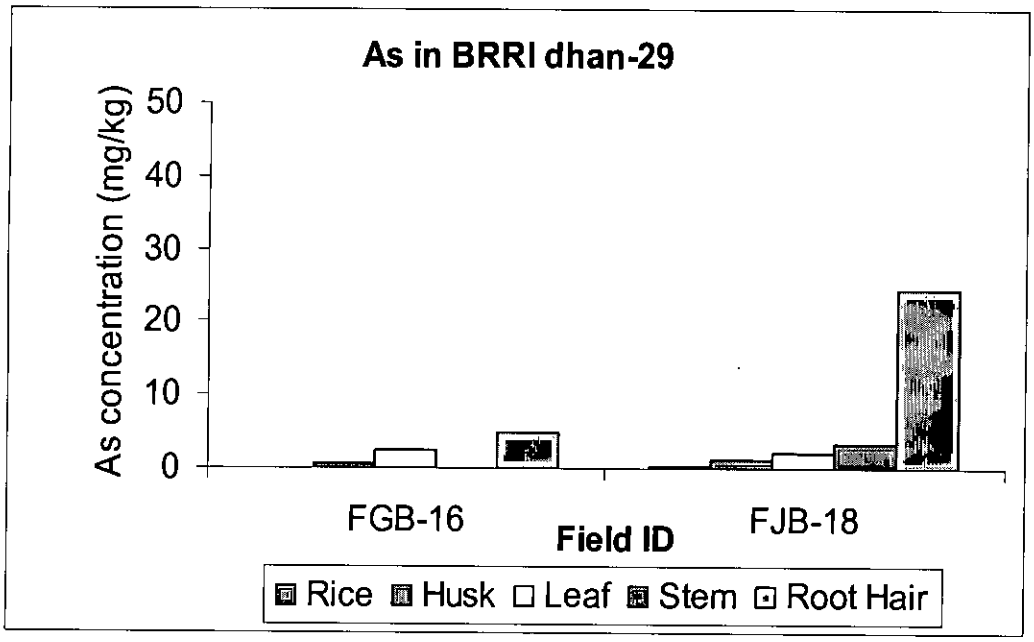


Figure 3.11: Arsenic accumulation in different parts of BR-29 rice plants.

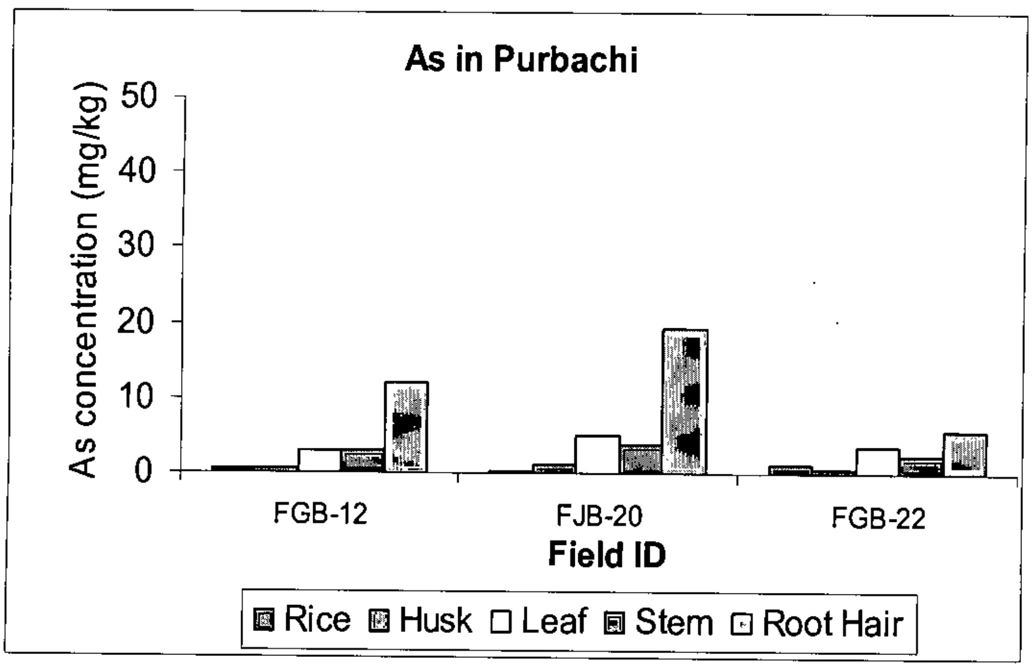


Figure 3.12: Arsenic accumulation in different parts of Purbachi rice plants.

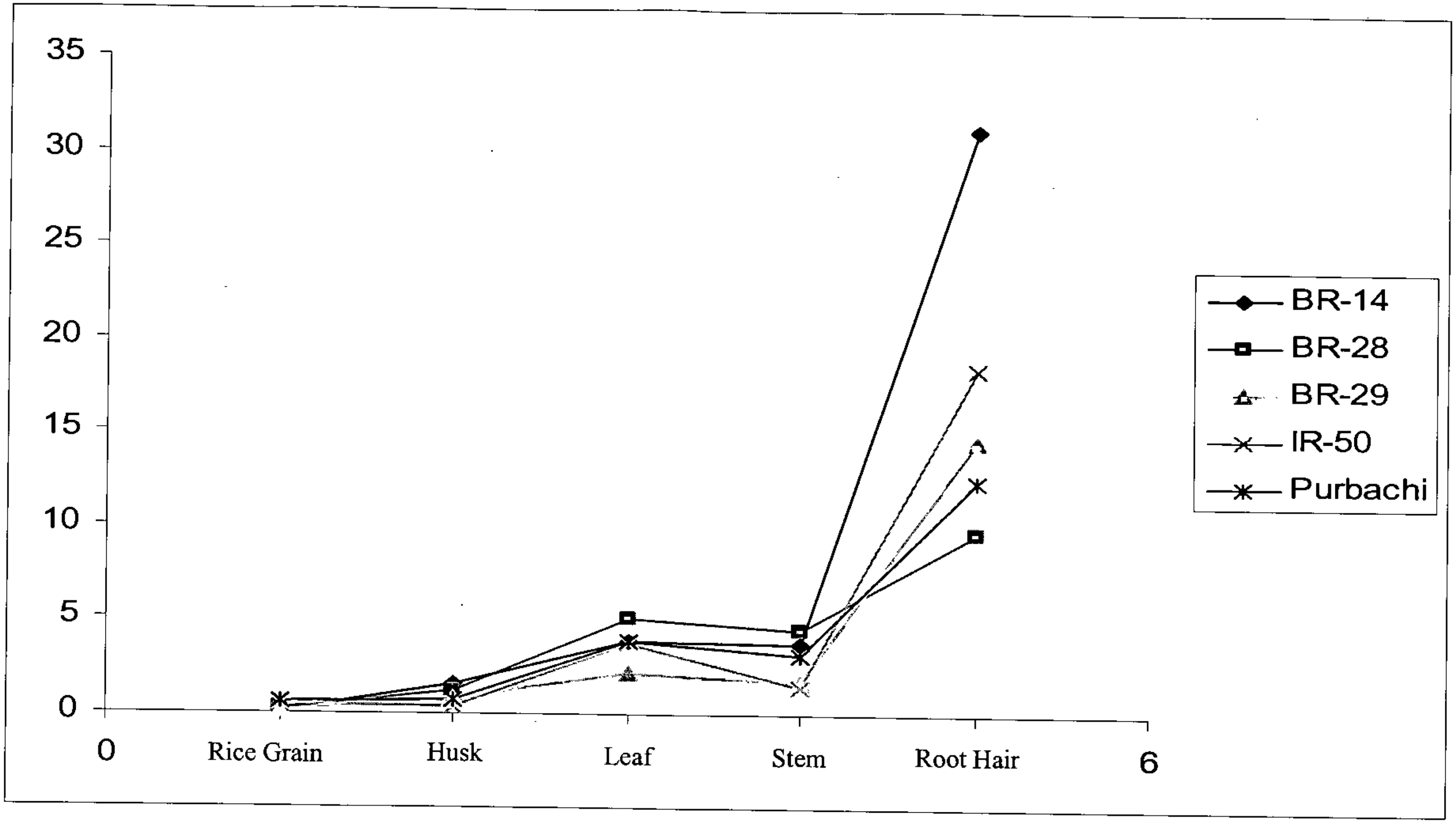
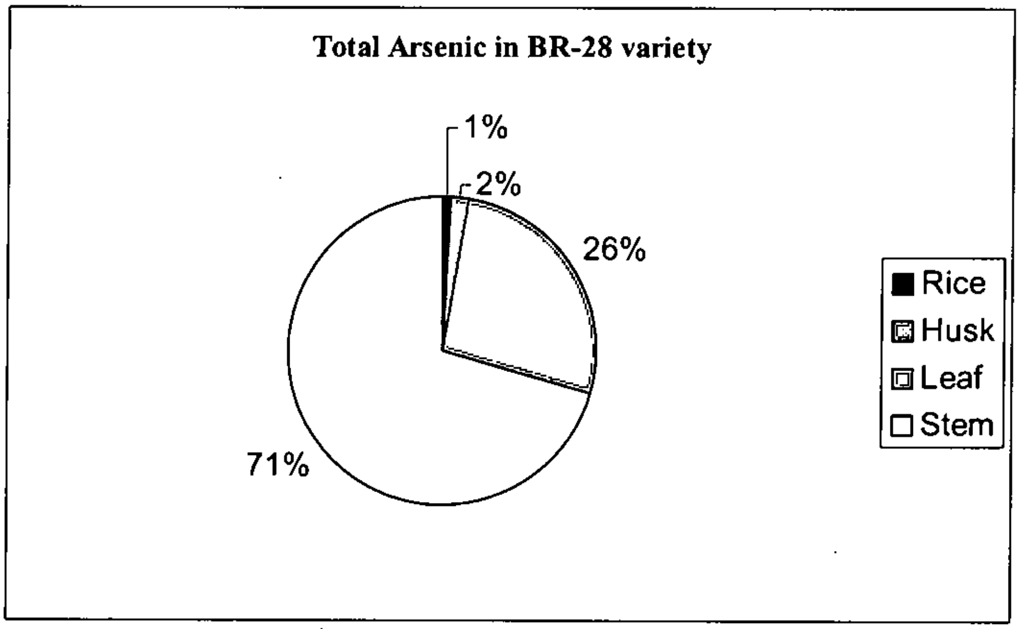


Figure 3.13: Arsenic concentration (mg/kg) in different parts of different rice varieties.

Due to technical constraints analysis for different species of arsenic (organic and inorganic) could not be conducted. Many researchers found arsenic accumulated in rice plants are both in organic and inorganic form. Chakraborti et al. (2001) showed 95% inorganic and 5% organic As species in rice. However, recent studies showed that DMAA could be a major component of total arsenic (up to 56%) in rice grain (Schoof et al., 1999; Heitkemper et al., 2001). In laboratory analysis highest arsenic concentration found in rice grain is 1.1 mg/kg which would be sum of organic and inorganic arsenic. But inorganic fraction is nontoxic or less toxic. Organic arsenic is about 100 times less toxic than inorganic arsenic (Nagy, G. et al., 1983). Deducting inorganic portion it could be assumed that toxic species of arsenic in rice grain within Australian Permissible Limit, 1 mg/kg.

Test results also show that leaf and stem contain high arsenic concentration. Average Arsenic concentration found 3.98 mg/kg in leaf and 3.32 mg/kg in stem, which are much higher than Australian Maximum Permissible Limit, 1 mg/kg. Abedin et al. (2002) reported the major arsenic species in rice straw was arsenate (87-100%), followed by arsenite (0-8%) and DMAA (0-5%). The rice straw (stem + leaf) contains a very small proportion of organic species (DMAA). Accumulated arsenic in straw would be mostly inorganic which could create potential health hazards for both cattle and human beings especially when the straw contains an elevated level of arsenic (maximum found in leaf 7.62 mg/kg).

To find out the ratio of arsenic accumulated in different parts of rice plant calculation has made in Appendix-C. In this Annexure samples of BRRI dhan-28 and BRRI dhan-29 varieties rice plants has taken into consideration for determination of total amount of absorbed arsenic. Total arsenic found in different parts of rice plants (except root hair) of these two varieties are graphically presented in pie charts of figure 5.14 - 5.15. Calculated results show that highest amount of arsenic accumulated in stem of a rice plant and the trend found is **Stem > Leaf > Husk > Rice grain**. The ration of total arsenic in Stem, Leaf, Husk and Rice grain in for BRRI dhan-28 and BRRI dhan-29 varieties rice plants are **71 : 26 : 2 : 1** and **65 : 31 : 3 : 1** respectively.



200845

Figure 3.14: Percentage of total Arsenic accumulated in different parts of a rice plant of BR-28 variety.

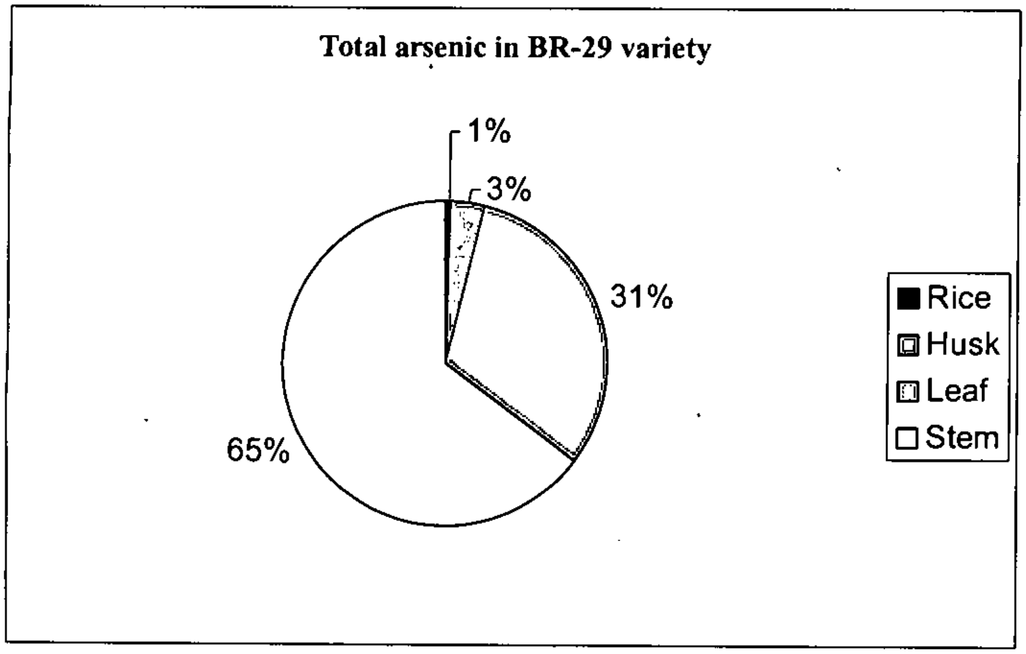


Figure 3.15: Percentage of total Arsenic accumulated in different parts of a rice plant of BR-29 variety.

3.3.3 Correlation among Arsenic Content of Water, Soil and Rice Plants

From the test results it is clear that rice plant absorbs arsenic from arsenic contaminated irrigation water. The level of arsenic concentration varies from one part to another parts of the rice plants grown in the study fields. To check the relationships between concentrations of arsenic in different parts of rice plant and corresponding irrigation water arsenic the following linear regression analysis were made.

(i) Analysis of arsenic concentration of rice grain, husk, leaf, stem and root hair with corresponding arsenic concentration of irrigation water;

(ii) Analysis of arsenic concentration of paddy (rice grain + husk) with corresponding arsenic concentration of irrigation water. To determine the arsenic concentration of paddy calculation has made using grain arsenic concentration and husk arsenic concentration considering the weight ratio of grain : husk to be 5 : 3; and

(iii) Analysis of total arsenic accumulated by a rice plant of BRRI dhan-28 variety with corresponding irrigation water arsenic. In statistical analysis total arsenic accumulated in a rice plant (excluding root) of BRRI dhan-28 variety has been taken from calculated results presented in Appendix-C.

Results of regression analyses mentioned above are presented in Figs. 3.16 – 3.22.

Similar linear regression analyses were conducted to check the relationships between concentrations of arsenic in rice plant and in corresponding field soils. Results of regression analysis for arsenic in rice grain and arsenic in paddy (rice grain + husk) are shown in Figs. 2.23 -2.24. Regression analysis was also conducted for arsenic in soil with corresponding arsenic in irrigation water. The relationship is shown in Fig. 2.25.

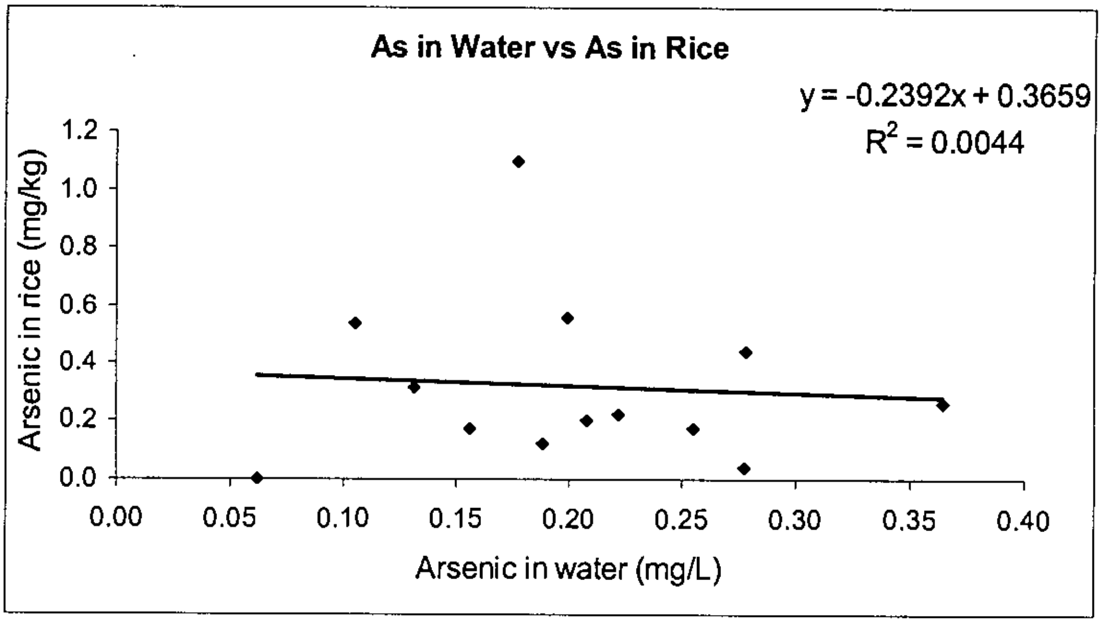


Figure 3.16: Arsenic concentration in rice grain (mg/kg) verses arsenic in irrigation water (mg/L).

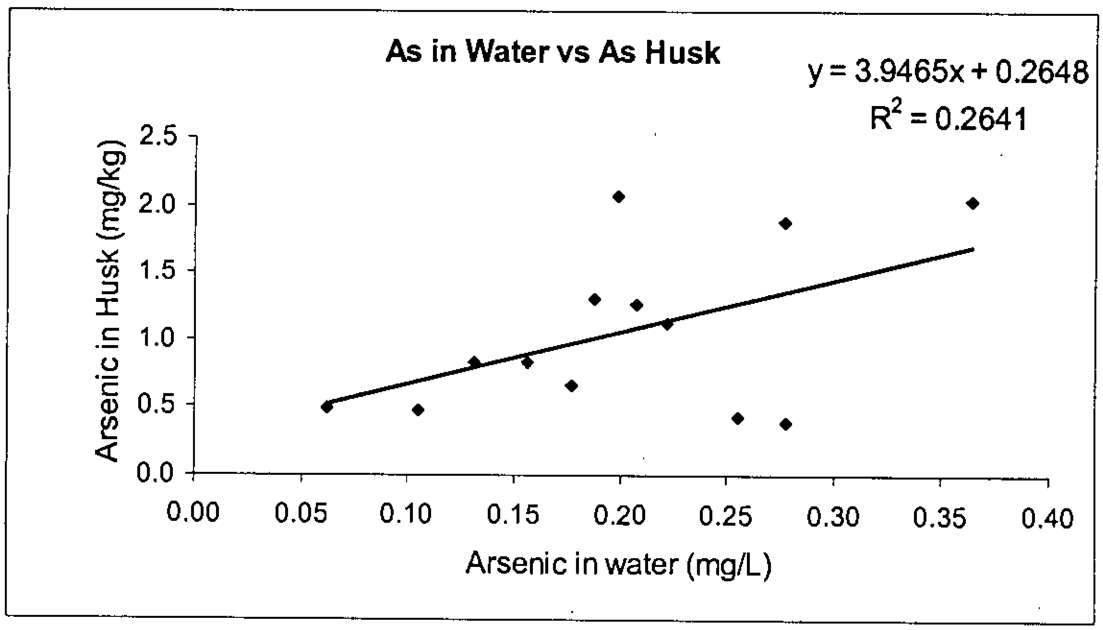


Figure 3.17: Arsenic concentration in husk (mg/kg) verses arsenic in irrigation water (mg/L).

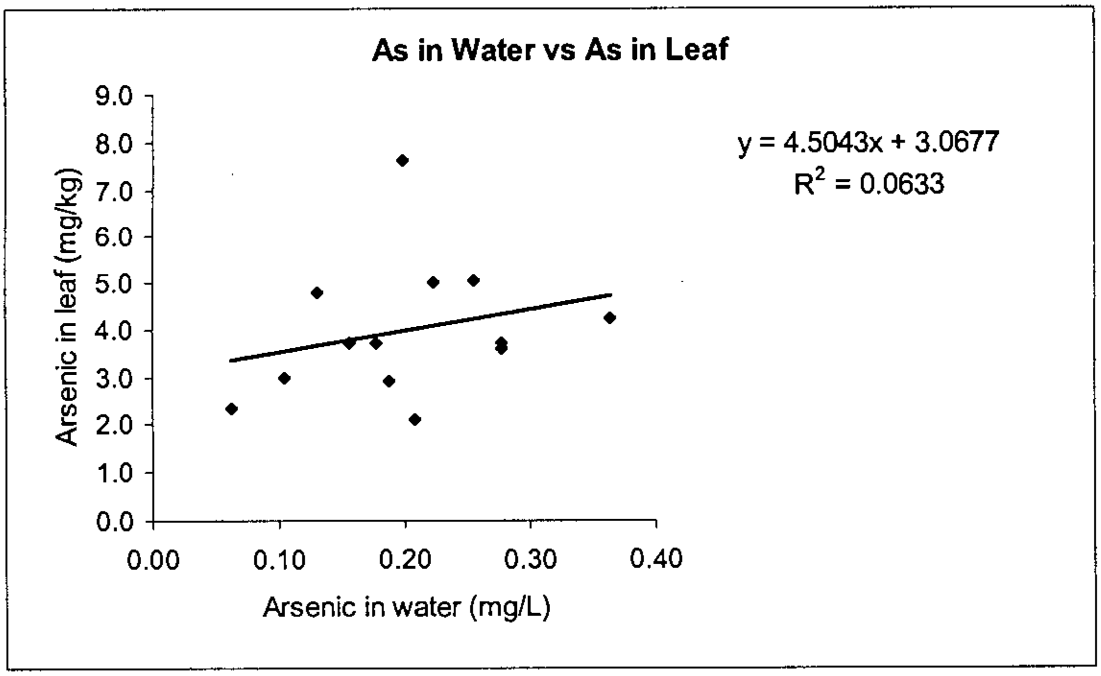


Figure 3.18: Arsenic concentration in leaf (mg/kg) verses arsenic in irrigation water (mg/L).

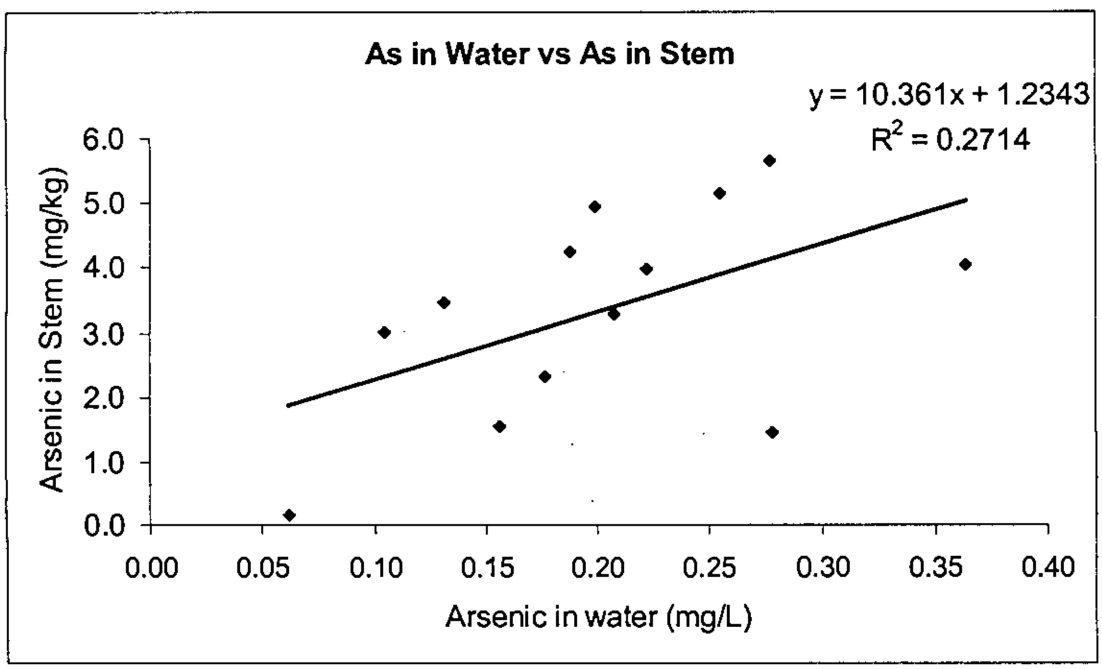


Figure 3.19: Arsenic concentration in stem (mg/kg) verses arsenic in irrigation water (mg/L).

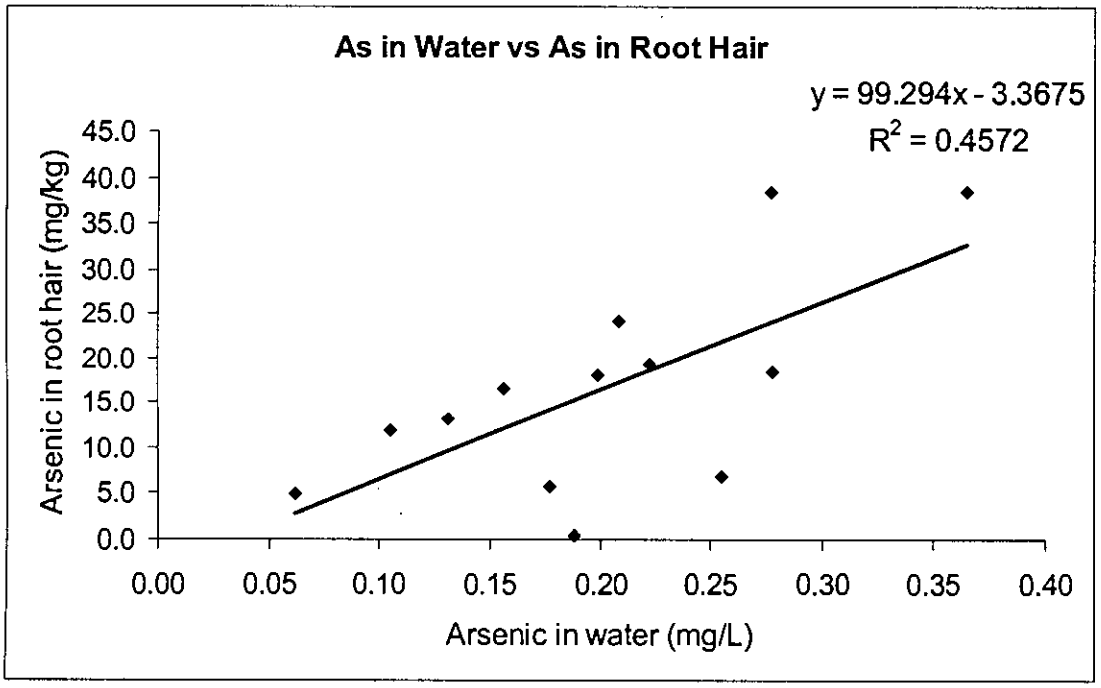


Figure 3.20: Arsenic concentration in root hair (mg/kg) verses arsenic in irrigation water (mg/L).

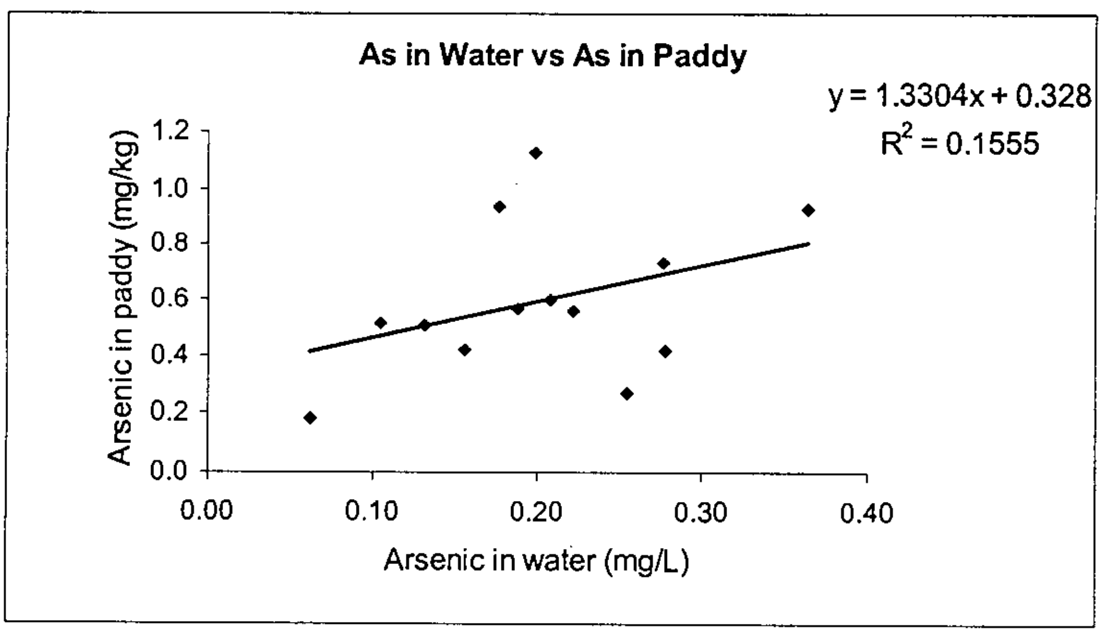


Figure 3.21: Arsenic concentration in paddy (mg/kg) verses arsenic in irrigation water (mg/L).

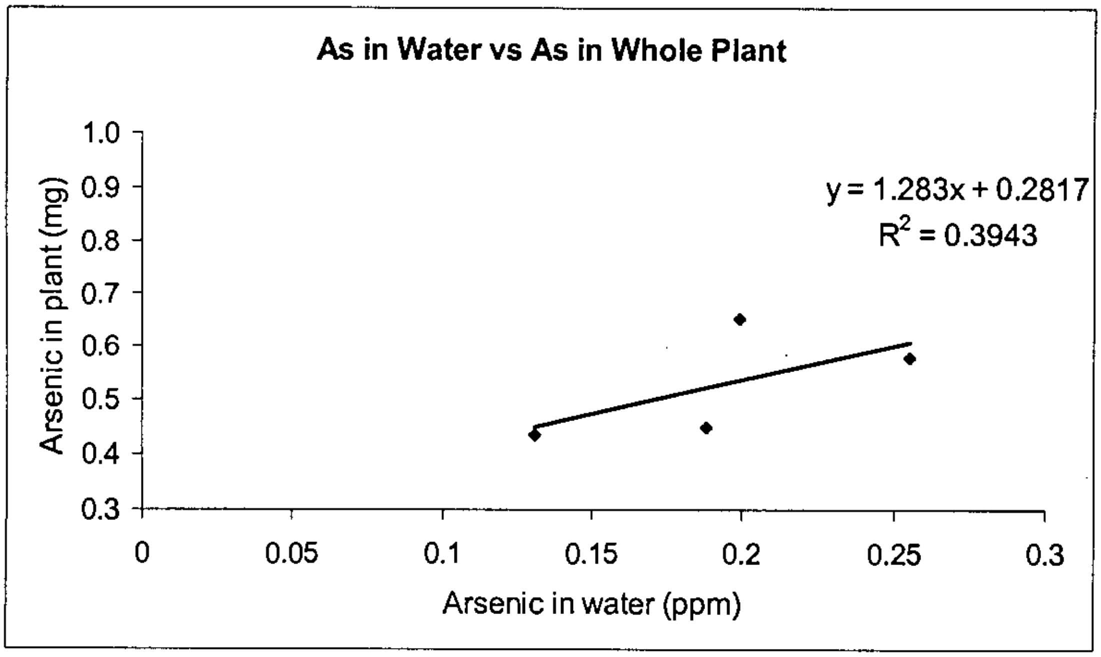


Figure 3.22: Total arsenic in a plant of BRRI dhan-28 variety (mg) verses arsenic in irrigation water (mg/L).

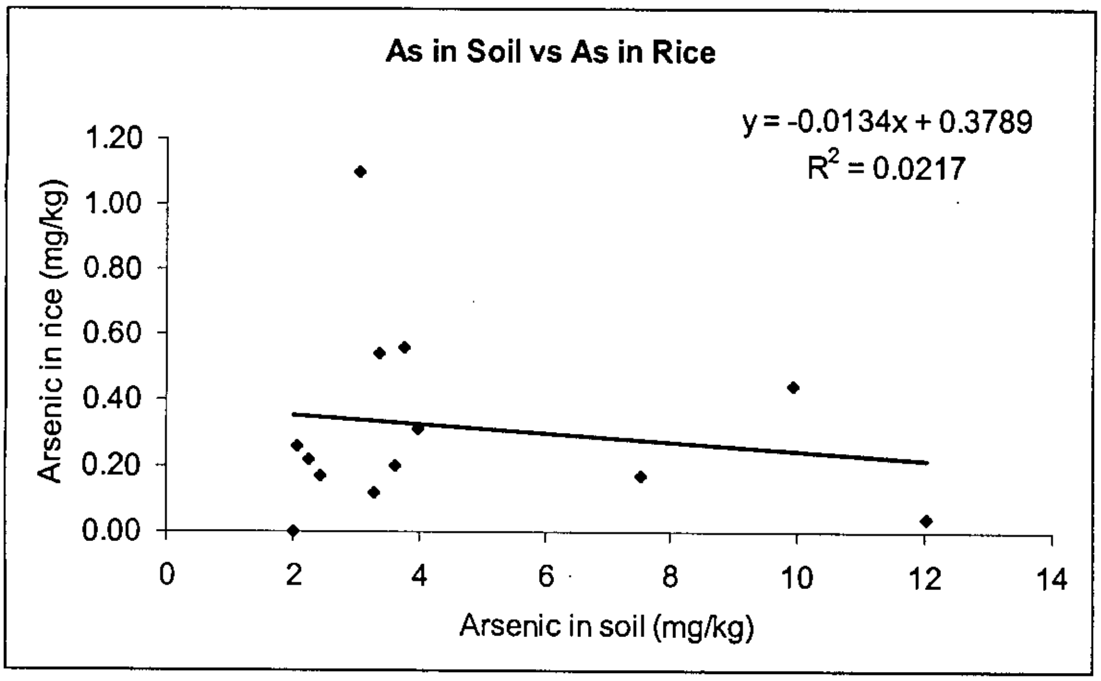


Figure 3.23: Arsenic concentration in rice (mg/kg) as a function of arsenic in soil (mg/kg).

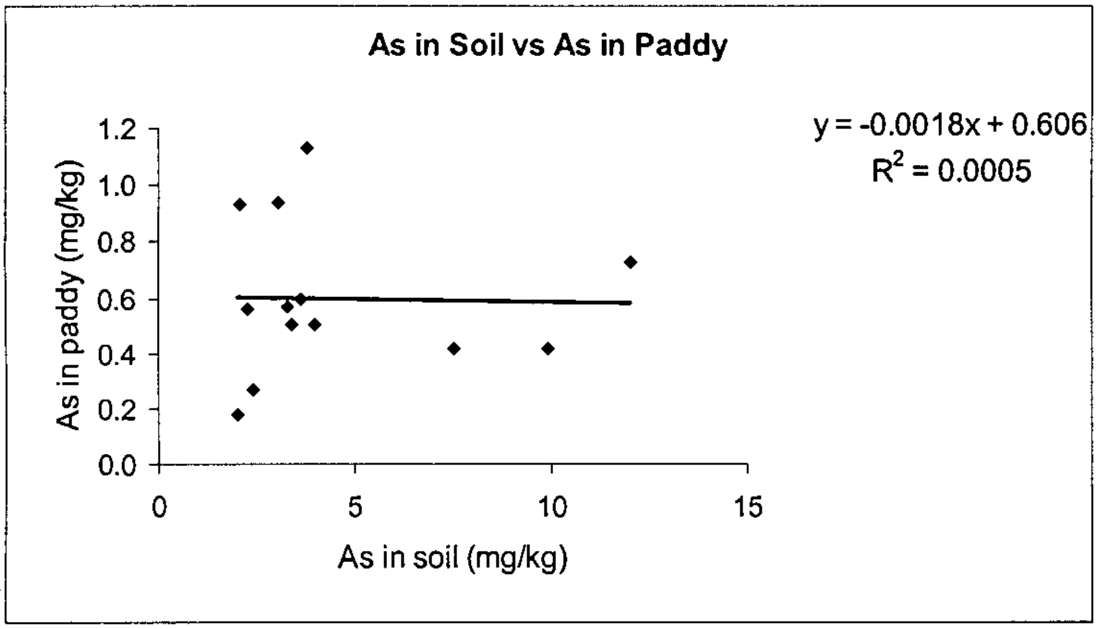


Figure 3.24: Arsenic concentration in paddy (mg/kg) as a function of arsenic in soil (mg/kg).

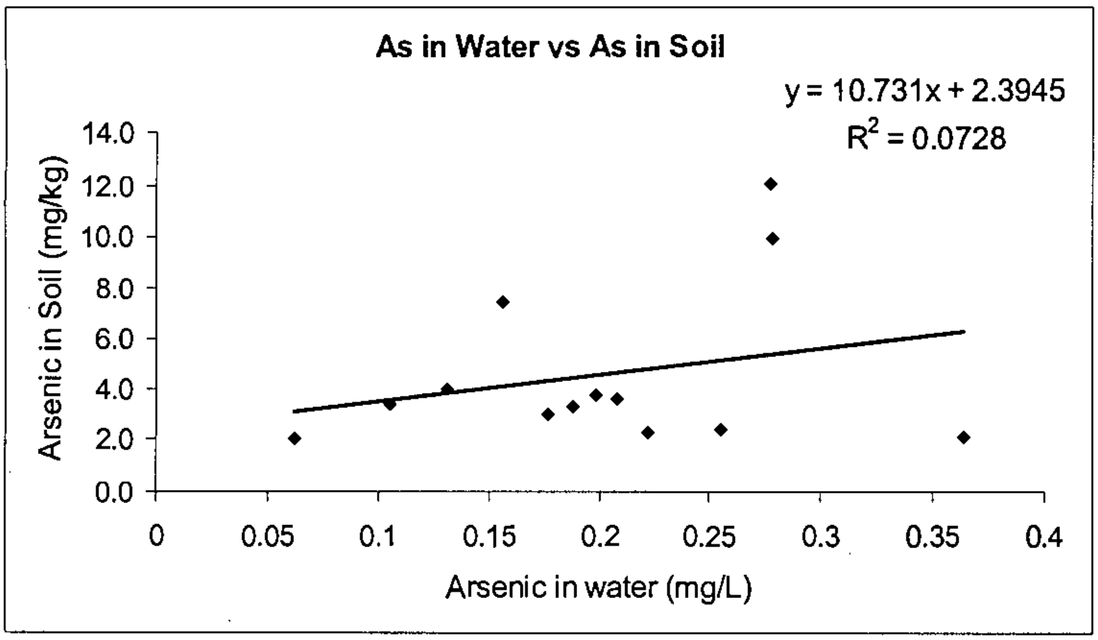


Figure 3.25: Arsenic in soil samples (mg/kg) verses arsenic in irrigation water (mg/L).

Linear Regression Analysis are conducted to assess the correlation between (i) arsenic concentrations in different parts of rice plants and irrigation water arsenic, (ii) soil arsenic and irrigation water arsenic, (iii) concentrations in different parts of rice plants and soil arsenic. Linear regression analyses show that arsenic concentrations in different parts of rice plant (except root hair) are poorly correlated with irrigation water arsenic or soil adsorbed arsenic. Root hair shows a moderate correlation (R = 0.45) with irrigation water arsenic. Similarly, from fig. 3.25 it is observed that there is no correlation between soil arsenic and corresponding irrigation water arsenic.

Test results and statistical analysis shows that arsenic is accumulated in rice plant and soil but it depends on many factors beyond arsenic concentration in water during practical cultivation in rice field. No linear relationship was found with irrigation water and/soil arsenic though some researchers (Yan Chu et al., 1995; Abedin et al., 2002) have established such linear relationships. So, it can be assumed that correlation between plant arsenic and irrigation water/soil arsenic in case of practical cultivation wouldn't be similar to the correlations established in green house experiments.

Recent studies showed that arsenic concentration in ground water of Bangladesh varies from time to time (Ali et al., 2003 and Rashid et al., 2004). Arsenic concentration in soil of rice field in Bangladesh also varies from time to time over the year and after flood it comes to the level nearby the initial concentration having during beginning of boro season (Ali et al.,2003). There is an evidence of arsenic methylation in paddy soil system (Takamatsu et al., 1982). Microbial methylation also produces volatile gases such as di- and tri-methyl arsine from soil. Depending on conditions, 17 – 60% of the total arsenic present in soils may be volatilized (NCCR, 1978). In addition to these there is an impact of green algae grown in boro rice field. This green algae accumulates arsenic at a significantly high concentration (Huq et al., 2005), which reduces the load of arsenic on rice plant. Due to these reasons poor correlations of arsenic in rice plant as well as soil adsorbed arsenic with respect to irrigation water arsenic were found.

3.4 Summary

Arsenic content found in different parts (rice grain, husk, leaf, stem and root hair) of rice plant grown in study fields using ground water with arsenic for irrigation purpose. Highest arsenic concentration found in root hair and least in rice grain (less than 1.0 mg/kg, Australian Maximum Permissible Limit. Arsenic content in straw (leaf + stem) were found more than 1.0 mg/kg, which poses health risk for cattle and human. Arsenic concentrations in soil samples were found up to 12 mg/kg. But insignificant correlations were found among arsenic content of water, soil and rice plant.

CHAPTER 4

NUTRIENT CONTENT IN RICE

4.1 Introduction

In this chapter percentage of protein and amylose content of 13 rice samples has been presented and probable adverse effect of arsenic contaminated irrigation water on nutrient content has been discussed. Rice samples collected from 13 study locations were of 5 varieties such as BR-14, BRRI dhan-28, BRRI dhan-29, Purbachi and IR-50. In order to determine the protein and amylose content of these rice samples, laboratory tests were conducted at the laboratory of Grain Quality and Nutrition Division of Bangladesh Rice Research Institute (BRRI), Gazipur. Percentage of protein contents was determined applying micro-Kjeldahl method and amylose content was determined using spectrometer at 620 nm.

4.2 Methodology

4.2.1 Site Selection and Sample Collection

Selected sites were in Barura and Debidwar upazila of Comilla district. The site selection procedures were expressed in article 3.2.1. Rice samples were collected from the study areas during harvesting; the procedure for rice sample collection was also described in article 3.2.2.

4.2.2 Protein Content Analysis

For analysing protein around 0.2 gm whole brown rice grain (actual weight should be taken) taken in Kjeldahl Flask and digested with concentrated H_2SO_4 (98%) in presence of catalyst namely called Digestion Mixture by heating in a digestion chamber. Digestion Mixture is a mixture of Potassium Sulfate, Copper Sulfate and Selenium powder in 50:10:1 ratio. Under this condition $(NH4)_2SO_4$ is formed reacting with H_2SO_4 and the protein nitrogen. Then digested mixture was cooled. Adding 25 ml of 40% NaOH the digested mixture is then steam distilled, which produces free ammonia. This ammonia is passed into Boric acid solution, forms a color complex, which is then back titrated with standard HCl of 0.03N. Blank solution is also digested and titrated as same procedures described above. Percentage of protein in rice is calculated using the formulas given bellow and a sample calculation for protein content has been made in Appendix- D.

Formula for percentage of Nitrogen is,

$$N_2\,(\%) = \frac{(\text{vol. of HCl for sample} - \text{vol. of HCl for blank}) \times \text{N of HCl} \times 0.014 \times 100}{\text{Weight of grain sample}}$$

Protein (%) = Percentage of Nitrogen x 5.95

Where, conversion factor for Nitrogen to protein of rice is 5.95

4.2.3 Amylose Content Analysis

For analysing amylose 100 mg of rice sample (fine powder form) is taken in 100 ml volumetric flask and 1 ml Ethanol (95%) is carefully added and spread over the whole sample. After some minutes 9.0 ml NaOH (1N) is added and heated the flask for 10 minutes in boiling Water Bath to gelatinize the starch and then cooled. Keep the sample overnight. Make the volume 100 ml adding distilled water and store it as stock solution. Take 5 ml of stock solution into a 100 ml volumetric flask and add 1 ml of 1N acetic acid and 2 ml of freshly prepared Iodine solution in the flask. Iodine solution is a mixture of iodine and potassium iodide. After mixing, make the volume up to the mark and determine percentage of transmittance with spectrometer at 620 nm. Similar procedures were used to determine the % of transmittance of standard solutions for determining standard curve. Standard curve for amylose content were plotted by computer and percentage of amylose were determined from standard curve. Sample calculation for amylose content in rice has given in Appendix- E.

4.4 Results and Discussions

4.4.1 Protein Content of Rice Grain

Protein contents in different rice varieties found in the laboratory experiment are shown in table 4.1.

Table 4.1 Protein content in rice grain samples with corresponding standard value.

Sl. No	Variety	Field ID	Protein content (%)	
			Measured	Standard (BRRI)
1	BR-14	FGB-15	7.3	7.5
2	BR-14	FGB-17	7.0	
3	BR-14	FJB-19	6.8	
4	BRRI dhan -28	FDD-6	6.2	8.6
5	BRRI dhan -28	FDB-13	7.0	
6	BRRI dhan -28	FGB-14	7.8	
7	BRRI dhan -28	FGB-21	6.7	
8	BRRI dhan -29	FGB-16	7.0	7.0
9	BRRI dhan -29	FJB-18	6.6	
10	IR-50	FGB-23	7.0	-
11	Purbachi	FGB-12	8.9	9.0
12	Purbachi	FJB-20	7.6	
13	Purbachi	FGB-22	8.7	

Test results show that all the samples tested contain protein less than standard value except one sample of BRRI dhan-29 (Sample ID FGB-16) that contain protein (7.0 %) equal to the standard level. The measured protein content found in laboratory experiments for the samples of different varieties (BR-14, BRRI dhan-28, BRRI dhan-29 and *Purbachi*) with corresponding standard values are presented by line charts in figures 4.1 – 4.4.

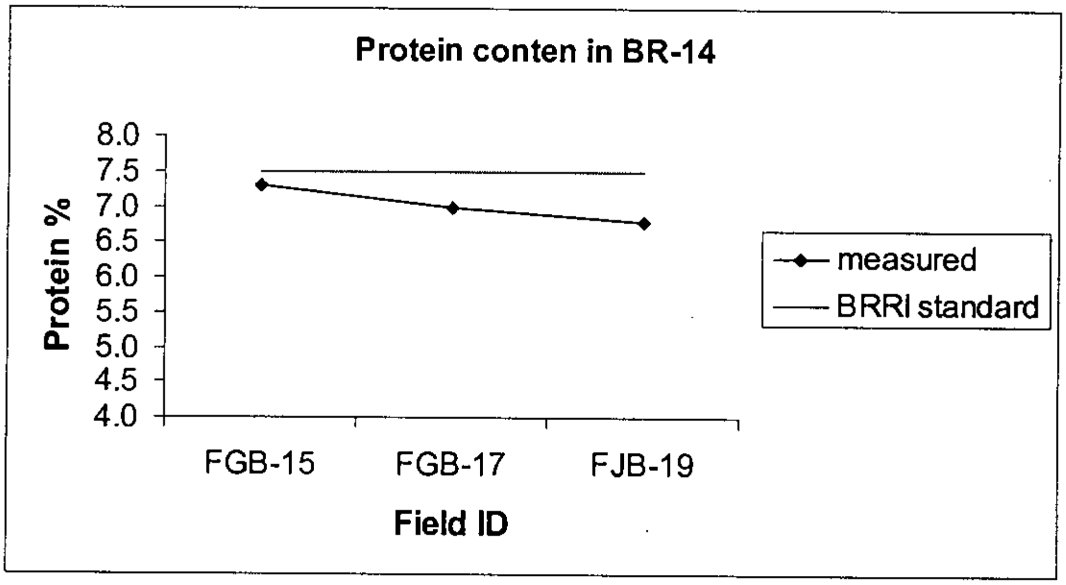


Figure 4.1: Protein content of BR-14 rice.

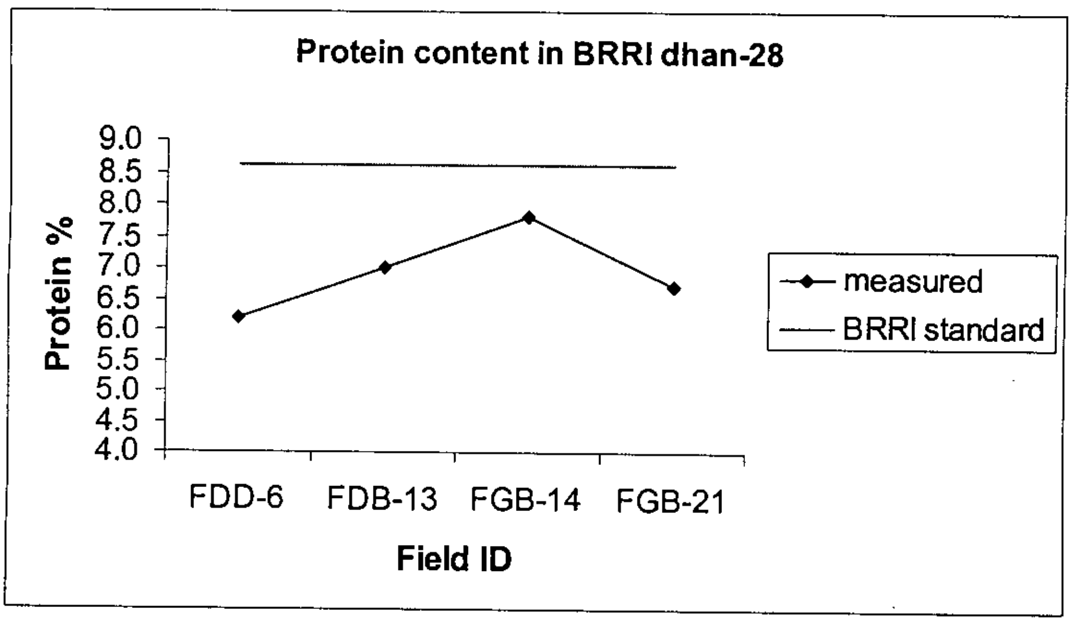


Figure 4.2: Protein content of BRRI dhan -28 rice.

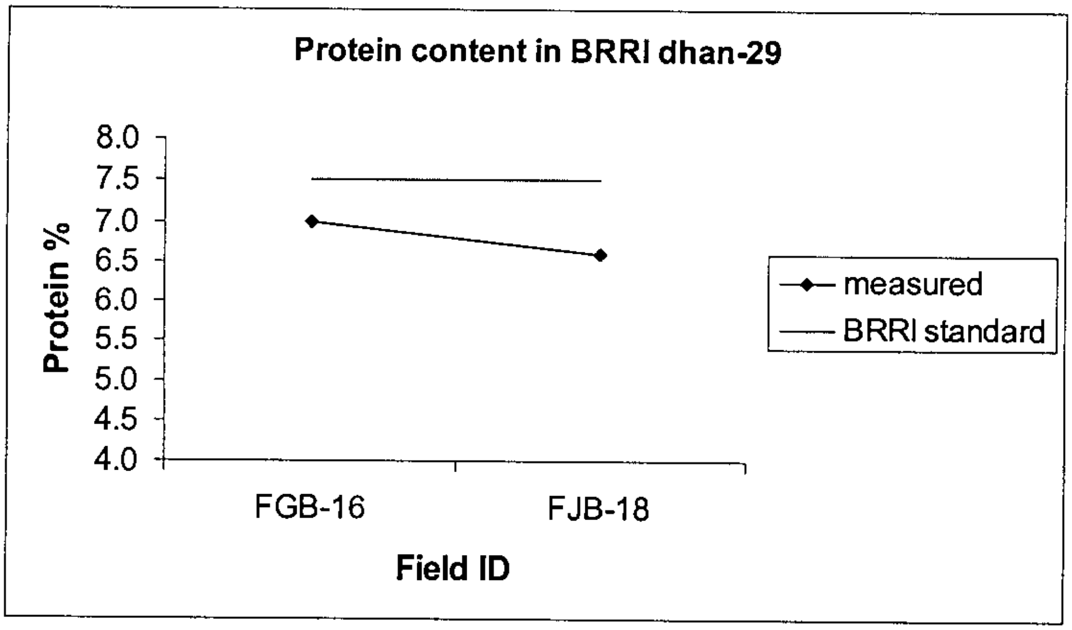


Figure 4.3: Protein content of BRRI dhan -29 rice.

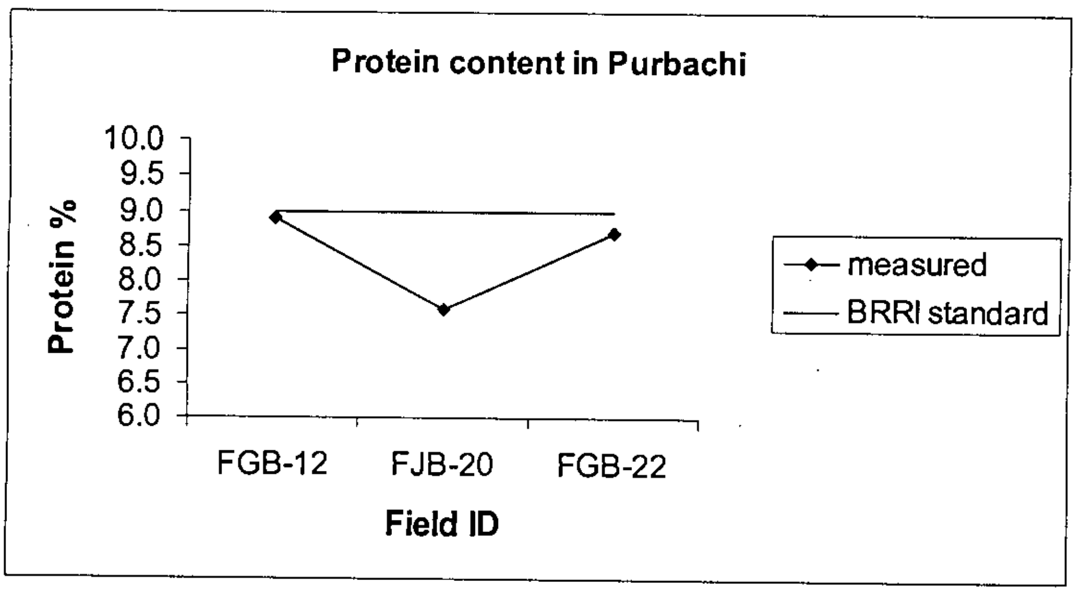


Figure 4.4: Protein content of Purbachi rice.

For BR-14, BRRI dhan-28 & BRRI dhan-29 varieties standard values for protein have been taken from BRRI standard values which are given in **Appendix–B**. For *Purbachi* variety (a China variety: original name **Cheng-chu-ai**) standard values for protein has been taken from BRRI Annual Report for 1989 and BRRI Annual Report for 1976-77 respectively. Out of 13 samples variations in nutrient contents for 12 samples could be compared with BRRI standard values but one sample of IR-50 variety (sample ID FGB-23) couldn't be compared with standard due to unavailable standard value for Protein.

Test results of protein content show that the protein contents in rice samples tested are lower than the standard values. Maximum negative variation in protein content found in BRRI dhan -28 variety, 27.9% with average 19.5% followed by Purbachi, 15.6% with average 6.7%; BR-14, 9.3% with average 6.2% and BRRI dhan-29, 5.7% with average 2.9%.According to the data available in Laboratory of BRRI protein content in rice may vary due to many factors. One of these factors is deficiency of nitrogen in soil. Application of urea fertilizer in rice field also plays a vital role on protein content. Presence of As in soil or soil solution or water cause adverse effect on growth and yield. Tsutsumi (1980), Milam et al. (1992), Tang and Miller (1991), Marin et al. (1992, 1993) reported about reduced shoot biomass and/or growth in rice grown either in soil or in solution culture with arsenic. But no reduction in dry matter production (Marin et al. 1993) has also been reported while arsenic concentrations in the growing media were low. However, in a study under PETRRA project Jahiruddin et al. (2004) found grain protein was adversely affected due to arsenic contamination.

4.4.2 Amylose Content of Rice Grain

Amylose contents in different rice varieties found in the laboratory experiment are shown in table 4.2. Test results show that the amylose content in rice samples of different varieties tested is less than the standard (BRRI) values except two samples of BR-14 varieties (Sample ID No. FGB-15 and FGB-17) having amylose content more than standard value which are 27.1% and 27.7% respectively. The comparative amylose contents found for different rice samples of different varieties with respect to standard values are presented by line charts of figure 4.5 – 4.8.

Table 4.2: Amylose content in different varieties of rice and corresponding standard value.

Sl. No	Variety	Field ID	Amylose content (%)	
			Measured	Standard (BRRI)
1	BR-14	FGB-15	27.1	27.0
2	BR-14	FGB-17	27.7	
3	BR-14	FJB-19	24.5	
4	BRRI dhan -28	FDD-6	27.4	28.0
5	BRRI dhan -28	FDB-13	25.0	
6	BRRI dhan -28	FGB-14	25.2	
7	BRRI dhan -28	FGB-21	26.5	
8	BRRI dhan -29	FGB-16	26.2	29.4
9	BRRI dhan -29	FJB-18	26.1	
10	IR-50	FGB-23	26.5	-
11	Purbachi	FGB-12	25.4	27.0
12	Purbachi	FJB-20	26.2	
13	Purbachi	FGB-22	26.9	

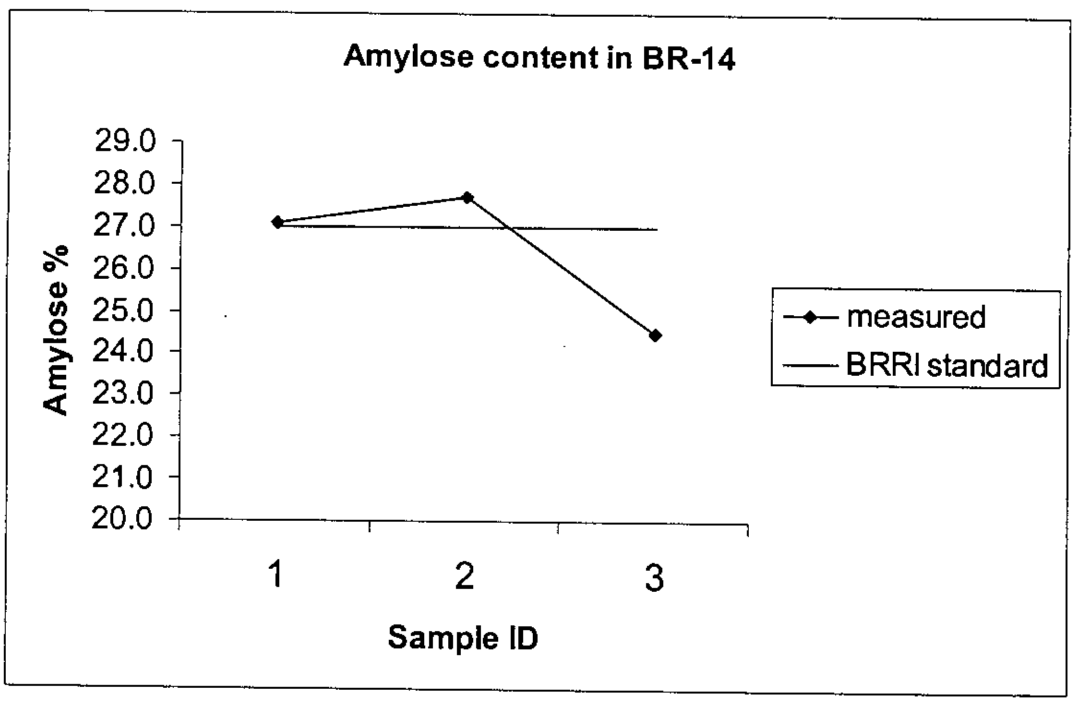


Figure 4.5: Amylose content of BR-14 rice.

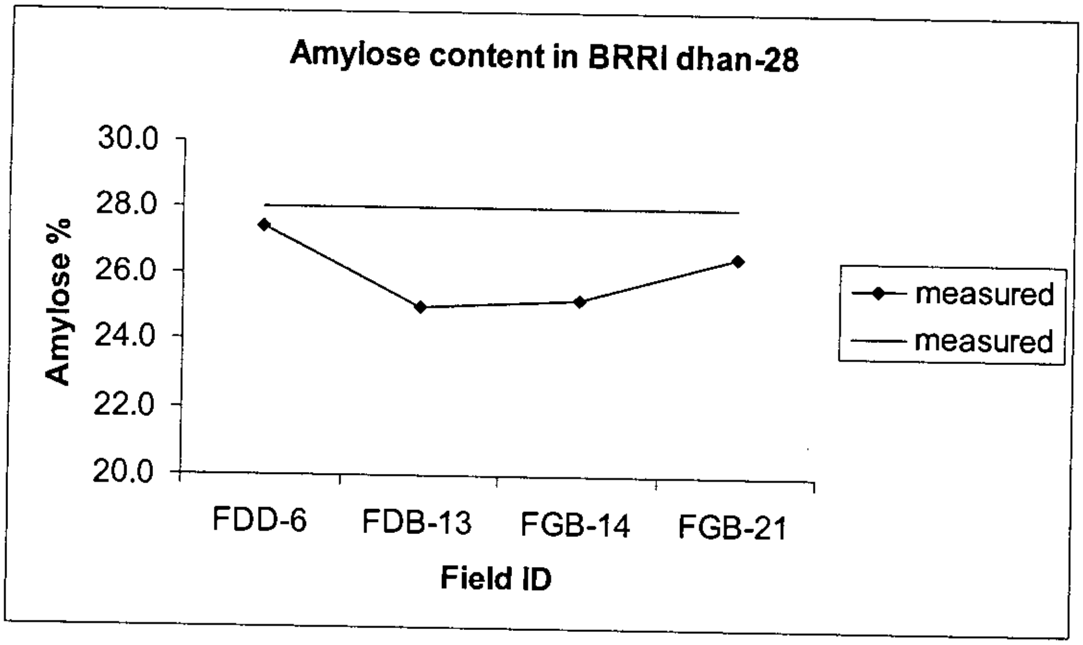


Figure 4.6: Amylose content of BRRI dhan -28 rice.

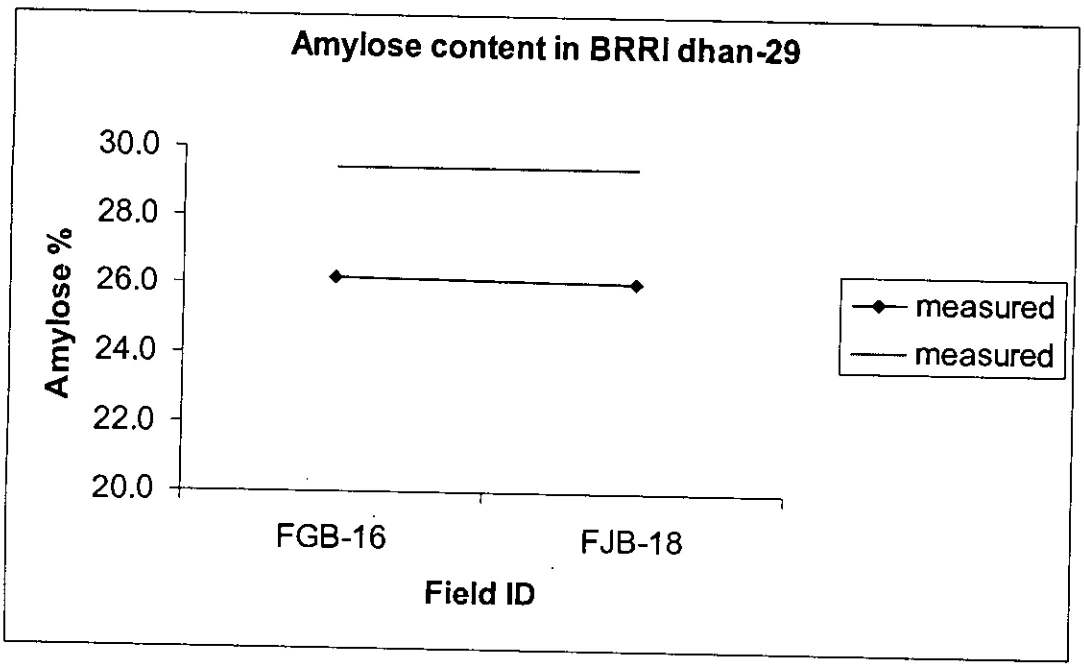


Figure 4.7: Amylose content of BRRI dhan-29 rice.

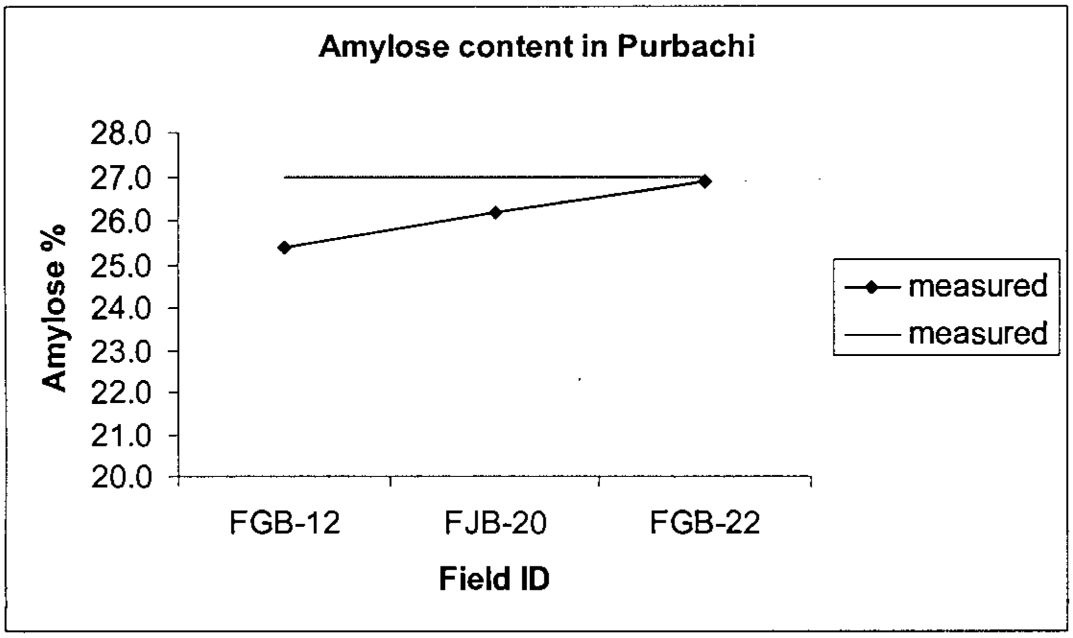


Figure 4.8: Amylose content of Purbachi rice.

Amylose contents found in laboratory tests have been compared with standard value. For BR-14, BRRI dhan-28 & BRRI dhan-29 varieties standard values for protein & amylose have been taken from BRRI standard values which are given in **Appendix–B**. For *Purbachi* variety (a China variety: original name **Cheng-chu-ai**) standard values for amylose has been taken from BRRI Annual Report for 1989 and BRRI Annual Report for 1976-77 respectively. Out of 13 samples variations in nutrient contents for 12 samples could be compared with standard values but one sample of IR-50 variety (sample ID FGB-23) couldn't be compared due to unavailable standard value for Amylose.

Test results of amylose content show that the amylose contents in rice samples tested are lower in most of the cases than the BRRI standard values only 2 samples of BR-14 variety found having amylose content more than standard value (0.4% & 2.6%). Maximum deficiency in amylose content found in BRRI dhan-29, 11.2% with average 11.1% followed by BRRI dhan-28, 10.7% with average 7.1%; Purbachi, 5.9% with average 3.1% and BR-14, 9.3% with average 2.1%. According to the data available in Laboratory of BRRI there is less scope of variation in amylose content. Presence of As in soil or soil solution or water cause adverse effect on growth and yield. Tsutsumi (1980), Milam et al. (1992), Tang and Miller (1991), Marin et al. (1992, 1993) reported about reduced shoot biomass and/or growth in rice grown either in soil or in solution culture with arsenic. But no reduction in dry matter production (Marin et al. 1993) has also been reported while arsenic concentrations in the growing media were low.

4.4 Summary

Protein content found in rice grain is less than the BRRI standard value. In case of amylose content only two samples found having amylose content less than the BRRI standard value. Percentage of deficiency of protein content in rice is more than that of amylose content. From the test result it can be assumed that there may be adverse effect of arsenic in irrigation water on nutrient content of rice.

However, the test results are only indicative of possible adverse effects of arsenic contaminated irrigation water on nutrient (protein & amylose) content. Further extensive research is necessary to draw such a conjecture.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the above discussions the following conclusions can be drawn:

1. Rice plant accumulates arsenic from the arsenic-contaminated irrigation water applied in rice field. Arsenic concentration varies from one part to another part of a rice plant. The pattern of arsenic concentration in different parts of rice plants found is **rice grain < husk < stem < leaf < root hair**.

2. Test results show that the arsenic accumulation in rice grain varies from variety to variety. Most of the varieties tested accumulate arsenic in an amount less than 1 mg/kg, which is the Australian maximum permissible limit (National Food Authority, 1993), except one sample of Purbachi variety that contained 1.1 mg/kg of arsenic.

3. The amount of total accumulated arsenic in different parts of BRRI dhan-28 and BRRI dhan-29 were calculated. The calculated result shows that the highest amount of arsenic accumulated in the stem of a rice plant and the trend found is **Stem > Leaf > Husk > Rice grain**. The ratio of total arsenic in Stem, Leaf, Husk and Rice grain in BRRI dhan-28 and BRRI dhan-29 varieties rice plants are **71 : 26 : 2 : 1** and **65 : 31 : 3 : 1**, respectively.

4. There is no definite correlation between arsenic content of water and soil and arsenic accumulated in rice grain, husk, leaf and stem of rice plant. But a moderate relationship ($R^2 \approx 0.45$) was found between arsenic accumulated in root hair sample and arsenic in irrigation water.

5. Arsenic concentration in soil samples varies from one field to another. But no relationship was found with arsenic in water.

6. Nutrient analysis shows that there is less protein content in rice with respect to the BRRI standard value, though protein development in grain depends on

many factors including fertilizer application. In case of amylase content the test results shows that there is less amylose content in rice grain than the BRRI standard value.

7. Maximum deficiency of protein content found was in BRRI dhan-28 (27.9%) variety followed by *Purbachi*, BR-14 and BRRI dhan-29. Deficiency of amylase content in the four tested varieties varies as BRRI dhan-29 > BRRI dhan-28 > *Purbachi* > BR-14.

5.2 Recommendations

For future experiments the author proposes a list of studies as given below:

1. Research works are needed to assess the level of accumulated arsenic for each HYV variety of Boro rice produced in the same field using irrigation waters with different concentrations of arsenic.

2. Research works may be conducted to determine the concentration of arsenic in milk and meat produced from cow, which is feeding arsenic contaminated straw.

3. Research works are needed to determine the level of accumulated arsenic in polished rice for different degrees of polishing.

3. Research works are needed to determine the level of accumulation of arsenic in pericarp (brown surface) of rice from where nutrient-enriched edible oil could be produced.

4. Research works are needed to find out the impact of irrigation water with arsenic on nutrient content of rice.

REFERENCES

Abedin, M.J. 2002. Arsenic Uptake, Metabolism and Toxicity in Paddy Rice (*Oriza sativa* L.), A thesis presented for the degree of Doctor of Philosophy at the University of Aberdeen, Scotland, United Kingdom. AB24 3UU.

Ahmad, Sk. F., 2000. Arsenic: Water Contamination and Health Hazard. ISBN: 984.31-0804-X. pp 144.

Ahmed K. M., Imam M. B., Akhter S. H., Hasan M. A., Alam M. M., Chowdhury S.Q., Burgess W. G., Nickson R., McAuthur J. J., Hasam M.K., Ravenscroft P. and Rahman M., 1998. Mechanism of arsenic release to groundwater: geochemical and mineralogical evidence. In International Conference on Arsenic Pollution of Groundwater in Bangladesh: Causes, Effects and Remedies, 8-12 February 1998, Dhaka Community Hospital, Dhaka, Bangladesh.

Ahmed, K. M., 2003. Geological and Hydrogeoloigcal Contrls on the Occurrences and Distribution of Arsenic in Bangladesh Groundwater. In: *Arsenic Contamination: Bangladesh Perspective* (Ed. M. Feroz Ahmed), ITN-Bangladesh, BUET, Dhaka.

Ahmed, M. F., 2001. Arsenic Contamination in Bangladesh: Severity of the Problem and Possible Consequences. Paper presented at the *Arsenic in the Asia-Pacific Region workshop 2001* held at Adelaide, South Australia during 20-23 Nov. 2001.

Ahmed, M. F., 2003. Arsenic Contamination of Groundwater in Bangladesh, In: *Arsenic Contamination: Bangladesh Perspective* (Ed. M. Feroz Ahmed), ITN-Bangladesh, BUET, Dhaka.

Ahmed, M. F., Ali, M. A. and Adeel, Z. [editors], 2003. Fate of Arsenic in the Environment, Bangladesh University of Engineering & Technology and United Nations University, February. ISBN 984-32-0507-3.

Alam M.G.M., Tokunaga, S. and Maekawa, T. 2001. Extraction of arsenic in a synthetic arsenic contaminated soil using phosphate. Chemosphere. 43, 1035-1041.

Ali, M. A., 2003. Fate of Arsenic in the Environment, In: *Arsenic Contamination: Bangladesh Perspective* (Ed. M. Feroz Ahmed), ITN-Bangladesh, BUET, Dhaka.

Alloway, B. J., 1990. Heavy metals in soil, Jhon Willey and Sons Inc.

Asher C.J. and Reay P.F. 1997. Arsenic uptake by barely seedlings. Aust. J. Plant Physiology 6. 459-466.

BADC (2002). Survey report on irrigation equipment and irrigated area in Boro/2001 season, Bangladesh Agricultural Development Corporation, March 2002.

Beever R.E. and Burns D.W.J., 1980. Phosphorous uptake, storage and utilization by fungi. Adv. Bot. Res. 8, 127-219.

Bennett, J., Chiriboga, J. Coleman and D. Waller (2000). Heavy metal in wild rice from northern Wisconsin. Science of the Total Environment 246: 261-269.

Berrow, M. L. and Reaves, G. A., 1984. In: *Proc Intl conf. Environ. Contamination*, 333-340. CEP Consultants Ltd. Edinburgh, UK.

BGS (British Geological Survey), 2000. Executive summary of the main report of Phase I, Groundwater Studies of Arsenic Contamination in Bangladesh, By British Geological Survey and Mott MacDonald (UK) for the Government of Bangladesh, Ministry of Local Government, Rural Development and Cooperatives, Department of Public Health Engineering, and Department of International Development (UK). (http://www.dainichi-consul.co.jp/english/article/DFID-sum.html).

BGS and DPHE, 2001. Arsenic Contamination of Groundwater in Bangladesh, Kinniburg, D.G. and Smedley. P. L. (Editors), Volume 2: Final Report. British Geological Survey Report WC/00/19.

Bhattacharya P., Larsson M., Leiss A., Jacks G., Sracek A. and Chatterjee D., 1998. Genesis of arseniferrous groundwater in the alluvial aquaifers of Bengal delta plains and strategies for low cost remediation. In International Conference on Arsenic Pollution of Ground water in Bangladesh: Causes, Effects and Remedies, 8-12 February 1998, Dhaka Community Hospital, Dhaka, Bangladesh.

Bolan, N.S., Kannathasan, M., Brooks, R.R., Robinson, B.H. and Buwig, C. (2001). Arseinic Accumulation by Watercress (*Lipidium sativum*) in the Taupo Volcanic Zone (TVZ): In Book of Abstract, Arsenic in the Asia-Pacific Region Workshop, 2001. p.p. 48-49.

Brown K.G., and Chen C.J. 1995. Significance of Exposure Assessment to Analysis of Cancer Risk from Inorganic Arsenic in Drinking water. Risk Analysis. 15(4). 475-484.

BRRI, 1977. BRRI Annual Report for 1976-77. Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

BRRI, 1990. BRRI Annual Report for 1989. Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

BRRI, 1999. BRRI Annual Report for Jan 1998 – June 1999. Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

BRRI, 2001. Annual Internal Review for July 2000- June 2001. X-Plant Physiology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh.

Burló, F., I. Guijarro, A.A. Carbonell-Barrachina, D Valero and F. Martínez-Sánchez (1999). Arsenic Species: Effects on and Accumulation by Tomato Plants. J. Agric. Food Chem. 47: 1247-1253.

Carbonell-Barrachina A.A., Aarabi M.A., DeLaune R.D., Gambrell R. P. and Patrick W. H. Jr., 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. Plant Soil 198, 33-43.

Carbonell-Barrachina A.A., Burló F, Valero D, López E., Martínez-Romero D. and martínez-Sánchez F., 1999. Arsenic toxicity and accumulation in Turnip as affected by arsenic chemical speciation. J. Agric. Food Chem. 47, 2288-2294.

Carbonell-Barrachina A.A., Burló-Carbonell F. and Mataix-Beneyto J. 1995. Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenic on plant growth and yield. J. Plant Nutr. 18, 1237-1250.

Chakrabarti, A.K., Saha K.C. 1987. Arsenical dermatosis from tubewell water in West Bengal. Indian J Med Res 1987; 85: 71-79.

Chakravarty, I., Sinha, R.K., and Ghosh, L., 2003. Arsenic in Food Chain – A Study on Both Raw and Cooked Food. In: *Arsenic Contamination: Bangladesh Perspective (M. F. Ahmed ed.)*, ITN-Bangladesh, BUET, Dhaka, Bangladesh.

Chakrobarti, D., Rahman, M.M., Paul, K. et al., 2001. Groundwater arsenic contamination in south east Asia with special reference to Bangladesh and West Bengal, India, Arsenic in the Asia Pacific Region Workshop, Adelaide, Managing Arsenic for the Future: 1-4.

Chen, K.P., Wu, H.Y. Epidermiological studies on black foot diseases: A study of source of drinking water in relation to the disease. J Formosan Med Assoc 1962;61:611-617.

Chen, Y., Ahsan, H., 2002. Cancer Burden Due to Arsenic Exposure from Drinking Water in Bangladesh. *Am J Public Health* (In press).

Clifford, D. A. and Zhang, Z. 1993. Arsenic chemistry and speciation. Proc. AWWA WQTC, Miami, Fla., Nov. 7-11.

Cox M.S., Bell P.F. and Kovar J.L. 1996. Differential tolerance of canola to arsenic when grown hydroponically or in soil. J. Plant Nutrl 19(12), 1599-1610.

Crecelcious, E. A. et al., 1974. Contamination of soils near a copper smelter by arsenic, antimony and lead. J. Water, air and soil pollut. vot. 3, pp. 337-342.

Dainichi 2001. URL.: http://www.dainichi-consul.co.jp/english/arsen.thm

Das D., Chatterjee A., Mandal B.K., Samanta G, Chanda B. and Chakroborti D., 1995. Arsenic in groundwater in six districts of West Bengal, India: the biggest arsenic calamity in the world, part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. Analyst 120. 917-924.

Das D., Samanta G, Mandal B.K., Chowdhury T., Chanda C. R., Chowdhury P.P., Basu G. K. and Chakraborti D., 1996. Arsenic in groudwater in six districts of West Bengal, India. Environ. Geochem. Health 18, 5-15.

Das, H. K. 2000. Bangladesh Arsenic: Bhayabahata O Sambhabbya Protikar. Bangla Academy, Dhaka. PP. 1-278.

Das, H. K., Chowdhury D.A., Rahman S., Obaidullah, Miah M. U., Sengupta P., Islam F. 2003. Arsenic Contamination of Soil and Water and Related Bio-hazards in Bangladesh. In: Fate of Arsenic in the Environment Ed. M. Feroze Ahmed et al. 2003. BUET-UNU, Dhaka, Bangladesh.

DPHE-BGS 2000. Groundwater studies for arsenic contamination in Bangladesh. Department of Public Health and Engineering and British Geological Survey. Pp. 11-49.

Duxbury, J. M., Mayer, A. B., Lauren, J. G. and Hassan, N., 2002. Arsenic Content of Rice in Bangladesh and Impacts on Rice Productivity, 4th Annual Conference on Arsenic Contamination in Groundwater in Bangladesh: Cause, Effect and Remedy, Dhaka, 12-13 January 2002.

Eisler, R. 1994. A review of Arsenic Hazards to Plants and Animals with Emphasis on Fishery and Wildlife Resources. In: Arseinc in the Environment, Part II: Human Health and Ecosystem Effects, ed. J.O. Nriagu, John Wiley & Sons, Inc. New York.

Environmental Protection Agency (EPA), 1980. Ambient water quality criteria for arsenic-1984. *US Environ. Prot. Agency Rep.* 440/5-84-033: 1-66.

Eriksen, N., Hamel, Zinia, B. K. N., 2001. A study of arsenic treatment technologies and leaching characteristics of arsenic contaminated sludge. In: Technologies for Arsenic Removal from drinking Water, ed. M. Feroze Ahmed et al. 2001. BUET-UNU, Dhaka, Bangladesh.

Farid, A.T.M., Roy, K.C., Hossain, K.M. and Sen, R. 2003. A Study of Arsenic Contaminated Irrigation Water and its Carried Over Effect on Vegetable. In: Fate of Arsenic in the Environment Ed. M. Feroze Ahmed et al. 2003. BUET-UNU, Dhaka, Bangladesh.

Fitz, W.J., Wenzel, W.W., Kollensberger, G., Nurmi, J., Mentler, A., Stipek, K., Fischerova, Z., Schweiger, P. and Ma, L.Q. 2001. Rhizosphere Studies on the Arsenic Hyperaccumulator *Pteris vittate* in Arsenic in the Asia Pacific Region Workshop, Adelaide, Managing Arsenic for the Future: 41-44.

Fowler B. A., 1977. Toxicology of Environmental Arsenic. In Toxicology of Trace Elements. Eds, R A Goyer, M A Mehlman, Wiley & Sons, New York, pp 79-122.

Frans R, Horton D. and Burdette L., 1988. Influence of MSMA on straighthead, arsenic uptake and growth response in rice (*Oryza sativa*). Arkansas AES. Rep. Ser. 302, 1-12.

Frederick, W. and Coleagues, 1994. Health implications of arsenic in drinking water. Journal AWWA, Sept. 1994.

Ghani, M A, M A Rashid and A L Shah., 2004. Arsenic contamination and its impact on food production and environment. Paper presented in the third South Asia Water Forum (SAWAF-iii), 13-15 July, 2004, Dhaka, Bangladesh.

Gilmour J.T. and Wells B.R., 1980. Residual effect of MSMA on sterility in rice cultivars. Agron. J. 72, 1066-1067.

Hartley-Whitaker J., Ainsworth G. , Vooijs R., Bookum T.W., Schat H. and Meharg A.A. 2001a. Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus*. Plant Physiol. 126, 299-306.

Hartley-Whitaker J., Ainsworth G. and Meharg A.A. 2001b. Copper- and arsenate-induced oxidative stress in *Holcus lanatus* L. clones with differential sensitivity. Plant Cell Environ. 24, 713-722.

Haswell, S. J., O'Neill, P., and Bancrofit, K. C., 1985. Arsenic speciation in soil-pore waters from mineralized and unmineralized areas of southwest England. *Talnta* 32: 69-72.

Heitkemper D.T., Vela N. P., Stewart K. R. and Westphal C.S., 2001. Determination of total and speciated arsenic in rice by ion chromatography and inductively coupled plasma mass spectrometry. J. Anal. Atomic Spect. 16, 299-306.

Helgensen, H. and Larsen, E.H. 1998. Bioabailability and speciation of arsenic in carrots grown in contaminated soil. Analyst. 123: 791-796.

Hinton, J. J. C., 1948. The distribution of vitamin B1 in the rice grain. Brit. J. Nutr. 2: 237.

Hironaka, H. and Ahmad, S.A., 2003. Arsenic Concentration of Rice in Bangladesh, Fate of Arsenic in the Environment. Ed. M. Feroze Ahmed et al. 2003. BUET-UNU, Dhaka, Bangladesh.

Hossain, A. F. M. A., 2003. An Approach to Locate Arsenic Sources, Its Propagation and Remedial Measures Using Mathematical Models, In: *Arsenic Contamination: Bangladesh Perspective* (Ed. M. Feroz Ahmed), ITN-Bangladesh, BUET, Dhaka.

Houston, 1978. Rice: Chemistry and Technology, 3rd edition, American Association of Cereal Chemists, Inc., St. Paul, Minnesota, USA.

Huq, S. M. I., 2005. Arsenic bioaccumulation in a green algae and its subsequent recycling in soils of Bangladesh, In: Natural Arsenic in Groundwater: Occurrences, Remediation and Management (Bundschuch, J. et al., ed.), A. A. Balkema Publishers, Leiden, The Netherlands.

Huq, S.M.I., Anisur Rahman, Nazma Sultana 2003. Extent and Severity of Arsenic Contamination in Soils. In: Fate of Arsenic in the Environment Ed. M. Feroze Ahmed et al. 2003. BUET-UNU, Dhaka, Bangladesh.

Huq, S.M.I., Quazi Afroz Jahan Ara, Khaleda Islam, Abduz Zaher and Ravi Naidu, 2001. The possible contamination from Arsenic through food chain. In: Groundwater Arsenic Contamination in the Bengal Delta Plain of Bangladesh. Proceedings of the KTH-Dhaka University Seminar, University of Dhaka, Bangladesh.

Jahiruddin, M., Islam, M. R., Shah, A. L., and Ghani, M. A., 2004. Effects of Arsenic Contamination on Yield and Arsenic Accumulation in Crops, In: *Proceeding of the workshop on Arsenic in the Food Chain: Assessment of Arsenic in the*

Water-Soil-Crop Systems (M A L Shah et al. Eds.), BRRI, Gazipur, Bangladesh.

Jameel, F., Miah, N. M. and Alam, M. N., 1999. Some Appropriate Technologies of Rice Cultivation. BRRI, Gazipur in collaboration with UNDP & FAO.

James P. Bennett J. P. Heavy metals in wild rice from northern Wisconsin. The Science of the Total Environment, 2000; 246: 261-269.

Johnson L.R. and Hiltbold A.E., 1969. Arsenic content of soil and crops following use of methanearsonate herbicides. Soil Sci. Soc. Am. Proc. 33. 279-282.

Juliano, B. O., 1978. The Rice Caryopsis and its Composition. *In*: Rice: Chemistry and Technology, 3rd edition, ed. D. F. Houston, American Association of Cereal Chemists, Inc., St. Paul, Minnesota, USA.

Juliano, J. B., and Aldama, M. J., 1937. Morphology of Oryza sativa Linnaeus. Philipine Agriculturist. 26:1.

Jung C. and Rothstein A., 1965. Arsenate uptake and release in relation to the inhibition of transport and glycolysis in yeast. Biochem. Pharmacol. 14, 1093-1112.

Kabata-Pendias A. and Pendias H.., 1984. Trace Elements in Soils and Plants. CRS Press, Inc., Boca Raton, Florida, USA, pp 51-68.

Kabir, Y., 2002. Report on "Food Gap Analysis on the basis of Nutritional Requirement of Different Food Items", Early Warning and Food Information System Project, Ministry of Food, Dhaka.

Kartinen, E.O. Jr., & Christopher, J.M., 1995. A Overview of Arsenic Removal Processes, Desalination, 103:79-88.

Kubota, Yoshihiro, 1998. Arsenic contamination of Drilling Core and Surface Geology in Samta Village, Possibility of Origin of Arsenic Supply, 3rd Forum on Arsenic Contamination of Groundwater in Asia, Miyazaki University, Japan, 22-23 November.

Leep, N.W. 1981. Effect of heavy metal pollution on plants. Vol. 1. Effects of Trace metal on Plant Function. Applied Science Publishers, London, UK.

Liebig-Jr, G.P. 1966. Arsenic. In Diagnostic criteria for plants and soils. Ec. H.D. Chapman. pp 13-23. University of California, Division of Agricultural Science, Riverside, CA.

Liu, G.L. and Gao, S.D. 1987. The effect of arsenic in red soil on crops. J. Soil Sci. (China) 18, 231-233.

Liu, J., Zheng, B., Aposhian, V., Zhou, Y., Chen, M., and Waalkes, M. P. 2002. Chronic Arsenic Poisoning from Burning High-Arsenic Containing Coal in GuiZhou, China. Environ. Health Perspectives. 110(2). Pp 119-222.

Mackenzie, F. T., Lnagrzy, R. J., and Patersnon, V., 1979. Global trace metal cycles and predictions. J. Int. Assoc. Math, Geol. vol. II, b pp. 99-142.

Macnair, M.R. 1993. The genetics of metal toleracne in vascular plants. New Phytol. 124, 541-559.

Maher, W. A., 1983. Inorganic arsenic in marine organisms. Mar. Pollut. Bull. 14: 308-310.

Marin, A.R., Masscheleyn, P.H. and Patrick, W.H. Jr. 1993a. Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice. Plant Soil 152, 245-253.

Marin, A.R., Pezeshki, S.R., Masscheleyn, P.H. and Choi, H.S. 1993b. Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosysnthesis of rice plants. J. Plant Nutr. 16, 865-880.

Marin, A.R., Pezeshki, S.R., Massscheleyn, P.H. and Choi, H.S. 1993b. Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. J. Plant Nutr. 16, 865-880.

Masscheleyn, P.H., Dlaune, R.D. and Patrick, W.H. Jr. 1991. Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. Environ. Sci. Technol. 25, 1414-1418.

McLaren, R.G., Megharaj, M. and Naidu, R. 2001. Fate of Arsenic in the Soil Environment, Arsenic in Arsenic in the Asia Pacific Region Workshop, Adelaide, Managing Arsenic for the Future: 27-28.

Meharg, A.A. and Macnair, M.R. 1990. An altered phosphate uptake system in arsenate tolerant *Holcus Lanatus*. New Phytol. 116, 29-35.

Meharg, A.A. and Macnair, M.R. 1992a. Suppression of the high affinity phosphate uptake system: a mechanism of arsenate tolerance in *Holcus Lanatus* L. J. Exp. Bot. 43, 519-514.

Meharg, A.A. and Macnair, M.R. 1992b. Polymorphism and physiology of arsenate tolerance in *Holcus Lanatus* L from an uncontaminated site. Plant Soil 146, 219-225.

Meharg, A.A. and Macnair, M.R. 1994. Relationship between plant phosphorus status and kinetic of arsenate influx in clones of *Deschampsia caspitosa* L. Beauv. that differ in their tolerance to arsenate. Plant Soil 162, 99-106.

Meharg, A.A. and Rahman, M.M. 2002. Arsenic Contamination of Bangladesh Paddy Field Soils: Implications for Rice Contribution to Arsenic Consumption. Environ. Sci. Tecnol., ASAP Artice 10.1021/es0259842 S0013-936X(02)05984-9.

Meyer, L.H. 1978. Food Chemistry, 3rd Edition, The AVI Publishing Company, Inc., Westport Connecticut, USA.

Milam, M.R., Marin, A., Sedberry, J.E. Jr, Bligh, D.P. and Sheppard, R. 1988. Effect of water management, arsenic, and zinc on selected agronomic traits and rice grain yield. In Annual progress report, Northeast Research Station and Macon Ridge Research Station, Baton Rouge, USA, pp 105-108.

Nagy, G. and Korom, I., 1993. Z. Hautkr., 58:961-964.

NAMIC- BAMWSP Tube Well Data Inventory, [URL: www.bamwsp.org].

National Food Authority, Australia, 1993. Australian Food Standard Code: March, 1993. Australian Govt. Pub. Service, Canberra.

Neshiwat L.F., Friedland M.L., Schorr-Lesnick B., Felman S., Glucksman W.J., Russo R.D. 1992. Hepatic Angiosarcoma. Am. J. Jed. 93, 219-222.

Nevens F., Fevery J., Van Steenbergen W., Sciot R., Desmet V., De Groote J. 1990. Arsenic and non-cirrhotic portal hypertension: A report of eight cases. J. Hepatol. 13, 376.

NGO Forum, 2002. Arsenic Pocket let, Ed. Dr. Abul Hasnat Milton, NGO Forum for drinking water supply and sanitation, Lalmatia, Dhaka, Bangladesh.

NHMRC, 1996. Australian Drinking Water Guidelines. National Health and Medical Research Council and Agric. and Resource Mining Council of Australia and New Zealand. Commonwealth of Australia, p. FS93.

Nickson, R.T., McArthur, J.M., Burgess, W., Ahmed, K.Z., Ravenscroft, P. and Rahman, M. 1998. Arsenic poisoning of Bangladesh groundwater. Nature 395, 338-338.

Nickson, R.T., McArthur, J.M., Ravenscroft, P., Burgess, W.G. and Ahmed, K.M. 2000. Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. Appl. Geochem. 15, 403-413.

Nissen, P. and Benson, A.A. 1982. Arsenic metabolism in fresh-water and terrestrial plants. Physiol. Platarum. 54, 446-450.

NRCC (National Research Council of Canada), 1978. Effects of Arsenic in the Canadian Environment. NRCC no. 15391, Ottawa, Canada.

Nriagu J. O., Lin T.S. Trace metals in wild rice sold in the United States. Science of Total Environment, 1995; 172: 223-228.

Nui, S., Cao, S., Shen, E. The status of arsenic poisoning in China. In: Abernathy CO, Calderon RL, Chappell WR, editors. Arsenic: Exposure and Health Effects. New York: Chapman and Hall, 1997.

Odanaka, Y., Tsuchiya, N., matano, O. and Goto, S. 1987. Absorption, translocation and metabolism of the arsenical fungicides, iron methanearsonate and ammonium iron methanearsonate, in rice plants, J. Pesticide Sci. 12, 199-208.

Onken, B.M. and Hossner, L.R., 1995. Plant uptake and determination of arsenic species in soil solution under flooded conditions. J. Environ. Qual. 24, 373-381.

Onken, B.M. and Hossner, L.R., 1996. Determination of arsenic species in soil solution under flooded conditions. Soil Sci. Soc. Am. J. 60, 1385-1392.

Paivoke, A. 1983. The long-term effects of lead and arsenate on the garden pea. Ann. Bot. Fenn. 20, 297-306.

Paliouris, G. and Hutchinson, T.C. 1991. Arsenic, cobalt and nickel tolerances in two populations of *Silence vulgaris* (Moench) Garcke from Ontario, Canada. Ney Phytol. 117, 449-459.

Parsons, R., 1974. STATISTICAL ANALYSIS: A Decision-Making Approach. @1974 Harper & Row, Publishers, Inc ., 10 East 53rd Street, New York, N.Y. 10022.

Pontius F. W., Brown K.G., and Chen C.J. (1994). Health Implications of Arsenic in Drinking water, J.AWWA, 86. pp. 52-63.

Ragaini, R. C., Ralston, H. R. and Roberts, N., 1977. Environmental trace metals contamination in Kellogg, Idahoo near a lead smelting complex. Environ. Sci. Technol. vol. II. pp. 773-781.

Rashid, M. A., Ghani, M. A., and Shah, A. L., 2004. Effect of Arsenic Contamination in Groundwater and its Spatial and Temporal Variation, In: *Proceeding of the workshop on Arsenic in the Food Chain: Assessment of Arsenic in the Water-Soil-Crop Systems* (M A L Shah et al. Eds.), BRRI, Gazipur, Bangladesh.

Raychowdhury T., Uchino T., Tokunaga H., and Ando M. (2002). Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. Food and Chemical Toxicology, 40 p. 1611 – 1621.

Roy Chowdhury, T., Basu, G.K., Samanta, G., Chanda, C.R., Mandal, B.K., Dhar, R.K., Biswas, B.K., Lodh, D., Ray, S.L. and Chakraborty, D. 1998. *In* International Conference on Arsenic Pollution of Groundwater in Bangladesh: Causes, Effects and Remedies, 8-12 February 1998, Dhaka Community Hospital, Dhaka, Bangladesh, pp 157-158.

Samanta, G., Chowdhury, T.R., Mandal, B.K., Chowdhury, U-K, Basu, G.K., Chanda, C.R., Lodh, D. and Chakraborty, D. 1999. Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India and Bangladesh. Microchem.J. 62, 174-191.

Sandberg, G. R., and Allen, I. K., 1975. A proposed arsenic cycle in an agronomic ecosystem. In E. A. Woolson (Ed.) *Arsenical pesticides,* Amercan Chemical Society, Washington DC, pp. 124-147.

Santos, J. K., 1933. Morphology of the flower and mature grain of Phillippine rice. Philippine J. Sci. 52: 475.

Schoof, R.A., Yost, L.J., Eickhoff, J., Crecelius, E.A., Cragin, D.W., Meacher, D.M. and Menzel, D.B. 1999. A market basket survey of inorganic arsenic in food. Food Chem. Toxicol. 37, 839-846.

Shah M Farouk, 2002. Keynote Paper, World Food Day Seminar, BARC, Dhaka, 16 October 2002.

Shah, A. L., Jahiruddin, M., Rahman, M. S., Rashid, M. A., Rashid, M. H., and Ghani, M. A., 2004. Arsenic Accumulation in Rice and Vegetables Grown under Arsenic Contaminated Soil and Water, In: *Proceeding of the workshop on Arsenic in the Food Chain: Assessment of Arsenic in the Water-Soil-Crop Systems* (M A L Shah et al. Eds.), BRRI, Gazipur, Bangladesh.

Sharma, A.K. and Tjell, J.C. 2003. Can Water Storage Habits Influence the Cancer Risk of Drinking Arsenic Contaminated Water? In: Fate of Arsenic in the Environment Ed. M. Feroze Ahmed et al. 2003. BUET-UNU, Dhaka, Bangladesh.

Sharples, J.M., mehagr, A.A., Chambers, S.M. and Cairney, J.W.G. 2000. Mechanism of arsenate resistance in the Ericoid mycorrhizal fungus *Hymenoscyphus ericae*. Plant Physiol. 124, 1327-1334.

Silver, S. and Misra, T.K. 1988. Plasmid-mediated heavy metal resistances. Annu. Rev. Microbiol. 42, 717-743.

Smith, L.H., Smith, J., Smith, E. and Naidu, R. 2001. Assessment of Soil-Plant Transfer of Arsenic from Agricultural Soils: In Book of Abstract, Arsenic in the Asia-Pacific Region Workshop, 2001. p.p. 156-157.

Takamatsu, T., Aoki, H. and Yoshida, T. 1982. Determination of arsenate, arsenite, monomethylarsonate and dimethylarsinate in soil polluted with arsenic. Soil Sci. 133, 239-246.

Talukder, M. H. R., 1982. Indegious Rice Variety, Bangladesh Rice Research Institute, Gazipur.

Tang, T. and Miller, D.M. 1991. Growth and tissue composition of rice grown in soil treated with inorganic copper, nickel, and arsenic. Commun. Soil Sci. Plant Anal. 22, 2037-2045.

Thanabalasingam, P., and Pickering, W. E., 1986. Arsenic sorption by humic aids. *Environ. Pollut.* 12B: 233-246.

The Bangladesh Pure Food Rules, 1967. Ministry of Health and Family Welfare, Government of Bangladesh.

Thompson, D.J., 1993. Chem. Biol. Interactions, 1993, 88:89-114.

Tsai S. M., Wang T.N., Ko, Y.C. 1999. Mortality for certain diseases in areas with high levels of As in drinking water. Arch. Environ. Health, 54, 186-193.

Tseng W.P., Chu H.M., How S.W., Fong J.M., Lin C.S., and Yeh S. 1968. Prevalence of Skin Cancer in an Endemic Area of Chronic Arsenicism in Taiwan. J. of the National Cancer Institute. 40(3). Pp 453-463.

Tseng, W.P., Chen, W.Y., Sung, J.L., Chen, J.S. A clinical study of Blackfoot disease in Taiwan: An endemic peripheral vascular disease. Mem Coll Med Natl Taiwan Univ 1961;7:1-18.

Tsutsumi, M. 1980. Intensification of arsenic toxicity to paddy rice by hydrogen sulphide and ferrous iron I. Induction of bronzing and accumulation in rice by arsenic. Soil Sci. Plant Nutr. 26, 561-569.

Ullah, S. M., 1998, Arsenic Contamination of Groundwater in Irrigated Soils of Bangladesh, International Conference on Arsenic Pollution of Groundwater in

Bangladesh: Causes, Effects and Remedies, Dhaka, Bangladesh, 2-12 February.

Ullrich-Eberius, C.I., Sanz, A. and Novacky, A.J., 1989. Evaluation of arsenate and vandate-associated changes of electrical membrane potential and phosphate transport in *Lemna gibba* GI. J. Exp. Bot. 40, 119-128.

UNICEF, 2001. Arsenic mitigation in Bangladesh. URL: *http://www.unicef.org/arsenic*

Vahter, M. and Marafante, E., 1988. Royal Society of Chemistry, London, 1988, p. 105-119.

Vahter, M., 1994. Review: Species Difference in the Metabolism of Arsenic Compounds, Applied Organometallic Chemistry, 8(3), pp. 175-182.

Viraraghavan, T., Subramanian, K.S. & Swaminathan, T.V. 1994. Drinking Water Without Arsenic: A Review of Treatment Technologies, Environmental Systems Reviews, 37.

WARPO (1999). Topic Paper 7, National Water Management Plan Project, Ministry of Water Resources, Govt. of Bangladesh.

Wells, B.R. and Gilmour, J.T. 1977. Sterility in rice cultivars as influenced by MSMA rate and water management. Agron. J. 69, 451-454.

WHO, 1981. Arsenic: Environmental Health criteria 18. Geneva, Switzerland. World Health Organization.

WHO, 1993. Guidelines for drinking Water Quality. World Health Organization, Geneva, p41.

WHO, 1996. Guidelines for drinking water quality. 2nd ed. Volume 2. p. 158-162.

WHO, 1999. Arsenic in drinking water URL: http://www.who.int/inf-fs/en/fact210.html

WHO, 2001. Arsenic in drinking water URL: http://www.who.int/inf-fs/en/fact210.html

WRI 2000. World Resources 2000-2001 – People and Ecosystem: The Fraying Web of Life, World Resources Institute, Washington, DC.

Wu M., Kuo T., Hwang Y., and Chen C. 1989. Dose-Response Relation between Arsenic concentration in Well water and Mortality from Cancer and Vascular Diseases. 130(6), pp 1123-1132.

Xie Z.M., Xu J.M., Zhang Y.M. and Luo Z.D. 2001. Some Research Advances in Arsenic Contamination and Remediation in China: In Book of Abstract, Arsenic in the Asia-Pacific Region Workshop, 2001. p.p. 97-99.

Xie, Z. and Huang, C. 1994. Relationship between lead, zinc and arsenic contents and rice tillering in the soil rice system. J. Zhejiang Agril. Univ. (China) 20, 67-71.

Xie, Z. and Huang, C.Y. 1998. Control of arsenic toxicity in rice plants grown on an arsenic-polluted paddy soil. Commun. Soil Sci. Plant Anal. 29, 2471-2477.

Yan-Chu, H. 1994. Arsenic distribution in soils. In Arsenic in the environment, Part I Cycling and characterization. Ed. J.O. Nriagu. pp. 17-49. John Wiley & Sons, Inc., New York, USA.

Yasui, A., Tsutsumi, C., and Toda, S., 1978. Selective determination of inorganic arsenic (III), (V) and organic arsenic in biological materials by solvent extraction-atomic absorption spectrophotometer. *Agric., Biol. Chem.* 42: 2139-2145.

Alloway, B. J., 1990. Heavy metals in soil, Jhon Willey and Sons Inc.

Crecelcious, E. A. et al., 1974. Contamination of soils near a copper smelter by arsenic, antimony and lead. J. Water, air and soil pollut. vot. 3, pp. 337-342.

Ragaini, R. C., Ralston, H. R. and Roberts, N., 1977. Environmental trace metals contamination in Kellogg, Idahoo near a lead smelting complex. Environ. Sci. Technol. vol. II. pp. 773-781.

Sandberg, G. R., and Allen, I. K., 1975. A proposed arsenic cycle in an agronomic ecosystem. In E. A. Woolson (Ed.) *Arsenical pesticides*, Amercan Chemical Society, Washington DC, pp. 124-147.

Mackenzie, F. T., Lnagrzy, R. J., and Patersnon, V., 1979. Global trace metal cycles and predictions. J. Int. Assoc. Math, Geol. vol. II, b pp. 99-142.

Clifford, D. A. and Zhang, Z. 1993. Arsenic chemistry and speciation. Proc. AWWA WQTC, Miami, Fla., Nov. 7-11.

Frederick, W. and Coleagues, 1994. Health implications of arsenic in drinking water. Journal AWWA, Sept. 1994.

Appendix A

Table-1: Arsenic in water samples of 13 finalized study fields in Comilla District.

Sl. No.	Field ID	Owner's name	Thana	Union	Village	Mouza	JL No.	Arsenic (ppb)
1.	FDD-6	Md. Habibur Rahman	Debidwar	North Dhamti	Dhamti	Dhamti	213	199
2.	FGB-12	Md. Omar Faruk Majumder	Barura	Galimpur	Jhapua	Jhapua	210	105
3.	FDB-13	Md. Abul Khair	Barura	Deora	Kaichcha Pukuria	Kaichcha Pukuria	216	131
4.	FGB-14	Md. Bappi Mia	Barura	Galimpur	Muggaon	Muggaon	190	188
5.	FGB-15	Arab Ali	Barura	Galimpur	Bauksar	Muggaon	190	156
6.	FGB-16	Ali Azam	Barura	Galimpur	Rari	Rari	208	62
7.	FGB-17	Md. Abdul Hakim	Barura	Galimpur	Bauksar	Muggaon	190	364
8.	FJB-18	Md. Lokman Mia	Barura	Jhalam	Shossia	Shossia	125	208
9.	FJB-19	Ali Ashraf	Barura	Jhalam	Haynna	Felua	123	277
10.	FJB-20	Abdul Latif	Barura	Jhalam	Jhur Pukuria	Felua	123	222
11.	FGB-21	Moulavi Ayub Ali	Barura	Galimpur	Bauksar	Muggaon	190	255
12.	FGB-22	Mafiz Muri	Barura	Galimpur	Shamishpur	Sripur	206	177
13.	FGB-23	Md. Sirajul Islam	Barura	Galimpur	Bauksar	Muggaon	190	278

Appendix B

Table-2: Standard Protein and Amylose content in different Rice Varity.

SL. No.	Variety	Protein %	Amylose %
1	BR1	8.8	25
2	BR3	8.0	26
3	BR8	7.2	27
4	BR9	7.7	27
5	BR10	8.4	26
6	BR11	8.2	26
7	BR12	7.4	27
8	BR14	7.5	27
9	BR15	7.4	26
10	BR17	7.0	27
11	BR18	8.0	27
12	BR19	7.5	26
13	BR20	8.5	25
14	BR21	8.7	25
15	BR22	7.8	26
16	BR25	9.1	25
17	BRRI Dhan 27	7.0	27
18	BRRI Dhan29	7.0	29.4
19	BRRI Dhan 30	8.7	26.7
20	BRRI Dhan 31	8.9	26.5
21	BRRI Dhan 32	7.0	26.3
22	BRRI Dhan 33	8.5	25
23	HBJB-11	9.0	20
24	HBJB-IV	10.5	25
25	HBJB-VI	9.0	22
26	HBJB-VIII	8.7	20
27	IR20	8.4	25
28	BR2	7.9	27
29	BR4	8.2	25
30	BR6	7.1	26
31	BR7	8.0	22
32	BR16	7.3	27
33	BR23	7.8	27
34	BR24	8.9	26
35	BR26	8.4	22.7
36	BRRI Dhan 28	8.6	28
37	BRRI Dhan 36	10.0	25.4
38	BRRI Dhan37	10.3	23.8
39	BRRI Dhan 39	8.4	26.6
40	Pajam	8.3	25
41	Nizersail	8.1	25
42	Latisail	8.9	26
43	BR5	9.1	26
44	BRRI Dhan 34	10.2	23
45	BRRI Dhan 38	8.8	22

Source: BRRI, 1999

Appendix C

Calculation of Arsenic in Rice Plant of BRRI dhan-28 and BRRI dhan-29 varieties

Arsenic concentration in Different parts of rice plant (mg/kg)

Sample ID	Variety	Rice	Husk	Leaf	Stem
FDD-6	BRRI dhan-28	0.56	2.07	7.62	4.94
FDB-13	BRRI dhan-28	0.31	0.83	4.79	3.47
FGB-14	BRRI dhan-28	0.12	1.31	2.89	4.24
FGB-21	BRRI dhan-28	0.17	0.43	5.04	5.13
Average		**0.29**	**1.16**	**5.09**	**4.44**
FGB-16	BRRI dhan-29	0.00	0.49	2.33	0.15
FJB-18	BRRI dhan-29	0.20	1.26	2.09	3.28
Average		**0.10**	**0.88**	**2.21**	**1.72**

Grain weight for a single rice plant of BRRI dhan-28 and BRRI dhan-29 varieties

Variety	No. of filled Spikelets/ m^2	No. of filled Spikelets/ plant	Weight of 1000 grain(g)	Weight of spikeletes (g)/plant	Weight of Brown rice (g)	Weight of Husk (g)
BR-28	33169.96	1326.7984	18.67	24.771326	15.482079	9.2892473
BR-29	36408.87	1456.3548	17.41	25.355137	15.846960	9.5081764

Weight of different parts of rice plant (g)

Variety	Stem	Leaf	Husk	Rice
BRRI dhan-28	84.63	27.68	9.2892473	15.482079
BRRI dhan-29	92.67	34.95	9.5081764	15.846960

* Considering Husk : Rice ratio 3:5 in grain

* No. of plants per sq. meter is 25 (considered as BRRI data)

* Data for plant weight collected from Plant Physiology Division of BRRI

Sample calculation for total accumulated arsenic in different parts of a rice plant

Arsenic in rice of **BRRI dhan-28** = As concentration. x Weight of rice

= 0.29 mg/kg x 15.482079 gm

= 0.29 x 15.482079 / 1000 mg

= 0.00448978 mg

= 4.48978 μg

Total accumulated arsenic by a single plant (excluding root hair) of BRRI dhan-28 & BRRI dhan-29 varieties (mg)

Variety	Rice(mg)	Husk(mg)	Leaf(mg)	Stem(mg)	Total As (mg)
BRRI dhan-28	0.00479942	0.00770987	0.1325872	0.2936661	0.4387626
	0.00866992	0.01922823	0.2109216	0.4180722	0.656892
	0.00185784	0.01216859	0.0799952	0.3588312	0.4528528
	0.00263194	0.00399427	0.1395072	0.43381338	0.5799468
Average	**0.00448978**	**0.01077524**	**0.1407528**	**0.37609572**	**0.5321135**
BRRI dhan-29	0.000	0.00465892	0.0814335	0.0139005	0.0999929
	0.0031694	0.01198008	0.0730455	0.3039576	0.3921526
Average	**0.0015847**	**0.0083195**	**0.0772395**	**0.15892905**	**0.2460728**

Ratio of Accumulated arsenic in different parts of a rice plant

For BRRI dhan-28

Rice : Husk : Leaf : Stem = 1: 2 : 26 : 71

BRRI dhan-29

Rice : Husk : Leaf : Stem = 1: 3 : 31 : 65

Appendix D: Calculation of protein in grain

Experimental procedure:

About 0.2 mg (actual weight noted) of whole grain was taken in Kjeldahl flask and digested with strong H_2SO_4 in presence of catalyst. During digestion $(NH_4)_2SO4$ formed from the protein nitrogen. The mixture was then steam distilled. Ammonia released from digested mixture collected into a conical flask containing boric acid and HCl. Before calculation of protein or nitrogen strength of HCl was measured by titration against a standard base (Sodium Borate of 0.03N). Then percentage of protein was calculated by equations –

$$\text{Nitrogen (\%)} = \frac{(\text{vol. of HCl for sample} - \text{vol. of HCl for blank}) \times \text{N of HCl} \times 0.014 \times 100}{\text{Weight of grain sample}}$$

Protein (%) = Percentage of Nitrogen x 5.95

Where, conversion factor for Nitrogen to protein of rice is 5.95

Sample calculation:

For sample **FGB-17**:

Weight of grain = 0.2014 gm
Strength of HCl = 0.0297N
Volume of HCl for blank = 0.30 ml
Volume of HCl for sample = 6.00 ml

$$\text{Nitrogen (\%)} = \frac{(\text{vol. of HCl for sample} - \text{vol. of HCl for blank}) \times 0.0297 \times 0.014 \times 100}{\text{Weight of grain sample}}$$

$$\text{Nitrogen (\%)} = \frac{(\text{vol. of HCl for sample} - \text{vol. of HCl for blank}) \times 0.04158}{\text{Weight of grain sample}}$$

$$\text{Nitrogen (\%)} = (6.00 - 0.30) \times 0.04158 / 0.2001$$
$$= 1.176$$

$$\text{\% of Protein} = \text{\% of Nitrogen} \times 5.95$$
$$= 1.176 \times 5.95$$
$$= 7.0$$

Table- A: Percentage of Protein content in rice grain.

Sl. No	Sample ID	HCl for Blank (ml)	HCl for sample (ml)	Sample weight (mg)	Nitrogen (%)	Protein (%)
1	FGB-15	0.30	6.25	0.2016	1.227	7.3
2	FGB-17	0.30	6.00	0.2014	1.176	7.0
3	FJB- 19	0.30	5.80	0.2001	1.143	6.8
4	FDD-6	0.30	5.40	0.2035	1.042	6.2
5	FDB-13	0.30	6.00	0.2014	1.176	7.0
6	FGB-14	0.30	6.60	0.1998	1.311	7.8
7	FGB-21	0.30	5.80	0.2030	1.126	6.7
8	FGB-16	0.30	6.00	0.2012	1.176	7.0
9	FJB-18	0.30	5.65	0.2005	1.109	6.6
10	FGB-23	0.30	6.00	0.2014	1.176	7.0
11	FGB-12	0.30	7.50	0.2001	1.496	8.9
12	FJB-20	0.30	6.50	0.2018	1.277	7.6
13	FGB-22	0.30	7.40	0.2019	1.462	8.7

Appendix E : Calculation of Amylose content in grain

Preparation of Standard Amylose Solution

40 mg Amylose has taken into a 100 ml flask and using ethanol, NaOH & Distilled water make the volume up to the mark. Concentration of standard solution is 0.4 mg/ml.

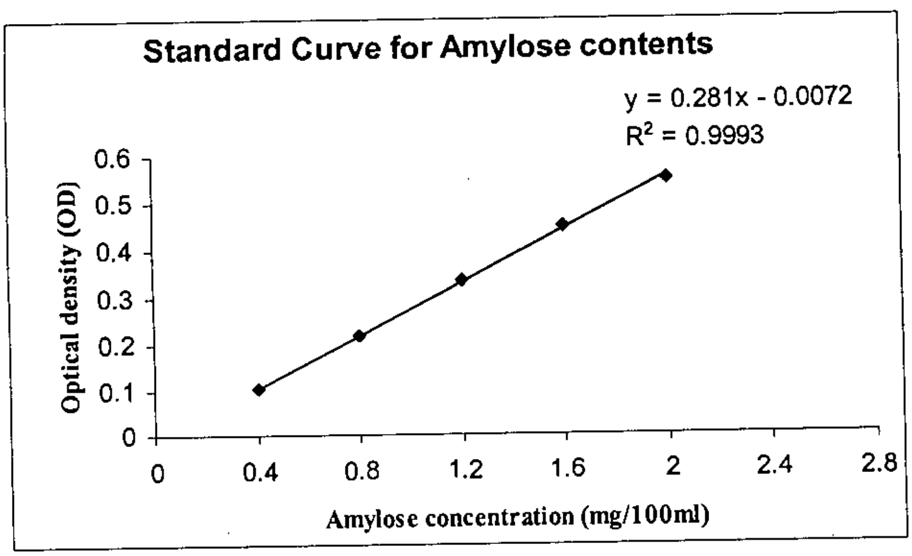
Preparation of Stock Solution

100 mg (0.1 gm) sample (fine powder) has taken in 100 ml flask and using ethanol, NaOH & Distilled water make the volume up to the mark.

Standard Curve Setting

Measure Optical Density (OD) using spectrophotometer at 620 nm for 1, 2, 3, 4 & 5 ml standard solution taking into 100 ml flasks and diluted up to the marks.

Concentration of diluted standard solution (mg/100 ml)	OD
0.4	0.103
0.8	0.216
1.2	0.334
1.6	0.448
2.0	0.549



Amylose content measurement

5 ml of each stock solution of 13 test samples have taken into 100 ml flasks separately and diluted with distilled water up to the volume. Co-factor would be 20. Using spectrometer OD of diluted stock solutions are measured. With the help of standard curve concentration of amylose in diluted solution (mg/100ml) calculated for corresponding mean OD. Finally, multiplying Co-factor with these measured amylose concentration percentage of amylose in grain has determined.

Sample Calculation

From equation of standard curve,

$$x = (y + 0.0072)/0.281$$

Where, x indicates the value of amylose content in diluted solution (mg/100ml)

y is the measured optical density

For the FDD-6, optical density 0.378,

the value of x is, $x = (0.378 + 0.0072)/0.281$

$= 1.37$

Therefore, percentage of amylose in grain is = Co-factor x 1.37

$= 20 \times 1.37 = 27.4$

Table-A: Percentage of Amylose content in grain

Sl. No	Sample ID	Mean Optical Density (OD)	Amylose in diluted stock solution (mg/100ml)	Amylose in grain (%)
1	FDD-6	0.378	1.370	27.4
2	FGB-12	0.350	1.270	25.4
3	FDB-13	0.344	1.250	25.0
4	FGB-14	0.347	1.260	25.2
5	FGB-15	0.374	1.355	27.1
6	FGB-16	0.361	1.310	26.2
7	FGB-17	0.382	1.385	27.7
8	FJB-18	0.360	1.305	26.1
9	FJB-19	0.337	1.225	24.5
10	FJB-20	0.361	1.310	26.2
11	FGB-21	0.365	1.325	26.5
12	FGB-22	0.371	1.345	26.9
13	FGB-23	0.365	1.325	26.5