Implications and prospects of microbial based integrated management of arsenic remediation in rice (*Oryza sativa* L.)

Thesis

SUBMITTED TO THE
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
FOR AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL MICROBIOLOGY

Submitted By

Nisha Bharti

Under the supervision of

Dr. Vinay Singh Baghel

Assistant Professor

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
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ESTABLISHED 1996

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
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CERTIFICATE

This is to certify that the thesis titled **"Implications and prospects of microbial based integrated management of arsenic remediation in rice (*Oryza sativa* L.)"** submitted by **Ms. NISHA BHARTI** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) Regulations – 1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

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Date: 18/12/2017

18/12/2017

Head of Department

Date: 13/12/2017

STUDENT DECLARATION

This is to certify that the material embodies in the present Ph.D. work entitled **"Implications and prospects of microbial based integrated management of arsenic remediation in rice (*Oryza sativa* L.)"** is my original research work done by me. It has not been submitted in part or full for any other diploma or degree in any other University. In this thesis, matter written, data presented plagiarism if any is the sole responsibility of the student Ms. Nisha Bharti. If any allegations/query/question/ copyright arises regarding the thesis myself Ms. Nisha Bharti will be solely responsible and answerable.

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ABBREVIATION

Anova	Analysis of pariance
APX	Ascorbate peroxidase
ASC	Ascorbate
As	Arsenic
As^{+3} /AsIII	Arsenite
As^{+5} /AsV	Arsenate
CAT	Catalase
Cys	Cysteine
CS	Cysteine synthase
DMA	Dimethylarsinic acid
DMRT	Duncan's multiple range test
DTNB	5, 5'- Dithiobistrinitro benzoic acid
dw	Dry weight
EC	Electrical conductivity
EDTA	Ethylene diamine tetraacetic acid
fw	Fresh weight
GDH	Glutathione dehydrogenase
GK	Glutamate kinase
GPX	Guaicol peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione-s-transferase
GW	Ground water
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
MBBr	Mono bromo bimane
MDA	Malondialdehyde
MMA	Monomethylarsonic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
NEM	N-Ethylmaleimide
NPT	Non-protein thiol
$O_2^{\bullet-}$	Superoxide radicals
$OH^{\bullet}$	Hydroxyl radicals
OPT	O-phathaldehyde
PCS	Phytochelatin synthase
PCs	Phytochelatins
PITC	Phenylisothiocynate

PMSF	Phenyl methyl sulphonyl fluoride
PVP	Polyvinyl pyrrolidone
ROS	Reactive oxygen species
SAT	Serine acetyltransferase
Se	Selenium
SeIV	Selenite
SeVI	Selenate
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TEA	Triethylamine
TFA	Trifluoroacetic acid
WB	West Bengal
GW	Ground water

General

h/hrs	Hours
min/mins	Minutes
d	Days
gm	Grams
µg	Microgram
mg	Milligram
kg	Kilogram

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Chapter 1

Introduction

1. INTRODUCTION

Environmental pollution of soil, water and air has become a major challenge for the existing population. Various kinds of pollutants are released into the environment by natural or anthropogenic means like mining, manufacturing, use of pesticides, chemicals, unsafe disposal of industrial effluents and other hazardous substances.

Arsenic (As) is metalloid, being the 20th most abundant element occurs naturally in the earth's crust. It is found in the atmosphere, soils and rocks, natural water, As-contaminated groundwater and organisms. More than 200 As-containing minerals exist; frequently As is associated with sulphur (S) in minerals such as arsenopyrites (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3). Mobilization of As occurs through combination of natural processes such as weathering reactions, biological activity and volcanic emissions as well as through a range of anthropogenic activities (Mandal and Suzuki, 2002). Use of arsenical products such as pesticides and herbicides as well as an additive to livestock feed, particularly for poultry and crop desiccants have increased mobilization of As in the environment. As-like other metals and metalloids stays in the soil for long periods of time, where it can either be taken up by plants or washed down into the groundwater, and present a risk to human health. However, the most important factor determining whether As in the soil gets into the plant-based food crops is the genetic makeup of the plant itself. As usually exists in two different forms: inorganic or organic. Inorganic form is more toxic to plants as

well as humans in comparison to organic form (Tripathi et al., 2007). Inorganic As (Asi) is well known for its carcinogenic effect on human (Tsuji et al., 2007). Most of the poppulation is exposed to As mainly due to drinking water and contaminated food.

Rice (*Oryza sativa*) is a staple food for a large part of the world's human population, especially in tropical Latin America, and East, South and Southeast Asia, making it the second-most consumed cereal grain, after maize. It is also the major crop in areas where severe As contamination occurs; it has been reported to accumulate up to 2 mg Kg^{-1} As in grains and up to 92 mg Kg^{-1} in straw. The diet of many rice consumers is, therefore, under threat from As contamination, which may pose a significant health risk (Kile et al., 2007; Mondal and Polya, 2008; Meharg et al., 2009). This is because paddy rice is rather efficient at As accumulation due to a combination of the anaerobic conditions prevailing in paddy soil, which leads to As(III) mobilization (Takahashi et al., 2004; Williams et al., 2007a,b; Xu et al., 2008), and the inadvertent uptake of As(III) through the rice silicic acid uptake pathway (Ma et al., 2008). This problem is further exacerbated by the widespread contamination of As in paddy fields as a result of irrigation with As-laden groundwater in South Asia (Meharg and Rahman, 2003). As contamination of paddy soils not only compromises food safety but also can cause substantial losses in rice production (Panaullah et al., 2009).

Rice plants acquire essential and beneficial elements from the soil through transporters. But, the selectivity of transporters is imperfect and they can also take up nonessential elements. In inorganic forms of As, As(III) is more toxic than

As(V) and they differ in their mode of toxicity as well as their transport in plants. Arsenate finds its way into plants through phosphate transporters (PHTs). Till date a number of phosphate transporters have been identified for the As(V) uptake in different plants. Arsenate enters into the cell via OsPHT1;1 (Kamiya et al., 2013), OsPHT1;8 (Wu et al., 2011) in rice and AtPHT1;1, AtPHT1;4, AtPHT1;5, AtPHT1;7, AtPHT1;8, AtPHT1;9 in *A. thaliana* (Shin et al., 2004; Catarecha et al., 2007; Remy et al., 2012; LeBlanc et al., 2013; Fontenot et al., 2015). Wang et al. (2016) compared As(V) tolerance of Aus variety Kasalath with *japonica* variety Nipponbare and found Kasalath to be more tolerant to As(V) than Nipponbare. This could be attributed to 2- to 3-fold higher expression of *OsPT2* and *OsPT8*. The *ospt8* mutants of both Kasalath and Nipponbare had a reduction of 33-57% in As(V) uptake and showed increased tolerance to As(V). Thus, OsPT8 was identified as an important transporter for As(V) uptake in rice. The root-to-shoot As(V) transport also occurs through different PHT proteins (Catarecha et al., 2007; Zhao et al., 2010; Mendoza-Cózatl et al., 2011; Wu et al. 2011). The regulators of phosphate transport viz., OsPHF1 (phosphate transporter traffic facilitator 1) and PHR2 (phosphate starvation response 2) also have an effect on As(V) uptake and transport (Wu et al., 2011). In contrast, As(III) and undissociated methylated As species are transported through aquaglyceroporins of various classes and more predominantly via nodulin 26-like intrinsic protein (NIP) class of aquaporin channels (Zhao et al., 2010; Mosa et al., 2012). A NIP class transporter, OsNIP2; 1 (Lsi1) is well known transporter for silicic acid (Si), which has a major role in As(III) uptake (Ma et al., 2008). AtNIP1;1, AtNIP1;2,

AtNIP5;1 (Kamiya and Fujiwara, 2009), AtNIP3;1 (Xu et al., 2008), AtNIP6;1 (Bienert et al., 2008), AtNIP7;1 (Isayenkov and Maathuis, 2008) facilitate As(III) uptake in *A. thaliana* and OsNIP1;1, OsNIP2;2 (OsLsi6), OsNIP3;1 (Ma et al., 2008), OsNIP3;2 (Bienert et al., 2008), OsNIP3;3 (Katsuhara et al., 2014) in rice. Recently, an important role of OsNIP3;2 in As(III) uptake by lateral roots was demonstrated in rice (Chen et al., 2017). Another aquaporin gene (Os07g26630) was upregulated in CN1646-2 and CN1646-5 but down regulated in Nayanmoni. Hence, there may be other aquaporin gene responsible for As uptake and transport, which may show variations in expression in different genotypes (Rai et al., 2015).

Once As enters into the plants, it exists mostly in its reduced form, i.e., As(III) that may get transported to vacuoles either as such via PvACR3 (As Compounds Resistance) in *Pteris vittata* (Indriolo et al., 2010) or after complexation with phytochelatins and then as PC-As(III) complexes via the members of ABC (ATP Binding Cassette) transporter family, ABCC1 and ABCC2 in *Arabidopsis* (Song et al., 2010) and rice (Song et al., 2014). There is a lot yet to be revealed about transporters involved in As loading from xylem to phloem and into seeds. Very recently, progress in this direction has been made and transporters for phloem loading of As in the form of As(III) have been identified as inositol transporters (INTs) known for inositol uptake in phloem in Arabidopsis. The disruption of inositol transporters (INT2 and INT4) in Arabidopsis resulted in decreased As in phloem, silique and seeds as compared to

wild-type plants (Duan et al., 2016). There is need to identify these transporters in rice also.

The uptake of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) has also been found to occur through Lsi1. However, Lsi2 was not found to be permeable to DMA (Li et al., 2009). Inorganic and organic As species differ in their mobility. Zhao et al. (2012) performed an experiment with radioactive As (^{73}As) for 2-4 days and found that out of total As(III) taken up by rice plants, only 10% reached to the shoots and only 3.3% to the grain. In contrast, the mobility of organic As species is greater than inorganic As species (Carey et al., 2010, 2011; Ye et al., 2010). This has been attributed to the phytochelatins (PCs) mediated complexation and storage of inorganic As (Raab et al., 2007; Moore et al., 2014). In the mobility of As, nodes act as a controlling point as they remain connected through their connections to both upper and lower nodes (Yamaji and Ma, 2014). Nodes regulate the As storage and its distribution to the rice grain (Yamaji and Ma, 2014; Zhao et al., 2014). Moore et al. (2014) found much higher concentration of As in the nodes than internodes and leaves. In agreement to earlier studies, Chen et al. (2015) confirmed that rice nodes limited the As(III) distribution into the grain by acting as As(III) filter. The ABCC transporter, localized in tonoplast of phloem cells in nodes, mediates PC-As(III) complex transport to vacuoles (Song et al., 2014). Knockout mutants of *osabcc1* showed higher As accumulation in grains but lower As in nodes than WT (Song et al., 2014). Since OsABCC1 is a vacuolar PC-As(III) transporter, it may sequester PC-As in vacuoles in nodes in WT but not in mutant. Moore et al. (2014) found

OsABCC1 localized in the phloem companion cells of the vascular bundle in nodes strengthening that OsABCC1 inhibits the translocation of As into grains by transporting PC-As complexes into vacuoles of phloem cells in nodes.

The regulation of expression and localization of transporters is also important in As tolerance. AtPht1;1 is regulated by transcription factor WRKY6 and WRKY45 to modulate As(V) uptake (Castrillo et al., 2013; Wang et al., 2014). Mohan et al. (2016) found As(V) tolerance in Arabidopsis mutants for cytokinin signaling. Cytokinin depletion was found to activate a coordinated activation of As(V) tolerance mechanisms that included increased synthesis of PCs and GSH. Hence, cytokinin plays regulatory role in As stress tolerance. Another regulator of NIP1;1 in Arabidopsis has been found to be a calcium-dependent protein kinase (CPK31). The mutant of *cpk31* improved tolerance of Arabidopsis plants similar to *nip1;1* mutation to As(III) and the double mutant *cpk31 nip1;1* had even greater tolerance to As(III) as compared to that of *cpk31* mutant (Ji et al., 2017). Hence, regulatory elements may affect transporter expression and activity to modulate As tolerance. There is need to identify such specific regulators in rice also.

The speciation of As is an important determinant of its uptake and transport in plants. Arsenate reductase (AR) is a crucial enzyme in plants regulating the conversion of As(V) to As(III). Several AR genes have been discovered in plants though with a questionable role /contribution in As(V) reduction (Zhao et al., 2009; Chao et al., 2014). In Arabidopsis, lately two AR genes have been identified namely ATQ1 (arsenate tolerance QTL1; Sánchez-Bermejo et al., 2014)

and HAC1 (High As Content1) (Chao et al., 2014). HAC1 has been found to reduce As(V) to As(III) in the outer cell layer of the root and to facilitate As(III) efflux from the roots to the soil (Chao et al., 2014). In rice also, two orthologous genes of HAC1 viz., OsHAC1;1 and OsHAC1;2 function as As(V) reductases (Shi et al., 2016). OsHAC1;1 and OsHAC1;2 both are expressed mainly in roots. However, their localization is different with OsHAC1;1 being abundant in epidermis, root hairs and pericycle while OsHAC1;2 being predominant in epidermis, outer cortex layers and endodermis. OsHAC1;1 also shows significant expression in stems and nodes (Xu et al., 2017). Xu et al. (2017) have recently identified HAC4 as an As(V) reductase from rice with expression in root elongation and maturation zone in epidermis and exodermis but no expression in leaves. The mutation of OsHAC1;1, OsHAC1;2 (Shi et al., 2016) and OsHAC4 (Xu et al., 2017) led to decrease in As(V) reduction in roots and consequently decreased As(III) efflux and increased As accumulation in shoots. In contrast, overexpression of these genes produced opposite effects.

Glutaredoxins (Grxs) are ubiquitous low molecular weight, cysteine-rich multifunctional proteins that take part in various cellular processes including maintenance and regulation of cellular redox state and protection under oxidative stress (Lillig et al., 2008). Recently, Verma et al. (2016a) characterized a Grx gene from rice (OsGrx) by cloning and expression of OsGrx_C7 and OsGrx_C2.1 in *Escherichia coli* and *Saccharomyces cerevisiae* mutant strains. It was found to result in increased tolerance to As(V) and As(III) presumably through increased As(V) reduction and As(III) extrusion. Over-expression of OsGrx_C7 and

OsGrx_C2.1 in *Arabidopsis thaliana* conferred As tolerance and reduced As accumulation in seeds and shoot tissues compared to WT plants. Thus, OsGrx_C7 and OsGrx_C2.1 are another important determinant of As-stress response in plants (Verma et al., 2016b). Hence, As(V) reduction is an important step of As detoxification in plants both for its onward transport and also for its complexation and storage (Figure 3). Further, As(V) reduction influences the grain As accumulation (Shi et al., 2016).

An important role of transpiration in As translocation from root-to-shoot has been revealed in *P. vittata.* It was observed by Wan et al. (2015) that subjecting the plants to shade to reduce transpiration by 28-67% decreased shoot As by 19-56%. They further compared ecotypes of *P. vittata* from moister and warmer habitat having high transpiration rate with ecotypes from drier and cooler habitat and found that ecotypes with higher transpiration also had higher As in shoot.

Microbes have been considered as a vital component on the earth and significantly contribute in number of biological, physical and chemical processes. Microbes present in rhizospeheric area of rice contaminated with As developed resistance against its toxic impact and influence the mobility, distribution and fate of As (Mukhopadhyay et al., 2002). In addition, microbes control the bigeochemical cycle of As exist in nature (Yin et al., 2011). Microbes transform the toxic As into less toxic one through the process of oxidation, reduction, methylation and voltalization (Bolan et al., 2014). Singh et al. (2016) also reported that the phosphate growth promoting bacteria (PGPB) facilitate the plant growth through

secretion and synthesis of siderophore, organic acids, inodle acetic acids, phyotcheletins and other metabolites Thus microbes directly or indirectly participate in the detoxification of As contamination in rice (Qin et al., 2009).

In the present study, As resistant bacteria were isolated from As contaminated sites of paddy field from West Bengal, India and experiments were carried out to investigate the role of isolated resistant bacteria at different concentration of As after that examined the morphology and biochemical parameters. In a recent study I have examined the role of As resistant bacteria on growth, translocation and accumulation of As and also show effect on biochemical parameters and tolerance responses in agriculture crop rice in pot experiments. At last I was trying to find out the impact of As and As-resistant bacteria alone and combination on translocation and accumulation of mineral nutrient and biogeochemistry of As. Further it was also prospects in term of growth, toxicity and yield of the plants.

In view of the above the following objectives has been taken for the study:

1. To isolate arsenic resistant bacteria from arsenic affected area of West Bengal, India.
2. Characterization of arsenic resistant bacteria.
3. Arsenic accumulation in rice and role of arsenic resistant rhizospheric bacteria.
4. Different study on arsenic exposed rice.

Chapter 2

Review of Literature

2. REVIEW OF LITERATURE

Heavy metals (HMs) are essentially those chemical elements which have a specific gravity five times that of water (Hossain et al., 2012). HMs are natural components of the Earth's crust. Under physiological conditions, HMs are classified as essential and nonessential elements for the organisms. Essential metals such as copper (Cu), zinc (Zn), cobalt (Co), manganese (Mn), molybdenum (Mo) etc. play very important role in physiological processes of living organisms such as co-factor in redox reactions and ligand interactions. Whereas non-essential metals such as arsenic (As), cadmium (Cd),lead (Pb), chromium (Cr), mercury (Hg) etc. are not required by organisms but they interfere the physiological processes by disturbing enzymatic reactions due to their reactivity with thiols or other groups (Rahman et al., 2012). The main threats to human health from heavy metals are associated with exposure to As, Cd, Cr and Pb (As is a metalloid, but is usually classified as a heavy metal). These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the World Health Organization (WHO). People are exposed to these potentially harmful heavy metals due to their presence in air, food, water and soil. Though these heavy metals are present in most foodstuffs, their exposure to human beings varies considerably due to differences in dietary habits. Similar to other biotic and abiotic stresses, heavy metal exposure also induces oxidative stress and modulates gene expression (Shri et al., 2009). Though various studies have been carried out to study molecular networks

involved in different heavy metal stresses in plants, there are no reports addressing the changes to genome-wide transcriptome in response to different heavy metal stresses.

2.1. Arsenic

In nature, As and its compounds are present almost everywhere. Arsenic in its natural state is found primarily in the form of sulphides in association with those of silver (Ag), Cu, iron (Fe), Pb, nickel (Ni)and antimony (Sb) in ores.Arsenic a ubiquitously present toxic element has recently gained much attention. It has been detected in the ground waters of several regions of the world, especially in West Bengal, India. The levels of As in these aquifers exceed the WHO drinking water guideline value of 10 µg/L (Tripathi et al., 2007) as well as the national regulatory standards (e.g. 50 µg/L in India and Bangladesh, Ahamed et al., 2006; Hoque et al., 2012). This is a threat to the health of millions as also to sustainable agriculture.

Arsenic is a toxic and carcinogenic metalloid whose occurrence is quite widespread due to its release in large amounts as a consequence of geological activities and/or anthropogenic activities (Arain et al., 2008, 2009; Baig et al., 2009; Dwivedi et al., 2010a). Clinical manifestations of As poisoning begin with various forms of skin disease and progress via damage to internal organs ultimately to cancer and death (Hossain, 2006). Melanosis and Keratosis diseases are common indicators of As poisoning. Melanosis results in the gradual change of complexions towards blackishness and or duskiness. Generally, the limbs are

first affected and subsequently the change affects all of the body. Melanosis is generally a pre cursor of cancer. The initial stages of Keratosis witnesses the hardening hand palms and footsoles and may gradually lead to gangrenous ulcer (Bridge and Husain, 2006).

Hazardous effect of As has become a global concern because of the ever increasing contamination in drinking water, soil, and crops in many regions of the world (Kahlown et al., 2002; Mukherjee et al., 2005; Farooqi et al., 2007; Awasthi et al., 2017).

Arsenic in aquifers is mostly of geologic origins, but in some locations anthropogenic inputs can be extremely important. The use of such aquifers with geogenicAs is leading to chronic health disorders in most of the affected regions of the world (Bhattacharya et al, 2007; Smedley and Kinniburgh, 2002). The impact of As toxicity is particularly alarming in Asia. In the Bengal Basin of Bangladesh and West Bengal, India (Bhattacharya et al., 2007; Mukherjee and Bhattacharya, 2001) over 100 million people at risk of cancer and other As-related diseases due to groundwater As poisoning. Therefore, As in groundwater is now touted as the largest environmental health disaster. Recent studies indicate the occurrence of geogenic As in the Central Gangetic Plains of Uttar Pradesh, Bihar, Jharkhand and the Brahmaputra valley in Assam, and several regions of Madhya Pradesh and Chattisgarh, India (Bhattacharya et al, 2007; Chakraborti et al., 2004).

The concentrations of As in shallow alluvial aquifers of West Bengal, India and Bangladesh is very high exceeding the recommended drinking water

standards (10 µg/L WHO, 1993; 50 µg/L in India and Bangladesh) and the regional standard of, and are a major health concern for the millions of people who rely on these aquifers for drinking water (Stollenwerk et al, 2007; Das et al., 1996).

Arsenic is derived from erosion of lithified sediments and crystalline rocks of the Himalayan range and adjacent regions (Stollenwerk et al, 2007; Breit et al., 2003). Sediment is deposited on floodplains and across the lower delta where it is subjected to intense chemical weathering. Variable redox conditions and mineral dissolution/precipitation redistribute As among the solid phases (Acharyya et al., 1999; Breit et al., 2003). The most widely accepted mechanism for high concentrations of As in groundwater is reduction of Fe(III) oxides and release of associated As to solution (Bhattacharya et al., 1997; Nickson et al., 1998; McArthur et al., 2001). Most high-As groundwater is found in wells 100 m deep, that are screened in Holocene alluvial and deltaic deposits (Ravenscroft et al., 2005; Kinniburgh et al., 2003; Stollenwerk et al, 2007). The reason for release of As at shallow depths has been attributed to organic matter presence, acting as catalyst at shallow depths to release As from iron-oxides (McArthur et al., 2004). The uneven distribution in groundwaters and absence of As in deep waters in West Bengal may be due to formation of palaeosol in some areas in pliestocene era which resisted percolation of As laden waters (Mc Arthur et al., 2010; Hoque et al., 2012).

Chakraborti et al (2003) reported As concentrations of 300–1,000 µg L^{-1} in groundwaters of West Bengal, Bangladesh, Bihar, and also high concentrations in

human urine (570- 2,349 μg kg^{-1}), hair (1,935-8,471 μg kg^{-1}) and nail (2,844-7,923 μg kg^{-1}). Bhattacharya et al (2009) reported As levels of 1,380 – 12,270 μg kg^{-1} in soils and 110 – 760 μg L^{-1} in groundwater. A later study (Chakraborti et al 2009) on As concentrations in varying depths of groundwaters of West Bengal revealed that As concentrations were lower in deeper aquifers. Chauhan et al (2009) reported As concentrations of upto 34.55 and 13 μg L^{-1} in shallow and deep groundwaters in Ballia, Uttar Pradesh, India. Chatterjee et al (2010) have reported high As consumption through drinking water **(**18 – 39 μg L^{-1})and through rice **(**80 – 200 μg kg^{-1} dw) (Table 2.1.). Even retting of jute is now said to be contributing to As contamination of surface waters (Majumder et al., 2013).

It is becoming apparent that ingestion of drinking water is not the only elevated source of As to the diet in Bengal delta. Irrigation of agricultural fields with As-contaminated ground- water has led to As build-up in soil (Meharg and Rahman, 2003), with subsequent elevation of As in crops grown on these soils (Roychowdhury et al., 2008; Alam et al., 2003). The As species reported in groundwater from Bengal delta is inorganic (Rasul et al., 2002; Tokunaga et al., 2005). Inorganic As contributed more than 90% of the total content of As in crops, grown on As-contaminated soil (Chowdhury et al., 2001; Zhao et al., 2006)

World over high concentrations have been reported in grains of various food crops (Meharg et al, 2009; Baig et al, 2010; Dwivedi et al., 2010, 2014; Mishra et al., 2016; Uraguchi et al., 2017; Awasthi et al., 2017).

2.2. Arsenic-resistant bacteria and there remediation approach

Soil microbial activities could affect the mobility and bioavailability of metal(loid)s (Singh et al. 2010). Especially, some rhizobacteria with the characters of synthesizing phytohormone, producing siderophore, fixingnitrogen, or solubilizing phosphorus and other nutrients (Passardi et al. 2004), could promote the growth ofplant (plant growth–promoting rhizobacterium, PGPR; Belimov et al. 2004). Inoculation of PGPR resulted inreduction of arsenic toxicity to Pi. *Calomelanos* (Jankonget al. 2007) and *P. Vittata* (Yang et al. 2011). Therefore, inoculation of arsenic-resistant PGPR with poplarmay improve the phytoremediation efficiency inarsenic-contaminated soils.

The natural abundance of arsenic in the environment, representatives from various bacterial genera have developed different resistance mechanisms for arsenic compounds (Mukhopadhyay et al., 2002; Rosen, 2002). While arsenate enters into the microbial cells via transmembrane phosphate transport proteins (Cervantes et al., 1994), arsenite enters at neutral pH via aquaglyceroporins (Wysocki et al., 2001). The bacterial resistance with regard to reduction of arsenate or oxidation of arsenite can be divided into two basic categories consisting of either detoxification reactions that confer arsenic resistance, or redox reactions that conserve the energy gained by the reactions for cell growth (Stolz et al., 2002; Silver and Phung, 2005).

Many researchers have reported that microbial application could reduce the toxicity of metal and metalloids and sustain plant growth at high toxic level of As (Huang et al. 2010; Singh et al., 2016). Microorganisms can transform As(III) to

less toxic form and contribute in natural attenuation of As pollution from the environment. Microbes present in As contaminated land adopt difference resistance mechanism to protect themselves against stress (Mukhopdhyay et al., 2002). Rhizospheric microbes altered the bio accessibility of As by changing soil pH, redox balance and precipitation etc. (Huang et al., 2010). Mukhopadhyay et al. (2002) has reported that the biogeochemical cycle of As depends on microbial mediated oxidation, reduction and methylation processes which influence the mobility and distribution of As species in the environment. In this way, microbes also control the stress induced oxidative damage response, growth, biomass and mineral content in paddy plant grown in As contaminated soil. Bacteria present in paddy field modulate the accessibility of As to the rice plants. Alteration in toxicity associated with As poisoning in rice is exhibited by reduced accumulation followed by diminished antioxidant responses and induced growth responses in rice (Singh et al., 2016).

Many arsenic resistant microbes were reported which can withstand high concentration of arsenic, can be potentially used for the bioremediation of arsenic from arsenic contaminated ground water. Chowdhury et al. (2009) isolated a novel strain, Planococcus KRPC10YT from arsenic contaminated bore-well of West Bengal, India which can tolerate up to 30 mM arsenate and 20 mM arsenite. Shivaji et al. (2005) found a novel arsenic-resistant strain, Bacillus arsenicus from arsenic contaminated soils in Chakdah district of West Bengal, India which was able to grow in the presence of 20 mM arsenate and 0.5 mM arsenite. But very limited works have been done toward bioremediation of arsenic using the arsenic

resistant bacteria. Purbasthali block of Burdwan, West Bengal, India is severely affected with arsenic. According to Roy et al. (2013), the arsenic concentration in the tube well water of this area is 0.076–0.205 ppm. But no research has been conducted to isolate arsenic resistant bacteria from this particular affected area and also to apply these bacteria in bioremediation of arsenic contaminated ground water till date. In this present study, two arsenic resistant bacteria are being reported which were resistant to very high concentration of arsenate and arsenite, from the arsenic contaminated water of Purbasthali and are also able to reduce arsenic concentration from contaminated water.

Bacterial populations associated with arsenic transformations have been characterized from diverse environments such as in oxic environments (Macur et al., 2004) and in anoxic sediments of lakes and rivers naturally contaminated with arsenic (Cummings et al., 1999; Oremland et al., 2005). The role of dissimilatory arsenate reducing bacteria in arsenic release into groundwater of sedimentary aquifers of Bengal delta has been proved recently (Islam et al., 2004). Similarly, As(V)-reducing bacteria have been also found to mediate the reduction of As(V) under highly aerobic conditions resulting in enhanced mobilization of arsenic from limed mine tailings (Macur et al., 2001). Microbial reduction of As(V) may occur via respiratory reduction, as microorganisms use As(V) as the terminal electron acceptor in anaerobic respiration (Mukhopadhyay et al., 2002; Stolz et al. 2002, 2006; Lloyd and Oremland, 2006), e.g. bacteria (*Sulfurospirillum barnesii, Bacillus arsenicoselenatis, Bacillus selenitireducens, Sulfurospirillum arsenophilum, Desulfotomaculum auripigmentum, Chrysiogenes arsenatis* and

Desulfomicrobium strain Ben-RB) (Newman et al., 1997b). Arsenic detoxification has been documented in *E. coli, Staphylococcus aureus* and *Staphylococcus xylosis*, and is controlled by ars genes that encode for As (V) (Cervantes et al., 1994). Microbial methylation allows the transformation of aqueous- or solid-associated inorganic As into gaseous arsines and leaves from the living medium, which is usually regarded as a detoxification (Jia et al., 2013). Demethylation may occur under oxic and anoxic conditions but is usually faster under oxic conditions (Huang et al., 2007). Elimination of the organic moieties not only increases the general toxicity of As but also decreases its mobility. Thus, demethylation is apparently not suitable for the purpose of remediation and therefore draws relatively few research interests. Only a mixed culture could perform the complete process of demethylation, demonstrating that monomethylarsonic acid demethylation to As(III) is a two-step process.

2.3. Arsenic uptake, speciation and transport *in planta*

Rice (*Oryza sativa* L.) is much more efficient in As accumulation than other cereals such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), with its As TF often approaching unity. The relatively high As accumulation in rice is due to two reasons: (a) enhanced As bioavailability under the anaerobic conditions of submerged paddy soils; and (b) the inadvertent uptake and transport of arsenite through the Si pathway, which is highly efficient in rice (Zhao et al., 2010).

Arsenite may be partly oxidized to arsenate in the rhizosphere because of the oxygen release through the arenchyma tissue of wetland plant roots. Moreover, ferrous iron is oxidized to form Fe hydroxide/oxyhydroxide precipitate [mostly ferrihydride: Fe(OH)3], which is then coated onto the root surface forming an Fe plaque. The Fe plaque was found to have a significant effect on the absorption ki-netics of As by rice roots, decreasing arsenate uptake but increasing arsenite uptake (Zhao et al., 2010). Although arsenate is the main species taken up by plants growing in aerobic soils, there is evidence of the presence of arsenite in the rhizosphere. The occurrence of arsenite is likely a result of arsenite efflux from roots. Arsenite effluxed by roots may be re-absorbed by the roots or oxidized to arsenate in the rhizosphere.

Arsenate, the dominant form of As in aerobic conditions, is taken up by plants via the phosphate transport systems since it is chemically analogous to phosphate (Abedin et al., 2002a,b). Deficiency or starvation of P can enhance As(V) uptake and accumulation by grasses (e.g. *H. lanatus*) (Meharg and Macnair, 1992a) and *P. vittata* (Wang et al., 2002). Uptake of As(III) is known to occur through aquaglyceroporins (Abedin et al., 2002a; Meharg and Jardine, 2003). Abedin et al. (2002a) showed that uptake of both As(III) and As(V) was hyperbolic rather than linear and there are two uptake systems for both As(V) and As(III) in roots. One system dominates at lower substrate concentrations (high affinity uptake systems) and another at high substrate concentrations (low affinity uptake systems). Both carriers obey saturation kinetics.

Uptake of organic species of As, DMA and MMA is also known to take place (Meharg, 2004), however role of any transporter has not yet been implicated. Marin et al. (1992, 1993a) found higher uptake of organic species (MMA and DMA) when rice plants were treated with these species in hydroponic culture. The phytoavailability (measured as long-term As uptake) of four As species in hydroponic systems, to *Spartinapatense*followed the trend: DMA < MMA ≈ As(V) < As(III) (Carbonell-Barrachina et al., 1998) while the uptake of As by rice in long-term hydroponic culture was DMA < As(V) < MMA < As(III) (Marin et al., 1992). Differences in translocation of the four As species to the shoots were also observed in both studies, with a greater proportion of MMA and DMA translocated to the shoots compared with As(V) and As(III). However, it has been shown for rice that the short-term uptake of MMA and DMA is considerably less than that of As(V) and As(III) (Abedin et al., 2002a). Recently it was demonstrated that plants are unable to methylate Asi and instead take up methylated As produced by microorganisms in the soil environment (Lomax et al., 2012).

Inside the plant, various transformations and distribution take place depending on the plant species. Arsenate in the plants is converted to As(III) and then methylated in the leaves, accompanied by the induction of As methyltransferase activities (Meharg and Hartley-Whitaker, 2002; Zhao et al., 2006). Koch et al. (2000) reported that the predominant As species in terrestrial plants are inorganic forms, of which up to 50% are present as As(III), whereas only small amounts of the methylated As compounds are present in some plants.

Studies on *P. vittata* have shown that almost all As in this plant is present as inorganic forms, with much greater amounts of As(III) in the fronds (47–80%) than in the roots (8.3%) (Ma et al., 2001; Zhang et al., 2002; Zhao et al., 2003). Lower terrestrial plants like fern species readily reduce As(V) to As(III) but show limited methylation of As (Meharg and Hartley-Whitaker, 2002).

Arsenite uptake is of particular importance for rice with their roots growing in anaerobic or semi-anaerobic environments. Ma et al. (2008) have identified OsNIP2;1, also named Lsi1 because of its primary function as a silicon (Si) transporter (Ma et al., 2006), as a major pathway for the entry of arsenite into rice roots. Expression of Lsi1 in *Xenopus laevis* oocytes and in yeast markedly increased the uptake of arsenite, but not of arsenate. These data indicate that arsenite shares the Si transport pathway for entry into rice root cells. Ma et al. (2008) showed that, in addition to Lsi1, three other NIP (nodulin26-like intrinsic proteins (NIPs), a subfamily of the plant aquaporin family) channel proteins in rice, OsNIP1;1, OsNIP2;2 (also named Lsi6) and OsNIP3;1, are also able to mediate arsenite influx into X. laevis oocytes expressing these genes (Zhao et al., 2008). Arsenite and undissociated methylated As species are transported through the nodulin 26-like intrinsic (NIP) aquaporin channels (Zhao et al., 2010; Mosa et al., 2012). Srivastava et al. (2013) observed down-regulation of aquaporins in *Brassica juncea* presumably to regulate AsIII levels, though alongwith, reduced growth, disturbed water balance and induced oxidative stress.

2.4. Arsenic accumulation in rice plants

Rice (*Oryza sativa*) is an important source of inorganic As (iAs) exposure for populations dependent on a staple rice diet (Meharg, 2004; Meharg and Rahman, 2003; Mondal and Polya, 2008; Ravenscroft, 2007) in South Asia. In the Bengal Delta a person consumes a minimum of approximately 300 g of rice per day as subsistence diet, which thus comprises > 80% of calorific intake (Spallholz et al., 2008). Thus, intake of iAs through rice has been proposed to be a concern in relation to rice consumption (Meharg et al., 2009; Stone, 2008). Arsenic concentrations of 80-2050 μg kg^{-1}dw have been reported in rice (Roychowdhary et al., 2008; Williams et al., 2005, Islam et al., 2004, Liu et al., 2004).

Various studies have concentrated on accumulation and speciation of As in rice plants, which is a major staple crop of world and is extensively cultivated in areas which are most severely contaminated with As, including India, Bangladesh, China and Taiwan. Accumulation of As in rice depends on many factors such as climate, soil type and rice variety. Onken and Hossner (1995) grew rice (*Oryza sativa*) in two soil types treated with up to 45 μg As(III) or As(V) g^{-1} for 60 days.

It has been observed recently that the application of organic matter and flooding led to an increase in accumulation of arsenic within rice grains (Norton et al., 2013). The As concentration of rice plants correlated with the mean soil solution As(V) concentration in the clay soil and to the mean soil solution As(III) for the silt loam. The rate of As uptake by plants increased as the rate of plant growth increased. Abedin et al. (2002a) studied uptake kinetics of different species of As in rice plants. DMA and MMA showed much lower rates of uptake than As(V) and As(III). Phosphate application did not affect As(III) uptake and

toxicity but reduced As(V) uptake and toxicity. DMA and MMA also showed restricted translocation with 0-5% of total As in rice straw being DMA. However, others show that DMA may be major component of As in rice grain (Schoof et al., 1999; Heitkemper et al., 2001). When irrigated with As(V) contaminated water, rice plants have been found to accumulate As to levels of 1.8 μg g^{-1}dw in seeds and up to 92 μg g^{-1} As in rice straw (Abedin et al., 2002a; Meharg and Rahman, 2003). Liu et al. (2004) studied the effect of P nutrition and iron plaque formation on As uptake by and translocation within the rice seedlings. Total uptake of As in –P plans (around 150–225 μg g^{-1} dw) was significantly higher than the +P plants (around 50–65 μg g^{-1} dw). In seedlings grown with P, most of the As accumulated in roots and shoots while in –P seedlings, most of As was retained in iron plaque. In –P +As plants, only 2-3% of the total As was taken up by plants, whereas in +P+As plants, 15-23% of total As was transported to shoots. This was due to more iron plaque formation on rice roots of –P plants than +P and greater retention of As in iron plaque.

Williams et al. (2005) conducted a survey of As speciation in different rice varieties from different parts of the globe. They found that USA long grain rice had the highest mean grain As level of 0.26 μg g^{-1}dw and the highest grain As (0.40 μg g^{-1}dw). The mean As level from Bangladeshi rice was 0.13 μg g^{-1}dw, in a range from 0.03 to 0.30 μg g^{-1}dw. However, large variability exists in the mean As values of other Bangladeshi rice surveys, 0.10-0.95 μg g^{-1}dw with the maximum level of As reported in a rice grain sample being 2.05 μg g^{-1}dw (Islam et al., 2004). European rice had a mean As level of 0.18 μg g^{-1}dw, in a range from

0.13 to 0.22 μg g^{-1}dw. Basmati rice from India possessed the lowest mean As level at 0.05 μg g^{-1}dw, with little variation, only in the range of 0.03 to 0.08 μg g^{-1}dw. The main As species detected in the rice grain were As(III), DMA^V, and As(V). In European, Bangladeshi, and Indian rice 64, 80, and 81%, respectively, of the recovered As was found to be inorganic. In contrast, DMA^V was the predominant species in rice from USA, with only 42% of the As being inorganic. In the pot experiments, in all treated plants, grain As rise sharply with increasing shoot As only up to a threshold concentration of 2 μg g^{-1}dw, beyond which grain As plateaus at higher shoot As concentrations. Extensive investigations indicate that As bioavailability in rice is highly dependent on As speciation, which in turn can vary depending on rice cultivar, As in irrigation water, and the presence and nature of As speciation in cooking water (Juhasz et al., 2006).

Su et al. (2010) have observed that across two As exposure forms, the shoot As concentration in rice was 2.2- and 4.6-fold higher than that in wheat and barley, respectively. In both arsenate and arsenite treatments, shoot As concentration followed the order of rice>wheat>barley. In the arsenate treatment, rice and wheat had similar concentrations of As in roots, which was 20–25% higher than that of barley. In the arsenite treatment, root As concentration was the highest in rice, which was 90% higher than that in wheat or barley. Among the three plant species tested, the efficiency of As distribution from roots to shoots follows the order of rice>wheat>barley, and between the two As exposure forms, arsenite>arsenate. The translocation efficiency in rice was 2.5–4.4 times that in barley, and 68–78% higher than that in wheat.

2.5. Role of iron plaque formation in arsenic uptake

Iron plaque (IP) formation occurs commonly on the surface of roots of aquatic and wetland plants. It is either amorphous or crystalline in structure, containing ferric hydroxides, goethite, and lepidocrocite (Bacha et al., 1977; Chen et al., 1980). It is composed mainly of ferrihydrite $(Fe^{+3})_2 .5H_2O$ (63%) and goethite FeO(OH) (32%), with minor levels of siderite Fe CO_3 (5%) (Hansel et al., 2001). The formation of IP is considered to be a consequence of the oxidation of ferrous (Fe^{II}) to ferric (Fe^{III}) iron and the precipitation of iron oxide on the root surface, involving radial oxygen loss from plants, and biological oxidation by microorganisms. (Levan et al., 1986; Emerson et al., 1999)The importance of IP in sequestration and translocation of elements has recently been envisaged for designing strategies for reducing the translocation of toxic metals and metalloids (Liu et al., 2004) in rice plants, which is a semi-aquatic plant showing varietal differences in IP formation (Dwivedi et al., 2010). This review presents a comprehensive compilation of the available literature on the role of IP in metal and metalloid sequestration, uptake, and translocation, discussing the possible implications of this natural plant phenomenon.

Root plaque may also contain a variety of other metals and metalloids, such as aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni) andPb. Under laboratory conditions IP formation was similar in structure and distribution, but differed in their chemical composition. In the presence of Fe and phosphorus (P), the plaque was composed of either iron

phosphate or iron oxide, with P adsorbed onto the surface and Fe forming iron phosphate complexes. However, under field condition, IP formed on the roots of *P. australis* had high levels of Al and silicon (Si) associated with Fe, while P was not present (Batty et al., 2000). Blute et al. (2004) found that As oxidation state microtomograms showed that arsenite (As^{III}) and arsenate (As^{V}) were distributed heterogeneously in cattail root IP.

2.5.1. Role of microbes in iron plaque formation

The discovery of both lithotrophic Fe-oxidizing bacteria (FeOB) (Emerson et al., 1999) and Fe-reducing bacteria (FeRB) on the roots of wetland plants indicates that plaque associated microbes may directly influence IP formation. Neubauer et al. (2007) determined that the FeOB *Sideroxydans paludicola* (strain BrT) affected the total rates of rhizospheric Fe^{II} oxidation and IP formation. They concluded that the FeOB increased Fe^{II} oxidation rates by 1.3 to 1.7 times compared to an environment free of FeOB. Emerson et al., 1999found that there was a positive correlation between cell numbers of FeOB and the total amount of Fe present in *S. australis* (long beak arrow head), but the same correlation was not found for *Leersia oryzoides* (rice cutgrass). Weiss et al., 2004 demonstrated that the percentage of poorly crystalline Fe^{III} was significantly correlated with the percentage of (FeRB). DNA analysis revealed that Fe-oxyhydroxide nodules predominantly contain encrusting FeOB, such as *Acidithiobacillus ferrooxidans,* however, in the surrounding organic rich silty sediments almost no FeOB were detected (Joseph et al., 2003). This distinctive microbial zoning in and around the

Fe-oxyhydroxide nodules suggests that FeOB activity probably plays a crucial role in the accumulation and preservation of Fe-oxyhydroxides in nodules in a reducing environment. Furthermore, Emerson et al., 1999 identified the presence of FeOB in the rhizosphere of four species of wetland plants in a diverse wetland environment, in which the Fe^{II} concentrations ranged from tens to hundreds of micromoles per liter and had a pH range of 3.5 to 6.8. Enrichments for neutrophilic, putatively lithotrophic FeOB were successful on roots from all four species; acidophilic FeOB were enriched only on roots from plants whose root systems were exposed to soil solutions with a pH of <4. Similarly in *S. australis* there was a positive correlation between cell numbers of FeOB and the total amount of Fe present. Weiss et al., 2007 characterized a new genus of FeOB, *Ferritrophic umradiciola,* from the rhizosphere of wetland plants. Wang et al.[63] concluded that As mobility was inhibited by bacteria, especially in the presence of anthraquinone-2,6-disulfonic acid, a chemical that acts as a electron shuttle. Küsel et al., 2003 summarized that microbial activity influences the cycling of Fe in the rhizosphere, which supported the plant growth of *J. bulbosus*. Moreover, Hohmann et al. (2010) demonstrated that Fe^{II} oxidizing bacteria effectively mobilized As during Fe^{II} oxidation, however, Xinjun et al., 2009concluded that microbial Fe reduction induced the formation of more crystalline Fe minerals, leading to As sequestration.

2.5.2. Role of iron plaque in rice with respect to arsenic accumulation

IP formation on rice roots has been extensively studied with special emphasis on As accumulation (Geng et al., 2005; Dwievi et al., 2010). Irrigation of paddy fields with As-contaminated ground water particularly in South-East Asia, has caused accumulation of As in both soils and plants, which poses long term risks to soil and human health. Rice is one of the most important staple crop with nearly half of the world's population dependent on it for food (Zhao et al., 2010) and it's contamination with As (a class 1 human carcinogen) is a major issue. Rice cultivation in areas contaminated with Asleads to high concentrations of As in grains. Recently, Zavala and Duxbury (2008) reported a range of As concentrations from 0.005 to 0.710 mg kg^{-1} in rice grains for different countries. In addition, geographical variation in total and inorganic As have been reported. Daily intake of inorganic As loaded rice grains may be a potential As exposure pathways for humans, leading to serious health hazards i.e. increasing the risk of bladder, lung, skin and prostate cancer. Rice was found to contribute 44% to average inorganic As intake in a study carried out in West Bengal, India (Mondal and Polya, 2008). In the above context, there has been global concern about the strategies to mitigate As hazard either through the development of rice cultivars, which sequester most of the As in their roots by manipulation of the rhizospheric zone, or by the selection of low grain arsenic accumulating cultivars (Norton et al., 2009; Tuli et al., 2010).

Amongst various strategies to reduce As accumulation, selection of rice cultivars on their basis to produce IP could be an important criteria. It is well known that paddy rice forms IP under laboratory and field conditions. IP has been

shown to alleviate Cu, Ni and Zn toxicities. IP also change the uptake and translocation of P, and As, during solution culture experiments. IP deposited on the rice roots is uneven from root tip to root base and follows the order root-tip>middle-section>root-base. Sequential extraction and XANES data showed that As in IP was sequestered mainly with amorphous and crystalline iron oxides, and that As^V was the predominant species. Considering the importance of IP in the sequestration of As (Liuet al., 2006) and their correlation with other adsorbed metals in plaque, a number of studies were carried out to find out its role as buffer or barrier. Recently, μXANES study indicated that Fe plaque does not directly intercept As supply to rice roots (Seyfferth et al., 2010).

Recently, Dwivedi et al. (2010) observed during field trials that As concentrations in IP on the roots were positively correlated with adsorbed Fe. Similarly a significant correlation was established between the concentrations of Fe and As in IP with about 75%-89% of total As concentrated in IP.As concentrations on the root surface were significantly higher in plants grown in nutrient solution without phosphorus (-P) than in plants with phosphorus (+P). However, As accumulation in shoots were significantly lower in –P plants than in +P plants. This indicated that IP enhanced As adsorption but reduced its translocation from roots to shoots. Meng et al. (2002) found that Fe hydroxides in soil or solution had a very strong binding affinity for As^V however Fe hydroxides reduced As translocation from roots to shoots. Hu et al. (2005) further concluded that As concentrations in DCB-extracts with no P addition were significantly higher than those with P fertilization. As^V associated with IP may be largely

'locked up' because of its high affinity with Fe oxides. Thus IP may act as a 'buffer' for As^V in the rhizosphere, leading to a lower influx into root cells and thus preventing the increased translocation of As to shoots. The presence of phosphate may enhance the desorption of As^V from IP to both the external solution and the apoplastic space, demonstrated by the reduced As^V concentration in DCB extracts with the addition of phosphate to the external solution. The increase in As^V in the apoplastic space may thus lead to its higher uptake into rice roots. However, the phosphate in the uptake solution did not have a significant effect on As^{III} uptake irrespective of the presence of IP. Therefore, there may be a possibility of the oxidation of As^{III} to As^V by the presence of IP or the release of oxygen/oxidants from rice roots. Nevertheless, there is little information available at present for the speciation of As in the rhizosphere of rice plants. In wetland plants, Hansel et al. (2002) found that As existed as a combination of $As^V$and As^{III}iron (hydr)oxide complexes in the IP with a majority being As^V (*c*. 82%). This suggests that a plant-induced oxic–anoxic interface may exist at the root surface. Concentrations of As in rice roots decreased with increasing rates of sulfur (S) applications, thus, S fertilization may also be important for the development approaches to reducing grain As accumulation in rice. Meng et al. (2002) concluded that iron hydroxides are less effective in removing As^{III} than As^V at a neutral pH. They further demonstrated that the apparent adsorption constants indicate the affinity of anions for iron hydroxide sites decreased in the following order arsenate>phosphate>arsenite>silicate>bicarbonate.

A significant genotypic variation was found among rice cultivars with respect to IP formation during field trials in the West Bengal region of India. During one of our studies involving simulated pot experiments it has been observed that As concentrations increased with increasing DCB-Fe extraction on the root surface. The maximum Fe in DCB extract of treated plants was found in Triguna (20737), followed by IET-4786 (13573) and IR-36 (11802) at 8 mg l^{-1} As^{V}supply. However, the total amount of As accumulated in rice grains was also maximum in Triguna followed by IR-36, while it was minimum for IET-4786. Further, the correlation analysis between the levels of As in IP and roots had a significant positive correlation [Triguna (*R*=0.995***), IR-36 (*R*=0.971**), PNR-519 (*R*=0.936*) and IET-4786 (*R*=0.919*)]. This study demonstrated that despite contribution of IP in restricting significant amounts of As on the root surface, As uptake and transport by plant may also be regulated by genotypic differences. It seems that IP may act as a source of As rather than a barrier for its uptake depending on the environmental factors and genotype. In a study on excised rice roots, Chen et al. (2005) have shown that the presence of IP may favor the influx of As^{III}. Further, the strong affinity of IP for Asmay create a gradient for As diffusion towards root surface, where the intra-conversion of As^{V} and As^{III} may occur due to existence of plant-induced oxicanoxic interface (Hansel., 2002). Hence, As speciation and availability in the rhizosphere and subsequent plant uptake seems to be regulated by genotypic characteristics as well as by environmental factors.

2.6. Effects of arsenic on growth, photosynthetic pigments and proteins under metal/metalloid exposure

The phytotoxicity of As is affected considerably by its chemical form. Inorganic arsenicals are considered more toxic than organic ones and toxicity of As decreases in order: arsine>inorganic As(III)>organic As(III)>inorganic As(V)>organic As(V)>arsonium compounds (Mandal and Suzuki, 2002). Arsenicals can cause damage to DNA by inhibiting enzymes involved in DNA repair. Arsenite is weak mutagen but a potent comutagen. Since As(V) is a phosphate analogue, it has been reported to replace P in the phosphate groups of DNA (Patra et al., 2004). Arsenate affects the phosphate metabolism of plants and disrupts energy flows due to replacement of P by As in ATP forming unstable ADP-As complexes. Arsenite behaves more as a metal ion and shows high affinity for –SH groups of proteins and enzymes; consequently disturbs the biochemical functioning of cell leading to cell death (Meharg and Hartley-Whitaker, 2002). The presence of As in irrigation water or soil at an elevated level has been reported to hamper normal growth of plants with the toxicity symptoms such as reduction of root and shoot biomass (Tang and Miller, 1991; Carbonell-Barrachina et al., 1998), root plasmolysis followed by root discolouration (Machlis, 1941), wilting and necrosis of leaf tips and margins (Odanaka et al., 1987; Machlis, 1941) and lower fruit and grain yield (Carbonell-Barrachina et al., 1998; Miteva, 1998, 2002). Elevated levels of As have been shown to be associated with needle abscission and death of fine roots in certain conifers, stunting of plant growth and sparse mycorrhizal development.

Abedin et al. (2002a,b) studied effect of various As concentrations on growth and yield of rice plants. They reported decrease in plant height, root biomass, number of filled spikelets, and grain yield though no significant effect on straw yield was noticed when rice plants were exposed to As(V) in concentrations range of 20-800 μg l^{-1}. However in a different study, supply of up to 500 μg As l^{-1} was found to have no significant effects on dry weight of shoots and roots in rice plants (Liu et al., 2004). Jha and Dubey (2004) investigated the effect of As(III)(25 and 50 μM) on two varieties (Malviya-36 and Pant-12) of rice for duration of 10-20 d upon sugar levels and activities of enzymes of sugar metabolism. Results showed an increase in reducing, non-reducing and total soluble sugars. An increased conversion of non-reducing to reducing sugars was observed concomitant with As toxicity. Among starch degrading enzymes, activity of α- and β-amylase was decreased while that of starch phosphorylase increased. Activity of sucrose degrading enzymes, acid invertase and sucrose synthase was increased, whereas the activity of the sucrose-synthesizing enzyme, sucrose phosphate synthase, was inhibited. Further, an increase in the level of RNA, proteins and proline accompanied with a decline in the level of free amino acid pool was noticed (Mishra and Dubey, 2006). There was a marked inhibition in activities of ribonuclease (RNase), protease and leucine aminopeptidase whereas the activity of carboxypeptidase was enhanced. In a pot experiment, Williams et al. (2005) found no effect on straw biomass but reduced husk and grain yield in rice plants exposed to 100 μg As g^{-1} soil. Arsenic exposure resulted

in increased grain C and N status but decreased C:N ratio and P status. Thus As exposure impairs physiology and yield of rice plants.

Liu et al. (2005) studied effect of different concentration of As(III) and As(V) (0-16 mg l^{-1}) on seed germination, relative root length (RRL), relative shoot height (RSH) and activities of α-amylase, β-amylase and total amylolytic activity in young seedlings of wheat varieties. Both As(III) and As(V) significantly decreased the germination percentage, RRL, RSH, α-amylase, β-amylase and total amylolytic activity.

Photosynthesis is one of the most sensitive processes. Multifacial impacts on photosynthetic apparatus of plants have been reported upon exposure to various heavy metals and metalloids. Long-term exposure results in reduced leaf growth, decreased photosynthetic pigments and disturbed chloroplast ultrastructure (Marin et al., 1993a; Parys et al., 1998; Knauer et al., 1999). Reduction in Hill reaction activity, uncoupling of non-cyclic phosphorylation and oxidative phosphorylation have been noticed. The carotenoid pigments, which are also essential components of the photosynthetic membranes in all plants, algae and cyanobacteria and serve an extraordinary variety of functions (Demmig-Adams et al., 1996), are also sensitive to metal/metalloid stress. Protein levels of metal/metalloid stressed plants generally decline (Srivastava et al., 2006), however in some cases mild exposures have been found to result in an increased level of protein due to induction of some stress proteins. Metal/metalloid stress is also known to induce synthesis of some amino acids like cysteine, proline and glutamic acids etc., the building blocks of stress proteins.

Jain and Gadre (1997) studied the effect of As on chlorophyll biosynthesis and reported decrease in chlorophylls as well as chlorophyllase activity, an enzyme responsible for chlorophyll degradation, in greening maize leaf segments in response to As(V) (10 to 1000 μM), however the decline in chlorophylls was higher than chlorophyllase activity and the effect increased with time. Protein content of plants was also reduced. They further studied ameliorative effect of exogenously supplied substrates. Percent decrease in chlorophylls was reduced by exogenous supply of substrates of chlorophyll biosynthesis like 2-oxoglutarate, glutamine, glycine and Na-succinate. Addition of thiol compounds, cysteine and DTNB reduced the effect of As, while GSH had no effect. Jain and Gadre (2004) found that both *in vivo* and *in vitro* supply of As(V) inhibited the ALAD activity in excised etiolated maize leaf segments during greening. Increasing concentration of ALA during assay increased the activity of ALAD to different extent in control and As exposed conditins. It was suggested that As inhibits ALAD activity by affecting its thiol groups and/or binding of ALA to the enzyme.

Mascher et al. (2002) investigated the effect of As(V) (5, 10 and 50 μg g^{-1} soil) and heavy metal mixture (5 μg Cd, 300 μg Zn and 10 μg As g^{-1} soil) on the photosynthetic pigments and protein of *T. pretense.* Chlorophyll and carotenoid contents decreased upon As(V) exposure, which was ameliorated upon Zn and Cd addition. Protein content also decreased with increasing As(V) concentration but not in heavy metal mixture. High soil levels of As have been found to provoke some changes in pigment concentration in green bean and tomatoes (Miteva,

2002) and they correspond to an alteration of the chloroplasts in cells (Miteva and Merakchiyska, 2002).

During As exposure (5 to 100 μg g^{-1} soil) to *P. sativum*and tomato plants, increased chlorophyll content was observed at lower concentration, but higher concentration resulted in decline in photosynthetic pigments (Miteva et al., 2005). Upon exposure of As (133 and 267 μM) to *P. vittata* and *P. ensiformis*, no change was observed in soluble protein content after 1 d, however protein level decreased significantly beyond 5 d at 267 μM As in *P. vittata* and at both concentrations in *P. ensiformis* (Singh et al., 2006). Level of chlorophyll and carotenoids increased upon exposure to 133 μM As(V)in *P. vittata* whereas they decreased in *P. ensiformis*.

The impacts of As on the chloroplast ultrastructure and Ca distribution in *P. vittata* were studied by histochemical methods, with an emphasis on the possible function of Ca in As detoxification (Li et al., 2006). *P. vittata* was exposed to As(V) (100-800 μg As g^{-1} soil) for 24 weeks in a greenhouse. The addition of As did not affect the chloroplast ultrastructure of young pinna, meanwhile most of the membrane systems of chloroplasts in mature pinna were severely damaged under high As stress. Calcium concentration in the fronds was not significantly affected, but it was significantly increased in the mature pinna by As addition, consistent with the position appearing As toxicity (Li et al., 2006).

2.7. Arsenic induced oxidative stress and response of antioxidants: molecular and enzymatic

2.7.1. Reactive oxygen species and lipid peroxidation

One of the primary reasons behind the metal or metalloid induced toxicity is the enhanced generation of ROS, the generation of whose can be initiated either directely by redox-active metals or indirectly by mediation of lipoxygenase in case of non-redox active metals (Van Assche and Clijsters, 1990; Meharg and Hartley-Whitaker, 2002; Singh et al., 2006). The increased production of free radicals and ROS causes oxidative stress leading to lipid peroxidation and membrance damage. Previous studies have shown that As uncouples the oxidative phosphorylation pathway in mitochondria by inhibiting the FB factor of the H1-ATPase1, thus promoting generation of $O_2^{\bullet-}$. It has also been shown that treatment with As(III) can produce extensive oxidation of intramitochondrial NAD(P)H transhydrogenase. NAD(P)H shortages may result in accumulation of GSSG and ROS. Furthermore, it is known that As(V) is reduced rapidly to As(III) via cytochrome and cytochrome oxidase, using oxygen as a final electron acceptor, a reaction that is catalyzed rapidly in plants such as corn, peas, melons, and tomatoes. During this reduction generation of $O_2^{\bullet-}$ is possible through reduction of oxygen (Mylona et al., 1998).

In response to metals like Cu and Ni, plants of hydrilla exhibited decrease in MDA level (Gupta et al., 1996; Sinha and Pandey, 2003). This was attributed to a decrease in polyunsaturated fatty acids (PUFA) through effect on their synthesis. Dhir et al. (2004) also reported that Cd exposure in *H. verticillata*. However,

exposure to Ni for long duration (6 d) produced significant increase in MDA content (Sinha and Pandey, 2003). MDA content also increased significantly with increase in Fe concentration and duration with maximum being 79% after 7 d at 5 μg ml^{-1} in study by Sinha et al. (1997). Simultaneously increase in K leakage was also observed. Similarly, a concentration duration dependent increase in MDA level and electrical conductivity (EC; a measure of ion leakage) was observed in response to Cu in *H. verticillata* plants in a recent study (Srivastava et al., 2006) with maximum increase in MDA and EC being 244 and 122%, respectively after 7 d at 25 μM Cu.

Hartley-Whitaker et al. (2001a) studied biochemical responses of As(V)-tolerant and non-tolerant plants of *H. lanatus*, obtained from As/Cu-contaminated and uncontaminated areas, to As exposure. Plants from contaminated habitat were less sensitive to metal exposure. Study revealed that rapid As influx in non-tolerant plants resulted in fast increase in lipid peroxidation causing severe oxidative stress. On the other hand, in tolerant plants this process occurred at a slow rate due to decrease in the rate of As influx, enabling them to maintain their constitutive functions. Srivastava et al. (2005) examined response of three fern species, *P. vittata, P. ensiformis*, and *Nephrolepis exaltata* to exposure of As. A significant increase in lipid peroxidation was observed in the frond tissues but very low in root and rhizome. The level of lipid peroxidation was significantly higher in *N. exaltata* and *P. ensiformis* fronds than in *P. vittata*. A higher increase in H_2O_2 and lipid peroxidation in *P. ensiformis*than *P. vittata*was also observed by Singh et al. (2006) upon exposure to 133 or 267 μM As(V).

2.7.2. Enzymatic antioxidants

To cope up with oxidative stress caused by metals, plants show stimulated activity of various antioxidant enzymes, which are localized in different compartments of the cell. Mylona et al. (1998) has hypothesized that As induces various detoxification enzymes. This occurs because the As exposure rapidly depletes the pool of GSH, leading to a rise in steady state concentration of ROS. This results in changes in the equilibrium of ROS and antioxidant enzymes that leads to induction of the antioxidant defense system including rise in total GSH levels.

Different plants show differential responses of antixodiant enzymes in response to various metals. In a study on *Hydrilla* (Panda and Khan, 2005), SOD activity showed a uniform increase in Cd and Cu-exposed (0-1000 μmol l^{-1}), whereas plants exposed to Cr and Zn (0-1000 μmol l^{-1}) demonstrated a uniform decline. Sinha et al. (1997) studied toxic effect of Fe on *Hydrilla* plants. Iron exposure showed an increase in SOD activity up to 1 μg ml^{-1} (112% higher than control) thereafter a decline occurred (104% at 5 μg ml^{-1} as compared to SOD activity at 1 μg ml^{-1}), however activity did not decline lower than control. Srivastava et al. (2006) studied responses of various antioxidant enzymes in *Hydrilla* upon exposure to Cu (0-25 μM) for 7 d. The activity of all enzymes viz., SOD, APX, GPX, CAT and GR increased significantly at lower exposures of Cu. After attaining their maximum levels, activities showed decline to varying levels.

Mylona et al. (1998) investigated the antioxidant responses induced by As(V) and As(III) in different tissues and in different developmental stages of

maize. Both CAT and SOD activity increased in response to low concentration (0.01 and 0.1 mM) of both As(V) and As(III) in developing embryos, whereas in germinating embryos both CAT and SOD showed increase up to 10 mM As(V)/As(III). Isozyme analysis showed differential responses of CAT-1 and CAT-2 isozymes to As(V) and As(III), while SOD-3 showed similar response. Modulation of mRNA transcripts of CAT, SOD and GST was also observed indicating that both As(V) and As(III) modulated the antioxidants at molecular level. GST overexpression was supposed to be induced to catalyze the conjugation of As to GSH.

Hartley-Whitaker et al. (2001a) found an increase in SOD activity in both As tolerant and non-tolerant plants of *H. lanatus* upon As exposure. In red clover, *T. pratense,* SOD activity reduced at lower As exposure (5 μg g^{-1} As), then increased at moderate concentration (10 μg g^{-1} As) and again decreased to level of control at higher concentration (50 μg g^{-1} As). Mixture of Cd and Zn with As also resulted in reduced SOD activities. At 10 μg g^{-1} of As, activity of peroxidase also increased significantly by 250% in shoots of plants (Mascher et al., 2002).

Cao et al. (2004) investigated the antioxidant metabolism in response to As (0-200 μg g^{-1}) in *P. vittata*. The activities of enzymatic antioxidants (SOD, CAT, APX, GPX) increased only at low As exposures, then their levels either declined or leveled off. Activities of the enzymes in different tissues were related to As concentrations with higher activities being in the fronds than in the roots except for CAT, in which the opposite was true. The As level-dependent relationship of the enzyme activities was also found in the fronds of different ages, they all were

greater in the young fronds than in the mature fronds at As concentrations ≤ 20 μg g^{-1}, and the reverse was true at As concentrations >20 μg g^{-1} except for APX. Increase in the peroxidase activity has been observed under As stress in green bean and tomato plants (Miteva and Peycheva, 1999) and in maize roots. Srivastava et al. (2005) noticed induced levels of SOD, CAT and APX in *P. vittata* upon As exposure. *P. ensiformis* and *N. exaltata* also showed induction in these enzymes but activity was lower than that being in *P. vittata*.

2.8. Thiolic constituents

Like enzymes, some compounds like AsA, proline, and particularly thiols, such as cysteine and GSH act as non-enzymatic antioxidants and play an important role in controlling ROS. GSH is predominant non-protein thiol (NP-SH) in the plant cell. High thiol content enables metabolites to function in free radical and ROS detoxification. ROS species are reductively detoxified by concomitant oxidation of sulfhydryl moieties to disulfides (Elstner et al., 1988; Elstner, 1991).

Gupta et al. (1995) reported significant increase in cysteine and NP-SH in response to Pb exposure. Concomitant with the increase in NP-SH, a rapid decline in total GSH was observed. Tripathi et al. (1996) also reported induction of Cys and NP-SH in *H. verticillata* upon exposure to Cd (2.5 and 10 μM) for a period of 7 d. While, Cd exposure resulted in the reduction of GSH levels, which was more pronounced at 10 μM Cd (52% reduction) than at 2.5 μM Cd (32% reduction) after 3 d of exposure period. Increase in cysteine and NP-SH has also been

observed at low Cu exposures (Gupta et al., 1996; Srivastava et al., 2006), however GSH content showed progressive decline simultaneous to increase in GSSG. Gupta et al. (1998) studied role of GSH and PCs in the detoxification of Hg in *H. verticillata*. Significant increase in cysteine and NP-SH was observed with the increase in concentration and duration. However, GSH content declined significantly. Sinha et al. (1997) demonstrated that Fe exposure resulted in an increase in cysteine up to 1 μg ml^{-1}, and then it decreased, whereas NP-SH level increased up to 5 μg ml^{-1} (233% higher than control). However, there was significant decline in GSH (53%) and increase in GSSG (225%) at 5 μg ml^{-1} Fe after 7 d. Level of cysteine in Ni exposed *Hydrilla* plants increased at all concentrations up to 4 d, however after 6 d cysteine level declined at higher exposures. NP-SH levels increased at all concentrations and durations (Sinha and Pandey, 2003).

Mascher et al. (2002) investigated the effect of As(V) (5, 10 and 50 μg g^{-1} soil) in *T. pretense* on GSH level. Arsenic exposure (5 and 50 μg g^{-1}) reduced the concentration of GSH by 19.8% and 28%, respectively, while application of 10 μgAs g^{-1} increased it by 8%. Exposure to mixture of heavy metals (5 μg Cd, 300 μg Zn and 10 μg As g^{-1} soil) reduced the GSH content of plants by 24%. In another study, *P. vittata*, grown in soil containing 0 to 200 μg As g^{-1} for 12 weeks, showed induction in thiol content which was correlated with As taken up by the fern and the induction was more in fronds as compared to roots. GSH content increased 3-fold in plants exposed to 200 μgAs g^{-1} soil (Cao et al., 2004).

Cai et al. (2004) investigated the role of thiols in the hyperaccumulation of As by the fern, *P. vittata.* They found significant increase in total thoils and NP-SH in As exposed plants. They also found the highly significant induction of an unidentified thiol in As-exposed plants which showed a strong positive correlation with the As accumulation and similar to As accumulation pattern, was mostly present in leaves, very low in rachises and was undetectable in roots.

Singh et al. (2006) studied effect of As(V) exposure (133 or 267 μM) on the levels of AsA and GSH and their reduced/oxidized ratios in *P. vittata* and *P. ensiformis*. Level of these parameters was greater in control plants of *P. vittata* than *P. ensiformis*. Upon As exposure, level of AsA increased significantly in *P. vittata* at all exposures while only at 133 μM after 5 and 10 d in *P. ensiformis* while the level of DHA increased to higher level in *P. ensiformis* than *P. vittata*. Level of GSH increased at 133 μM in *P. vittata* while in *P. ensiformis*, it declined except after 1 d. Ratios of AsA/DHA and GSH/GSSG were significantly higher in *P. vittata* than *P. ensiformis* at various exposures.

Chapter 3

Materials and Methods

3. MATERIALS AND METHODS

3.1. Isolation and screening of As resistance bacteria

The different arsenic contaminated paddy fields *viz*., Chinsurah in district Hoogly was selected as the least As affected site. District Bardhaman is situated in the Gangetic alluvial zone where the block Purbasthali–I was selected as severely As affected. While Birnagar of district Nadia was highly As contaminated site. Soil sample were collected in polythene bag and carried to the laboratory for analysis. For the isolation of bacteria, 1 gm soil samples were dissolved in 1 ml distilled water, vertexd and leave for 10 min to settle down. Add 100µl of water from settled sample in appendorf tube containing 900 µl of distilled water. Mix the appendorf tube having the dilution 10^{-1}. After 2-5 dilution in similar way, 25 µl of sample were spread on presterlized petridishes. After 1-2 d of incubation, bacterial colonies were formed on agar plates. These plates were further utilized for the isolation of single colony of bacterium.

The isolated strains were cultured in nutrient broth media. For the analysis of As resistance, isolated bacteria were subjected to treatment by different concentrations of As(III) for 24 h at 37 ^{0}C incubation.

Sampling site and sample code

S.No.	Sample sites	Sample code
1	Chinsurah, Hooghly	BBAU/CH
2	Purbasthali, Bardhaman	BBAU/PB
3	Birnagar, Nadia	BBAU/BN

3.2. Physico-chemical analysis of soil samples

Physico-chemical characteristics of soil like pH and electrical conductivity (EC) were measured by the pH meter . Phosphorus content was measured by the method of Olsen (1954). Nitrogen content was measured by Page et al. (1982). Total organic carbon (TOC) by Walkley and Black (1934). Water holding capacity (WHC) was measured by measuring percentage of moisture retain by soil (Soil Testing Procedure Manual, 2008).

3.2.1. Soil Analysis

3.2.1.1. Available nitrogen

The available N in soil was estimated by using Kjeldhal method (Jackson, 1973).

Reagent Preparation

a) 2.5% sodium hydroxide: 25 gNaOH dissolved in 1 litre distilled water

b) 0.1 N HCl.

c) 1 % Boric acid: 1 g dissolved in 100 ml distilled water

d) Indicator Solution

100 mg bromocresol green indicator dissolved in100 ml methanol

100 mg methyl red indicator dissolved in 100 ml methanol

e) Receiver solution

Receiver solution is prepared by dissolving 10 ml Bromocresol green and 7 ml Methyl red in 1 litre Boric acid

In 0.25 g of soil, 1 g ferrous ammonium Sulphate (FAS) dissolved in 10 ml of distilled water was added. The samples were run in Kel Plus auto distillation unit. 2.5% alkali is added to the sample and treated with steam. The resulted ammonical nitrogen was collected in receiver solution (boric acid and indicator solution) that turns green in colour in presence of nitrogen.

Titration

Distilled sample obtained from Kjel plus was titrated with 0.1 N HCl. The blue green colour of sample was turned to pale pink colour at the end point. Burette reading was noted.

$$\textbf{\% Nitrogen} = \frac{\text{BR of sample} - \text{BR of blank } \mathbf{x} \text{ Normality of HCl} \mathbf{x}\ 1.401}{\text{Weight of sample}}$$

3.2.1.2. Total organic carbon

The total organic carbon (TOC) in soil was estimated by following Walkley and Black (1947). In 0.5 g of soil, 5 ml of 1 N $K_2Cr_2O_7$, followed by 10 ml of conc. H_2SO_4 was added. After cooling, 25 ml of distilled water was added. The mixture

was left for 10 min and 3 drops of ferroin indicator was added and titrated against 0.5 N FAS. After adding indicator sea green colour was turned to brown colour at the end point. Burette reading was noted.

$$\textbf{TOC} = \frac{\text{BR of blank} - \text{BR of sample X } 0.9}{\text{Weight of soil sample}}$$

3.2.1.3. Available phosphorus

The available phosphorus in soil was estimated by following Olsen method (Jackson, 1967). For extraction of soluble P, 20 ml of 5% $NaHCO_3$ was added to 2.5 g of soil, followed by 1 spoon of charcoal. Then sample was left on shaker for 30 min and filtered. The available P in the filtrate was determined colorimetrically by the phosphomolybdate ascorbic acid method. 500 ml of composite reagent (Murphy–Riley color-developing solution) was prepared by adding 250 ml of 2.5 M H_2SO_4, 75 ml of 4% ammonium molybdate solution [$(NH_4)_6Mo_7O_{24}.4H_2O$], 50 ml of ascorbic acid solution (2.64 g of L-ascorbic acid in 50 ml of DW), and 25 ml of antimony potassium tartrate solution (0.0727 gm of antimony potassium tartrate in 25 ml of DW). A total volume of 500 ml was diluted by adding 100 ml of deionized water and mixed on a magnetic stirrer and kept in an amber bottle. In 10 ml of aliquot, one to two drops of 0.25% (w/v) p-nitrophenol was added to adjust the pH resulting in a yellow solution. Lower the pH by adding 0.25 M H_2SO_4 until the solution just turns color less. 8 ml of the Murphy and Riley color-developing solution was added and volume was raised up to 50 ml with deionized water. The solution was stirred and left for 15 min for color development and

absorption was taken at 880 nm. The standard curve was constructed using the absorbance values from standards of known P concentration.

3.2.1.4. Soil pH and electrical conductivity

The pH and EC of soil were measured by ion meter (Orion, USA) while, water holding capacity was measured hydrometrically.

3.3. Germination of rice seedlings

Rice seeds (Satya) were first sterilized in 0.1 % $HgCl_2$ solution for 60sec, washed with deionized water and soak in distilled water for 24 h. The deionized seed were spread in pre-sterilized soaked blotting paper in a tray. The germination was carried out in seed germinator with constant temperature, light and humidity (16-hour light period with a light intensity of 350 mmol $m^{-2}s^{-1}$; 25/20^{o}C day/night temperatures; and 60% relative humidity). After germination, rice seedlings were transferred to light in controlled laboratory condition for growth using Hewitt nutrient media (Liu et al., 2004) for 10 days before application into pot experiment.

3.4. Experimental design

A pot experiments were performed in the month of June. Two Arsenite [As(III)] concentration i.e., low dose [50µM As(III)] and high dose [100 µMAs(III)] were applied in rice seedlings. Inoculums of bacterial strain (100 ml) were prepared in sterile distilled water. The bacterial inoculums were applied in

As(III) treated plant. A system devoid of As and bacterial inoculant served as control. The mass culture of isolated bacterial strain was prepared in Erlenmeyer flask containing 300 ml nutrient media. The mass cultures were incubated at 37 ^{0}C at 150-200 rpm in a orbital shaker. After the growth pellet were prepared by centrifuging the culture at 10,000 rpm for 20 min. Bacterial inoculums were prepared by dissolving collected biomass in sterile distilled water. All the experiments were performed in pots and in three replicates.

3.5. Biochemical analysis of rice plant

3.5.1. Estimation of growth characteristics and pigment

Growth parameters in the form of root, shoot length (cm) and fresh weights (g) were measured by metric scale and weighing balance respectively. Photosynthetic pigments of treated and untreated plants (100 mg) were extracted in 4 ml of 80% chilled acetone (v/v) and centrifuged at 10,000 g for 10 min at 4ºC. The absorbance of the extract was taken at 480, 510, 645 and 663 nm as per procedure of Arnon (1949).

Chlorophyll contents were calculated according the following formula:

Total Chlorophyll (mg g^{-1}fw): $[\{20.2\ (A_{645}) + 8.02\ (A_{663})\} \times V / \{d \times 1000 \times W\}$

Chlorophyll a (mg g^{-1}fw): $[\{12.7\ (A_{663}) - 2.63\ (A_{645})\}] \times V / \{d \times 1000 \times W\}$

Chlorophyll b (mg g^{-1}fw): $[\{22.9\ (A_{645}) - 4.68\ (A_{663})\}] \times V / \{d \times 1000 \times W\}$

Carotenoid content in this extract was calculated by the formula given by Duxbury and Yentsch (1956).

Carotenoid (mg g^{-1}fw): $[\{7.6\ (A_{480}) - 1.49\ (A_{510})\} \times V] / \{ d \times 1000 \times W \}$

where, A_{480}, A_{510}, A_{645}, A_{663} = *absorbance at these wavelengths*

V = volume of final extract (ml)

W = weight of plant sample (g)

d = width of the cuvette (1 cm)

3.5.2. Estimation of Protein

Protein was estimated by the method of Bradford (1976). The Bradford method of protein measurement is based on the binding of a dye, Coomassie Blue G, to the protein. The binding of dye shifts the absorption maximum (λ max) of dye from red to blue. A solution of dye is mixed with phosphoric acid and ethanol, and mixed with protein solution. The aborbance of blue color is measured at 595 nm. To determine protein concentration, a standard curve is first generated using known concentrations of bovine serum albumin.

3.5.3. Lipid peroxide/ estimation of malondialdehyde content

Lipid peroxidation was determined by estimation of the malondialdehyde (MDA) content following the method of Heath and Packer (1968) with slight modification. Plant material (500 mg) was homogenized in 5 ml of 0.1% TCA. The homogenate was centrifuged at 10,000 g for 5 min. For every 1 ml of aliquot, 4 ml of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added. Mixture was heated at 95 ºC for 30 min and then cooled quickly on ice bath. After centrifugation of the mixture at 10,000 g for 15 min, absorbance of the

supernatant was read at 532 and 600 nm. Correction of non-specific turbidity was made by subtracting the absorbance at 600 nm from the absorbance at 532 nm. The level of MDA was calculated using the extinction coefficientof 155 mM^{-1} cm^{-1} and is expressed as μmol MDA g^{-1}fw.

3.5.4. Measurement of hydrogen peroxide

The plant material (500 mg) was homogenized in 1 ml of ice cold 50 mM potassium phosphate buffer (pH 7.0) containing 0.1% Triton X-100 and 1% PVP (w/v) (Milosevic and Slusarenko, 1996). The homogenate was centrifuged at 15,000 × g at 4°C for 20 min. The supernatant was collected and used for the assay of H_2O_2 according to Pick (1986). The assay was based on horseradish peroxidase-dependent oxidation of phenol red by H_2O_2 leading to the formation of a compound at alkaline pH, which exhibited significant absorbance at 600 nm. The reaction mixture (total volume 460 μl) consisted of 50 μl plant extract, 0.52 mM phenol red solution in 50 mM potassium phosphate buffer (pH 7.0) and horseradish peroxidase (final concentration 43.5 unit ml^{-1}). The mixture was shaken for 10 min at 25 °C and centrifuged at 10,000 × g for 10 min. Then 100 μl of the supernatant was mixed with 1 ml of 1 N NaOH and the absorbance was measured at 600 nm. The amount of H_2O_2 was calculated using the extinction coefficient of 19.8 x 10^3 M^{-1} cm^{-1} and is presented as mmol H_2O_2 g^{-1}fw.

3.5.5. Cysteine Estimation

Cysteine content in control and metalloid-exposed plants was estimated following the method of Gaitonde (1967). Plant material (300 mg) was homogenized in 5% chilled perchloric acid and centrifuged at 10,000 × g for 10 min at 4 ºC. Cysteine content was measured in supernatant using acid-ninhydrin reagent. For preparation of every 10 ml of acid ninhydrin reagent, 250 mg of ninhydrin was dissolved in 6 ml glacial acetic acid and 4 ml HCl. Reaction mixture (3 ml) contained one ml each of supernatant, glacial acetic acid and acid ninhydrin reagent. Mixture was heated for 15 min at 95ºC, and then cooled rapidly to room temperature and absorbance was recorded at 560 nm. Cysteine content was calculated from the standard curve prepared using known concentrations of cysteine (L-cysteine hydrochloride, Sigma) and is expressed as nmol g^{-1}fw.

3.5.6. Estimation of Glutathione (Reduced and Oxidized)

The level of GSH and GSSG was measured by following the protocol of Hissin and Hilf (1976). Plant material (500 mg) was frozen in liquid nitrogen homogenized in 0.1 M sodium phosphate buffer (pH 8.0) containing 25% meta-phosphoric acid. The homogenate as centrifuged at 20000g for 20 min at 4^{o}C and total glutathione (GSSG and GSH) content was determined fluorometrically in the supernatant after 15 min incubation with o-phthaldialdehyde (OPT). Fluorescence intensity was recorded at 420 nm after excitation at 350 nm by using the Hitachi fluorescence spectrophotometer (F-7000).

The reaction procedure followed is given below:

GSH Assay	**GSSG Assay**
0.5 ml supernatant	0.5 ml supertant
4.5 ml buffer (pH 8.0)	200 µl NEM (0.04 M)
100 µl of mixture	Add 4.3 ml NaOH (0.1 N)
1.8 ml buffer (pH 8.0)	
100 µl OPT solution	
	100 µl of mixture
	1.8 ml NaOH (0.1 N)
	100 µl OPT solution
Fluorescence determined at 420 nm (excitation at 350 nm)	Fluorescence determined at 420 nm (excitation at 350 nm)

Known concentrations of GSH and GSSG (L-glutathione reduced and oxidized, Sigma) were used to prepare a standard curve for calculating the GSH and GSSG levels. The content of GSH and GSSG is expressed as µmol g^{-1} fw.

3.5.7. Antioxidant enzyme assay

Plant material (200 mg) was homogenized in 100 mM chilled potassium phosphate buffer (pH 7), containing 0.1 mM EDTA and 1% PVP (w/v) at 4ºC. Homogenate was squeezed through four layers of cheese cloth and extract thus obtained was centrifuged at 10000 *g* for 15 min at 4 ºC. Supernatant thus obtained was used to measure the activities of various enzymes were expressed as units mg^{-1} protein.

3.5.7.1. Superoxide dismutase

The activity of SOD (EC 1.15.1.1) was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of NBT. The 3 ml reaction mixture contained 40 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 100 µM EDTA and a suitable aliquot of enzyme extract. Riboflavin was added at the end. The test tubes were shaken and placed 30 cm below light source consisting of 15-W fluorescent lamp. Switching on the light started the reaction and after 30 minutes switching off the light terminated the reaction. A tube containing protein kept in dark served as blank while a tube kept in light with no enzyme served as control. The absorbance of the solution was taken at 560 nm. Activity of SOD was measured by subtracting NBT reduction in light with protein from NBT reduction in light without protein. One unit of activity is the amount of protein required to inhibit 50% initial reduction of NBT under light. The activity of the enzyme is expressed as units mg^{-1} protein.

3.5.7.2. Catalase

Catalase (CAT; EC 1.11.1.6)activity, extraction was done in buffer containing TrisHCl (pH 7), 1mM EDTA, 1mM PMSF and 0.3 gg^{-1}fw PVP. Activity was measured by method of Aebi (1974). The assay system comprised of 50 mM Na_2PO_4 buffer (pH 7), 20 mM H_2O_2 (Є=0.04 cm^2µmole-^{1}) and suitable aliquot of enzyme in final volume of 3 ml. Decrease in the absorbance was taken at 240 nm. Enzyme activity was expressed as µmoles H_2O_2 degradation min^{-1} mg^{-1} protein.

3.5.7.3. Assay of Glutathione Reductase

Activity of GR was assayed by following the method of Smith et al. (1988). The reaction mixture contained 1.0 ml of 0.2 M potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 0.5 ml 3 mM DTNB in 0.01 M phosphate buffer (pH 7.5), 0.25 ml H_2O, 0.1 ml 2 mM NADPH, 0.05 ml enzyme extract and 0.1 ml 20 mM GSSG. The components were added in the order as above directly to a cuvette and the reaction was initiated by the addition of GSSG. The increase in absorbance was monitored for 5 min at 412 nm. The rate of enzyme activity was calculated using standard curve prepared by known amounts of GR (Sigma, USA). Activity of enzyme is expressed as units mg^{-1} protein (1 unit = 1 µmole of GSSG reduced min^{-1}).

3.6. Estimation of As

Harvested plant samples were separated and oven dried till the constant weight at 70^0C for 24 h. 100 mg dried root and shoot were digested at 80 ^{0}C in HNO_3 (Dwivedi et al., 2010). After digestion, samples were allowed to cool and dissolved in 0.6% HNO_3 and filtered and maintained to 10 ml with distilled water. Analysis of As contents was estimated with the help of Atomic Absorption Spectrophotometer (AAS).

3.7 Translocation factor

Translocation ratio (TR) was calculated by the formula:

$TR = (Metal_{Shoot}) / (Metal_{Root})$

Where: $Metal_{shoot}$ = metal content in shoot

$Metal_{root}$ = metal content in root

3.8. Estimation of mineral content in rice

Phosphorus content in plant sample was analyzed calorimetry by stannous chloride methods (Jackson, 1973). Other mineral content (Fe, Zn, Mn Cu, Co and Se) in grain of rice was estimated by digestion method. 0.5 g oven dried grain were taken and digested with 3 ml of HNO_3 (Dwivedi et al. 2010), filtered in Milli-Q water (10 ml) and stored at 4°C. The elemental quantification was done with the help of Atomic Absorption spectrophotometer.

3.9. Quality control and Quality assurance

Standard reference materials (Hi media, Germany) of media and metals were used for the calibration and quality assurance with repeated analysis of quality control samples (n = 3). The results were under the certified values(± 2.82).

3.10. Statistical analysis

All the experiments were performed in three replicate (n=3). All the data were subject to Analysis of variance (ANOVA) post hoc Duncan's multiple range test (DMRT) to test the significant difference between treatments ($p \leq 0.05$).

Chapter 4

Results and Discussion

4. RESULTS AND DISCUSSION

4.1. Physico-chemical analysis of soil sample

Physicochemical analysis of soil sample collected from different As contaminated sites of West Bengal, India showed that out of three selected sites, maximum pH was found in the site Purbasthali with high level of electrical conductivity (159.7 µs/cm), porosity (99.42%) and total organic carbon (1.56%) as compared to other sites. The water holding capacity (WHC) was high in the site Chinsurah whereas minimum WHC was recorded in site Birnagar. In the case of inorganic nutrients such as N and P, it was maximum found in the site Chinsurah. The maximum contamination of As in all the studied site were reported in site Birnagar which was 17.18 mg/kg (Table 4.1).

Table 4.1: Physico-chemical properties of arsenic contaminated soils collected from different selected sites at West Bengal, India.

Parameters	**Chinsurah, Hoogly**	**Purbasthali, Bardhaman**	**Birnagar, Nadia**
pH	7.62±0.77	8.54±0.45	7.79±0.45
EC µs/cm	81.54±8.02	159±51.19	153±34.15
WHC (%)	88.54±6.43	79.53±3.56	73.32±6.43
Porosity (%)	85.10±5.49	99.42±6.32	90.54±2.33
Total Organic Carbon (%)	0.73±0.02	1.56±0.17	1.51±0.14
Available-Nitrogen (%)	0.60±0.07	0.42±0.03	0.52±0.04
Available phosphorus (mg kg^{-1})	924.54±10.34	805.41±11.02	587.45±9.04
Total As (mg kg^{-1})	10.53±1.24	14.89±4.56	17.18±6.75

All the values are mean of triplicates ±S.D. ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values at a particular treatment.

4.2. Isolation and screening of arsenic resistant bacteria

The different paddy fields *viz*., Chinsurah, Hoogly, Purbasthali, Bardhaman and Birnagar, Nadia of West Bengal are contaminated with arsenic ground water. Soil sample were collected in polythene bag and carried to the laboratory for analysis. For the isolation of bacteria, 1 gm soil samples were dissolved in 1 ml distilled water, vertexd and leave for 10 min to settle down. Add 100µl of water from settled sample in appendorf tube containing 900 µl of distilled water. Mix the appendorf tube having the dilution 10^{-1}. After 2-5 dilution in similar way, 25 µl of sample were spread on presterlized petridishes. After 1-2 d of incubation, bacterial colonies were formed on agar plates. These plates were further utilized for the isolation of single colony of bacterium.

From all the three sites total fifteen isolates (five from each site sample) has been isolated and screened for arsenic resistance on different concentrations (25-200mM) and BBAU/CH shows growth on maximum concentration which were taken for further study (Table 4.2).

4.3. Growth responses in rice with bacteria under arsenic stress

Rice plants treated with different dose of As and bacterial inoculants showed alteration in growth (Table 4.3). Results showed that the root length in rice plant treated with high dose of As(III) reduced from 12.53 cm to 5.91 cm and with low dose, it was 8.43 cm in comparison to control. In the case of shoot length approx. 50% reduction was observed at high dose of As(III). Root shoot length in rice plant treated with bacterial inoculants increased 18.2% and 15.6%,

respectively as compared to control. In the case of rice plant treated with the combination of bacterial strain and As improvement in root shoot length was observed in comparison to rice treated with As alone. However, no significant improvement root length was observed under bacterial inoculated with different indication of dose of As. Reduction in root and shoot length is clearcut indication of As induced toxicity. It may be due to As decrease the uptake and translocation of other micro element and nutrients by adsorption on root cell, cell membrane damage and reduced availability of nutrient required for the growth of the plants (Kumar et al., 2016).

Table 4.2: Growth responses of As(III) resistant bacteria isolated from different sites under various concentration of arsenic.

S. No.	Strains	25 mM	50 mM	100 mM	200 mM
1	BBAU/CH	+++	+++	++	+
	BBAU/C1	+++	+++	+	–
	BBAU/C2	+++	+++	+	–
	BBAU/C3	+++	+++	+	–
	BBAU/C4	+++	+++	+	–
2	BBAU/PB	+++	++	+	–
	BBAU/P1	++	++	–	–
	BBAU/P2	++	++	–	–
	BBAU/P3	++	++	–	–
	BBAU/P4	++	++	–	–
3	BBAU/BN	++	++	+	–
	BBAU/B1	++	++	–	–
	BBAU/B2	++	++	–	–
	BBAU/B3	++	++	–	–
	BBAU/B4	++	++	–	–

+++=best growth; ++normal growth; +=weak growth; –=growth absent

Table 4.3: Root, shoot length and biomass of rice plants treated with bacterial inoculums (BBAU/CH) and As(III).

Treatments	**Length (cm)**		**Biomass (cm)**	
	Root	**Shoot**	**Root**	**Shoot**
Control	12.53^{d}±1.40	26.35^{c}±2.89	1.59^{c}±0.15	3.07^{c}±0.37
Bacteria	14.82^{d}±1.10	30.46^{d}±3.17	2.09^{d}±0.22	3.99^{d}±0.12
50 µMAs(III)	8.43^{b}±0.90	21.95^{b}±2.54	1.22^{b}±0.14	2.36^{b}±0.34
100 µM As(III)	5.91^{a}±0.36	13.91^{a}±2.94	0.81^{a}±0.07	1.76^{a}±0.18
50 µM As(III)+ Bacteria	11.57^{c}±0.52	24.5^{c}±3.25	1.40^{c}±0.16	2.65^{b}±0.14
100 µM As(III)+Bacteria	11.12^{c}±0.82	25.35^{c}±1.67	1.25^{b}±0.13	2.71^{b}±0.45

The biomass content of rice plant also decreased approx 50% at high dose of As(III) in root and shoot as compared to control while increased in rice treated with bacterial inoculants in root (30.4%) and shoot (29.96%) as compared to control. In the case of rice plants supplemented with As(III) +bacterial inoculants, biomass of root and shoot increased as compared to rice plant treated with As(III) alone. Enhanced growth in rice by the inoculation of bacterial strain may be due to high uptake, biotransformation of As, and sequestration by the plants (Mahmood et al., 2016). In addition, bacteria induced production of IAA, siderophore, phosphate solubilization, mineral uptake enhance the growth of the plants (Hare et al., 2017; Srivastava et al., 2013).

4.4. Effect on photosynthetic pigment

Arsenic induced reduction and destruction of chlorophyll machinery has been observed in rice plant treated with low and high dose of As(III) which was more prominent with high concentration of As(III). However, increased chl a, chl b

and total chl content was observed in shoot inoculated with bacterial strain as compared to control (Table 4.4). In the case of rice plant co-supplemented with high dose of As(III)+bacterial inoculants, chlorophyll content increased from 0.122-0.190 mg/g. Similarly under low dose it was increased from 0.128-0.210 mg/g. Decreased chlorophyll content in the present study is ascribed to formation of reactive oxygen species which damage photosystem and thus chlorophyll formation. Bacteria form colony around the root tissue inhibiting the entry of the As resulted into high growth of plants (Ryan et al., 2008).

Table 4.4: Photosynthetic Pigments of rice plants treated with bacterial inoculums (BBAU/CH) and As(III).

Treatments	**Photosynthetic Pigments**			
	Chl a	**Chl b**	**Total chl**	**Carotenoid**
Control	0.171^{b}±0.014	0.182^{b}±0.013	0.099^{b}±0.008	0.071^{a}±0.006
Bacteria	0.249±0.022^{c}	0.235^{d}±0.021	0.134^{d}±0.014	0.069^{a}±0.005
50 µM As(III)	0.1350.012^{a}	0.132^{a}±0.010	0.082^{a}±0.007	0.090^{b}±0.009
100 µM As(III)	0.122^{a}±0.009	0.128^{a}±0.011	0.062^{a}±0.006	0.101^{c}±0.007
50 µM As(III) + Bacteria	0.195^{b}±0.020	0.210^{c}±0.020	0.115^{c}±0.008	0.083^{ab}±0.009
100 µM As(III)+ Bacteria	0.190^{b}±0.015	0.148^{a}±0.011	0.110^{a}±0.011	0.081^{ab}±0.008

All the values are means of 3 replicate (n=3) ± S.D. ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values between treatments (DMRT, $p \leq 0.05$).

The carotenoid content in rice plant treated with high and low dose of As(III) increased 42.2% and26.7% respectively as compared to control. However no significant change was observed in rice treated with bacterial inoculants only. In

the case of rice supplemented with As(III) and bacterial strain, 19.8% and 17.8% reduction was observed with high and low dose of As(III) respectively as compared to their respective control i.e., rice treated with As(III) only. Carotenoid, act as antioxidant, protect the plant cell from photoxidation and ROS by scavenging free radicals (Pinto et al., 2003). In the present study, high carotenoid content under As show toxicity whereas decrease carotenoid content by co-supplementation of As and bacteria reflects tolerance responses (Hare et al. 2017).

4.5. Effect on MDA and hydrogen peroxide

Rice plant treated with different concentration of As and bacterial strain showed cellular toxicity by increasing the MDA content which was significantly increased in the case of high dose of As as compared to control (Fig. 4.1A). It was observed that rice treated with high dose of As(III) and bacteria separately showed 30.9% increased in MDA level while no significant change observed with rice inoculated with bacteria only. In the case of rice plant root and shoot treated with combination of low dose of As(III) and bacterial inoculants, MDA content decreased by 12.05% and 30.34% respectively while with high dose of As(III)+bacterial inoculants it showed 14.2% and 20% as compared to their As(III) control. Malonyldialdehyde is the byproduct of lipid peroxidation developed due to peroidation of membrane lipid under stress. High level of MDA in the present study under As(III) clearly demarcated cellular toxicity as a consequence of membrane damage and electrolyte leakage (Shri et al., 2009). Increased MDA level under As stress have been reported by many authors.

Decrease MDA level by the inoculatio of bacteria in As treated rice in the present study exhibited tolerance response of bacteria. It might be due to low accumulation of As inside cell by the bacteria (Singh et al., 2016).

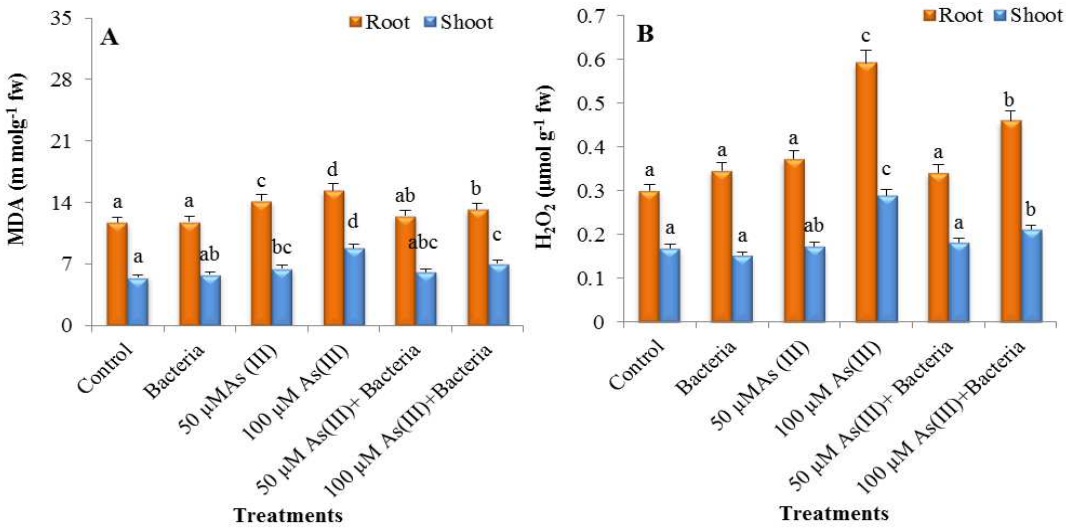

Fig. 4.1: Effect on the MDA (A) and H_2O_2 (B) content in rice plant treated with As(III) and bacterial strain (BBAU/CH). All values are means ± S.D. One-way ANOVA was performed and significant differences in different parameters were tested by DMRT. Identical superscripts denote no significant difference between means according to DMRT ($P \leq 0.05$).

H_2O_2 content maximum increased in higher concentration of arsenic in root then shoot. Alone arsenic resistant bacteria no significant change in H_2O_2 content. When Combination of As and Bacteria decrease the H_2O_2 content, maximum in 50 µM As. H_2O_2 content in rice root and shoot treated with high As(III) showed increased level (0.592 and 0.289 mmol/g fw) in comparison to control (0.300 and 0.169 mmol/g fw). In the case of rice treated with low dose of As(III), no significant change was observed. H_2O_2 content in Rice plant treated with bacterial inoculants increased in the root while decreased in shoot as compared to control.

The co-application of bacterial strain and different doses of As, recovered the level with respect to rice treated with As alone. Overall results showed that inoculated bacteria reduced the toxic level of As by decreasing the H_2O_2 content in rice treated with low dose of As and bacterial inoculants (0.342 mmol/g fw) in comparison to As control (0.592 mmol/g fw) (Fig. 4.1B). Hydrogen peroxide is a reactive oxygen species formed under stress disturb the homeostasis of the plant cell leads to oxidation of protein, lipid breakdown, DNA damage and denature of cell structure and function (Nagajyoti et al., 2010). High H_2O_2 content in rice root and shoot in present study revealed the toxicity exert by the As which counter balance by the application of combination of As(III)+bacterial strain. Similar results were also reported by Singh et al. (2016). Bacterial inoculation may itself act as an antioxidant (Singh et al., 2016; Hare et al., 2017) that might have contributed in reducing oxidative stress. Besides, As resistant bacteria supplementation might be also prevented the As induced oxidative damage in rice plants and may play important role for sustainable production of rice.

4.6. Thiol compounds (Cys, GSH, GSSG and GSH/GSSG ratio)

Thiolic legends presented in Cys, GSH and GSSG in Figure 4.2A-D. Cysteine content in rice root and shoot treated with high dose of As increased (107.26 and 191.25 nmol/g fw) in comparison to control root and shoot (69.20 and 128.8 nmol/g fw). In the case of rice plant treated with bacterial inoculants also increased cysteine content in root and shoot. However, no significant change was observed in the case of root. Rice plant treated with combination of bacterial

inoculants and low dose As(III) showed decreased cysteine content in root in comparison to As while increased in shoot. In the case of high dose +bacterial inoculants cysteine content increased to 131.08 and 254.95 nmol/g fw respectively in root and shoot in comparison to As(III) alone. Cystein is important amino acids and acts as antioxidants inside plant cell. Increased cysteine content by the As(III) presented tolerance response of the plant (Kumar et al., 2014).

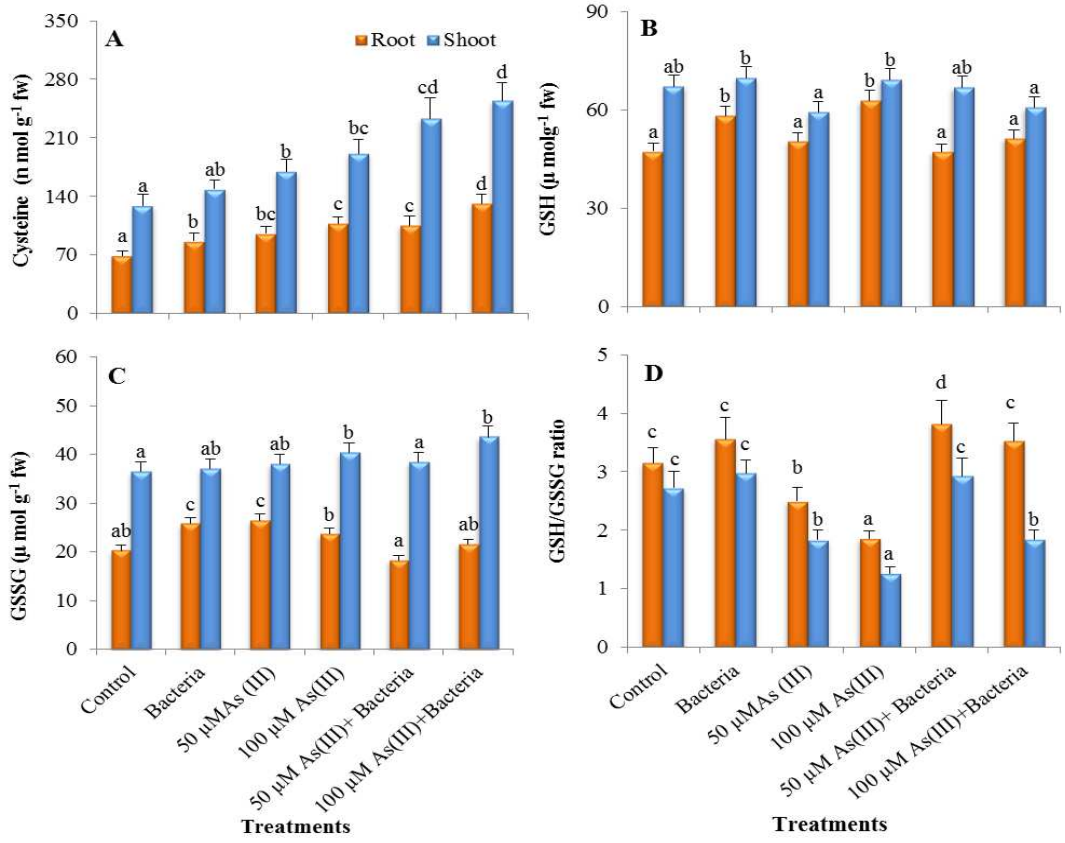

Fig. 4.2: Effect on the cysteine (A), GSH (B), GSSG content (C), GSH/GSSG (D) ratio and PCs in rice plant treated with As(III) and bacterial strain (BBAU/CH).All values are means ± S.D. One-way ANOVA was performed and significant differences in different parameters were tested by DMRT. Identical superscripts denote no significant difference between means according to DMRT ($P \leq 0.05$).

The glutathione (GSH) content in rice root treated with high dose of As(III) increased by 32.5% while no significant change was observed under low As(III). In the case of rice shoot treated with high dose of As(III) showed increased GSH value from 67.29-69.20 μmol/g fw and with low As(III), GSH content was decreased from 67.29-59.50 μmol/g fw in comparison to control. Rice plant treated with bacterial inoculants singly showed increased GSH content in comparison to control. In the case of rice plant treated with the combination of As(III) and bacterial inoculants, showed reduced GSH content in comparison to As(III) alone. The oxidized glutathione (GSSG) content in rice root treated with different combination of As and bacterial inoculants either singly or in combination showed similar trends. GSH protect the plant against metal stress through forming complex and sequestration into the vacuole (Wirtz et al., 2010). Increased GSH level in the present study provide defense. Inside the cell GSH and GSSG are in redox balance and altered due to stress. Increased GSH level reduced the production of GSSG which further converted to GSH in the presence of enzyme glutathione reductase maintain redox balance (Ahsan et al., 2008).

4.7. Effect on antioxidant enzymes

Antioxidant enzymes like SOD, CAT and GR presented in Figure 4.3A-C. Superoxide dismutase activity in rice plant treated with singly and combination of As(III) and bacterial inoculants showed increased response which was more in the case of rice treated with high dose of As(III) separately. The SOD activity was increased by 13.2% in root treated with bacterial inoculants.

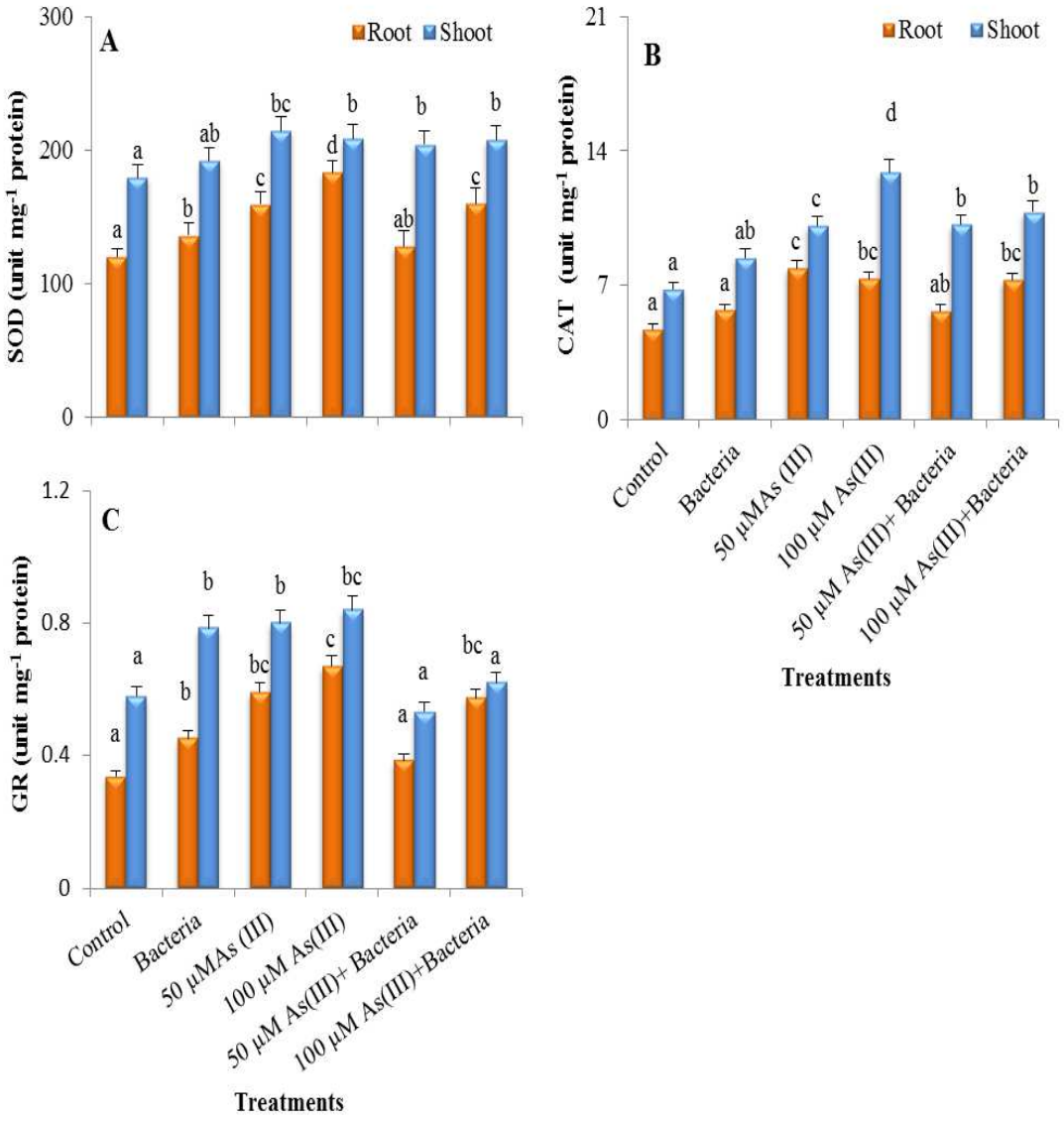

Fig. 4.3: Effect on the antioxidant enzymes [SOD (A), CAT (B) and GR (C)] activities content in rice plant treated with As(III) and bacterial strain (BBAU/CH). All values are means ± S.D. One-way ANOVA was performed and significant differences in different parameters were tested by DMRT. Identical superscripts denote no significant difference between means according to DMRT ($P \leq 0.05$).

In the case of high dose of As(III), the SOD activity in root and shoot was increased by 52.5% and 16.12% as compared to control. Rice plant treated with

combination of As(III) + bacterial inoculants showed maximum reduction in SOD activity in root (128.16 U/ mg protein) as well as shoot (204.33 U/mg protein) under low dose of As(III) as compared to As(III) only. Superoxide dismutase is the first line of defence in enzymatic antioxidant defence system (Mishra et al., 2011). Increased SOD activity under stress have been reported by many authors (Tripathi et al., 2008; Shri et al., 2009; Kumar et al., 2016). SOD dismutase superoxide radical into peroxide radical. Bacterial inoculated positive response is ascribed to immobilization and reduced uptake of As by the plants render tolerance to the plants (Singh et al., 2016).

The catalase activity was increased with increased concentration of As(III) in rice root (7.33 Unit/ mg protein) as compared to control (4.73 Unit/mg/ protein). A marked increased (21.7% and 24.1%) in catalase activity was also observed in rice root and shoot inoculated with bacteria. In the case of rice root and shoot treated with the combination of As(III)+bacterial inoculants, reduction in catalase activity was observed in comparison to rice treated with As(III) alone. Catalase breakdown H_2O_2 generated inside the cell in water and oxygen molecule. Increased catalase activity in rice inoculated with bacteria might be due to production of enzyme by the bacteria itself around the rhizospheric area of the plants (Liu and Huang, 2002).

Rice plants treated with bacterial inoculants and As(III) separately and in combination showed alteration in GR activity. In the case of rice root treated with high dose of As(III), GR activity increased from 0.34-0.67 while with low As(III), it was 0.58-0.84. Rice treated with high dose of As(III)+ bacterial inoculants,

showed reduction in activity in root and shoot by 14.9% and 26.19% respectively. Rice plant treated with the combination of As(III) and bacterial inoculants showed reduced GR activity in rice root and shoot in comparison to As(III) control reflecting bacterial induced tolerance responses. Glutathione reductase is an important enzyme maintains the redox balance of the cell under various stress (Mishra et al., 2011; Rai et al., 2011). Increased activity of GR in present study under bacteria and As both clearly reflects toxicity which ultimately increased the GSSG level of the cell. Increased GSSG level disturb the pH, redox imbalance and thus metabolic system inside the cell which balance by the GR converting the high GSSG content to GSH (Tripathi et al., 2013). In this way GR provide tolerance to the plants.

4.8. Accumulation and translocation of As

Rice plant treated with low dose of As(III) accumulated 868.08 mg/kg dw As in its root followed by shoot (262.19 mg/kg dw). In the case of high dose the accumulation was 1451.4 and 455.48 mg/kg dw respectively in the root and shoot. Further rice plant supplemented with high dose of As(III)+ bacterial inoculants, showed approx. 50 % reduction in accumulation in root while in shoot reduction was decreased from 455.48- 269.02 mg/kg dw as compared to rice plant treated with As(III) alone. A concentration dependent accumulation of As was found in rice in the order of root>shoot (Table 4.5). High accumulation of As in root might be due to compartmentalization of As in the root vacuole (Shri et al., 2009). However, bacterial mediated reduced uptake of As in rice is due to the binding of

the As to bacteria functional groups and chelation with bacterial extracellular polymers and exudates (Glick, 2010). Bacterial mediated modulation in As uptake is also reported by Srivastava et al. (2013).

Table 4.5: Arsenic accumulation and translocation factor of rice plants treated with bacterial inoculums (BBAH/CH)and As(III).

Treatments	**As (mg kg^{-1} dw)**		**TF**
	Root	**Shoot**	
Control	-	-	-
Bacteria	-	-	-
50 µMAs(III)	868^{b}±26.79	262^{b} ±23.38	0.32
100 µM As(III)	1451^{c}±141	455^{c} ±50.39	0.30
50 µM As(III)+ Bacteria	434^{a}±18.07	203^{a}±14.34	0.47
100 µM As(III)+Bacteria	798^{b} ±32.50	269^{b} ±29.22	0.37

All the values are means of 3 replicate (n=3) ± S.D. ANOVA significant at $p \leq 0.05$. Different letters indicate significantly different values between treatments (DMRT, $p \leq 0.05$).

The translocation factor in rice plant treated with As(III) presented lower value (<1). Maximum translocation of As was observed in the case of rice plant treated with low dose of As(III)+ bacteria (0.49) while minimum translocation (0.30) was observed with high dose of As(III) only. Translocation factor are important parameter to measure hyperaccumulation potential of plant. Present study reveals lower value of TF (<1) signifies reduce translocation of As to above ground tissue. Reduced TF value at high concentration of As clearly demarcated its phytostabilization potential in growing soil condition. This may ascribed to change in physiochemical and biological structure of soil due to plants and

bacterial exudates (Burd et al., 2000). In bacterial inoculated rice plants with reduced TF may be due to reduced uptake of toxic element facilitated by bacteria present around the root (Jia et al., 2014).

4.9. Growth characteristics of matured rice plants

Rice plants supplemented with As resistant bacterial strains showed positive growth in form of root length, shoot length and biomass with respect to control. Significantly increased were observed in root length (17%) and shoot length (9%) with BBAU/BN strain. Concentration dependent significant reduction in growth was observed in rice treated with AsIII (5 ppm) and AsIII (10 ppm). However, in combination of bacteria and As(III), plant showed positive response which was more pronounced in the case of As treated rice supplemented with bacteria strain. Results demarcated that As resistant bacteria play significant role in plant to overcome the toxic impact of As (Fig. 4.4). Decreased growth of rice plant under As stress have been reported by many authors (Ahsan et al., 2010; Tuli et al., 2010; Kumar et al., 2014). Degeneration of root tissue, membrane peroxidation and photosynthetic machinery under As are the primary cause of reduced growth (Kumar et al., 2014). Growth recovery in rice inoculated with Arsenic and bacteria are the results of uptake of As by microbes and reduced translocation (Singh et al., 2016; Rajkumar and Freitas, 2008). Hare et al. (2017) reported that microbes enhances the solubility of P through the process of mineralization and bacterial mediated nitrification also contribute for the better growth response in rice inoculated with bacteria.

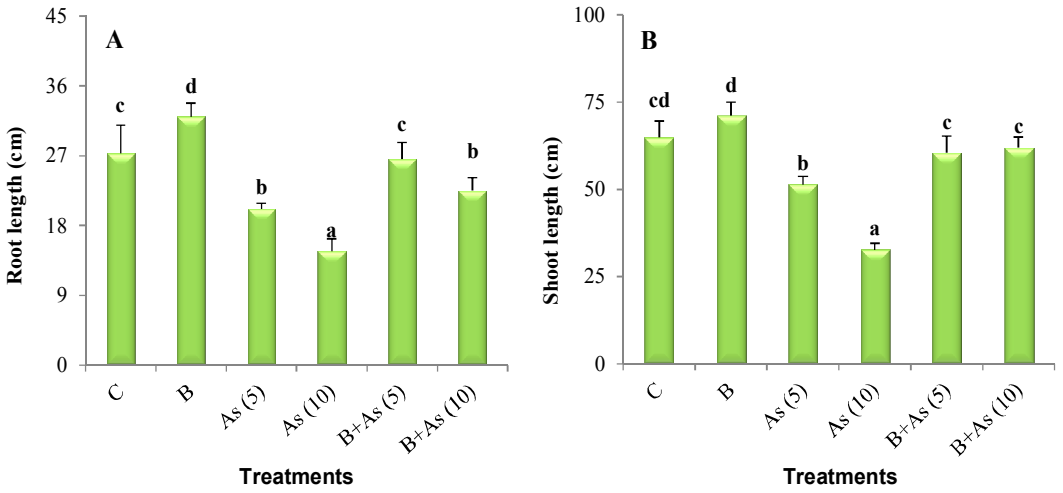

Fig. 4.4: Growth characteristics (root and shoot length) of rice plants treated with different concentration of As(III) and bacteria. All value are means ±SD. ANOVA pos hoc DMRT has done to analyse the significant difference. Identical superscript denotes o significant change.

Application of As(III) and bacterial inoculants in rice plant either singly and in combination showed alteration in number of tiller, panicle length and yield. Rice plant treated with high dose of As(III) showed 27.71%, 33.26% and 34.21%reduction in number of tiller, panicle length and yield, respectively, in comparison to control. However, rice inoculated with bacteria only, 16.17% increased in tiller number was observed. In the case of rice plant treated with combination of high dose of As(III)+bacteria, number of tiller increased from on average 9.31 to 12.42%, while panicle length showed similar change i.e., approx. 30% increased in comparison to their As(III) control. In the case of yield it was increased by 17.56%. Further, rice plants treated with low dose of As(III)+bacteria, the yield was increased by 11.11% as compared to rice treated with As(III) alone (Fig. 4.5).

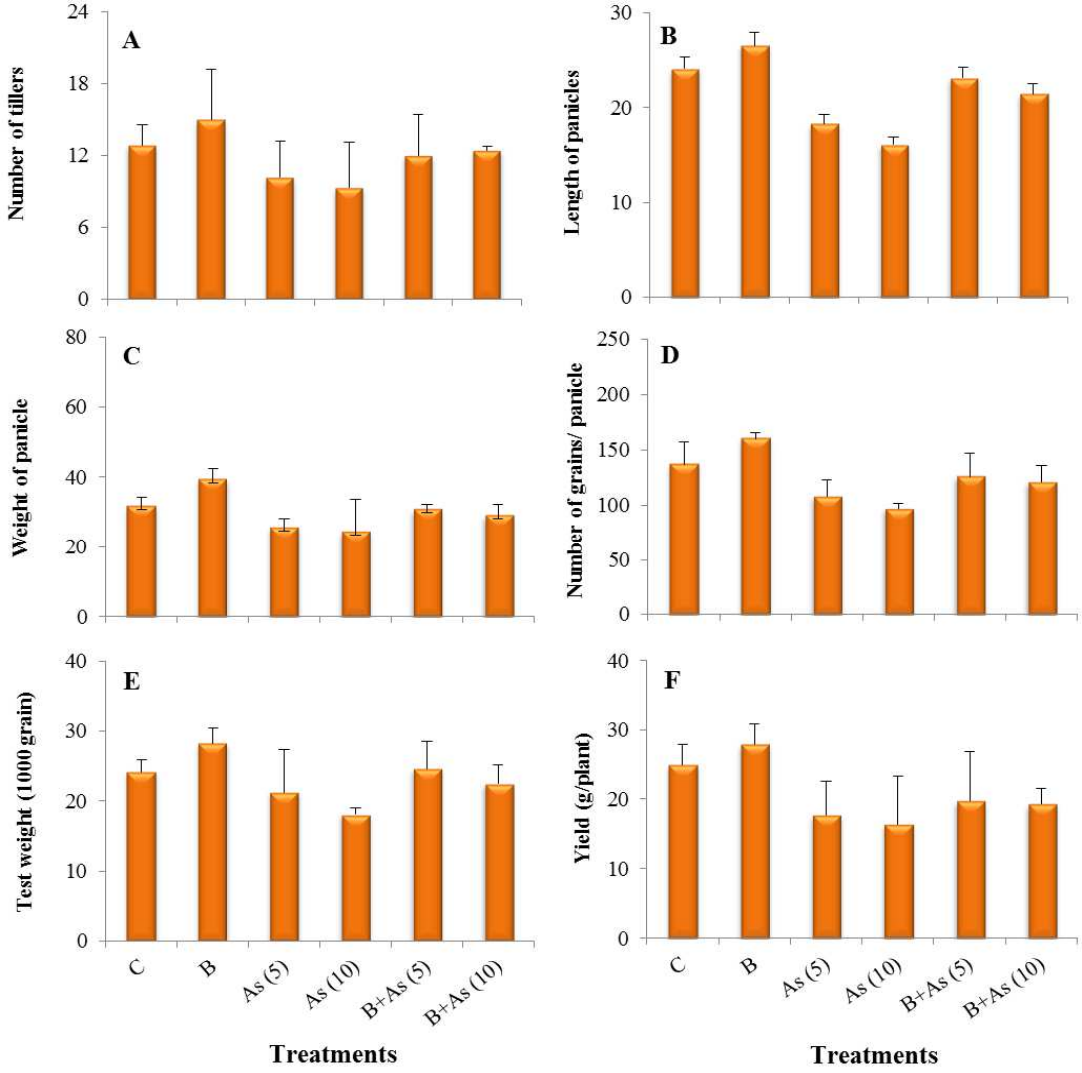

Fig. 4.5: Response of growth and yield attributing characters of rice plants treated with different concentration of As(III) and bacteria.

4.10. Arsenic accumulation

Rice plant treated with different dose of As(III) showed that rice root accumulated high content of As than shoot which increased with increasing the concentration of As(III). Results showed that rice root accumulated 1508 µg kg^{-1} dw As and 2578 µg kg^{-1} dw at 5 and 10 ppm, respectively. In the case of shoot it was 400 and 688 µg kg^{-1} dw, respectively in comparison to control. Arsenic

treated rice supplemented with bacterial inoculants showed approx. 50% reduction in accumulation which was 738 and 1385 µg kg^{-1} dw, respectively, as compared to their respective control. The accumulation of As in husk and grain was 166 and 95.06 µg kg^{-1} dw at low dose of As(III). In the case of rice plant treated with combination of bacterial and high dose of As(III), 37.92% reduction was observed in grain while in husk it was 40.97% as compared to therice treated with As(III) alone (Table 4.6). In the presented study high accumulation of As in the root was in accordance with study of many authors (Kumar et al., 2014; Mishra et al., 2016; Hare et al., 2017). However, reduction in As accumulation to the grain level after the supplementation of microbial inoculants may ascribed tomicrobial mediated transformation (oxidation, reduction and methylation) which leads to the reduction in mobility and distribution of As species in the environment (Yin et al., 2011). Microbes present in soil secrets various organic acids which changes the pH and thus redox balance of the surrounding enabling sequestration of metals and inhibition of the translocation in the root (Silver and Fung, 2005).

Table 4.6: Arsenic accumulation in root, shoot, husk and grain rice plants.

Treatments	**Arsenic accumulation**			
	Root	Shoot	Husk	Grain
Control	-	-	-	-
Bacteria	-	-	-	-
As (5)	1508 ^{b}±104	400 ^{b}±18.31	166 ^{b}±13.40	95.06 ^{b}±4.48
As (10)	2578 ^{c}±252	688 ^{c}±62.63	349 ^{c}±40.16	192.52 ^{d}±26.23
Bacteria + As (5)	738^{a}± 63	232 ^{a}±16.45	114 ^{a}±18.97	66.08 ^{a}±5.37
Bacteria + As (10)	1385 ^{b}± 108	308 ^{a}±33.51	206 ^{b}±31.59	119.50 ^{c}±17.63

All value are means ±SD.

4.11. Effect on mineral elements in grain of rice

Results showed that concentration of all the studied element (Fe, P, Zn, Mn, Cu, Co, and Se) decreased under As(III) stress (Fig. 4.6). However, increasing trends were observed in the mineral content in rice grain inoculated with bacteria. Maximum reduction was observed in Se(39.25%) while minimum in Cu (16.62%) in rice plant treated with high dose of As(III) as compared to control. In the case of rice grain inoculated with bacterial strain maximum increased was observed in Co (40.47%) followed by Cu (34.88%), Mn (30.41%), P (30.01%), Fe (26.94%), Zn (22.31%) and Se (16.51%) as compared to control. Further, rice plant treated with the combination of As and bacterial inoculants showed decreasing trend as compared to their As(III) control. Maximum reduction was observed in Zn (52.5%) in rice grain cosupplementedwith high dose of As (III)+bacteria and minimum with Mn (7.44%) Results revealed that rice plant treated with AsIII (5 and 10) supplemented with bacterial strain showed positive response in the form of increased mineral content. Reduction in mineral uptake by the rice plant is the clearcut demarcation of As induced toxicity. Williamson et al. (2009) also reported the minimal uptake of Se, Zn and Ni in the grain of rice under As stress. Reduction in mineral content in rice grain in present study may be due to arsenic induced minimizing transportation, metal interaction, competition, root cell degeneration and reducing metabolic processes (Siedlecka, 1995). He However, increased mineral content by bacteria supplementation is the results of acquisition of As by microbes and As transformation into less toxic form (Rajkumar et al., 2012). Decreased mineral content is also caused by ion

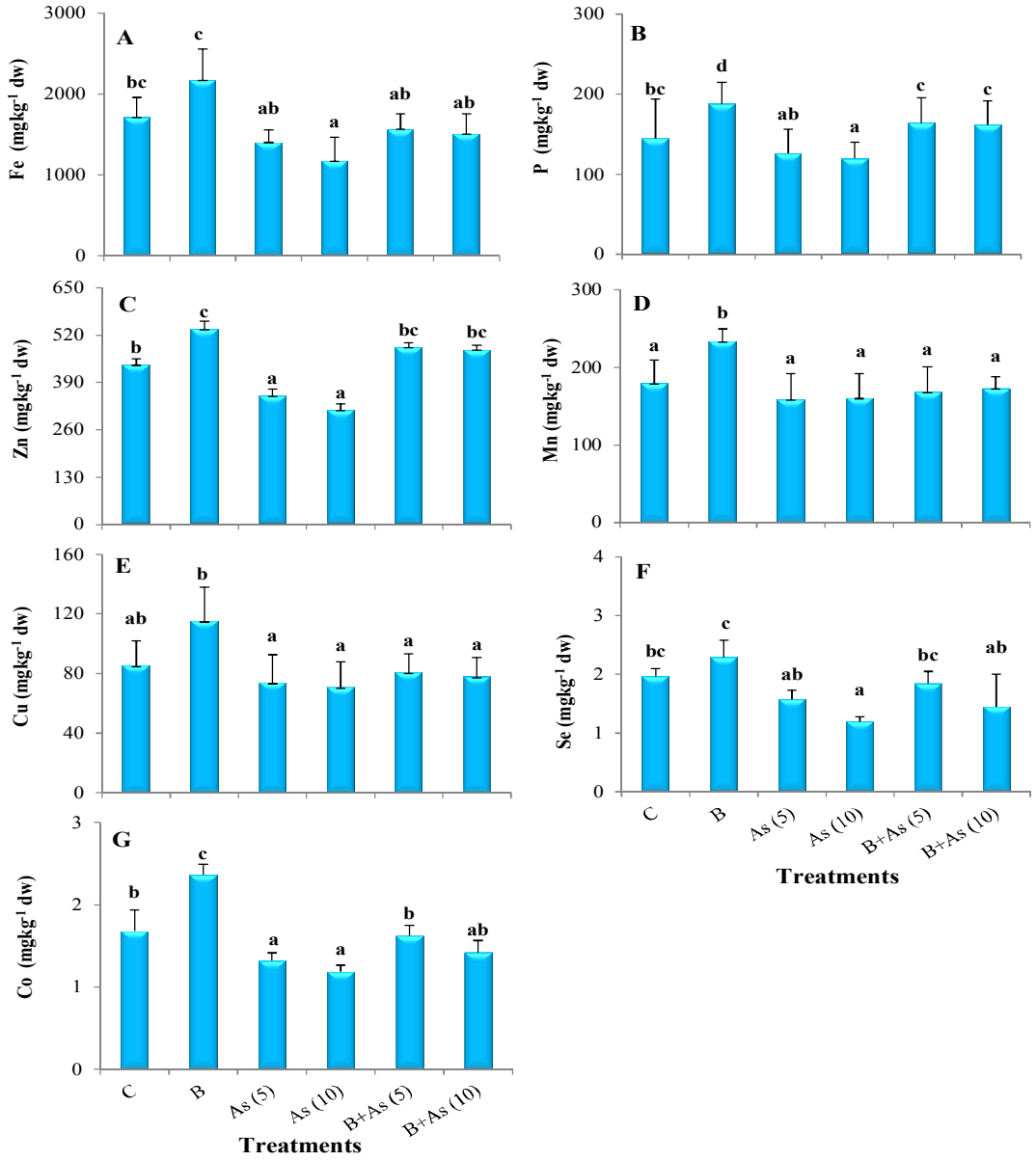

Fig. 4.6: Accumulation of different mineral nutrients (Fe, P, Zn, Mn, Cu, Se and Cu) in rice grain treated with different concentration of As(III) and bacteria. All value are means ±SD. ANOVA pos hoc DMRT has done to analyse the significant difference. Identical superscript denotes o significant change.

leakage from damaged root and immobilization of elements in root resulting into strong deficiency in shoot (Li et al., 2006). A number of author have been reported the lower translocation of mineral under heavy metal stress in plants (Williamson et al., 2009; Dwivedi et al., 2010).

Chapter 5

Summary and Conclusion

5. SUMMARY AND CONCLUSION

Arsenic (As) is naturally occurring well known deadlier non threshold carcinogen of natural and anthropogenic origin. Rice (*Oryza sativa* L.) is a staple diet for more than half of the world's population. Rice is particularly efficient in accumulating As and paddy fields in South and South-East Asia, which are highly contaminated with As. Particularly, in West Bengal, India, where millions of people depend on rice as subsistence diet are getting affected. It is also a threat to sustainable agriculture in affected areas.

The main objective of this study was rhizospheric microbes grown in As contaminated soil of West Bengal, India were isolated and characterized for their As resistant potential and bioremediation potential. The work entailed studying the effects of As on plant metabolism, uptake and toxicity along with the understading the role of antioxidant and thiol metabolism in alleviation of deleterious effects of As in rice plants. Further, the study evaluated the potential of arsenic resistant bacteria supplementation on lowering the As level in rice plants.

Physicochemical analysis of soil sample collected from different As contaminated sites of West Bengal, India showed that out of three selected sites, The water holding capacity (WHC) was high in the Chinsurah site. In the case of inorganic nutrients such as N and P, it was maximum found in the site Chinsurah site . The maximum contamination of As in all the studied site were reported in site Biranagar site which was 17.18 mg/kg.

The species of As significantly reduced the root length; the maximum being during AsIII exposure in IET-19226. Shoot length of BRG-12 and IET-19226 increased at lower concentration of AsIII and AsV followed by a decline at higher doses.. Photosynthetic pigments showed significant decline in chlorophyll *a, b* and chlorophyll total at As exposures with more effect being in BRG-12 in comparison to IET-19226. Carotenoid content showed increase at low concentrations but decrease in higher concentration in IET-19226. The maximum induction in protein content was observed in IET-19226 at equimolar (10 μM) concentration of AsV and AsIII, however, maximum decline in protein level was observed in BRG-12 at higher exposure.

Rice plants treated with different dose of As and bacterial inoculants showed alteration in growth. Shoot length approx. 50% reduction was observed at high dose of As(III). In the case of rice plant treated with the combination of bacterial strain and As improvement in root shoot length was observed in comparison to rice treated with As alone. However, no significant improvement root length was observed under bacterial inoculated with different indication of dose of As. Reduction in root and shoot length is clear cut indication of As induced toxicity. It may be due to As decrease the uptake and translocation of other micro element and nutrients by adsorption on root cell, cell membrane damage and reduced availability of nutrient required for the growth of the plants.

Arsenic induced reduction and destruction of chlorophyll machinery has been observed in rice plant treated with low and high dose of As(III) which was more prominent with high concentration of As(III). However, increased chl a , chl

b and total chl content was observed in shoot inoculated with bacterial strain as compared to control.

Rice plant treated with different dose of As(III) showed that rice root accumulated high content of As than shoot which increased with increasing the concentration of As(III). Results showed that rice root accumulated 1508µg/kg dw As and 2578 µg/kg dw at 5 and 10 ppm, respectively. In the case of shoot it was 400 and 688 µg/kg dw, respectively in comparison to control. Arsenic treated rice supplemented with bacterial inoculants showed approx. 50% reduction in accumulation which was 738 and 1385 µg/kg dw, respectively, as compared to their respective control. The accumulation of As in husk and grain was 166 and 95.06 µg/kg dw at low dose of As(III). In the case of rice plant treated with combination of bacterial and high dose of As(III), 37.92% reduction was observed in grain while in husk it was 40.97% as compared to the rice treated with As(III) alone.

The concentration of all the studied element (Fe, P, Zn, Mn, Cu, Co, and Se) decreased under As(III) stress. However, increasing trends were observed in the mineral content in rice grain inoculated with bacteria. Maximum reduction was observed in Se (39.25%) while minimum in Cu (16.62%) in rice plant treated with high dose of As(III) as compared to control. In the case of rice grain inoculated with bacterial strain maximum increased was observed in Co(40.47%) followed by Cu (34.88%), Mn(30.41%), P(30.01%), Fe (26.94%), Zn(22.31%) and Se(16.51%) as compared to control. Further, rice plant treated with the combination of As and bacterial inoculants showed decreasing trend as compared

to their As(III) control. Maximum reduction was observed in Zn (52.5%) in rice grain cosupplemented with high dose of As(III)+bacteria and minimum with Mn(7.44%) Results revealed that rice plant treated with AsIII (5 and 10) supplemented with bacterial strain showed positive response in the form of increased mineral content.

Rice plant treated with different concentration of As and bacterial strain showed cellular toxicity by increasing the MDA content which was significantly increased in the case of high dose of As as compared to control. It was observed that rice treated with high dose of As(III) and bacteria separately showed 30.9% increased in MDA level while no significant change observed with rice inoculated with bacteria only. In the case of rice plant root and shoot treated with combination of low dose of As(III) and bacterial inoculants, MDA content decreased by 12.05% and 30.34% respectively while with high dose of As(III)+bacterial inoculants it showed 14.2% and 20% as compared to their As(III) control.

H_2O_2 content in rice root and shoot treated with high As(III) showed increased level (0.592 and 0.289 mmol/g fw) in comparison to control (0.300 and 0.169 mmol/g fw). In the case of rice treated with low dose of As(III), no significant change was observed. H_2O_2 content in Rice plant treated with bacterial inoculants increased in the root while decreased in shoot as compared to control. The co-application of bacterial strain and different doses of As, recovered the level with respect to rice treated with As alone. Overall results showed that inoculated bacteria reduced the toxic level of As by decreasing the H_2O_2 content

in rice treated with low dose of As and Bacterial inoculants (0.342 mmol/g fw) in comparison to As control (0.592 mmol/g fw).

Thiolic legends presented in Cys, GSH and GSSG in figure 2 (A-D). Cysteine content in rice root and shoot treated with high dose of As increased (107.26 and 191.25 nmol/g fw) in comparison to control root and shoot (69.20 and128.8 nmol/g fw). In the case of rice plant treated with bacterial inoculants also increased cysteine content in root and shoot. However, no significant change was observed in the case of root. Rice plant treated with combination of bacterial inoculants and low dose As(III) showed decreased cysteine content in root in comparison to As while increased in shoot. In the case of high dose +bacterial inoculants cysteine content increased to 131.08 and 254.95 nmol/g fw respectively in root and shoot in comparison to As(III) alone. Cystein is important amino acids and acts as antioxidants inside plant cell.

The glutathione (GSH) content in rice root treated with high dose of As(III) increased by 32.5% while no significant change was observed under low As(III). In the case of rice shoot treated with high dose of As(III) showed increased GSH value from 67.29- 69.20 µmol/g fw and with low As(III), GSH content was decreased from 67.29-59.50 µmol/g fw in comparison to control. Rice plant treated with bacterial inoculants singly showed increased GSH content in comparison to control. In the case of rice plant treated with the combination of As(III) and bacterial inoculants, showed reduced GSH content in comparison to As(III) alone. The oxidized glutathione (GSSG) content in rice root treated with

different combination of As and bacterial inoculants either singly or in combination showed similar trends.

Superoxide dismutase activity in rice plant treated with singly and combination of As(III) and bacterial inoculants showed increased response which was more in the case of rice treated with high dose of As(III) separately. The SOD activity was increased by 13.2% in root treated with bacterial inoculants. In the case of high dose of As(III), the SOD activity in root and shoot was increased by 52.5% and 16.12% as compared to control. Rice plant treated with combination of As(III)+ bacterial inoculants showed maximum reduction in SOD activity in root (128.16 U/ mg protein) as well as shoot (204.33 U/ mg protein) under low dose of As(III) as compared to As(III) only.

The catalase activity was increased with increased concentration of As(III) in rice root (7.33 Unit/ mg protein) as compared to control (4.73 Unit/mg/ ptotein). A marked increased (21.7% and 24.1%) in catalase activity was also observed in rice root and shoot inoculated with bacteria. In the case of rice root and shoot treated with the combination of As(III)+bacterial inoculants, reduction in catalase activity was observed in comparison to rice treated with As(III) alone.

Rice plants treated with bacterial inoculants and As(III) separately and in combination showed alteration in GR activity. In the case of rice root treated with high dose of As(III), GR activity increased from 0.34-0.67 while with low As(III), it was 0.58-0.84. Rice treated with high dose of As(III)+ bacterial inoculants, showed reduction in activity in root and shoot by 14.9% and 26.19% respectively. Rice plant treated with the combination of As(III) and bacterial inoculants showed

reduced GR activity in rice root and shoot in comparison to As(III) control reflecting bacterial induced tolerance responses.

The present study evaluated the growth, mineral content and biochemical effects of arsenic exposures with inoculation of arsenic resistant bacteria in rice plant. The results of the study the following concluding remarks:

- In the present study, rhizospheric microbes grown in As contaminated soil of West Bengal, India were isolated and characterized for their As resistant potential and bioremediation potential.
- Physicochemical analysis of soil sample collected from different As contaminated sites of West Bengal, India showed that out of three selected sites, maximum pH was found in the site purbosthali with high level of electrical conductivity (159.7 µs/cm), porosity (99.42 %) and total organic carbon (1.56 %) as compared to other sites.
- The water holding capacity (WHC) was high in the site chinsurah whereas minimum WHC was recorded in site birnagar.
- The maximum contamination of As in all the studied site were reported in site birnagar which was 17.18 mg/kg.
- Out of three selected bacteria from different site, bacterial isolates (BBAU/CH) showed good growth, high tolerance and resistant to the level of 100 mM of As(III).
- Arsenic treated rice plant inoculated with isolated bacterial strain (BBAU/CH) showed increased growth of the rice plant in term of root length, shoot length, fresh weight and chlorophyll content.

- Reduced translocation and accumulation root (2 fold) and shoot (1.5 fold) of As in rice inoculated with bacterial strain was observed.
- Rice inoculated with bacterial strain exhibited reduced lipid peroxidation, H_2O_2 production, induces antioxidants (cysteine, GSH, and GSSG) and enzymatic activities (SOD, CAT, and GR) were observed in rice root and shoot.
- The mineral status in grain of As(III) (high dose) treated rice exhibited alteration in mineral content and showed maximum reduction was observed in Se (39.25%) while minimum in Cu (16.62%).
- Bacterial strain (BBAU/CH) may be used as potent bioremediator for rice grown in As contaminated land or area.

Further, financial support and opportunity is needed for the detailed study and research.

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Annexures

Arsenopyrites

Realgar

Orpiment

Fig. 1: Arsenic naturally occurs in different mineral forms such as arsenopyrites (FeAsS), realgar (As_4S_4) and orpiment (As_2S_3).

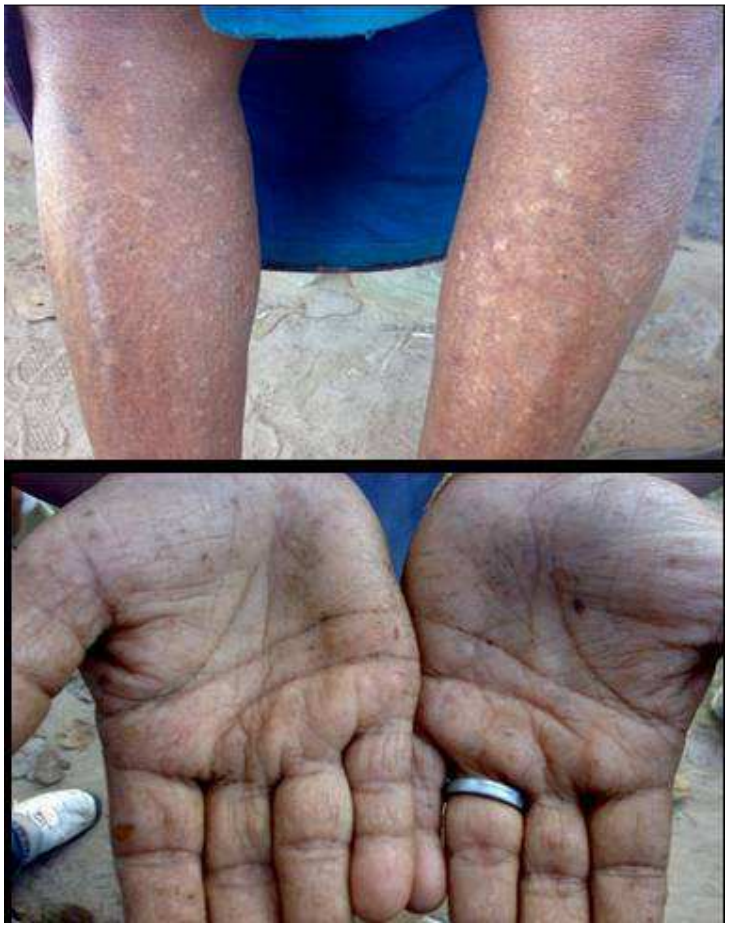

Fig. 2: Some external signs of arsenic toxicity

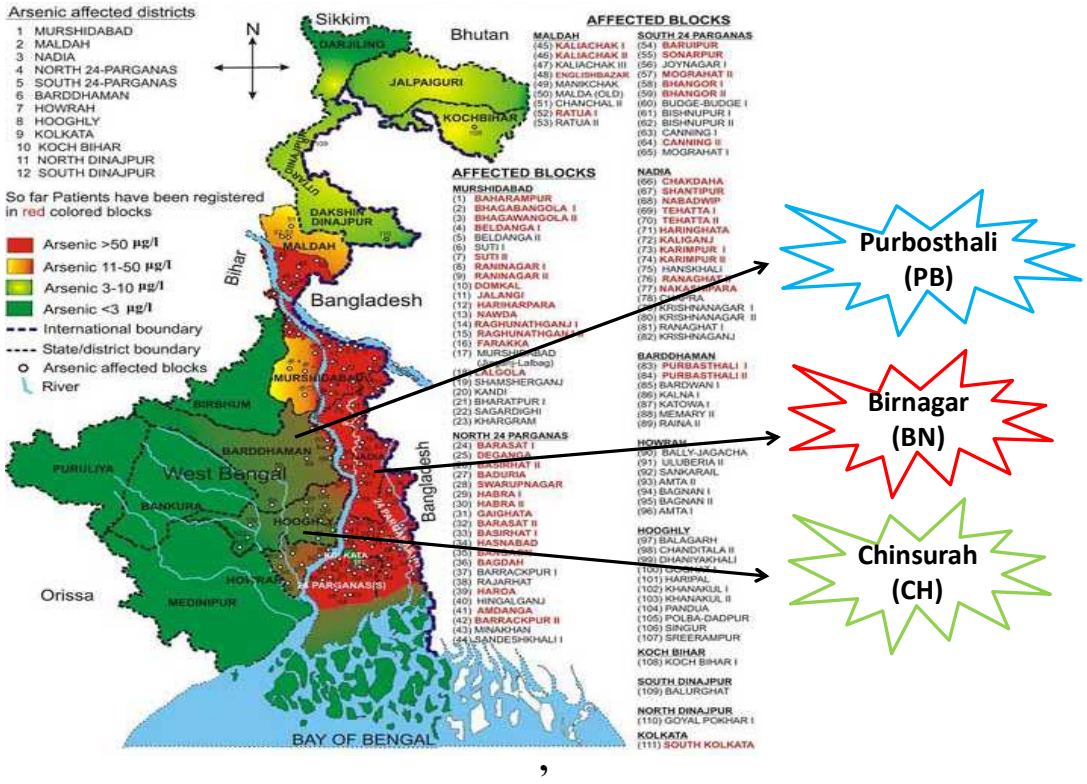

Fig. 3: Ground water arsenic contamination and sampling site in West Bengal.

Fig. 4: Rice plants germination in control condition.

Fig. 5: Figure showing the isolates bacteria

Hewitt Medium Composition

S.No.	Chemicals	g L^{-1}	Stock 100% (mll^{-1})
1	Ammonium nitrate (NH_4NO_3)	0.352	10
2	Potassium sulfate (K_2SO_4)	0.348	10
3	Calcium chloride ($CaCl_2$)	0.588	10
4	Magnesium sulfate ($MgSO_4$)	0.369	10
5	Potassium nitrate (KNO_3)	0.141	10
6	Monopotassium phosphate (KH_2PO_4)	0.176	10
7	Ethylenediaminetetraacetic acid (EDTA)	0.0202	0.5
8	Ferrous sulphateheptahydrate ($FeSO_4.7H_2O$)	0.0139	0.5
9	Boric acid (H_3BO_4)	0.778	1
10	Zinc sulfate ($ZnSO_4$)	0.287	1
11	Manganese sulfate ($MnSO_4$)	0.845	1
12	Sodium molybdate (Na_2MoO_4)	0.121	1
13	Cobalt sulfate ($CoSO_4.7H_2O$)	0.031	1
14	Copper sulfate ($CuSO_4$)	0.2496	1

Nutrient agar medium

Nutrient agar medium was used for the isolation of arsenic resistant bacteria from various soil and sediment samples the composition is as follows:-

Composition	Amount g/L distilled water
Beef extract	3.0
Peptone	5.0
Agar	15.0
NaCI	8.0
Ph	7.0

Nutrient broth

Nutrient broth was used for the preparation of bacterial suspension.The composition is as follows:-

Composition	**Amount g/L Distilled water**
Beef extract	3.0
Peptone	5.0
NaCI	8.0
pH	7.0

List of Publications

LIST OF PUBLICATIONS

- **Nisha Bharti**, Vishvas Hare, Rupali Mishra and Vinay Singh Baghel (2017). Microbial based integrated management of arsenic toxicity in rice (*Oryza sativa* L.) grown under arsenic contaminated soil of West Bengal, India. International Journal of Current Trends in Science and Technology, 7(12): 20420-20430.
- **Nisha Bharti**, Vishvas Hare, Rupali Mishra and Vinay Singh Baghel (2017). Role of bacteria on the growth and mineral content in rice (*Oryza sativa* L.) plant under arsenite stress. International Journal of Current Trends in Science and Technology