Variation of arsenic accumulation in paddy plant during its post monsoon cultivation in West Bengal, India: with special reference to its effects on photosynthetic pigments and a study of arsenic metabolizing bacterial population with a scope of bioremediation

Thesis submitted for the partial fulfilment of the degree

of

Master of Technology in Environmental Biotechnology

by

MEENAKSHI MUKHERJEE

Registration No.: **141028** of 2017-18

Examination Roll No.: **M4EBT19008**

Class Roll No.: **001730904004**

Jadavpur University

Under the guidance of

DR. TARIT ROYCHOWDHURY

School of Environmental Studies

Jadavpur University

Kolkata- 700032

To Whom It May Concern

It is hereby recommended that this thesis entitled "**Variation of arsenic accumulation in paddy plant during its post monsoon cultivation in West Bengal, India: with special reference to its effects on photosynthetic pigments and a study of arsenic metabolizing bacterial population with a scope of bioremediation**" is prepared and submitted for the partial fulfilment of the continuous assessment of **Master of Technology** in **Environmental Biotechnology** course of Jadavpur University by **Meenakshi Mukherjee**, a student of the said course for the session 2017 – 2019, under my supervision and guidance. It is also declared that no part of this thesis has been presented or published elsewhere.

Dr. Pankaj Kumar Roy
Director
School of Environmental studies
Jadavpur University, Kolkata-700032

Dr. Tarit Roychowdhury
Thesis Supervisor
Associate Professor
School of Environmental studies
Jadavpur University, Kolkata-700032

Dr. Pankaj Kumar Roy
Dean
Faculty of Interdisciplinary Studies, Law and Management (FISLM)
Jadavpur University
Kolkata-700032

Declaration

The thesis entitled "**Variation of arsenic accumulation in paddy plant during its post monsoon cultivation in West Bengal, India: with special reference to its effects on photosynthetic pigments and a study of arsenic metabolizing bacterial population with a scope of bioremediation**" is prepared and submitted for the partial fulfilment of the continuous assessment of Master of Technology in Environmental Biotechnology course of Jadavpur University for the session 2017-19.

Meenakshi Mukherjee

Examination Roll No.:M4EBT19008

Class Roll No.: 001730904004

Registration No.: 141028 of 2017-18

Date:

Place: School of Environmental Studies

Jadavpur University

Kolkata-700032

CERTIFICATE OF APPROVAL

This foregoing thesis is hereby approved as a credible study of an engineering subject carried out and presented in a manner satisfactorily to warranty its acceptance as a prerequisite to the degree for which it has been submitted. It is understood that by this approval the undersigned do not endorse or approve any statement made or opinion expressed or conclusion drawn therein but approve the thesis only for purpose for which it has been submitted.

Final Examination for evaluation of Thesis

_____

_____

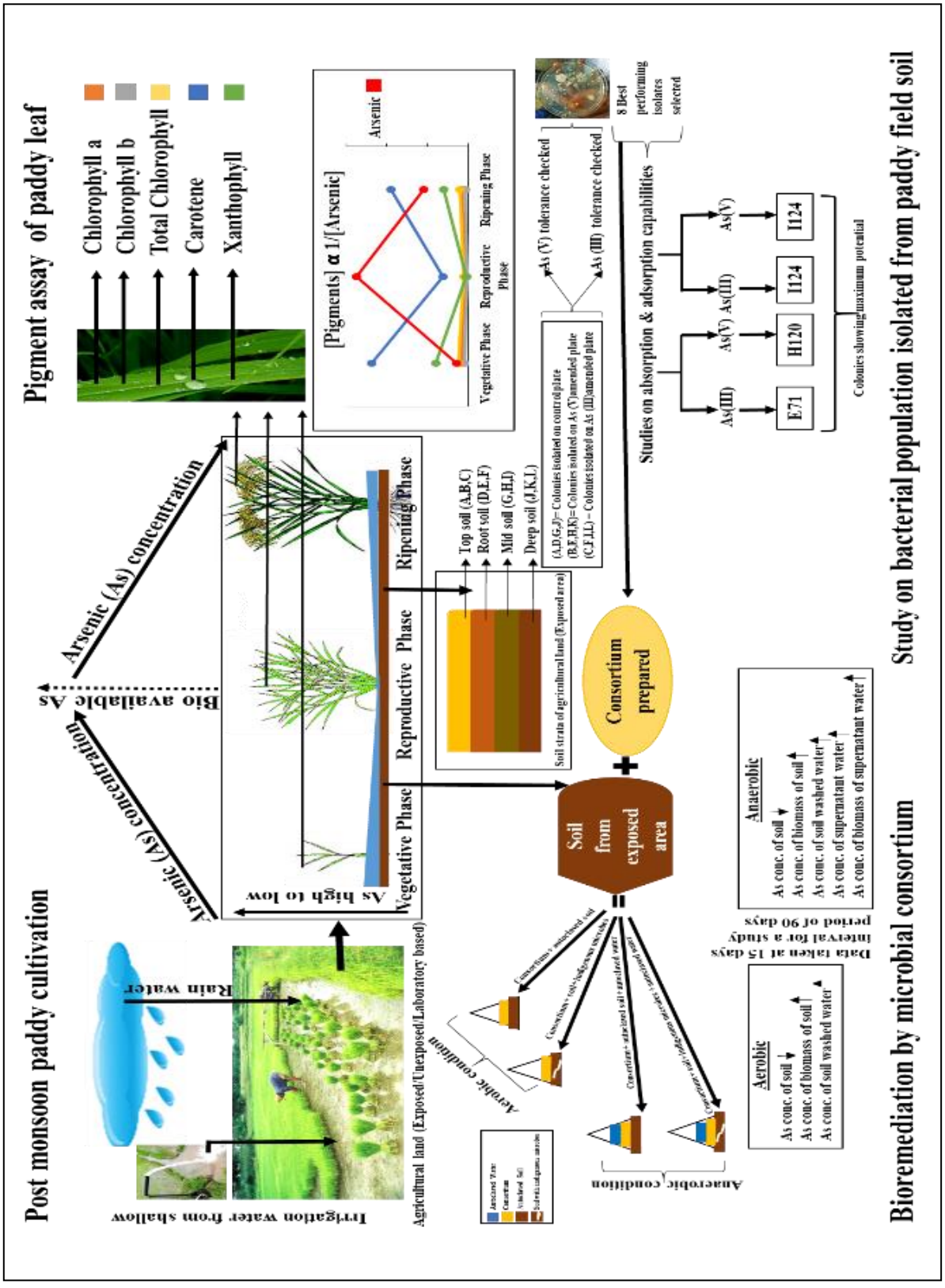
(Signature of the Examiners)

Acknowledgement

I would like to express my sincere gratitude to each and every one of those who have helped me with this project. I am grateful to **Dr. Tarit Roychowdhury**, Associate Professor, School of Environmental Studies, Jadavpur University, for giving me the permission to carry out this project and for his excellent guidance, patience, support and encouragement. I am deeply indebted to **Dr. Joydeep Mukherjee**, Professor, School of Environmental Studies, Jadavpur University and **Dr. Subarna Bhattacharya**, Professor, School of Environmental Studies, Jadavpur University and **Dr. Reshmi Das**, Professor, School of Environmental Studies, Jadavpur University who encouraged me while carrying out my work. I am grateful to **Dr. Soma Ghosh**, **Nilanjana Roy Chowdhury, Madhurima Joardar, Shresthashree Swain**, **Antara Das** and **Ayan De** (Scholars) for helping me out in times of need. I am also thankful to my class-mates Bishal Ghosh and Swarnendu Shekhar Das for their assistance, criticisms and their useful insights. I would also like to thank Debasish Maity, Nilu Das and Pijush Sarkar. Last but not the least, this research project would not have been possible without the confidence, endurance and support of my parents and family.

Meenakshi Mukherjee

Graphical Abstract



Abstract

The trends of Arsenic (As) accumulation in different parts of paddy plant *(Oryza sativa*) was observed from a highly As contaminated field of Teghoria and a moderately As contaminated field of Madhusudankathi, in Gaighata block of North 24 Parganas during post monsoon cultivation period (August-November).The results were compared with control field of Pingla in west Medinipur along with two pot studies which were carried out with moderately contaminated soil (of Madhusudankathi) and uncontaminated water and uncontaminated soil and uncontaminated water respectively. Among the three phases of cultivation; Vegetative, Reproductive and Ripening phase, maximum accumulation was observed during the Reproductive Phase. Besides, in each phase, As accumulation in different parts of paddy plant followed the Translocation theory showing maximum accumulation in the roots>stem> leaf>pedicle>grain. Pigment assays were done with the leaves of these paddy plants at different phases of post monsoon cultivation from the same three fields. Trends of metabolic activity estimated by the change in pigment concentration were observed for Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll. In case of all the pigment assays, the trends showed that the concentration of total chlorophyll, carotene and xanthophyll varied inversely with the As accumulated during the cultivation period. 149 bacterial colonies were isolated from top soil, root soil, mid soil and bottom soil of paddy fields of Madhusudankathi in Gaighata block of North 24 Parganas during the month of September. All the soil samples were subjected to dilution plating on nutrient agar medium under three conditions i.e. i) control ii) amended with As^{+3} iii) amended with As^{+5} and their CFU count suggested that both As^{+3} and As^{+5} resistant bacteria predominate in root soil. It was followed by As tolerance study for both As^{+5} and As^{+3}over a wide range of concentrations varying from 50 mM to 600 mM for As^{5+} and 5 mM to 15 mM for As^{+3} from which 8 colonies showing maximum tolerance were selected for absorption and adsorption study. Maximum absorption of 10 µg of As^{+3} by E71 and 14 µg of $As+^{5}$ by H120, respectively while maximum adsorption of 0.127 µg of As^{+3} and 0.1321 µg of As^{+5} was shown by I124, respectively. Since the selected strains were capable of remediating As, the 8 colonies were mixed to form a consortium and their efficiency of remediation was checked by mixing them with high and moderately contaminated soil in aerobic as well as anaerobic conditions. It was seen that with time, the consortium was capable of reducing the soil As by absorbing the same into its biomass. Therefore, this consortium can be efficiently used to arrest As preventing it from further being translocated in the paddy plant.

Contents

Tables and Figures

1. Introduction

1. Introduction

Contamination of groundwater, either from anthropogenic or natural sources have several social impacts. It has now turned out to be a major environmental concern in different parts of the world. Millions of people in various countries are exposed to very high levels of Arsenic (As) via intake of As contaminated groundwater. Elevated level of As in groundwater has been well documented in Chile, Mexico, USA, China, Argentina, and Hungary as well as in the Indian States of West Bengal, Bangladesh, and Vietnam (Ravenscroft et al., 2009). Around 150 million people around the world are estimated to be globally affected with an increasing prospect because new affected areas are continuously being discovered (Shankar & Shanker., 2014). Arsenic, a well-known carcinogen, is considered as one of the world's most hazardous chemicals. Excessive and long term (such as 5–10 years) human intake of toxic inorganic As through drinking water and food may result in Arsenicosis, a common name generally used for As related health problems like skin disorders, skin cancers, internal cancers (in bladder, kidney, and lung), diseases of the blood vessels of the feet, possibly diabetes, increased blood pressure, and also reproductive disorders (Chakraborti et al., 2004). In terrestrial environment, the inorganic forms of As (such as trivalent arsenite and pentavalent arsenate) in general are more prevalent and toxic than the organic forms. Arsenic also exerts detrimental effects on general protein metabolism with high toxicity by reacting with sulfhydryl groups existing in the cysteine residues (Finnegan & Chen., 2012). Arsenic is ubiquitous in our environment. Soil contamination by As often leads to ground water contamination and As toxicity in plants, human and animals. Besides origin of As from geo-chemical sources, human activities have caused major accumulation of As in soil through use of As based pesticides, manufacturing of As based compounds, smelting of As ores, mining processes, fuel utilization etc. (Bissen & Frimmel., 2003). Plants represent the primary route by which As enters terrestrial food webs. Arsenate and arsenite are two major forms of As available in the soil, both of which are inter-convertible. Arsenate is taken up via carrier mediated phosphate (PO_4^{-3}) translocation system and arsenite is taken up by plants through aquaporins. Arsenite after being taken up by plants, inhibits cellular functions, causes damage to root membranes, induces oxidative stress and may even cause cellular death. Arsenite is regarded to be more toxic than arsenate for plants in terms of root length and shoot height etc. Depression of plant growth and development has often been observed as a result of high levels of As additions to soils and groundwater in As contaminated areas (Adriano., 2001). It is not considered as an essential element for plant growth. Plants vary considerably in their tolerance to high levels of soil As. Plants take up As from the solution phase of soils.

Bioaccumulation of As is hazardous to humans and animals due to its possible relationship with cancer, arteriosclerosis, chronic liver disease etc. The highest concentrations of As are found in plant roots, intermediate levels in vegetative tissue and in the lowest levels in reproductive tissue. Toxicity to animals and humans is usually due to the ingestion of As accumulated plant materials (Eisler., 2004).

1.1 Arsenic contamination all over the world

Arsenic contamination of groundwater is a form of groundwater pollution which is often due to the naturally occurring high concentrations of As in deeper levels of groundwater. It is a high-profile problem because of the use of deep tube-wells for water supply in the Ganges Delta, causing serious As poisoning to large numbers of people (Shankar & Shanker., 2014). A 2007 study found that more than 137 million people in more than 70 countries are probably affected by As poisoning of drinking water (Paul et al., 2015). The problem became a serious health concern after mass poisoning of water in Bangladesh. In many countries throughout the world, including the US, Asia, Thailand, China, Pakistan, Bangladesh, Chile, Argentina and Nepal As contamination of ground water is reported (Ravenscroft et al., 2009). In India, the states of West Bengal, Jharkhand, Bihar, Assam, Uttar Pradesh, Manipur and Chhattisgarh are reported to be mostly affected by As contamination of groundwater above the permissible level. West Bengal (WB) is an As endemic state in India, with 9 out of 18 districts exposed to groundwater contaminated with As (of geological origin) above the WHO's maximum permissible limit (10 mg/L) including Malda, Murshidabad, Nadia, North 24 Parganas, South 24 Parganas, Burdwan, Kolkata, Howrah and Hoogly (Bhattacharya., 2019).

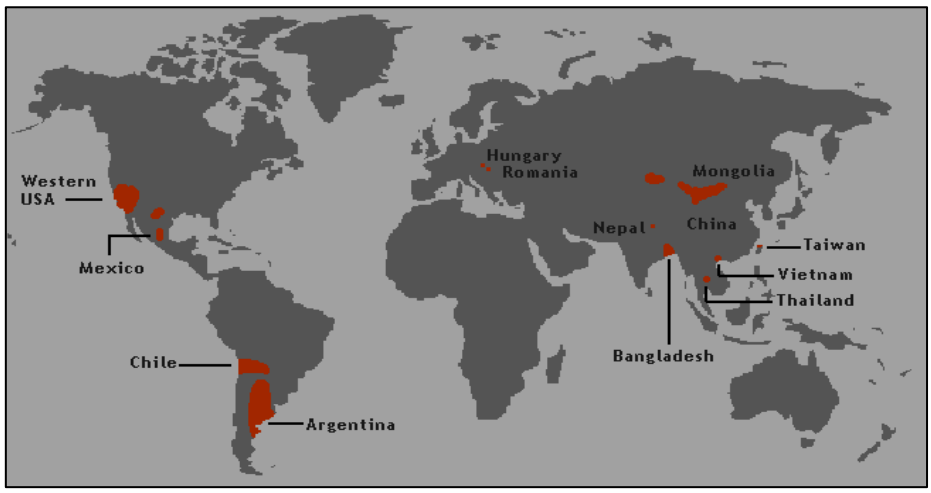


Fig 1.1: World Arsenic contamination scenario

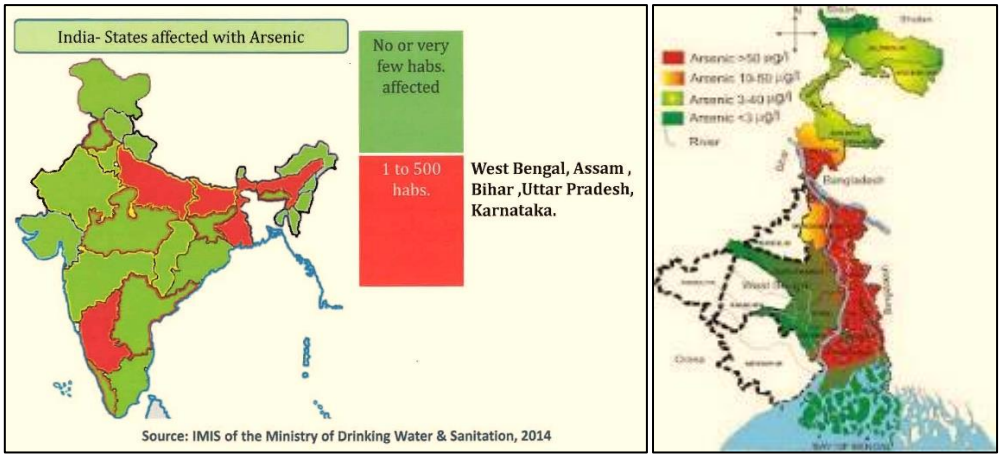


Fig 1.2: Arsenic contamination in India with special reference to West Bengal

1.2 Occurrence of Arsenic in the environment

Arsenic is widely distributed in nature despite its low crustal abundance (0.0001%) and is commonly associated with ores of metals. Anthropogenic point sources which contribute to the As in environment include smelter slag, coal combustion, runoff from mine tailings, hide tanning waste, pigment production for paints and dyes etc. (Oremland & Stolz., 2003). Elemental As is rarely found (Cullen & Reimer., 1989). Arsenate [$As^{+}5$ as $H_2AsO_4^{-}$ and $HAsO_4^{2-}$] is the predominant form of inorganic As in aqueous, aerobic environments whereas arsenite [As^{+3} as $H_3AsO_3^{0}$ and $H_2AsO_3^{-}$] is more prevalent in anoxic environments. Arsenate adsorbs strongly to the surface of several common minerals, such as ferrihydrite and alumina, due to which its hydrologic mobility is constrained, while Arsenite adsorbs less strongly and to fewer minerals making it the more mobile oxyanion (Bhattacharya et al., 2002). Some of the different minerals possessing As are tabulated below.

Table 1.1: Minerals possessing Arsenic (Cullen & Reimer., 1989)

Name of the minerals	Composition of Arsenic-Bearing Minerals
Arsenolite	As_2O_3
Arsenopyrite	FeAsS
Cobaltite	CoAsS
Haematolite	$(Mn, Mg)_4Al(AsO_4)(OH)_8$
Niccolite	NiAs
Realgar	AsS

Arsenolite Arsenopyrite Cobaltite Realgar Haematolite Niccolite

Fig 1.3: Minerals containing Arsenic

1.2.1 Arsenic geochemistry

Since it has the properties of both metals and non-metals, As is a metalloid which belongs to group V of the periodic table. It is the 20th most common element on the earth's crust and is found bound to over 200 different mineral compounds (WHO, 2001). Arsenopyrite (FeAsS) is the most common mineral containing As. Though As exists in the environment in several oxidation states like -3, 0, +1, +3 and +5, in water chemistry, it is generally found in its inorganic form mostly as oxy-anions of trivalent As^{+3} as well as pentavalent As^{+5}; which is less toxic (Yu et al., 1983).

1.2.1.1 Arsenic speciation in soil

Arsenic is present in soil in mainly two forms, organic and inorganic; however, the natural presence of the inorganic forms (As^{+3} and As^{+5}) are higher compared to the organic forms i.e. monomethy larsenic acid (MMA) and dimethyl arsenic acid (DMA) (Abedin et al., 2002). Redox reactions i.e. oxidation and reduction processes largely control the speciation of inorganic As in soil; As^{+5} predominates under aerobic (oxidizing) conditions while As^{+3} predominates under anaerobic (reducing) conditions (Yamaguchi et al., 2011). Microbial activity can influence As speciation via various mechanisms such as redox reactions with Fe and As and via (de)methylation of As species (Mahimairaja., 2005, Fitz & Wenzel., 2002). In an experimental paddy field, during non-flooded condition, 30% of total As was present as As^{+3} whereas under flooded conditions, 70% of total As was present as As^{+3} (Takahashi et al., 2004). It has also been reported that As was mainly present as As^{+5} (95% of total soluble As) with a relatively low solubility under oxidizing conditions and under reducing conditions, solubility of As increased sharply as As^{+3} became the predominant specie (Masscheleyn et al., 1991).

1.2.1.2 The role of iron hydroxides

Iron (Fe) redox chemistry is a very important factor in regulating As speciation since the behavior of (FeOOH) is highly dependent on redox reactions. Arsenic, released during the weathering of sulphide minerals is generally adsorbed onto the surface of iron oxy-hydroxides (that precipitated under oxidizing conditions). However, redox processes in the sediments, triggered the reductive dissolution of iron oxides that transferred substantial amount of As in aqueous phases through biogeochemical cycle (Smedley & Kinniburgh., 2002). Under anaerobic condition, FeOOH readily dissolves and As is released into the soil solution mainly as As^{+3} while under aerobic condition, FeOOH is relatively insoluble and serves as the sink of As. Though both As^{+3} and As^{+5} adsorb to Iron (oxy) hydroxides in soil, As^{+5} binds the strongest (Takahashi et al., 2004, Masscheleyn et al., 1991). Also, in lowland paddy fields, the close dynamic relationship between As and Fe is highly aided by microbial activity (Zobrist et al., 2000). During non-flooded period, As concentration in soil water is lower than that in irrigation water due to sorption to FeOOH. Therefore, under flooded condition, plants are more exposed to higher As concentration in soil water due to remobilization based on the concentration in applied irrigation water (Takahashi et al., 2004). FeOOH is mainly present in the clay size soil fraction (<2 μm) and clayey soils therefore generally have a higher As content compared to more sandy soil. Sandy soil is more toxic than clayey soil because As is more tightly bound in clayey soil (Mahimairaja., 2005, Fitz & Wenzel., 2002). Other sorption substrates such as carbonate minerals and manganese oxides (MnO) can also be relevant under specific soil conditions (Mahimairaja., 2005).

1.2.1.3 The role of pH

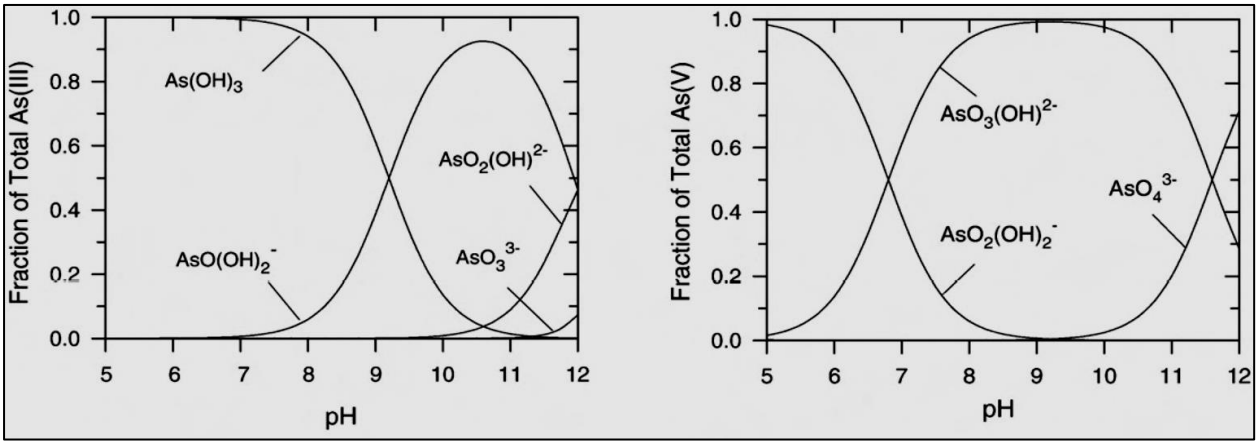


Fig 1.4: Effect of pH on speciation of As^{+3} and As^{+5} (Sharma & Sohn., 2009)

The adsorption of As^{+3} increases with increasing pH, in particular above pH 8.5, whereas the reverse occurs for As^{+5}. The adsorption maximum in case of As^{+3} on FeOOH lies around pH 7-8.5, whereas for As^{+5} the maximum is found at pH 4 approximately (Mahimairaja., 2005, Masscheleyn et al., 1991).

1.2.2 Groundwater Arsenic contamination

Apart from dry and wet deposition or precipitation of As by several natural and anthropological sources such as rock erosion, mining activity, volcanic eruption, forest fire etc., the main causes of aquifer contamination in the Ganga-Meghna-Brahmaputra plain are the percolation of rainwater through As rich mineral ores and the alluvial sediments which consists of sand, silt, clay, minerals and organic matter deposited by the rivers originating from the Himalayas (Nickson et al., 1998). Thus, the occurrence of As in groundwater in the Bengal delta plain and Gangetic plains has been recognized as of geological origin with sprawl out resulting from the mobilization under natural hydro geological conditions.

In most studied areas, high As content in the groundwater is dependent on three main factors: high As concentration in the parent rock, bio-geochemical triggers to mobilize As from the sorbed phase to groundwater and sufficient time for the As to accumulate and retain in the aquifer and not flush away (Smedley & Kinniburgh., 2002). There are a number of processes for mobilization of As in groundwater namely, (i) mineral dissolution, (ii) desorption of As under alkaline and oxidizing conditions, (iii) desorption and dissolution of As under reducing conditions, (iv) reduction in bond strength between As and holt mineral surface and (v) reduction of oxide mineral surface area (Smedley & Kinniburgh., 2002). Oxidation of sulphide minerals (pyrite-FeS_2) was advocated strongly by several investigators in West Bengal as the cause of groundwater arsenic contamination in the shallow aquifers (Das et al., 1994, Mandal et al., 1998). The lowering of water table due to over exploitation of groundwater for irrigation is the cause of release of As. A recent research study explained that desorption or dissolution of As from iron oxides could be the process on regional distributions of As in water (Smedley & Kinniburgh., 2002).

1.2.3 Distribution, speciation and mobility of Arsenic in environment

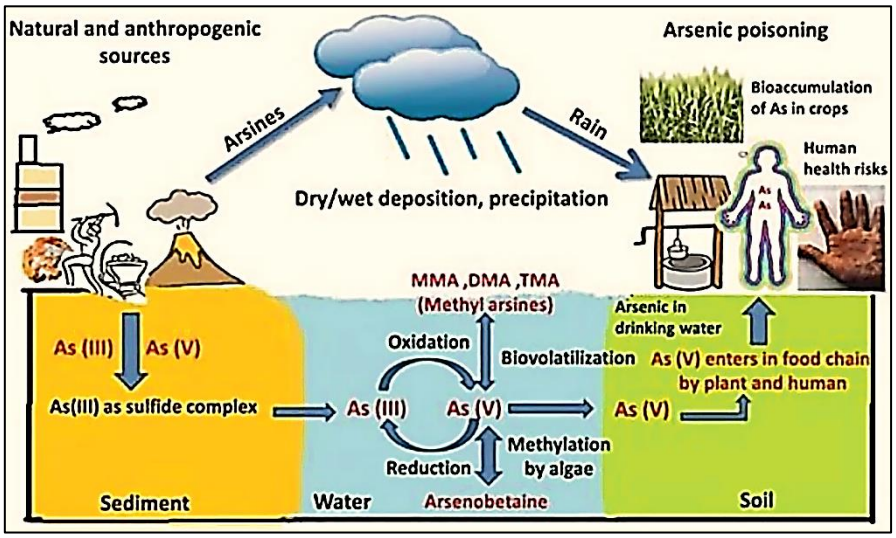


Fig 1.5: Bio-geochemical cycle of Arsenic (Arora et al, 2018)

The combined processes of recharging of groundwater from rainfall, groundwater flow, sediment water interaction, infiltration of irrigation return water (which is As rich due to the use of As containing pesticides, wood preservatives, etc. and the pumping of As rich groundwater for agriculture purpose), the oxidation of natural or anthropogenic organic matter together with the reductive dissolution of ferric iron and manganese oxides has played a key role in the evolution of groundwater As contamination (Sikdar & Chakraborty., 2008). Some scientists explained that the excessive use of water for irrigation and excessive use of fertilizers have caused the mobilization of phosphate (PO_4^{-3}) from fertilizers down below the shallow aquifers, which have resulted in the mobilization of As for the anion exchange onto the reactive mineral surfaces. Of late, a new hypothesis based on the displacement of As by dissolved bicarbonate as an alternative mechanism for the genesis of high As groundwater has been proposed (Smedley & Kinniburgh., 2002). Among a few hypotheses proposed to elaborate the possible mechanism of As groundwater contamination, most scientists have agreed to two hypotheses: (i) oxidation of arsenopyrite or As rich pyrite in soil strata, and (ii) reductive dissolution of As from soils. However, based on As geochemistry, three hypotheses describing the probable mechanisms of As mobilization in groundwater specially in West Bengal (WB) and Bangladesh, have been suggested (Bose & Sharma., 2002). These are: (i) Mobilization of As by the oxidation of As bearing pyrite minerals: Insoluble As bearing minerals, such as Arsenopyrite (FeAsS), are rapidly oxidized when exposed to the atmosphere, realizing soluble As^{+3}, sulfate (SO_4^{-2}), and ferrous iron (Fe^{+2}). The dissolution of these As-containing minerals

is highly dependent on availability of oxygen and the rate of oxidation of sulfide. Microbially mediated reactions partially oxidized the released As^{+3} to As^{+5}. The chemical reaction is given by:

$$7\ FeAsS + 13\ Fe^{+3} + 8\ H_2O \rightarrow 14\ Fe^{+2} + SO_4^{-2} + 13\ H^+ + H_3AsO_4\ (aq.)$$

(ii) The dissolution of As rich iron oxyhydroxides (FeOOH) due to onset of reducing conditions in the subsurface. Under oxidizing conditions and in the presence of Fe, inorganic species of As are mostly retained in the solid phase through interaction with FeOOH coatings on soil particles. The onset of reducing conditions in such environments may lead to the dissolution of FeOOH coatings. Fermentation of peat beneath the subsurface releases organic molecules (e.g., acetate) to drive reducing dissolution of FeOOH, resulting in release of Fe^{+2}, As^{+3}, and As^{+5} present on such coatings. The chemical reaction is given by:

$$8FeOOH - As(s) + CH_3COOH + 14\ H_2CO_3 \rightarrow 8\ Fe^{+2} + As(d) + 16\ HCO_3^- + 12\ H_2O$$

where As(d) is dissolved As and As(s) is sorbed As.

(iii) The release of As, sorbed to aquifer minerals by competitive exchange with phosphate ($H_2PO_4^-$) ions that migrate into the aquifers from the application of fertilizers to subsurface soil. The second mechanism regarding dissolution of FeOOH under reducing conditions is considered as the most probable reason for excessive accumulation of As in groundwater (Ghosh & Singh., 2009).

Moreover, As also undergoes bio methylation by certain algae and certain bacteria which first reduces the As^{+5} to As^{+3}, followed by the addition of methyl group to produce organic forms of As i.e. monomethyl arsenic acid (MMA) and dimethyl arsenic acid (DMA) (Abedin et al., 2002). Arsenic may be lost from the soil by the formation of volatile As components (Abedin et al., 2002, Mahimairaja et al., 2005). As summarized by WHO (2001), volatilization of As to Arsine gas can contribute a removal of 12 to 35 % of As per year. As^{+5} is first reduced to As^{+3} followed by further reduction to Arsine gas, which is volatile. However, the extent to which this process is relevant to water-logged paddy fields with their distinct soil parameters is still unknown.

1.3 Toxicity of Arsenic

1.3.1 Effects on human health

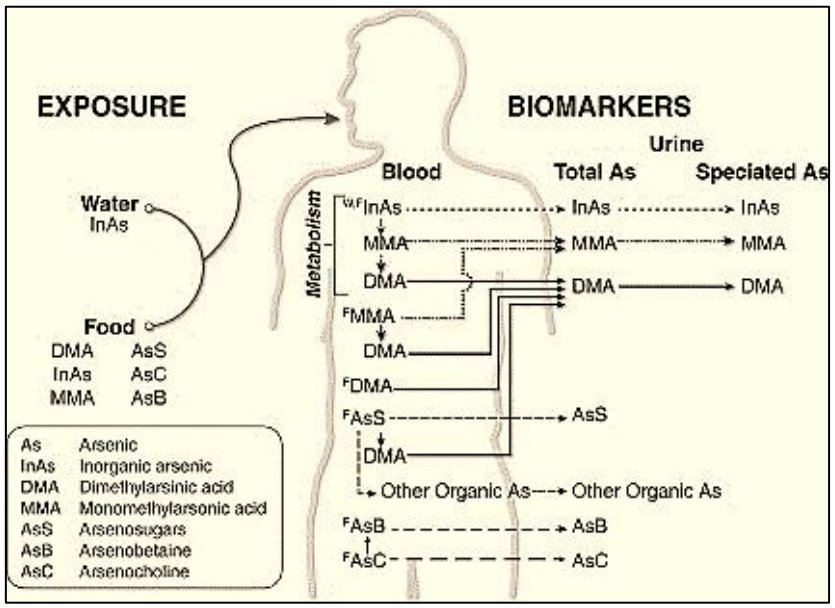


Fig 1.6: Mechanism of toxicity of Arsenic on human body (Tsuji et al., 2015)

Biologically, the trivalent Arsenite (As^{+3}) is more toxic than the pentavalent Arsenate (As^{+5}) significantly, including the ability to induce amplification of genes in mammalian cells. Once absorbed, As combines with Haemoglobin rapidly and localises in blood within 24 hrs and then redistributes itself to various organs like the liver, kidney, lung, spleen and gastro-intestinal (GI) tract, with lesser accumulation in muscles and nervous tissue. After accumulation of a small dose of As, it undergoes methylation primarily in the liver to mono-methyl arsinic acid (MMA) and dimethyl arsinic acid (DMA) which are excreted along with the residual inorganic As in the urine (Kapaj et al., 2006). The most common specie of inorganic As, As^{+3} and As^{+5} in the environment show their toxicity in different levels. After entering the cell, As^{+5} substitutes for phosphate in phosphorylation, which leads to the production of unstable arsenical by products like ADP–As^{+5} which causes the disruption of ATP (adenosine 5-triphosphate) synthesis (Rosen et al., 2011). These unstable arsenical by products spontaneously hydrolyse to ADP and As^{+5}, preventing ATP production. Likewise, other metabolic processes like ATP dependent transport, glycolysis, pentose phosphate pathway (PPP) and signal transduction pathways (two component and phosphor lay systems, chemotaxis, etc.) are also disturbed. On the other hand, As^{+3} has a very strong affinity for sulphydryl groups (Silver & Phung., 2005). Arsenite reacts with cysteine groups and also glutathione, thioredoxin and glutaredoxin which are present on the active sites of many enzymes, which control intracellular redox homeostasis, DNA synthesis and repair, sulphur

metabolism, protein folding and xenobiotic detoxification. Arsine gas binds to red blood cells (RBC), causing haemolysis by damaging the membranes (Čerňanský et al., 2009).

Table 1.2: Types of Arsenic toxicity and its symptoms

Type of toxicity	Symptoms
Acute toxicity	Gastrointestinal (GI) signs: Vomiting (often with blood) and severe cholera like diarrhea (may be with blood or rice-water like); dehydration and hypovolemic shock
	Cardiovascular effects: Myocardial dysfunction, ventricular dysrhythmias, diminished systemic vascular resistance, capillary leakage
	Central nervous system (CNS) manifestations: Seizures, encephalopathy, coma, and cerebral edema
	After an acute poisoning Alopecia and Mees lines might occur subacutely
Chronic toxicity	Manifests as a classic dermatitis (hyperkeratosis, "dew drops on a dusty road" appearance), peripheral neuropathy (usually painful, symmetrical paresthesia with stocking-glove distribution), Melanosis, Keratosis, Arsenicosis.
	Hepatic and renal damage (multiorgan involvement)
	Obliterative arterial disease of lower extremities (blackfoot disease)

Fig 1.7: Dermatological effects of Arsenic (Published in: **Scientific India (Health)** Dheeraj Pandey & Amit Kumar Singh (2018).

1.3.2 Effects on plants

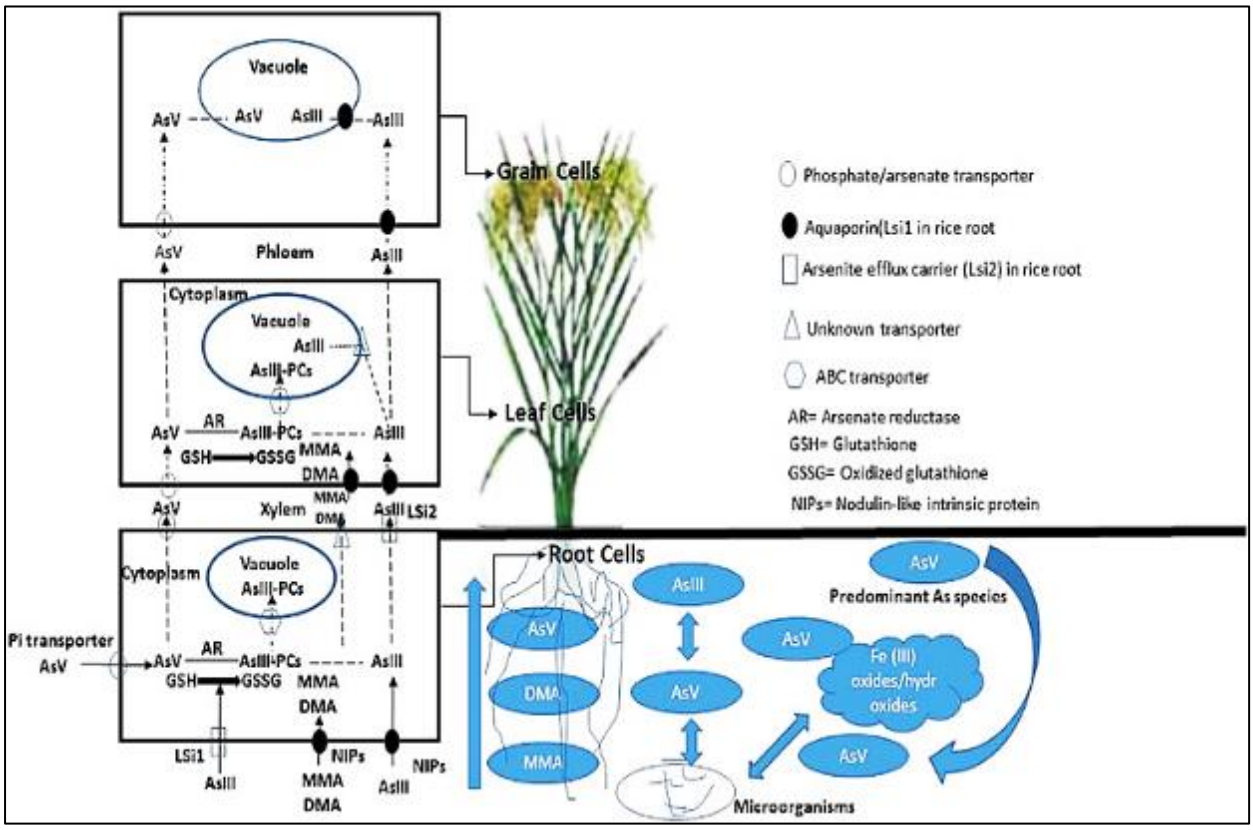


Fig 1.8: Mechanism of uptake of Arsenic by plants (Islam et al., 2016)

One of the very first symptoms of injury by sodium arsenite is wilting, caused by loss of turgor, and this immediately suggests alteration in the membrane integrity. The arsenate symptoms involve chlorosis, but not loss of turgor rapidly (at least in the early expression of toxicity), and the contact action is more subtle. Arsenate is known to uncouple phosphorylation (Hossain., 2006). Thus, coupled phosphorylation of adenosine diphosphate (ADP) is abolished as the energy of adenosine triphosphate (ATP) is not available and the plant slowly succumbs. Trees planted on the old soils that have accumulated lead arsenate exhibit a red or brown discoloration along the leaf margins and then throughout the leaves by midsummer. The discolored tissues slowly die and drop out, giving the leaves a shot-hole like appearance. Also, defoliation occurs and may be complete by late summer time. The injury appears first in older leaves, young leaves on shoot tips may stay normal. Yields of fruit are generally reduced, and the trees are usually stunted in growth (Shaibur & Kawai., 2009).

1.4 Arsenic in food chain

In the rural affected areas of the GMB plain, As contaminated drinking water is not just an elevated source of As exposure, but also the As contaminated groundwater which is mainly used for irrigation purposes is also responsible for the entry of As in the food chain. Rice being a kharif crop, requires heavy rainfall for its cultivation. But during the dry season, the paddy cultivation is solely dependent on irrigation by groundwater in rural Bengal, since the sources of surface water regimes become dry during that period (Rahman et al., 2007).

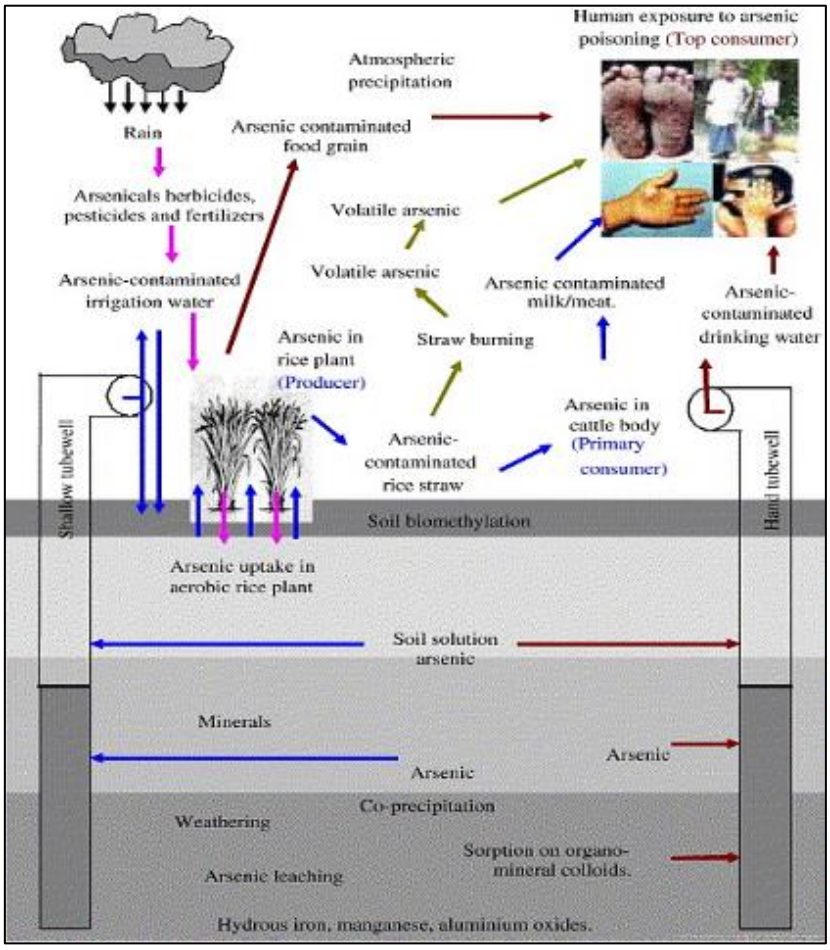


Fig 1.9: Arsenic accumulation through food chain (Rahman et al., 2008)

Prolonged use of As contaminated groundwater for irrigation practice in a particular land, results in a huge amount of As accumulation in the soil and eventually in the entire paddy plant via its root system. According to the translocation theory (Liu et al., 2004, Abedin et al., 2002), the As translocates and accumulates in paddy grains, which is the edible part of paddy. The grain is marketed either as raw or as sunned rice which is directly prepared by hulling, or parboiled rice that is prepared by light boiling of paddy using contaminated water, followed by mechanical hulling to obtain parboiled rice. This post-harvesting treatments of the grain using As contaminated water causes additional increment of As in parboiled rice. According to a

study, carried out in the fields of Deganga, Norh 24 Parganas, half boiled whole grain showed an increase of 43% in As concentration from that of raw whole grain, and a final increase of 61% was noticed in the full boiled whole grain. A concurrent increase of As concentrations in the water at half boiled and full boiled stages were also observed. An increase of 1.2% and 7.56% of As concentrations were found in half boiled and the full boiled water samples (Chowdhury et al., 2018, 2019). Such increment in As concentrations in the water samples after cooking of rice samples has been reported previously (Ohno et al., 2009). Besides the As contaminated groundwater used for drinking purpose, rural population is also exposed to a huge amount of As accumulating through different cereals and vegetables grown in the affected zones. This has been proved by observing the As concentrations in urine, hair and nail of the population exposed to As contamination in six affected districts of West Bengal (Das et al., 1995).

1.5 Remediation of Arsenic

To prevent further calamities associated with As toxicity, development of mitigation strategies for its remediation is extremely important and an absolute need of the hour (Purakayastha., 2011). Among several ways of remediation including physical processes like immobilization, stabilization etc. and chemical process like coagulation, co-precipitation, oxidation, ion-exchange etc., bioremediation (which includes phytoremediation and microbial remediation), is an emerging alternative and has good public acceptance (Lim et al., 2014).

1.5.1 Phytoremediation

Phytoremediation, the use of green plants to remove or degrade contamination from soils and surface waters, has been proposed as a cheap, sustainable, effective, and environmentally friendly approach, alternative to the conventional remediation technologies (Raskin and Ensley., 2000). Phytoremediation is advantageous as this technique helps in treating a variety of contaminants over a diverse range of environment. Phytoremediation also does not require skilled technicians or highly expensive equipment, making it easier to implement. Akhtar et al., 2013 found that, ferns are the hyper As accumulator plants that are capable of absorbing very high concentrations of As from water as well as soil.

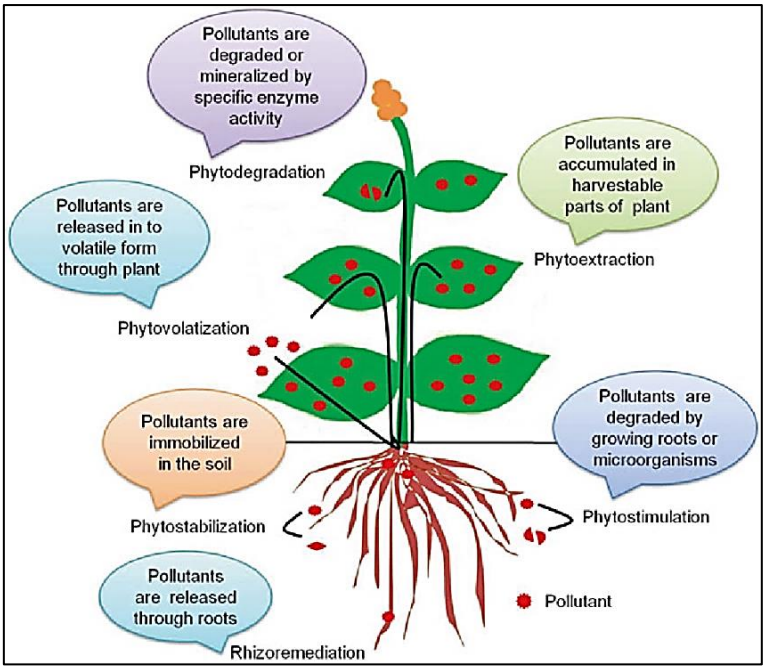


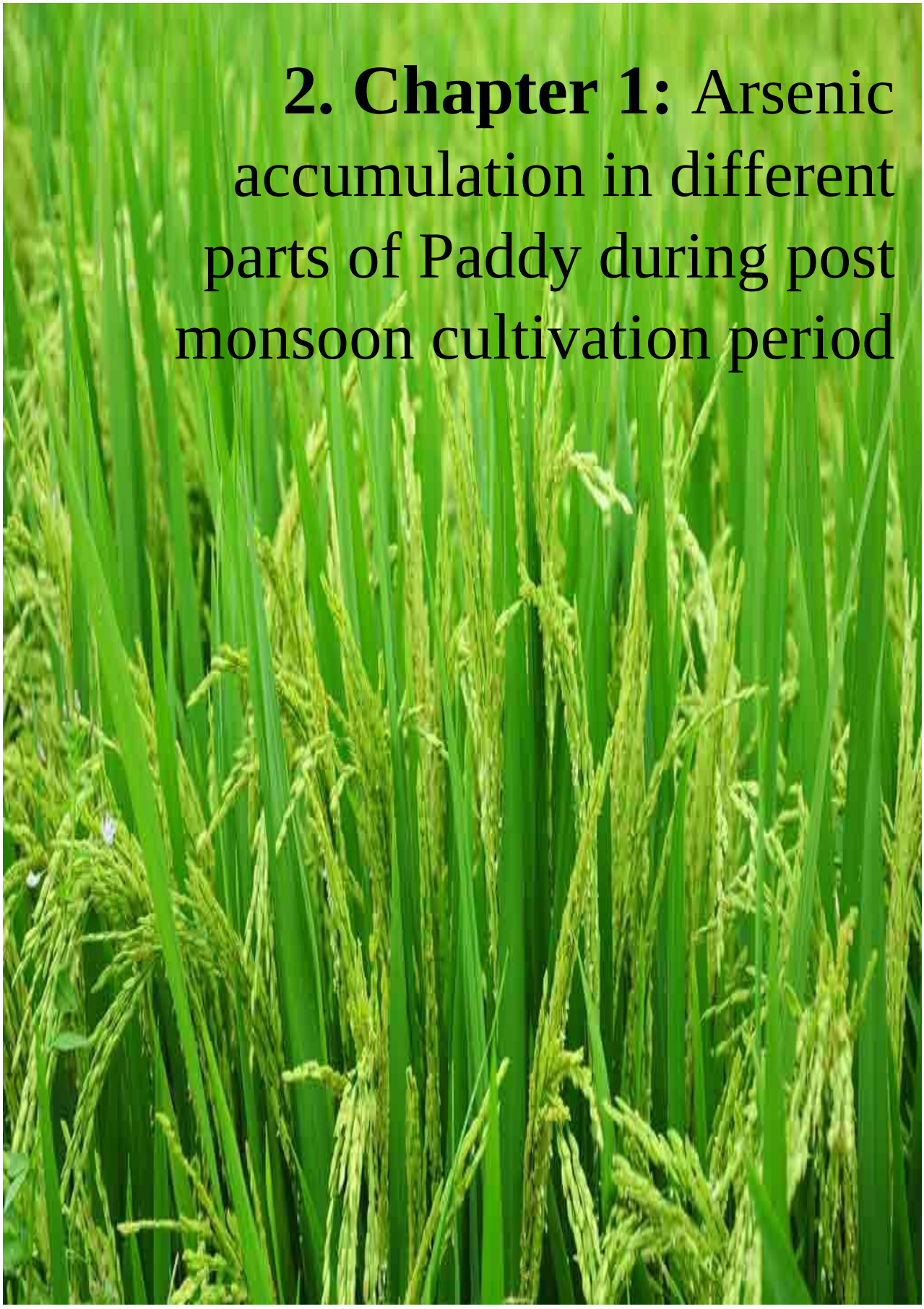
Fig 1.10: Mechanisms of phytoremediation

Several findings have already been done with various species of ferns, weeds and several aquatic and terrestrial macrophytes for removal of As from different ecosystems like soil (Bagga et al., 2001, Cao et al., 2003, Fayiga., 2005 etc.), water (Alvarado et al., 2008, Farnese et al., 2014 etc.), wetlands (Favas et al., 2012, Sandhi et al., 2017 etc.) and mines (Bleeker et al., 2002, Mkandawire et al., 2004 etc.). Several experiments have been done hydroponically in laboratory environment for removal of As by macrophytes (Artus et al., 2006). Many reviews of different strategies of phytoremediation of As have been published (Yadav et al., 2018, Mc Grath et al.,2003, Rahman et al., 2011, etc.) based on either the mechanism of phytoremediation (Yadav et al., 2018) or the potential of As accumulation or removal (Rahman et al.,2011). A wholesome overview of the plants (aquatic as well as terrestrial macrophytes, ferns, weeds, flowering plants as well as food-crop plants) that can be used as potent phytoremediators in different ecosystems (soil of mines and wetlands, contaminated water) as well as laboratory hydroponic conditions and their mechanism of removal of As have been already reported .There are various mechanisms of phytoremediation like Phytoextraction (which includes Phytoaccumulation, Phytosequestration and Phytoabsorption) , Phytostabilization or Phytoimmobilization, Phytovolatilization, Phytodegradation and Phytofiltration (Yadav et al., 2018, Akhtar et al.,2013).

1.5.2 Bioremediation

Bioremediation by microbes (bacteria, fungi, yeast, algae) is an effective method which is mainly concerned with the use of natural or engineered microbes which are capable of oxidizing, reducing, volatilizing or immobilizing As through bio-sorption, bio-methylation, bio-stimulaton, bio-augmentation, complexation and siderophore-based amelioration (Lievremont et al., 2009). It is advantageous because it includes simple, natural processes which are highly specific, less expensive, can be carried out on site, as well as causes complete degradation of a wide variety of contaminants (Leung., 2004). Bio Stimulation is the stimulation of indigenous microbial populations in ground water or soil which may be done in-situ or ex-situ. In Native soil, bacteria having bioremediation capacities may be stimulated by adding nutrients to the soil. For example hydrocarbon biodegradation in soil can be restricted by many factors like nutrients, pH, oxygen, temperature, moisture, soil properties and contaminant presence (Atagana 2008, Bundy et al., 2002). Bio stimulation may involve modification of the environment to stimulate the existing bacteria capable of bioremediation. This process can be performed by the addition of several types of limiting nutrients and electron acceptors, like phosphorus, oxygen, nitrogen, or carbon (e.g. in the form of molasses), that are otherwise available in low enough quantities to sustain the microbial activity (Rhykerd et al., 1999, Elektorowicz, 1994, Piehler et al., 1999). Further it can improve pollutant degradation by optimizing conditions like addition of nutrients, aeration, pH and temperature control. The primary advantage of biostimulation is that bioremediation will be undertaken by the already present native microorganisms that are well-suited to the subsurface environment, and are well distributed spatially in the subsurface. The challenge is the delivery of additives in a manner that allows the additives to be available readily to the subsurface microorganisms is based on the local geology of the subsurface. From the 1970s, bioaugmentation, i.e. microorganisms to supplement the indigenous populations, has been proposed as an alternate strategy for the bioremediation of any contaminated environment. Bioaugmentation involves the introduction of microorganisms isolated from contaminated areas and genetically modified to support the remediation of contaminated sites. The rationale for this approach is that indigenous microbial populations may not be capable of degrading a wide range of potential substrates present in complex mixtures such as, petroleum (Leahy and Colwell., 1990) or that they may be in a stressed state as a result of the recent exposure to the spill. Other situations under which bioaugmentation may be considered are when the indigenous hydrocarbon degrading population is low, the speed of decontamination is the primary factor, and when seeding may

reduce the lag period to start the bioremediation process (Forsyth et al., 1995). For this approach to be successful in the field, the seed microorganisms must be able to degrade most contaminated components, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and move through the pores of the sediment to the contaminants (Atlas, 1977). The study of microbes in bioremediation systems makes possible the selection of microorganisms with potential for the degradation and production of compounds with biotechnological applications. Successful bio augmentation treatments depend on the use of inoculum containing of the microbial strains or microbial consortium which have been well adapted to the site, to decontaminate the site. Foreign microorganisms can be applied successfully but the efficiency depends on their ability to compete with indigenous microorganisms, together with predators and various abiotic factors.



2. Chapter 1: Arsenic accumulation in different parts of Paddy during post monsoon cultivation period

2.1 Aim and objective of study

The main objective of the study of paddy plants *(Oryza sativa)* during post-monsoon cultivation period (July-November) (2018-19) are as follows:

1. To study the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the highly contaminated field of Teghoria in Gaighata block of North Parganas, West Bengal.
2. To analyse the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the moderately contaminated field of Madhusudankathi in Gaighata block of North 24 Parganas, West Bengal.
3. To evaluate the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the control field of Pingla, West Medinipur, West Bengal.
4. To compare the variation of As accumulation in different parts of Paddy plant during post monsoon period in highly contaminated field (Teghoria) and moderately contaminated field (Madhusudankathi) of Gaighata, with the control field (Pingla) of West Medinipur.
5. To verify if the Translocation theory holds good in case of As accumulation in paddy plants grown in highly contaminated field (Teghoria) and moderately contaminated field (Madhusudankathi) of Gaighata, as well as the control field (Pingla) of West Medinipur.
6. To estimate the variation of As accumulation in different parts of paddy plant cultivated by Pot culture using SRI (System of rice intensification) technique, one with non-contaminated soil and non-contaminated water (Control 1) and the other with As contaminated soil and non-contaminated water (Control 2).
7. To compare the trends of As accumulation in different parts of paddy plants grown in field conditions with that grown in pot culture using SRI technique (i.e. Control 1 and Control 2).
8. To interpret the overall As and Iron (Fe) accumulation patterns in the 3 different phases of growth of the paddy plant: Vegetative phase, Reproductive phase and Ripening phase in case of highly contaminated field, moderately contaminated field, control field as well as plants grown in pot culture.

2.2 Literature review

In Bengal delta, groundwater Arsenic (As) contamination has been termed as the largest mass poisoning in history (Smith et al., 2000). More than 500 million population reported has been exposed to groundwater As contamination in a significant part of the Ganga–Meghna–Brahmaputra (GMB) plain, covering an area of 569 749 sq. km (Chakraborti et al., 2004). In West Bengal, rice is the major staple crop which is cultivated in different agro-ecosystems throughout the year in uplands as well as rainfed lowlands. The countries that receive moderate rainfall like India and Bangladesh practice two processes of paddy cultivation: Boro (the premonsoon cultivation, irrigated with groundwater) and Aman (the post-monsoon cultivation, irrigated with rain water) (Chowdhury et al., 2018). It has been widely reported that during pre-monsoon cultivation, the As from the contaminated groundwater used for irrigation, generally causes phytotoxicity by translocating through the root of the paddy plant in the rhizosphere showing accumulation in the other parts (root, shoot, grain etc.) (Rahman et al., 2007, Liu et al., 2006). Monsoon rice which is typically grown during the months of July- October accounts for 69% of the total rice production. During this period, As in top layer of soil is reduced because of seasonal flooding. During monsoon flooding, the topsoil As concentration decreases due to temporal variability that causes diffusion of As in floodwater followed by lateral removal with the receeding floodwater. Arsenic transport to the deeper soil layer by infiltration due to flooding, results in reduced As concentration in top soil (Shrivastava et al., 2017). Microbial methylation of As into volatile Arsine gas, also causes loss of As into the atmosphere (Miah et al., 2005). Therefore, non-flooded soil, irrigated by As contaminated groundwater, during pre-monsoon cultivation, results in higher accumulation of As and therefore, poses higher exposure and threat to human health. Since the monsoon flood water removes large amount of As from the top layer of soil in paddy fields, it provides a potential window for future rice production in our country (Linda et al., 2010). Various trends of As accumulation reported during pre-monsoon as well as monsoon cultivation of paddy have been tabulated in Table 2.1.

Table 2.1: Arsenic accumulation trends reported during pre-monsoon and monsoon cultivation

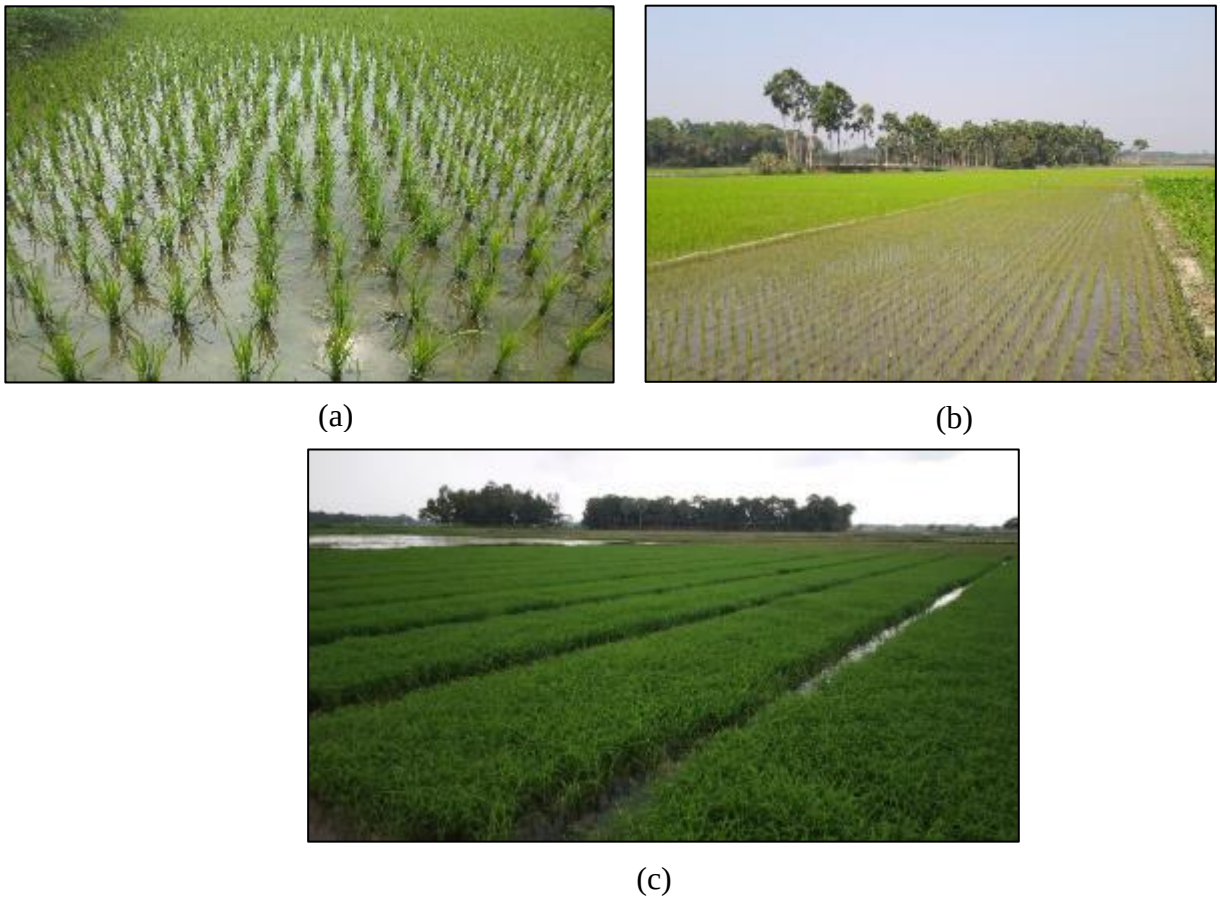
Sl No.	Season of cultivation	Arsenic accumulation in different parts of Paddy plant	Reference
1	Post monsoon	In Murshidabad district, mean As concentration in the leaf, stem and root were 246 µg/kg, 297 µg/kg and 996 µg/kg respectively whereas that in soil collected from surface and root of plants were 14200 µg/kg and 13700 µg/kg, respectively.	Roychowdhury et al., 2005
2	Post monsoon	In Bengal deltaic region, As accumulation in the surface soil and root soil were 19400 ± 0.38 µg/kg and 27170 ± 0.44 µg/kg respectively in 2013 and the pattern of translocation was root>straw>husk>grain. Mean As concentration in rice grain in 2013 and 2014 were 1400 µg/kg and 1600 µg/kg respectively, both above the permissible limit (1000 µg/kg).	Shrivastava et al., 2017
3	Post monsoon	The As concentration in 6 different types of soil were 6500 µg/kg, 15100 µg/kg, 5000 µg/kg, 6200 µg/kg, 6900 µg/kg and 9800 µg/kg respectively. The As concentration in the straw grown were 11700 µg/kg, 10200 µg/kg, 3400 µg/kg, 6500 µg/kg, 3800 µg/kg and 700 µg/kg in 2005 and 10200 µg/kg, 13400 µg/kg, 4900 µg/kg, 6300 µg/kg, 4200 µg/kg and 700 µg/kg in 2006 respectively whereas that in polished rice were 230 µg/kg, 228 µg/kg, 140 µg/kg, 201 µg/kg, 140 µg/kg and 73 µg/kg in 2005 and 268µg/kg, 30 µg/kg, 142µg/kg, 257µg/kg, 141µg/kg and 68 µg/kg in 2006 respectively.	Bogdan & Schenk (2008).
4	Pre monsoon	Ten fields were studied in Deganga block of North 24 Parganas, where the mean accumulation of As in leaf, stem and root were 6120 µg/kg, 21850 µg/kg and 1278900 µg/kg for vegetative phase, 2540 µg/kg, 5080 µg/kg and 40510 µg/kg in reproductive phase and 3870 µg/kg, 7850 µg/kg and 233230 µg/kg for ripening phase, respectively.	Chowdhury et al., 2018
5	Pre monsoon	The mean As concentrations ± standard deviations (SD) values were 172900 ± 64.8 µg/kg (65000-277.0 µg/kg) in grain; 2599000 ± 1742 µg/kg (428000-6006 µg/kg) in straw; 110,173,000 ± 86,794 µg/kg (19,588,000-380,550 µg/kg) in root ; and 15,421,000 ± 4160 µg/kg (9135,000-25,838 µg/kg) in soil, showing translocation pattern as root > soil > straw > grain.	Ma et al., 2017

6	Pre monsoon	Arsenic accumulation in grain and soil were 500±0.02 µg/kg and 60,000 µg/kg respectively while that in rice straw was 20600±0.52 µg/kg at initiation stage and 23700±0.44 µg/kg at maturity stage. As concentration in rice husk was 1600 ±0.20 µg/kg i.e. (straw>husk>grain).	Rahman et al., 2008
7	Pre monsoon	In Koudikasa village mean As concentration in the soil, rice grain, husk, straw and root were 126000 ± 28 µg/kg, 470 ± 0.07 µg/kg, 830 ± 0.15 µg/kg, 4200± 0.5 µg/kg and 276000 ± 21 µg/kg, respectively.	Patel et al., (2016)
8	Pre monsoon	Arsenic concentration in root soil, root, rice straw, husk and grain varied from 31000 ±1 µg/kg to 197000±4 µg/kg, 36000 µg/kg to 201000 µg/kg, 7700 µg/kg to 197000 µg/kg, 1300 µg/kg to 7500 µg/kg and 260 µg/kg to 740 µg/kg, respectively.	Abedin et al., 2002
9	Pre monsoon	Arsenic accumulation followed the trend, root > straw > husk > leaf >grain. Accumulation ranged from 8000± 0.52 µg/kg to 395000 ± 12.90 µg/kg in root, 2350 ± 0.07 µg/kg to 72570 ± 6.62 µg/kg in straw 1210 ± 0.07 µg/kg to 33290 ± 1.92 µg/kg in leaf, 350 ± 0.03 µg/kg to 3800 ± 0.40 µg/kg in husk and 170 ± 0.01 µg/kg to 1090 ± 0.05 µg/kg in grain.	Liu et al., 2009
10	Pre monsoon	The As accumulation in soil, root, straw and grain ranged from 1380±0.108 µg/kg to 12270±0.094 µg/kg, 7190±0.166 µg/kg to 18630±0.155 µg/kg, 1170±0.014 µg/kg to 4150±0 µg/kg and 250±0.014 µg/kg to 730±0.009 µg/kg, respectively; As accumulation followed the trend, root > straw > husk > grain.	Bhattacharya et al., 2009
11	Pre monsoon	Arsenic concentration in soil ranged from 67000 µg/kg to 438000 µg/kg. The mean As content in grains was measured at 200 µg/kg, 1183 µg/kg being the highest. The mean total As level in root was 244000 µg/kg and in straw 4400 µg/kg. In comparison, regression of topsoil As levels with root ($r^2 = 0.93$) was more significant compared to rice grain ($r^2 = 0.00$) and straw ($r^2 = 0.56$) and the mean As level in rice from root to grain showed a decreasing trend.	Lin et al., 2013

2.3 Materials and methods

2.3.1 Study area

The study area includes the highly As contaminated agricultural field of Teghoria and the moderately contaminated field of Madhusudankathi, situated in Gaighata block of North 24 Parganas, West Bengal. These fields were chosen based on the high As concentration in the groundwater as well as in the urine, hair and nail samples of the population living in Gaighata district as reported by Roychowdhury., 2010. Pingla, situated in west Medinipur was chosen as control field based on the reports by Samanta et al., 2007 and Chakraborti et al., 2009. Besides, two experimental pot studies where paddy was grown using SRI technique, one with non-contaminated soil and non-contaminated water (Control 1) and the other with As contaminated soil and non-contaminated water (Control 2) were carried out.



(a) (b)

(c)

Fig 2.1: Post-monsoon cultivation of paddy in (a) Teghoria (Gaighata) (b) Madhusudankathi (Gaighata) (c) Pingla (Medinipur)

2.3.2 Reagents used

a) Concentrated Nitric acid (HNO_3) (69% v/v) (Merck)
b) Hydrogen peroxide (H_2O_2) (30% v/v) (Merck)
c) Potassium iodide (Merck)
d) Hydrochloric acid (HCl) (37 % v/v) (Merck)
e) Sodium borohydride ($NaBH_4$) (Merck)
f) Sodium hydroxide pellets (NaOH) (Merck)
g) Sodium acetate (CH_3COONa) (Merck)
h) Acetic acid (CH_3COOH) (Merck)
i) Hydroxylamine hydrochloride ($NH_2OH.HCl$) (Merck)
j) Liquor ammonia (NH_4OH) (25%) (Merck)
k) Ortho-phenanthroline ($C_{12}H_8N_2$) (Merck)
l) Double distilled water

All chemicals used were of analytical grade.

2.3.3 Methodology

Monsoon paddy cultivation (Aush and Amon)

During monsoon period, paddy cultivation starts with the vegetative phase when the initial seedling gets planted and continues till the stem starts elongating in size. The reproductive phase, starts with pedicle initiation and ends with flowering. Lastly, in the ripening phase paddy grains appear, get matured and is ready to be harvested (Chowdhury et al., 2018). In case of Aush dhan, grown in Madhusudankathi, the cultivation period is of 90 days, the vegetative phase lasts for 10-20 days, reproductive phase for 45-55 days and ripening phase upto 85-90 days. In case of Amon dhan, grown in Teghoria as well as Pingla, the cultivation period is of 120 days, the vegetative phase lasts for 10-20 days, reproductive phase for 55-65 days and ripening phase upto 110-120 days. The shallow tube wells with an average depth of 20 ft (~6 m) acts as the source of irrigational water whenever the monsoon downpour is insufficient to keep the field water logged (upto 6 inches) for Aush as well as Amon cultivation.

2.3.3.1 Sample collection, preparation, and preservation

To assure statistical significance of the observed values, the samples were methodically collected from all the three agricultural fields in triplicates. The triplicate samples were collected from three points equidistant from each other covering the whole field. Distance between two points was 40 ft in Madhusudankathi, 30 ft in Teghoria and 50 ft in Pingla. Accumulation in various parts of the plant was analyzed by individual estimation of As concentration in grain, pedicle, leaf, stem and root at various phase of cultivation i.e. during initial or vegetative phase (10-20days), intermediate or reproductive phase (45-55 days for Aush and 55-65 days for Amon) and final or ripening phase (85-90 days for Aush and 110-120 days for Amon). However, grain and pedicle appeared only in the reproductive phase of cultivation. Moreover, water samples being used for cultivation together with the surface soil and root soil surrounding the plant were also collected to estimate their As concentration. Paddy plant and soil samples were collected from approximately same locations of the field during all three phases to minimize variations due to geo-physicochemical features of the soil.

Fig 2.2: Sample collection from the field of Teghoria (in the order of Bijdhan to ripened plant with grain of final stage from left to right)

Fig 2.3: Sample collection from the field of Madhusudankathi (in the order of Bijdhan to ripened plant with grain of final stage from left to right)

Fig 2.4: Sample collection from the field of Pingla (in the order of Bijdhan to ripened plant with grain of final stage from left to right)

Fig 2.5: Sample collection from the pot study (Control 1& Control 2) (in the order of Bijdhan to ripened plant with grain of final stage from left to right)

At each phase of cultivation, paddy plant collected from the field was manually segregated, to obtain the individual parts of the plant (i.e. grain, pedicle, leaf, stem and root) for further processing. The individual parts of the paddy plant obtained, were then washed properly using de-ionized water and sonicated to remove the dirt present on its external surface. Next, the plant parts were dried in hot air oven at 50°C for 2 days. These samples were then subjected to hot plate digestion for further estimations. Both the root soil and surface soil collected, after drying were ground to fine particles using mortar and pestle, and screened before digestion. Lastly, all the samples were preserved in sterile polyethylene zip lock packets for further analysis. The water logged were collected individually from each of the 3 agricultural field, stored in sterile polyethylene bottles, preserved by adding 0.1% (v/v) concentrated HNO_3 and stored at 4°C for analysis. Details of sample collection, preservation, storage and preparation have been already described earlier (Roychowdhury et al., 2002a, 2002b, 2005; Roychowdhury., 2008a, 2008b, 2010).

(a) (b)

(c) (d)

Fig 2.6: Sample preparation (a) segregation of different parts after washing (b) drying in hot air oven (c) dried samples in watch glass (d) grinding of soil in mortar and pestle

2.3.3.2 Total digestion

Approximately, 0.01-0.2 g (dry weight) of the dried samples were weighed in a beaker. Concentrated HNO_3 and H_2O_2 (30% v/v) were added in a ratio of 2:1 and left overnight for digestion. The beakers were then placed over hot plate at 90°C for 1 hr till the evaporation was complete. The evaporated samples were then adjusted to a final volume of 5-10 ml with double distilled water and filtered through a vaccum filter (Millipore 0.45 mm). The filtered samples were then stored at 4 °C until analyzed. Details of sample digestion methodology have already been described (Das et al., 1995; Roychowdhury., 2010).

(a) (b) (c)

(d) (e) (f)

Fig 2.7: Steps of acid digestion (a) weighing of dried parts (b) addition of HNO_3 and H_2O_2 (c) hot plate digestion (d) volume make up with double distilled water (e) preparation of filtration (f) vaccum filtration

2.3.3.3 Arsenic Analysis

The samples were prepared with required dilutions and KI and HCl added for reduction and analysis of total As was performed through FI-HG-AAS (Flow injection hydride generation atomic absorption spectrometry) method using VGA (vapor generation assembly) principle following the protocol mentioned in Roychowdhury, 2008 b. A Varian Model AA 140 AAS (USA) was used for estimation of total As. Details of the instrumentation, optimization conditions and methodology of FI-HG-AAS system have already been reported by Das et al., 1995; Samanta et al., 1999; Roychowdhury., 2008b, 2010.

(a) (b) (c)

Fig 2.8: Estimation of Arsenic (As) (a) lots of stock samples (b) preparation of samples from stock by dilution (c) samples prepared by addition of KI and HCl

2.3.3.4 Iron Analysis

For spectrometric analysis of iron, sodium acetate-acetic acid buffer (pH 4-5), 10% hydroxylamine hydrochloride (for reduction of ferric to ferrous solution), 1:1 NH_3 solution (for adjustment of pH) and 0.25% of Ortho-phenanthroline solution were used. The iron content of the digested root, root soil and surface soil samples were analysed by a colorimetric method by following the protocol mentioned by Fries et al., (1977), Roychowdhury (2008a), Roychowdhury et al., (2010), Chakraborti et al., (2016). A Thermo Scientific Orion Aquamate 8000 UV-VIS spectrophotometer (USA) was used for spectrometric analysis of iron.

Fig 2.9: Estimation of Iron (Fe)

2.4 Results and discussion

2.4.1 Field: Madhusudankathi (Gaighata)

2.4.1.1 Arsenic concentration in different parts of paddy and soil

Table 2.2: Arsenic concentration in different parts of paddy and soil in Madhusudankathi

Lot no.	Spot No.	As concentration (µg/kg)						
		Grain	Pedicle	Leaf	Stem	Root	Root soil	Surface Soil
Seedling	-	-	-	3728	5779	101316	18785	-
1	1	-	-	1518	2590	49231	46354	9180
	2	-	-	710	1728	44805	35959	8953
	3	-	-	1617	3690	58879	55568	9605
2	1	-	-	1120	3486	92024	36366	26262
	2	-	-	1014	2619	127878	44610	27388
	3	-	-	1720	2878	230120	45090	32330
3	1	1493	2787	12886	17369	250000	18418	36618
	2	4527	5996	15768	19964	251020	29920	18535
	3	3879	6451	12429	30893	253774	25720	23529
4	1	1058	2600	3053	5142	65086	10215	8685
	2	780	2314	4190	7316	52113	10134	5702
	3	1194	2951	3333	9675	56087	6325	6768
5	1	375	2876	7167	9952	30973	8076	7517
	2	102	2914	4673	9760	16055	7692	7350
	3	542	3007	9661	10079	55140	8152	9817

The As concentrations in different parts of paddy and soil of 5 lots of samples from the field of Madhusudankathi, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.3.

Table 2.3: Phase wise variation of As in Madhusudankathi (90 days cultivation period)

Interval of days	Phase	Average As concentration (µg/kg)				
		Leaf	Stem	Root	Root soil	Surface soil
10-20 days	Vegetative Phase	1426	2832	50972	24686	9246
45-55 days	Reproductive Phase	13694	22742	200803	43991	27444
80-90 days	Ripening Phase	5464	8654	58153	8432	7640

From Table 2.3, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 13694 µg/kg, 22742 µg/kg, 200803 µg/kg, 43991 µg/kg and 27444 µg/kg in leaf, stem, root, root soil and surface soil respectively. The variation of As accumulation in leaf, stem and root and its translocation in different parts during the 3 phases of monsoon cultivation have been represented in Fig 2.10 and Fig 2.11 respectively.

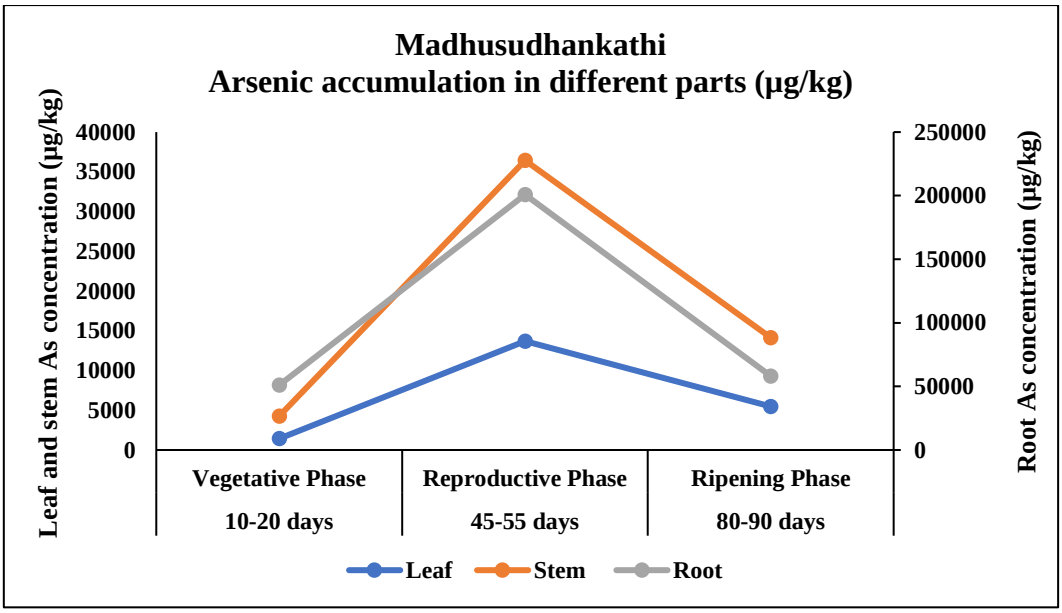


Fig 2.10: Phase wise variation of As in leaf, stem and root in Madhusudankathi

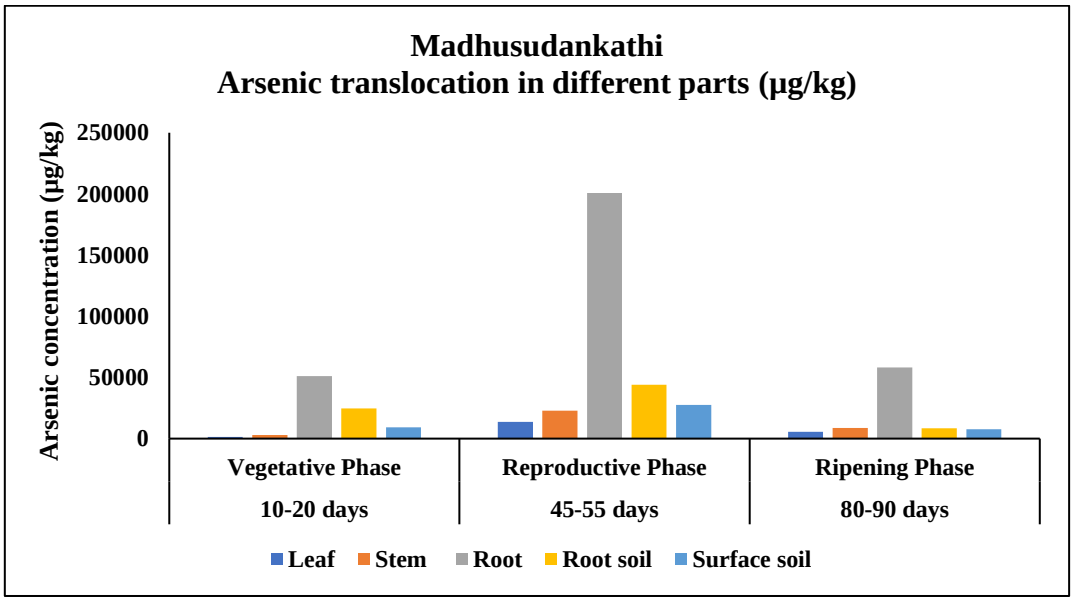


Fig 2.11: Phase wise As translocation in different parts of paddy and soil in Madhusudankathi

Therefore, it was observed that As accumulation in the different parts, ie. grain, pedicle, leaf, stem and root follows Translocation theory and the As concentration is in the order grain<pedicle<leaf<stem<root. Moreover, maximum accumulation of As was observed in root, stem, leaf, root soil as well as surface soil in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. The seasonal flooding during the vegetative phase, mainly causes dilution if As in the top soil, making the As less available for translocation through the root. The paddy plants accumulate As more and more with time and by the time it reaches Reproductive phase, the As accumulation becomes maximum in all the parts. Also, during Reproductive phase due to less rainfall, groundwater from shallow tube well is used for irrigation with As concentration of 8.63 μg/L to maintain the water logged condition, causing an increase in the soil As and consequently higher As translocation through the root and accumulation in different parts. During the Ripening phase, the paddy plants on reaching their saturation, fail to accumulate As any further due to their lowered accumulation efficiency. So, during Ripening phase, the As accumulation decreases steadily in the different plant parts as well as the soil.

Table 2.4: Correlation matrix of As accumulation in different parts of paddy (Madhusudankathi)

	Leaf	**stem**	**Root**	**Root soil**	**Surface soil**
Leaf	1				
stem	0.999181	1			
Root	0.95929	0.969933	1		
Root soil	0.694609	0.723152	0.869503	1	
Surface soil	0.920325	0.9354	0.99333	0.920658	1

Table 2.4 represents the Correlation matrix of As accumulation during monsoon cultivation period among different parts of the paddy plants collected. It is observed that a very strong correlation of 0.999181 exists between leaf and stem. The root shows a strong and positive correlation with the leaf as well as the stem of 0.95929 and 0.969933 respectively as well as with the root soil and surface soil of 0.869503 and 0.9933 respectively. The root soil shows a moderate correlation of 0.694609 and 0.723152 with leaf and stem respectively and a strong correlation of 0.920658 with surface soil.

2.4.1.2 Iron- Arsenic interaction in root and soil

The As and Fe estimated in the root, root soil and surface soil of all the 5 lots of Madhusudankathi are tabulated below in Table 2.5.

Table 2.5: Variation of As and Fe in root and soil of Madhusudankathi (90 days cultivation period)

Lot No.	Spot No.	Root		Root soil		Surface soil	
		As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)
Seedling	-	101316	23980	18785	18222	-	-
1	1	49231	29077	46354	15582	9180	10260
	2	44805	33896	35959	9760	8953	10902
	3	58879	12617	55568	16250	9605	13969
2	1	92024	32773	36366	25756	26262	23333
	2	127878	44091	44610	29507	27388	23600
	3	230120	51777	45090	37040	32330	32989
3	1	250000	22204	18418	34204	36618	26779
	2	251020	20823	29920	22030	18535	17198
	3	253774	34559	25720	40965	23529	32142
4	1	65086	13470	10215	16328	8685	18388
	2	52113	12042	10134	23529	5702	23053
	3	56087	14000	6325	13417	6768	12516
5	1	30973	12412	8076	19019	7517	23806
	2	16055	12615	7692	19032	7350	22952
	3	55140	13365	8152	17990	9817	23151

The As and Fe concentrations in the root of paddy and root soil of 5 lots of samples from the field of Madhusudankathi, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.6.

Table 2.6: Phase wise variation of As and Fe in root and root soil of Madhusudankathi

Interval of days	Phase	Root		Root soil	
		As (µg/kg)	Fe (µg/kg)	As (µg/kg)	Fe (µg/kg)
10-20 days	**Vegetative Phase**	50972	25197000	24686	1591600
45-55 days	**Reproductive Phase**	200803	40651000	43991	31583000
80-90 days	**Ripening Phase**	58153	19423000	8432	18219000

From Table 2.6, it is definite that the As and Fe concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of

200803 µg/kg and 43991 µg/kg in root and root soil respectively and Fe concentrations of 40651000 µg/kg and 31583000 µg/kg in root and root soil respectively. The As and Fe interaction in root and root soil is graphically shown in Fig 2.12 and Fig 2.13 respectively.

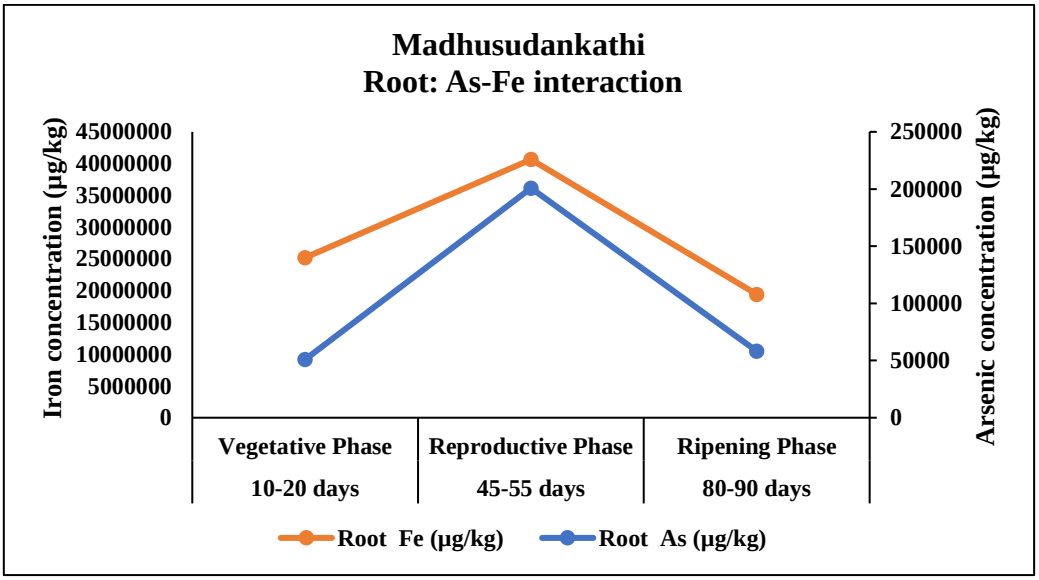


Fig 2.12: Phase wise As and Fe interaction in the root (Madhusudankathi)

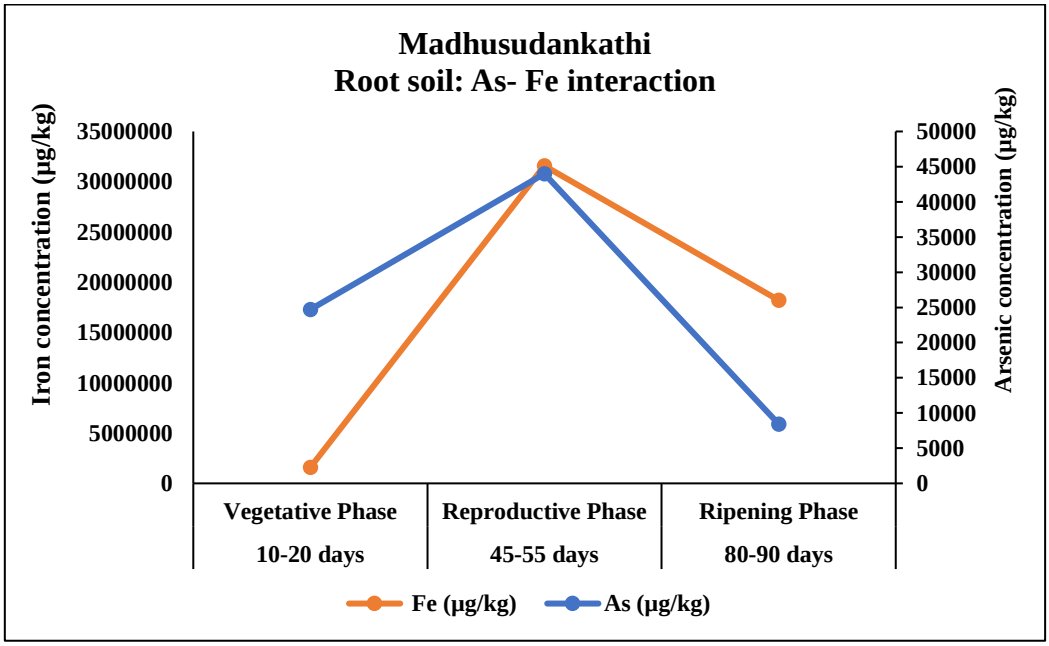


Fig 2.13: Phase wise As and Fe interaction in the root soil (Madhusudankathi)

Therefore, it was observed that As and Fe has a direct relationship in root and root soil in case of monsoon cultivation. Moreover, maximum accumulation of As and Fe in root and root soil was observed in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. During vegetative phase, topsoil As and Fe concentration decreases due to temporal variability which causes diffusion of As in floodwater followed by lateral removal with

receding floodwater. Whereas during Reproductive phase, groundwater from shallow tube well is used for irrigation with As and Fe concentration of 8.63 µg/L and 11950 µg/L respectively to maintain the water logged condition, causing an increase in the root soil As and Fe and consequently higher As and Fe translocation into the root. During the Ripening phase, the root of paddy plants on reaching their saturation, fail to accumulate As and Fe any further due to their lowered accumulation efficiency. So, during Ripening phase, the As and Fe accumulation decreases steadily in the root as well as root soil.

Table 2.7: Correlation matrix of As and Fe in root and root soil (Madhusudankathi)

	Root As (µg/kg)	**Root Fe (µg/kg)**	**Root soil As (µg/kg)**	**Root soil Fe (µg/kg)**
Root As (µg/kg)	1			
Root Fe (µg/kg)	0.952739204	1		
Root soil As (µg/kg)	0.869502707	0.978459469	1	
Root soil Fe (µg/kg)	0.855728725	0.658098148	0.488487791	1

Table 2.7 represents the Correlation matrix of As and Fe accumulation during monsoon cultivation period in root of the paddy plants and root soil collected. It is observed that a very strong correlation of 0.95274 exists between As and Fe of root. The As of root soil has a strong correlation of 0.86951 and 0.97486 with As and Fe of root respectively. The Fe of root soil has a strong correlation of 0.85573 with As of root and a moderate correlation of 0.658098 and 0.48849 with Fe of root and As of root soil respectively.

2.4.2 Field: Teghoria (Gaighata)

2.4.2.1 Arsenic concentration in different parts of paddy and soil

Table 2.8: Arsenic concentration in different parts of paddy and soil in Teghoria

Lot no.	Spot No.	As concentration (µg/kg)						
		Grain	Pedicle	Leaf	Stem	Root	Root soil	Surface Soil
Seedling	-	-	-	5052	6842	505172	88730	-
1	1	-	-	4351	6336	188941	130218	124323
	2	-	-	2769	5598	239640	141344	104058
	3	-	-	3096	9723	219276	69410	68728
2	1	-	-	3602	9027	302236	153654	169267
	2	-	-	3119	5374	329192	121058	160994
	3	-	-	3912	9348	343275	82054	108856
3	1	-	-	14557	16457	312682	101517	105273
	2	-	-	14167	16010	312477	101985	85127
	3	-	-	8352	14715	297169	132922	69688
4	1	2774	4507	12564	19193	173043	79371	87870
	2	1590	3214	10712	17495	331915	48571	45167
	3	940	2113	9056	15634	310309	40140	37150
5	1	595	1680	7098	9592	140000	99031	33279
	2	109	1005	5952	15299	164400	71455	19329
	3	270	1876	6585	17293	206771	27407	20708

The As concentrations in different parts of paddy and soil of 5 lots of samples from the field of Teghoria, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.9.

Table 2.9: Phase wise variation of As in Teghoria (120 days cultivation period)

Interval of days	Phase	Average As concentration (µg/kg)				
		Leaf	Stem	Root	Root soil	Surface soil
10-20 days	Vegetative Phase	3475	7568	215952	113657	99036
55-65 days	Reproductive Phase	12359	16045	318740	137356	116534
110-120 days	Ripening Phase	9545	14058	205878	60886	40584

From Table 2.9, it is evident that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 12359 µg/kg, 16045 µg/kg, 318740 µg/kg, 137356 µg/kg and 116534 µg/kg in leaf, stem, root, root soil and surface soil respectively. The variation of As accumulation in leaf, stem and root and its translocation in different parts during the 3 phases of monsoon cultivation have been represented in Fig 2.14 and Fig 2.15 respectively.

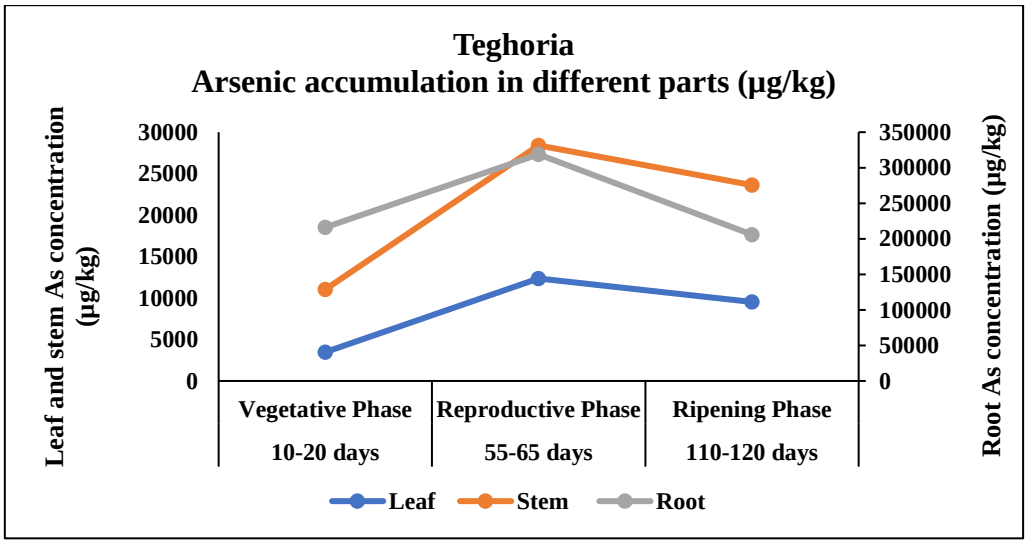


Fig 2.14: Phase wise variation of As in leaf, stem and root in Teghoria

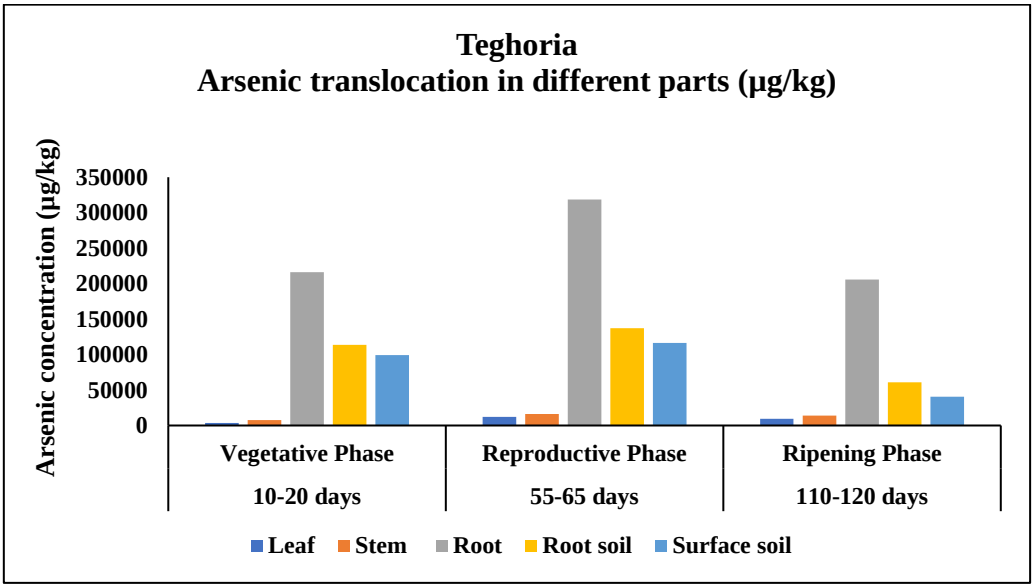


Fig 2.15: Phase wise As translocation in different parts of paddy and soil in Teghoria

Therefore, it was observed that As accumulation in the different parts, ie. grain, pedicle, leaf, stem and root follows Translocation theory and the As concentration is in the order grain<pedicle<leaf<stem<root. Moreover, maximum accumulation of As was observed in root,

stem, leaf, root soil as well as surface soil in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. During vegetative phase, the monsoon flood water removes large amount of As from the top layer of soil in the paddy field, making the As less available for translocation through the root. The paddy plants accumulate As more and more with time and by the time it reaches Reproductive phase, the As accumulation becomes maximum in all the parts. Moreover, groundwater from shallow tube well is used for irrigation with As concentration of 10.44 μg/L to maintain the water logged condition, causing an increase in the soil As and consequently higher As translocation through the root and accumulation in different parts of the plant. During the Ripening phase, the paddy plants on reaching their saturation, fail to accumulate As any further due to their lowered accumulation efficiency. So, during Ripening phase, the As accumulation decreases steadily in the different plant parts as well as the soil.

Table 2.10: Correlation matrix of As accumulation in different parts of paddy plant (Teghoria)

	Leaf	**Stem**	**Root**	**Root soil**	**Surface soil**
Leaf	1				
Stem	0.996036883	1			
Root	0.687424996	0.620107	1		
Root soil	0.098845317	0.009948	0.790648	1	
Surface soil	0.013264397	-0.07572	0.73531	0.996326	1

Table 2.10 represents the Correlation matrix of As accumulation during monsoon cultivation period among different parts of the paddy plants collected. It is observed that a very strong correlation of 0.99604 exists between leaf and stem. The root shows a positive and moderate correlation with the leaf as well as the stem of 0.687425 and 0.620107 respectively as well as with the root soil and surface soil of 0.790648 and 0.73531 respectively. The root soil shows a poor correlation of 0.098845 and 0.009948 with leaf and stem respectively and a strong correlation of 0.996326 with surface soil. The surface soil shows poor correlation with leaf and negative correlation with stem.

2.4.2.2 Iron- Arsenic interaction in root and soil

The As and Fe estimated in the root, root soil and surface soil of all the 5 lots of Teghoria are tabulated below in Table 2.11.

Table 2.11: Variation of As and Fe in root and soil of Teghoria (120 days cultivation period)

Lot No.	Spot No.	Root		Root soil		Surface soil	
		As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)
Seedling	-	505172	25632	88730	39260	-	-
1	1	188941	30609	130218	22782	124323	22391
	2	239640	34964	141344	24018	104058	24507
	3	219276	47072	69410	21308	68728	25282
2	1	302236	77485	153654	53142	169267	46802
	2	329192	59472	121058	41489	160994	40472
	3	343275	68448	82054	43929	108856	41144
3	1	312682	42927	101517	47235	105273	37873
	2	312477	64633	101985	44773	85127	46287
	3	297169	40991	132922	45076	69688	56823
4	1	173043	17523	79371	30378	87870	28133
	2	331915	15128	48571	32515	45167	30128
	3	310309	19958	40140	28293	37150	26293
5	1	140000	11522	99031	35766	33279	41991
	2	164400	20559	71455	30857	19329	39968
	3	206771	35567	27407	22142	20708	30968

The As and Fe concentrations in the root of paddy and root soil of 5 lots of samples from the field of Teghoria, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.12.

Table 2.12: Phase wise variation of As and Fe in root and root soil of Teghoria

Interval of days	Phase	Root		Root soil	
		As (µg/kg)	Fe (µg/kg)	As (µg/kg)	Fe (µg/kg)
10-20 days	**Vegetative Phase**	215952	37548000	113657	22703000
55-65 days	**Reproductive Phase**	318740	68468000	137356	45941000
110-120 days	**Ripening Phase**	205878	34780000	60886	29992000

From Table 2.12, it is definite that the As and Fe concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 318740 µg/kg and 137356 µg/kg in root and root soil respectively and Fe concentrations of 68468000 µg/kg and 45941000 µg/kg in root and root soil respectively. The As and Fe interaction in root and root soil is graphically shown in Fig 2.16 and Fig 2.17 respectively.

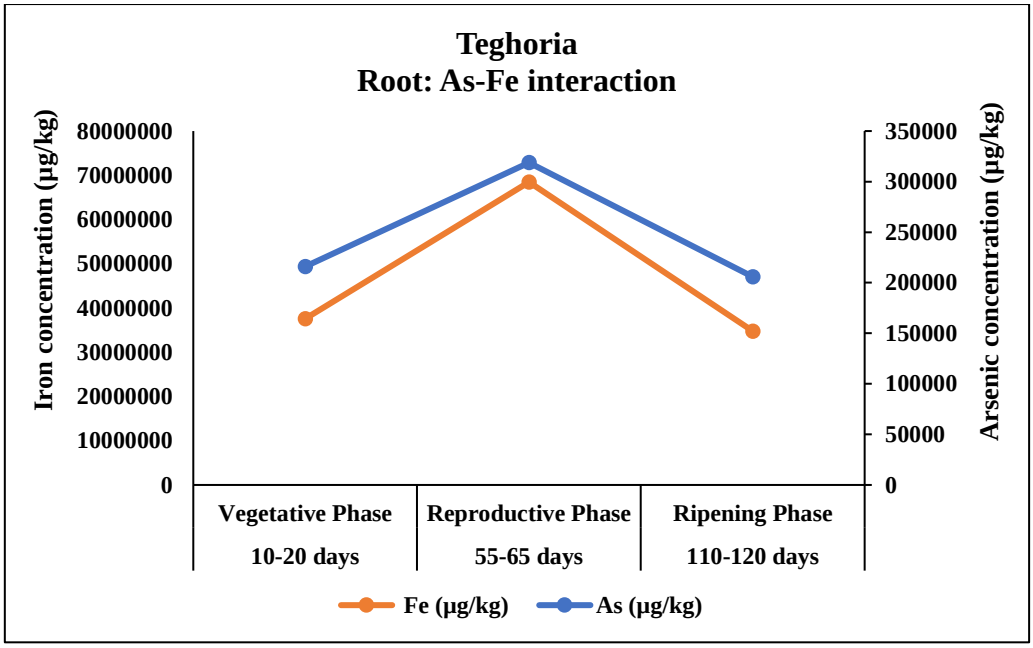


Fig 2.16: Phase wise As and Fe interaction in the root (Teghoria)

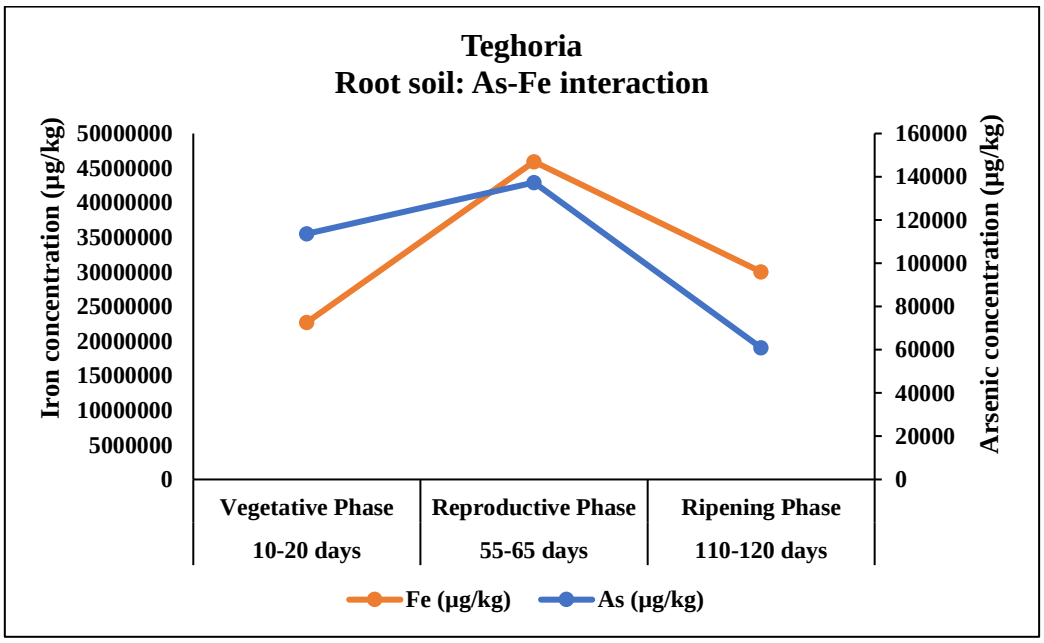


Fig 2.17: Phase wise As and Fe interaction in the root soil (Teghoria)

Therefore, it was observed that As and Fe has a direct relationship in root and root soil in case of monsoon cultivation. Moreover, maximum accumulation of As and Fe in root and root soil was observed in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. During vegetative phase, topsoil As and Fe concentration decreases due to temporal variability which causes diffusion of As in floodwater followed by lateral removal with receding floodwater. Whereas during Reproductive phase, groundwater from shallow tube well is used for irrigation with As and Fe concentration of 10.44 µg/L and 12540 µg/L respectively

to maintain the water logged condition, causing an increase in the root soil As and Fe and consequently higher As and Fe translocation into the root. During the Ripening phase, the root of paddy plants on reaching their saturation, fail to accumulate As and Fe any further due to their lowered accumulation efficiency. So, during Ripening phase, the As and Fe accumulation decreases steadily in the root as well as root soil.

Table 2.13: Correlation matrix of As and Fe in root and root soil (Teghoria)

	Root As (µg/kg)	**Root Fe (µg/kg)**	**Root soil As (µg/kg)**	**Root soil Fe (µg/kg)**
Root As (µg/kg)	**1**			
Root Fe (µg/kg)	**0.999977784**	**1**		
Root soil As (µg/kg)	**0.79064751**	**0.786548773**	**1**	
Root soil Fe (µg/kg)	**0.923991164**	**0.926519664**	**0.496410192**	**1**

Table 2.13 represents the Correlation matrix of As and Fe accumulation during monsoon cultivation period in root of the paddy plants and root soil collected. It is observed that a very strong correlation of 0.999978 exists between As and Fe of root. The As of root soil has a moderate correlation of 0.790647 and 0.786549 with As and Fe of root respectively. The Fe of root soil has a strong correlation of 0.92399 and 0.92652 with As and Fe of root respectively and a moderate correlation of 0.49641 with As of root soil.

2.4.3 Pingla (West Medinipur)

2.4.3.1 Arsenic concentration in different parts of paddy and soil

Table 2.14: Arsenic concentration in different parts of paddy and soil in Pingla

Lot no.	Spot No.	As concentration (µg/kg)						
		Grain	Pedicle	Leaf	Stem	Root	Root soil	Surface Soil
Seedling	-	-	-	2007	3458	8483	10332	7049
1	1	-	-	1553	1840	8984	2028	-
	2	-	-	1225	1441	7389	2219	-
	3	-	-	1264	2166	8602	2663	-
2	1	-	-	2619	3187	28499	1522	1920
	2	-	-	1630	1894	35838	3510	2337
	3	-	-	1408	3248	60833	1562	1527
3	1	-	-	4280	4419	36433	3095	-
	2	-	-	5040	5541	37251	4041	-
	3	-	-	2915	4094	39456	4689	-
4	1	-	-	1755	4283	8587	3719	-
	2	-	-	3419	3620	15147	4465	-
	3	-	-	2295	3664	8106	3768	-
5	1	318	1663	2070	2854	8640	4416	-
	2	213	1907	1994	2009	10810	2700	-
	3	197	1762	2234	2103	8679	4633	-
6	1	198	1011	2018	2143	8700	7490	-
	2	212	982	1403	1543	11305	6340	-
	3	262	1075	1480	1643	13615	7927	-

The As concentrations in different parts of paddy and soil of 6 lots of samples from the field of Pingla, West Medinipur were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.15.

Table 2.15: Phase wise variation of As in Pingla (120 days cultivation period)

Interval of days	Phase	Average As concentration (µg/kg)			
		Leaf	Stem	Root	Root soil
10-20 days	Vegetative Phase	1617	2296	8325	2250
55-65 days	Reproductive Phase	4078	4685	39718	3963
110-120 days	Ripening Phase	1988	2534	10399	3916

From Table 2.15, it is evident that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 4078 µg/kg, 4685 µg/kg, 39718 µg/kg and 3963 µg/kg in leaf, stem, root and root soil

respectively. During the 3 phases of monsoon cultivation, the variation of As accumulation in leaf, stem and root and its translocation in different parts have been represented in Fig 2.18 and Fig 2.19 respectively.

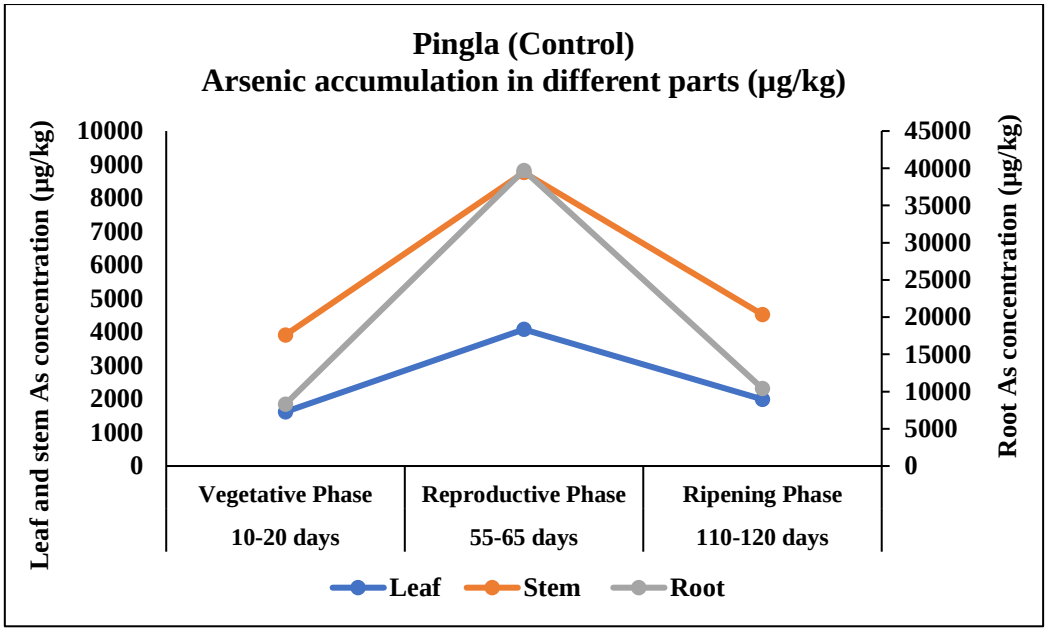


Fig 2.18: Phase wise variation of As in leaf, stem and root in Pingla

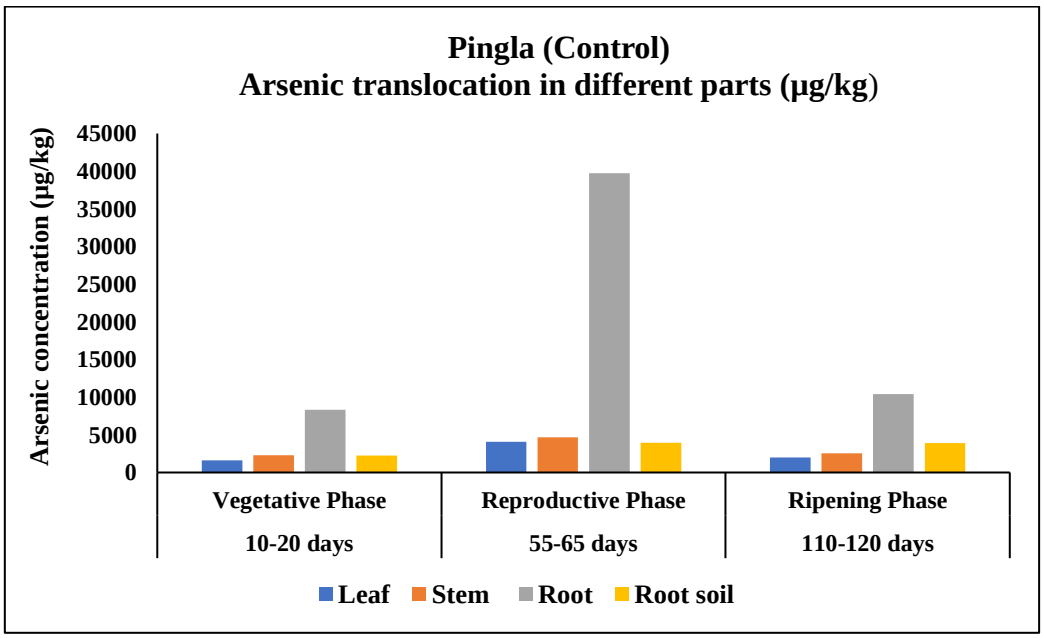


Fig 2.19: Phase wise As translocation in different parts of paddy and soil in Pingla

Therefore, it was observed that the Translocation theory holds well in the different parts of paddy, ie. grain, pedicle, leaf, stem and root and the As concentration is in the order grain<pedicle<leaf<stem<root. Moreover, maximum accumulation of As was observed in root, stem, leaf, root soil as well as surface soil in the reproductive phase in case of monsoon

cultivation unlike pre-monsoon cultivation. During vegetative phase, As transport to deeper soil layer by infiltration due to flooding results in reduced As concentration in top soil, making the As less available for translocation through the root. The paddy plants accumulate As more and more with time and by the time it reaches Reproductive phase, the As accumulation becomes maximum in all the parts. Also, during this phase, groundwater from shallow tube well is used for irrigation with As concentration of 9.27 μg/L to maintain the water logged condition, causing an increase in the soil As and consequently higher As translocation through the root and accumulation in different parts of the plant. During the Ripening phase, the paddy plants on reaching their saturation, fail to accumulate As any further due to their lowered accumulation efficiency. So, during Ripening phase, the As accumulation decreases steadily in the different plant parts as well as the soil.

Table 2.16: Correlation matrix of As accumulation in different parts of paddy plant (Pingla)

	Leaf	**Stem**	**Root**	**Root soil**
Leaf	1			
Stem	0.998764235	1		
Root	0.996707502	0.999505469	1	
Root soil	0.634959754	0.595780266	0.57023	1

Table 2.16 represents the Correlation matrix of As accumulation during monsoon cultivation period among different parts of the paddy plants collected. It is observed that a very strong correlation of 0.99876 exists between leaf and stem. The root shows a positive and a very strong correlation with the leaf as well as the stem of 0.996707 and 0.999505 respectively. The root soil shows a moderate correlation of 0.63496, 0.59578 and 0.57023 with leaf and stem and root respectively.

2.4.3.2 Iron- Arsenic interaction in root and soil

The As and Fe estimated in the root, root soil and surface soil of all the 6 lots of Pingla are tabulated below in Table 2.17.

Table 2.17: Variation of As and Fe in root and soil of Pingla (120 days cultivation period)

Lot No.	Spot No.	Root		Root soil		Surface soil	
		As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)
Seedling	-	8483	28401	10332	13293	7049	15275
1	1	8984	31703	2028	11892	-	-
	2	7389	29246	2219	10667	-	-
	3	8602	27306	2663	11178	-	-
2	1	28499	61251	1522	17898	1920	23955
	2	35838	57903	3510	28655	2337	23683
	3	60833	59583	1562	21922	1527	16234
3	1	36433	60882	3095	24538	-	-
	2	37251	71231	4041	20631	-	-
	3	39456	70198	4689	20584	-	-
4	1	8587	61016	3719	14248	-	-
	2	15147	82548	4465	14229	-	-
	3	8106	72839	3768	12903	-	-
5	1	8640	37953	4416	9172	-	-
	2	10810	31996	2700	10368	-	-
	3	8679	30692	4633	11356	-	-
6	1	8700	14174	7490	10854	-	-
	2	11305	13558	6340	9083	-	-
	3	13615	13020	7927	6132	-	-

The As and Fe concentrations in the root of paddy and root soil of 6 lots of samples from the field of Pingla, west Medinipur were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.18.

Table 2.18: Phase wise variation of As and Fe in root and root soil of Pingla

Interval of days	Phase	Root		Root soil	
		As (µg/kg)	Fe (µg/kg)	As (µg/kg)	Fe (µg/kg)
10-20 days	**Vegetative Phase**	8325	29783000	2250	11246000
55-65 days	**Reproductive Phase**	39718	66383000	3963	22321000
110-120 days	**Ripening Phase**	10399	23565000	3916	10927000

From Table 2.18, it is evident that the As and Fe concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 39718 µg/kg and 3936 µg/kg in root and root soil respectively and Fe concentrations of

66383000 µg/kg and 22321000 µg/kg in root and root soil respectively. The As and Fe interaction in root and root soil is graphically shown in Fig 2.20 and Fig 2.21 respectively.

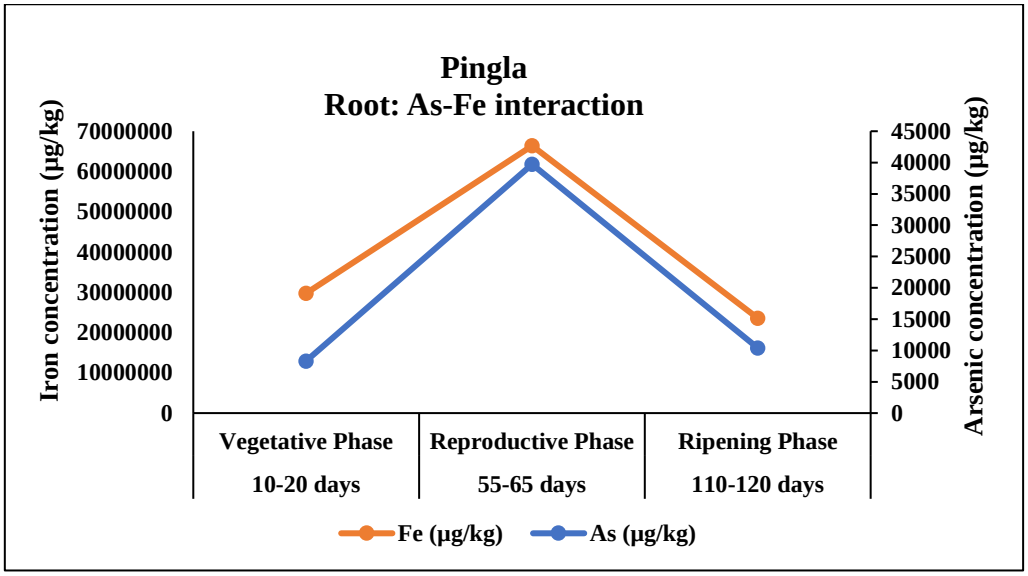


Fig 2.20: Phase wise As and Fe interaction in the root (Pingla)

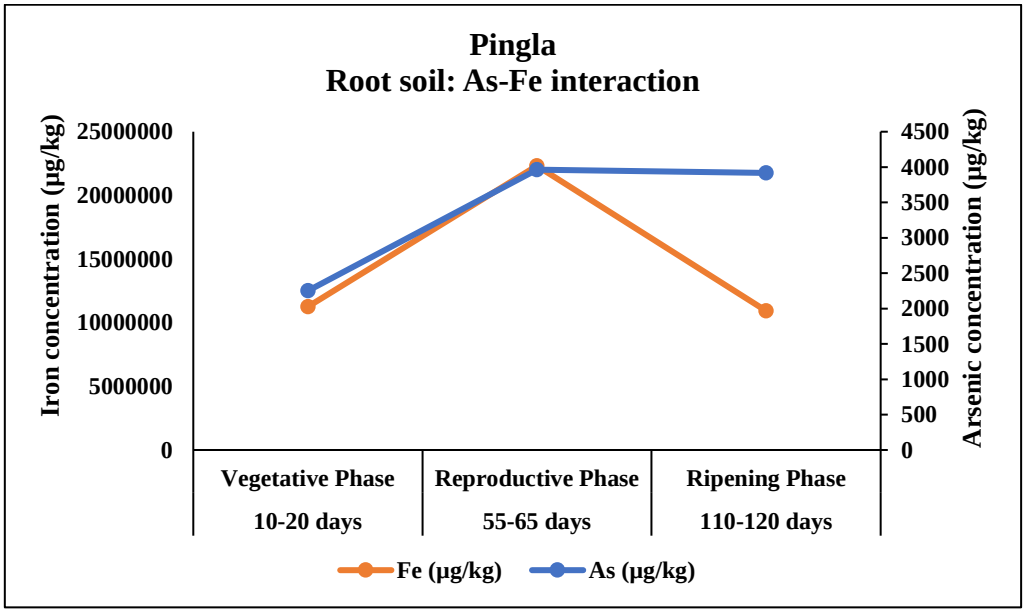


Fig 2.21: Phase wise As and Fe interaction in the root soil (Pingla)

Therefore, it was observed that As and Fe has a direct relationship in root and root soil in case of monsoon cultivation. Also, maximum accumulation of As and Fe in root and root soil was observed in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. During vegetative phase, topsoil As and Fe concentration decreases due to temporal variability which causes diffusion of As in floodwater followed by lateral removal with receding floodwater. Whereas during Reproductive phase, groundwater from shallow tube well is used for irrigation with As and Fe concentration of 9.27 µg/L and 16850 µg/L respectively

to maintain the water logged condition, causing an increase in the root soil As and Fe and consequently higher As and Fe translocation into the root. During the Ripening phase, the root of paddy plants on reaching their saturation, fail to accumulate As and Fe any further due to their lowered accumulation efficiency. So, during Ripening phase, the As and Fe accumulation decreases steadily in the root as well as root soil.

Table 2.19: Correlation matrix of As and Fe in root and root soil (Pingla)

	Root As (µg/kg)	**Root Fe (µg/kg)**	**Root soil As (µg/kg)**	**Root soil Fe (µg/kg)**
Root As (µg/kg)	1			
Root Fe (µg/kg)	0.981262503	1		
Root soil As (µg/kg)	0.570230282	0.40126547	1	
Root soil Fe (µg/kg)	0.996500392	0.993933857	0.499568347	1

Table 2.19 represents the Correlation matrix of As and Fe accumulation during monsoon cultivation period in root of the paddy plants and root soil collected. It is observed that a very strong correlation of 0.98126 exists between As and Fe of root. The As of root soil has a moderate correlation of 0.57023 and 0.40126 with As and Fe of root respectively. The Fe of root soil has a strong correlation of 0.99650 and 0.993934 with As and Fe of root respectively and a moderate correlation of 0.49957 with As of root soil.

2.4.4 Pot study (Control 1 and Control 2)

2.4.4.1 Arsenic concentration in different parts of paddy and soil

Table 2.20: Arsenic concentration in different parts of paddy and soil in Control 1

Lot No.	As concentration (µg/kg)						
	Grain	Pedicle	Leaf	Stem	Root	Root soil	Surface Soil
Seedling	-	-	1287	4548	57059	6128	6991
1	-	-	1558	2569	22047	3885	1823
2	-	-	761	1160	21429	4235	2487
3	224	992	1684	3580	35208	8048	7263
4	148	949	1136	1163	20210	4233	4962

Table 2.21: Arsenic concentration in different parts of paddy and soil in Control 2

Lot No.	As concentration (µg/kg)						
	Grain	Pedicle	Leaf	Stem	Root	Root soil	Surface Soil
Seedling	-	-	3468	3476	75856	6928	6774
1	-	-	1639	3039	34174	1298	2587
2	-	-	1230	2606	30442	5252	3360
3	318	5253	6840	8278	78138	9276	7580
4	216	853	1312	1374	12500	4279	5538

The As concentrations in different parts of paddy and soil of 4 lots of samples from the Control pots 1 and 2 were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.22 and 2.23 respectively.

Table 2.22: Phase wise variation of As in Control 1 (90 days cultivation period)

Interval of days	Phase	Average As concentration (µg/kg)				
		Leaf	Stem	Root	Root soil	Surface soil
10-20 days	Vegetative Phase	1156	1865	21738	4060	2155
45-55 days	Reproductive Phase	1684	3580	35208	8048	7263
80-90 days	Ripening Phase	1136	1163	20210	4223	4962

Table 2.23: Phase wise variation of As in Control 2 (90 days cultivation period)

Interval of days	Phase	Average As concentration (µg/kg)				
		Leaf	Stem	Root	Root soil	Surface soil
10-20 days	Vegetative Phase	1435	2823	32308	3924	2974
45-55 days	Reproductive Phase	6840	8278	78138	9276	7580
80-90 days	Ripening Phase	1312	1374	12500	4279	5538

From Table 2.22 and 2.23, it is evident that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1684 µg/kg, 3580 µg/kg, 35208 µg/kg, 8048 µg/kg, 7263 µg/kg and 6840 µg/kg, 8278 µg/kg, 78138 µg/kg, 9276 µg/kg, 7580 µg/kg in leaf, stem, root, root soil and surface soil of Control 1 and Control 2 respectively. During the 3 phases of monsoon cultivation, the variation of As accumulation in leaf, stem and root and its translocation in different parts have been represented in Fig 2.22 and Fig 2.23 for Control 1 and Fig 2.24 and 2.25 for Control 2 respectively.

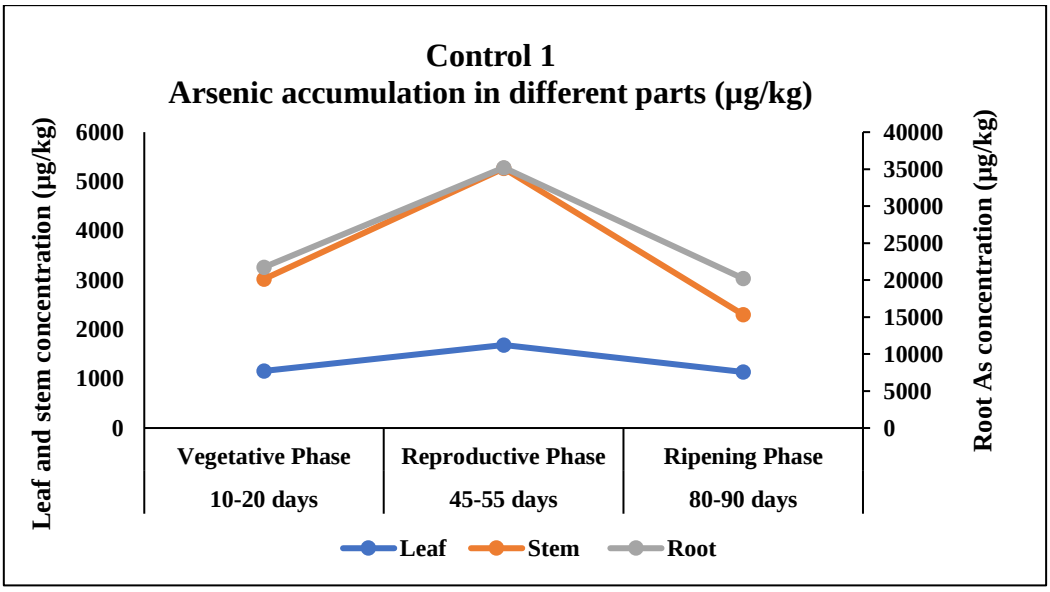


Fig 2.22: Phase wise variation of As in leaf, stem and root in Control 1

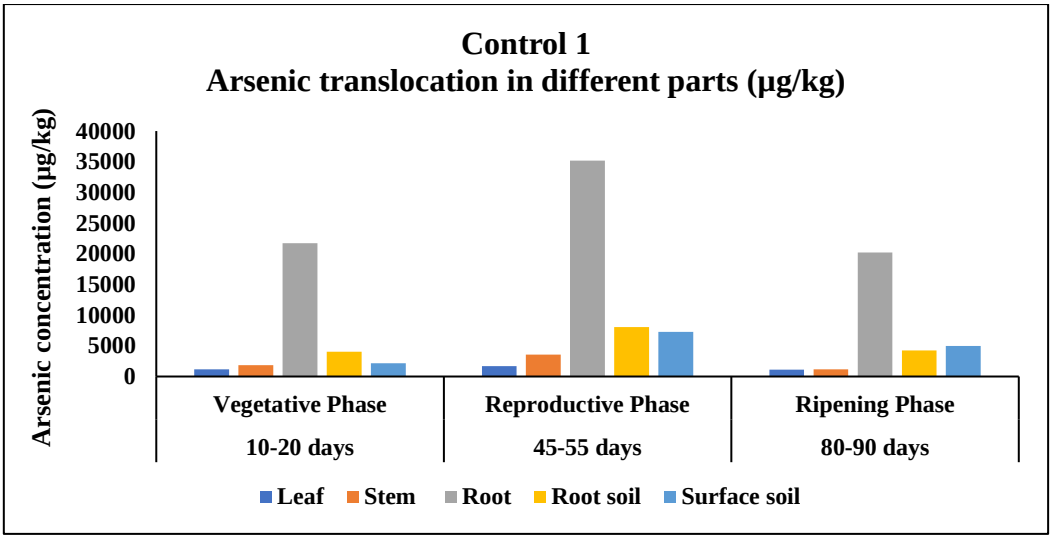


Fig 2.23: Phase wise As translocation in different parts of paddy and soil in Control 1

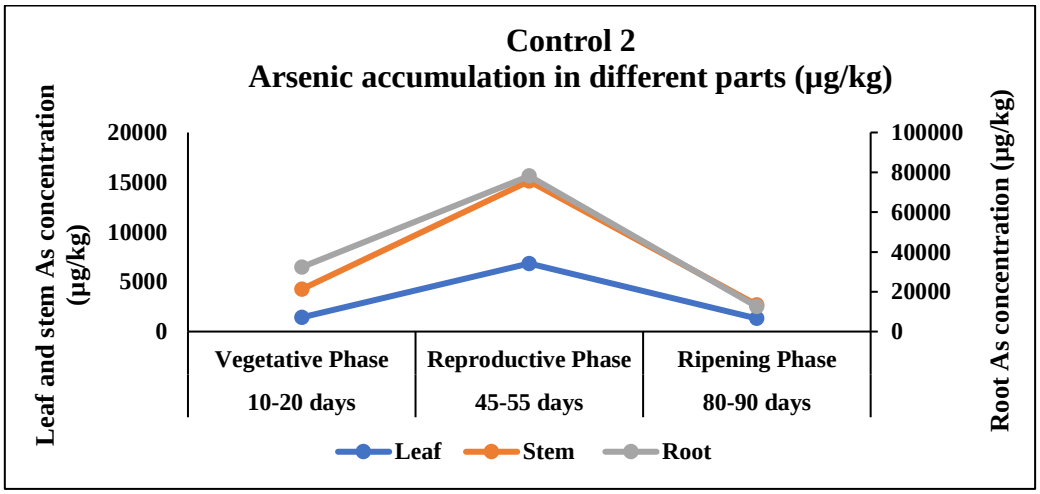


Fig 2.24: Phase wise variation of As in leaf, stem and root in Control 2

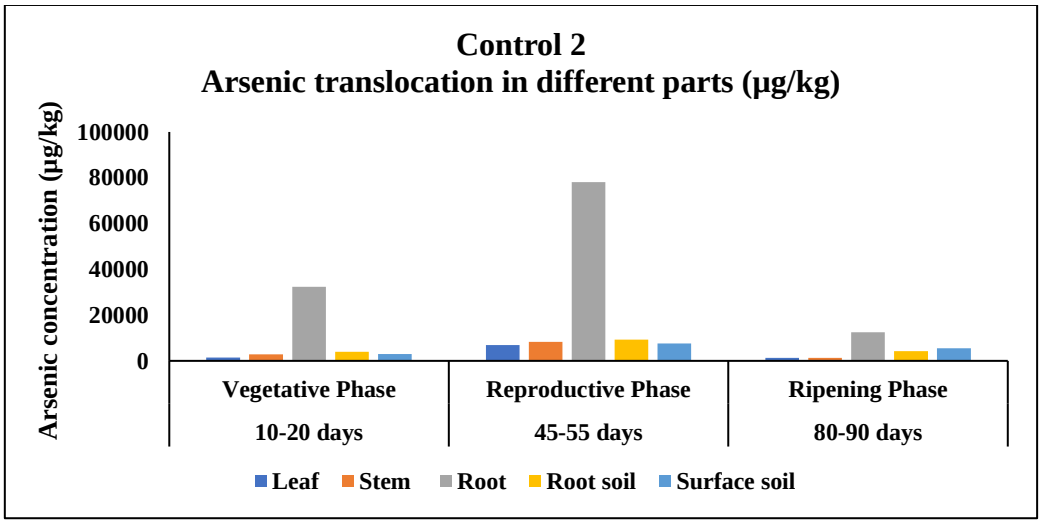


Fig 2.25: Phase wise As translocation in different parts of paddy and soil in Control 2

Therefore, it was observed that the Translocation theory holds well in the different parts of paddy, ie. grain, pedicle, leaf, stem and root and the As concentration is in the order grain<pedicle<leaf<stem<root. Moreover, unlike pre-monsoon cultivation, maximum accumulation of As was observed in root, stem, leaf, root soil as well as surface soil in the reproductive phase in case of monsoon cultivation. The paddy plants accumulate As more and more with time and by the time it reaches Reproductive phase, the As accumulation becomes maximum in all the parts. During the Ripening phase, the paddy plants on reaching their saturation, fail to accumulate As any further due to their lowered accumulation efficiency. So, during Ripening phase, the As accumulation decreases steadily in the different plant parts as well as the soil.

Table 2.24: Correlation matrix of As accumulation in different parts of paddy plant (Control 1)

	Leaf	**Stem**	**Root**	**Root soil**	**Surface soil**
Leaf	1				
Stem	0.967914	1			
Root	0.998169	0.98134	1		
Root soil	0.997668	0.948508	0.991714	1	
Surface soil	0.817976	0.647181	0.781688	0.85533	1

Table 2.25: Correlation matrix of As accumulation in different parts of paddy plant (Control 2)

	Leaf	**Stem**	**Root**	**Root soil**	**Surface soil**
Leaf	1				
Stem	0.983689	1			
Root	0.961303	0.995178	1		
Root soil	0.996895	0.96647	0.936624	1	
Surface soil	0.820552	0.704355	0.631335	0.863013	1

Table 2.24 and 2.25 represents the Correlation matrix of As accumulation during monsoon cultivation period among different parts of the paddy plants collected from Control 1 and Control 2 pots. In case of Control 1, it is observed that a very strong correlation of 0.967914 exists between leaf and stem. The root shows a positive and a very strong correlation with the leaf as well as the stem of 0.99817 and 0.98134 respectively. The root soil shows a strong correlation of 0.997668, 0.948508 and 0.991714 with leaf and stem and root respectively. The surface soil shows a strong correlation of 0.817976 and 0.85533 with leaf and root soil and a moderate correlation of 0.647181 and 0.781688 with the stem and root respectively. In case of Control 2, it is observed that a very strong correlation of 0.983689 exists between leaf and

stem. The root shows a positive and a very strong correlation with the leaf as well as the stem of 0.961303 and 0.99517 respectively. The root soil shows a strong correlation of 0.996895, 0.96647 and 0.93662 with leaf and stem and root respectively. The surface soil shows a strong correlation of 0.82055 and 0.86301 with leaf and root soil and a moderate correlation of 0.70435 and 0.63134 with the stem and root respectively.

2.4.4.2 Iron- Arsenic interaction in root and soil

The As and Fe estimated in the root, root soil and surface soil of all the 4 lots of Control 1 and control 2 are tabulated below in Table 2.26 and Table 2.27 respectively.

Table 2.26: Variation of As and Fe in root and soil of Control 1 (90 days cultivation period)

Lot No.	Root		Root soil		Surface soil	
	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)
Seedling	57059	45588	6128	29294	6991	23277
1	22047	10750	3885	18284	1823	18098
2	21429	34921	4235	31703	2487	22653
3	35208	29478	8048	39785	7263	24939
4	20210	13862	4233	18350	4962	16573

Table 2.27: Variation of As and Fe in root and soil of Control 2 (90 days cultivation period)

	Root		Root soil		Surface soil	
Lot No.	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)	As conc. (µg/kg)	Fe conc. (mg/kg)
Seedling	75856	65582	6928	36602	6774	27640
1	34174	10799	1298	11688	2587	14095
2	30442	19679	5252	15948	3360	15709
3	78138	25134	9276	18747	7580	22025
4	12500	12732	4279	12082	5538	13415

The As and Fe concentrations in the root of paddy and root soil of 4 lots of samples from the pots of Control 1 and Control 2 were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 2.28 and 2.29 respectively.

Table 2.28: Phase wise variation of As and Fe in root and root soil of Control 1

Interval of days	Phase	Root		Root soil	
		As (µg/kg)	Fe (µg/kg)	As (µg/kg)	Fe (µg/kg)
10-20 days	**Vegetative Phase**	21738	10750000	4060	18284000
45-55 days	**Reproductive Phase**	35208	32196000	8048	35744000
80-90 days	**Ripening Phase**	20210	13862000	4223	18350000

Table 2.29: Phase wise variation of As and Fe in root and root soil of Control 2

Interval of days	Phase	Root		Root soil	
		As (µg/kg)	Fe (µg/kg)	As (µg/kg)	Fe (µg/kg)
10-20 days	**Vegetative Phase**	32308	10799000	3924	11688000
45-55 days	**Reproductive Phase**	78138	22406000	9276	17348000
80-90 days	**Ripening Phase**	12500	12732000	4279	12082000

From Table 2.28 and 2.29, it is evident that the As and Fe concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 35208 µg/kg and 8048 µg/kg in root and root soil respectively and Fe concentrations of 32196000 µg/kg and 35744000 µg/kg in root and root soil respectively for Control 1 and As concentrations of 78138 µg/kg and 9276 µg/kg in root and root soil respectively and Fe concentrations of 22406000 µg/kg and 17348000 µg/kg in root and root soil respectively for Control 2. The As and Fe interaction in root and root soil in Control 1 and Control 2 are graphically shown in Fig 2.26, Fig 2.27, Fig 2.28 and Fig 2.29 respectively.

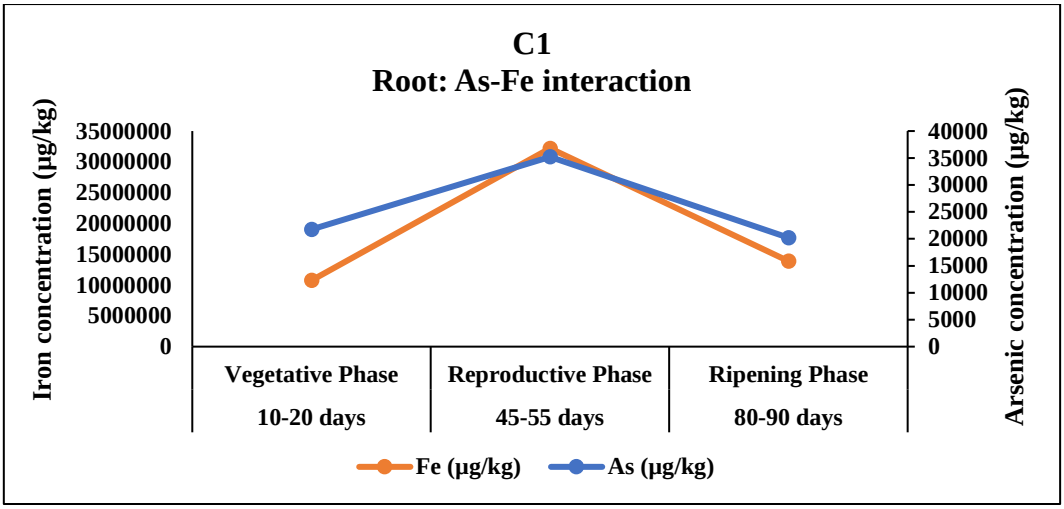


Fig 2.26: Phase wise As and Fe interaction in the root (Control 1)

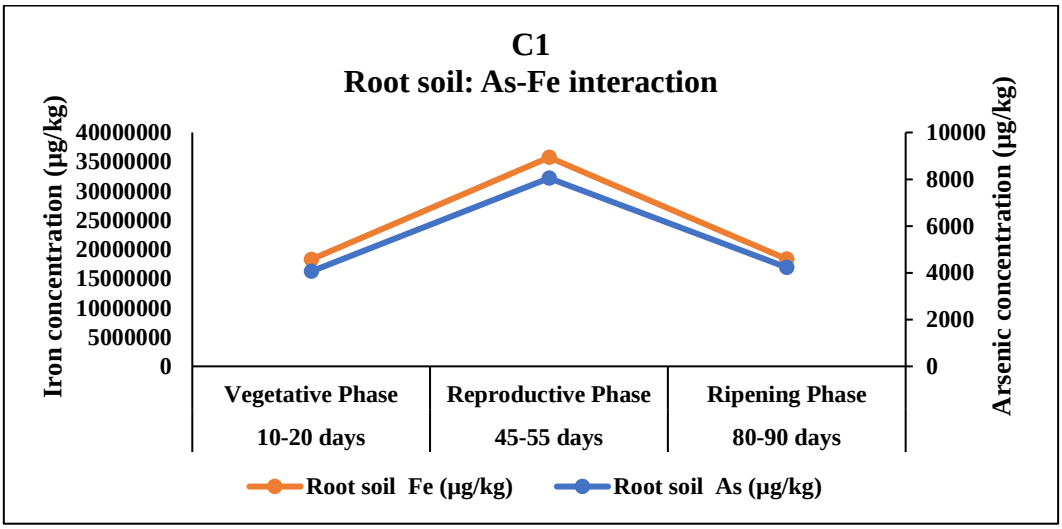


Fig 2.27: Phase wise As and Fe interaction in the root soil (Control 1)

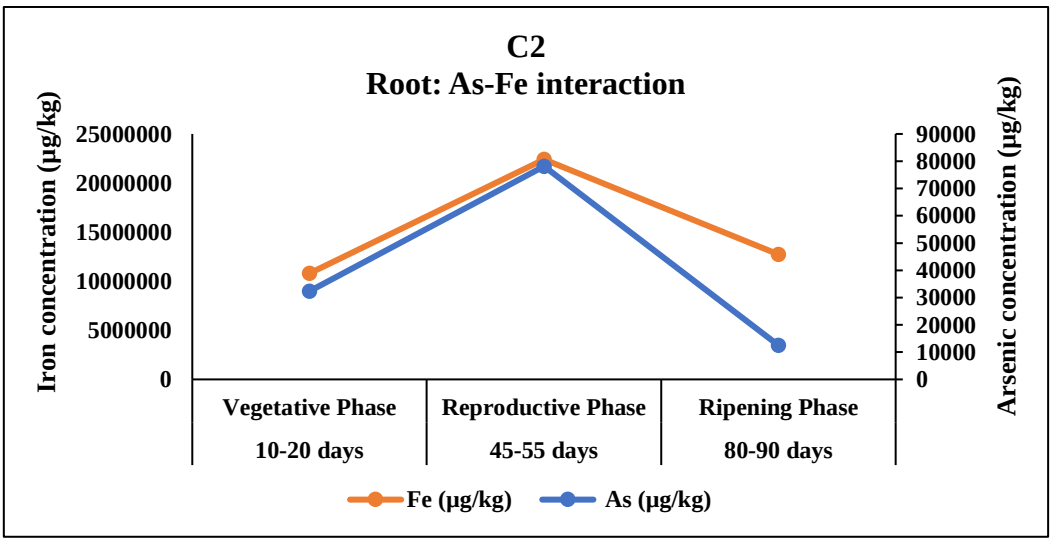


Fig 2.28: Phase wise As and Fe interaction in the root (Control 2)

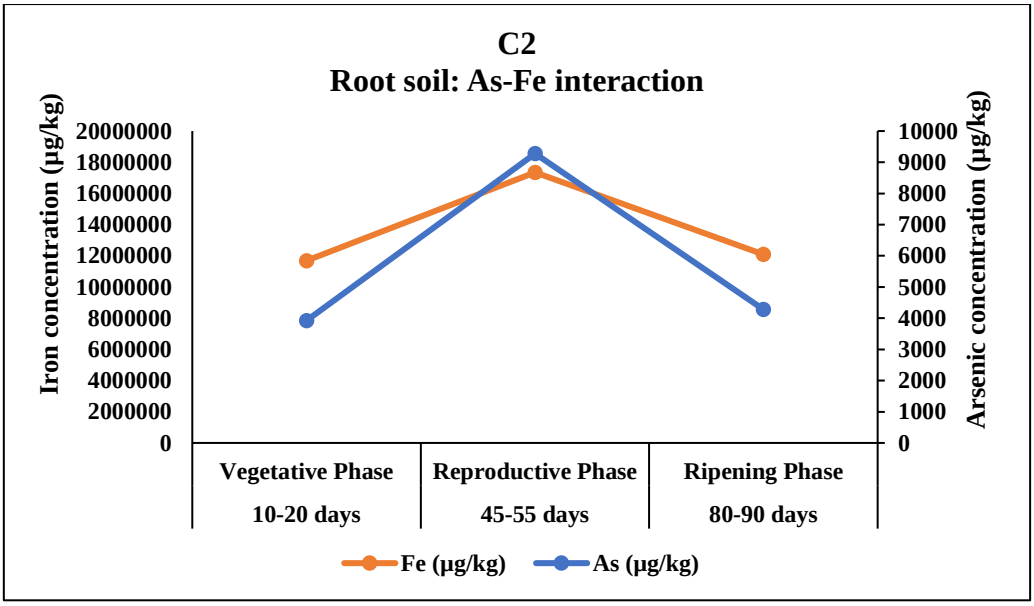


Fig 2.29: Phase wise As and Fe interaction in the root soil (Control 2)

Therefore, it was observed that As and Fe has a direct relationship in root and root soil in case of monsoon cultivation for both Control 1 and Control 2. Also, maximum accumulation of As and Fe in root and root soil was observed in the reproductive phase in case of monsoon cultivation unlike pre-monsoon cultivation. During vegetative phase, topsoil As and Fe concentration decreases due to As transport to deeper soil layer by infiltration due to flooding (Shrivastava et al, 2017). During Reproductive phase, the accumulation efficiency in plants reaches the maximum causing an increase in the root As and Fe from root soil. During the Ripening phase, the root of paddy plants on reaching their saturation, fail to accumulate As and Fe any further due to their lowered accumulation efficiency. Microbial methylation of As into volatile Arsine gas also causes loss of As into the atmosphere (Miah et al, 2005). So, during Ripening phase, the As and Fe accumulation decreases steadily in the root as well as root soil.

Table 2.30: Correlation matrix of As and Fe in root and root soil (Control 1)

	Root As (µg/kg)	**Root Fe (µg/kg)**	**Root soil As (µg/kg)**	**Root soil Fe (µg/kg)**
Root As (µg/kg)	1			
Root Fe (µg/kg)	0.97426064	1		
Root soil As (µg/kg)	0.991714217	0.995146984	1	
Root soil Fe (µg/kg)	0.995397456	0.991379599	0.999460827	1

Table 2.31: Correlation matrix of As and Fe in root and root soil (Control 2)

	Root As (µg/kg)	**Root Fe (µg/kg)**	**Root soil As (µg/kg)**	**Root soil Fe (µg/kg)**
Root As (µg/kg)	1			
Root Fe (µg/kg)	0.898422321	1		
Root soil As (µg/kg)	0.93662388	0.995327963	1	
Root soil Fe (µg/kg)	0.935556799	0.99561628	0.999995399	1

Table 2.30 and 2.31 represents the Correlation matrix of As and Fe accumulation during monsoon cultivation period in root of the paddy plants and root soil collected from Control 1 and Control 2 respectively. In case of Control 1, it is observed that a very strong correlation of 0.97426064 exists between As and Fe of root. The As of root soil has a very strong correlation of 0.991714 and 0.995147 with As and Fe of root respectively. The Fe of root soil has a strong correlation of 0.995397, 0.9913796 and 0.9994608 with As and Fe of root and As of root soil respectively. In case of Control 2, it is observed that a strong correlation of 0.898422 exists between As and Fe of root. The As of root soil has a very strong correlation of 0.936624 and 0.995328 with As and Fe of root respectively. The Fe of root soil has a strong correlation of 0.9355568, 0.9956163 and 0.9999954 with As and Fe of root and As of root soil respectively.

2.5 Conclusion and future scopes

2.5.1 Conclusion

Arsenic translocation in paddy plant and the mechanism of accumulation of As in different parts have been explained keeping in mind the water-logged condition prevailing due to the monsoon rainfall. The As accumulation in different parts of paddy plant in the fields of Madhusudankathi, Teghoria (Gaighata block) and Pingla (Medinipur) as well as in the pot studies of Control 1 and Control 2 follow a similar trend. In all cases, the maximum As accumulation is noticed in the Reproductive phase than the Vegetative or Ripening Phase. This trend is exactly the opposite of what is noticed in case of Pre-monsoon cultivation of Paddy (Chowdhury et al, 2018). The Translocation theory holds good in all the cases with maximum accumulation in the roots, followed by stem, leaf, pedicle and grain. The maximum As accumulation has been observed during Reproductive phase in the roots of paddy plants in Teghoria (318740 μg/kg) which is highly As affected, followed by Madhusudankathi (200803 μg/kg) which is moderately As affected, located in Gaighata block, North 24 Parganas. The As accumulation in control field of Pingla, West Medinipur showed root accumulation of 39718 μg/kg during Reproductive phase, which is almost 10 times less than the fields of Gaighata. During Reproductive phase, the As accumulation in the roots of paddy in Control 1 is 25308 μg/kg which is grown on uncontaminated soil, irrigated with uncontaminated water whereas that in Control 2 is 78138 μg/kg which is grown in uncontaminated soil but contaminated water. Therefore, it is evident that the water used for irrigation from shallow tube wells play an important role in the translocation and accumulation of As in paddy.

During the harvesting phase, the As concentration in the grain was found to be 389 μg/kg in Teghoria (highly As affected), followed by 375 μg/kg in Madhusudankathi (moderately affected), in Gaighata block, North 24 Parganas. The As accumulation in the grain of control field, Pingla, Medinipur was lower compared to that of Gaighata, i.e. 224 μg/kg whereas that in case of pot studies by SRI technique, Control 1 and Control 2 were found to be 148 μg/kg and 216 μg/kg respectively. The As accumulation in the grains of Control 1 in the final phase was least as uncontaminated water was used for irrigation unlike that in Control 2. Therefore, irrigation by uncontaminated water and pot cultivation by SRI technique has been proved to be advantageous compared to water-logged fields. The As-Fe interaction study in the root and root soil of all the fields as well as the pot study shows that Fe has a direct relationship with As i.e. maximum Fe accumulation in root and soil occurs in the Reproductive phase as in

the case of As. Moreover, the Translocation of As in different parts and the As-Fe interaction in root and root soil are statistically significant as in 95% cases, positive and very strong correlation is established between the parameters.

2.5.2 Future scopes

a) Sequential digestion of the root soil collected and preserved from the different fields as well as the 2 Control pots could be done to get a clear idea about the leaching of As and Fe throughout the different phases of monsoon cultivation. Sequential digestion of the soil basically deals with sonication of weighed soil sample in double distilled water, followed by acid digestion with 2:1 HNO_3 and H_2O_2. This is followed by digestion with Aqua regia (1:3 HNO_3 and HCl and finally digestion with Hydrofluoric acid (HF). The samples generated in each step is estimated for As and Fe to understand the leaching process better during the three phases of cultivation.

b) SEM (Scanning electron microscope) study of the root soil collected and preserved from the different fields as well as the 2 Control pots could be used to get a physical image of Fe plaque formation if any, throughout the monsoon cultivation period.

3. Chapter 2: Biochemical assay of leaves of paddy plant during post monsoon cultivation period

3.1 Aim and objective of study

The main objective of the biochemical study of leaves of paddy plants *(Oryza sativa)* during post-monsoon cultivation period (July-November) (2018-19) are as follows:

1. To study the variation of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the highly contaminated field of Teghoria in Gaighata block of North Parganas, West Bengal.
2. To evaluate the variation of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the moderately contaminated field of Madhusudankathi in Gaighata block of North 24 Parganas, West Bengal.
3. To analyse the variation of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend of As accumulation in different parts of paddy plant throughout the post-monsoon cultivation period in the control field of Pingla, West Medinipur, West Bengal.
4. To compare the variation of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend of As accumulation in different parts of Paddy plant during post monsoon period in highly contaminated field (Teghoria) and moderately contaminated field (Madhusudankathi) of Gaighata, with the control field (Pingla) of West Medinipur.
5. To estimate the variation of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend with As accumulation in different parts of paddy plant cultivated by Pot culture using SRI (System of rice intensification) technique, one with non-contaminated soil and non-contaminated water (Control 1) and the other with As contaminated soil and non-contaminated water (Control 2).
6. To compare the trends of pigments like Chlorophyll a, Chlorophyll b, total Chlorophyll, Carotene and Xanthophyll with the trend of As accumulation in different parts of paddy plants grown in field conditions with that grown in pot culture using SRI technique (i.e. Control 1 and Control 2).

3.2 Literature review

At a higher concentration, As is toxic to most varieties of plants. Arsenic inhibits plant growth and development and interferes with its metabolic processes through As induced phytotoxicity (Marin et al., 1993). Plants exhibit toxicity symptoms like inhibition of seed germination (Abedin and Meharg., 2002a); decrease in shoot growth (Cox et al., 1996); decrease in plant height (Marin et al., 1992; Carbonell-Barrachina et al., 1995; Abedin et al., 2002b; Jahan et al., 2003); decrease in tillering (Kang et al., 1996, Rahman et al., 2004); reduction in root growth (Abedin and Meharg., 2002a); lower fruit and grain yield (Carbonell-Barrachina et al., 1995, Kang et al., 1996, Abedin et al., 2002b); and sometimes, leads to death (Baker et al., 1976, Marin et al., 1992) when exposed to excess As either in soil or in solution culture. But the effect of As on photosynthesis, the basis of plant bio-chemical system has been less studied. As almost all of the above-mentioned adverse effects of As on physiological and agronomical parameters are related to the basic photochemical reaction in plants, photosynthesis, it is important to measure the Chlorophyll a and b, the major photosynthetic pigments, contents in rice leaf to justify their correlations with rice growth and yield. Accoring to Rahman et al., 2007, a study was undertaken to evaluate the effects of soil As concentrations on photosynthetic pigment, i.e.Chlorophyll, and correlations with growth and yield of five popular and widely cultivated Boro rice (*Oryza sativa*) varieties in Bangladesh.

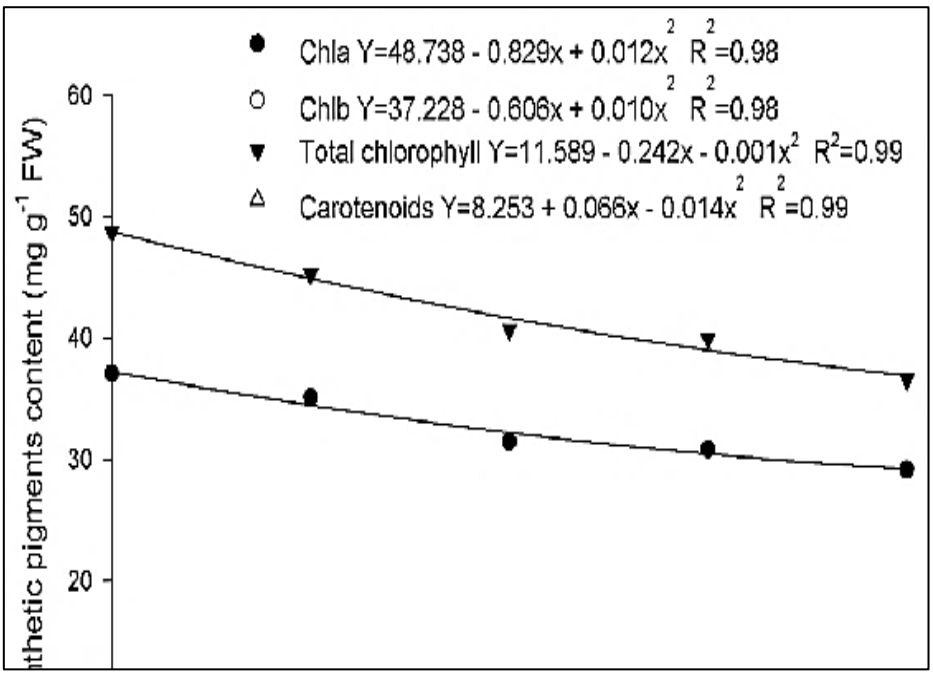


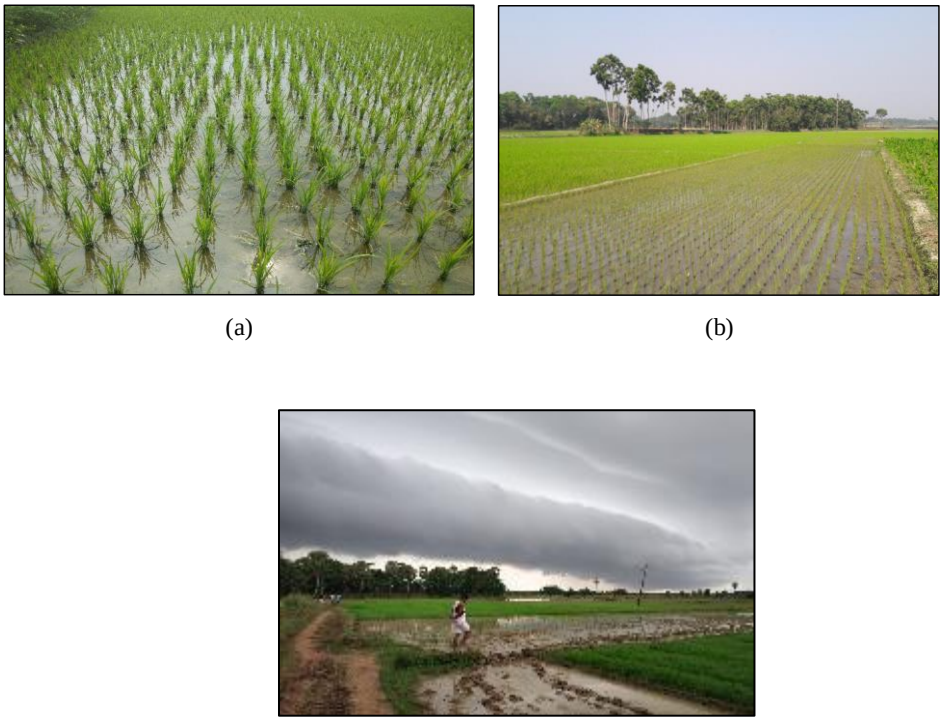
Fig 3.1: Relationship between As and photosynthetic pigments (Farnese et al, 2014)

Both Chlorophyll a and b contents in rice leaf was reported to decrease significantly ($p < 0.05$) with the increase of soil As concentrations. Significant correlations were observed between Chlorophyll

content and rice growth and yield suggesting that, As toxicity affects photosynthesis which ultimately results in the reduction of rice growth and yield. Another study conducted by Choudhury et al; 2009, on the effect of As^{+5} on the growth and metabolism in rice seedlings; showed As was more toxic for root growth, than for shoot growth, where root hairs were fewer and short, roots were characteristically stubby, brittle and root tips gradually turned brown. Arsenic caused damage to the root epidermal cells and aerenchymatous cortex. The level of total Chlorophyll, Chlorophyll a, Chlorophyll b and fluorescence intensity were decreased in As treated rice seedlings. Both Chlorophyll a, Chlorophyll b contents and also intensity of Chlorophyll fluorescence in rice leaf were decreased with the increasing concentrations of As, accompanied by pale green coloration of leaves. The other accessory photosynthetic pigments, such as Carotene and Xanthophyll, contents also declined in rice seedlings on As exposure. According to a previous work by Miteva and Merakchiyska., 2002, increased As concentrations resulted in an alteration of the chloroplast shape with concaving membrane bending and partial destruction, along with the changes in the accumulation and flow of assimilates leading to the decrease of Chlorophyll contents in rice leaf. The higher plants produce carbohydrate using Chlorophyll a. Thus the Chlorophyll a contents are directly associated, with the carbohydrate production. It may be concluded that the reduction of growth and yield in rice might be partially the result of reduced chlorophyll content in rice leaf due to As toxicity.

3.3 Materials and methods

3.3.1 Study area



(a) (b)

(c)

Fig 3.2: Post-monsoon cultivation of paddy in (a) Teghoria (Gaighata) (b) Madhusudankathi (Gaighata) (c) Pingla (Medinipur)

The study area includes the highly As contaminated agricultural field of Teghoria and the moderately contaminated field of Madhusudankathi, situated in Gaighata block of North 24 Parganas, West Bengal. These fields were chosen based on the high As concentration in the groundwater as well as in the urine, hair and nail samples of the population living in Gaighata district as reported by Roychowdhury., 2010. Pingla, situated in west Medinipur was chosen as control field based on the reports by Samanta et al., 2007 and Chakraborti et al., 2009. Besides, two experimental pot studies where paddy was grown using SRI technique, one with non-contaminated soil and non-contaminated water (Control 1) and the other with As contaminated soil and non-contaminated water (Control 2) were carried out.

3.3.2 Reagents used

a) Acetone ($(CH_3)_2CO$) (99.5% v/v) (Merck)
b) Sodium carbonate (Na_2CO_3) (SRL)
c) Cyclohexane (C_6H_{12}) (SRL)
d) Methanol (CH_3OH) (99.8 % v/v) (SRL)
e) Double distilled water

All chemicals used were of analytical grade.

3.3.3 Methodology

3.3.3.1 Estimation of Chlorophyll a, Chlorophyll b and Total Chlorophyll

Chlorophyll a, Chlorophyll b and total Chlorophyll was estimated by spectrophotometric analysis as mentioned by Arnon., 1949. For this, the leaves were first washed three times with double distilled water, dried and kept in a desiccator. The leaves were then cut into small pieces leaving away the midribs. Approximately, 1 g of freshly cut sample was then taken in a mortar and grinded by a pestle with 25 ml of 80% acetone (v/v) (with a pinch of Na_2CO_3 added). The extract was then centrifuged at 1000 rpm in a Laboratory centrifuge (REMI) and the supernatant was collected. The supernatant was then estimated spectrophotometrically at 645 nm and 663 nm using Orion Aquamate 8000 UV-VIS spectrophotometer (Thermo Scientific). The chlorophyll contents were expressed in terms of mg chlorophyll present g^{-1} fresh weight.

Formulae:

Chlorophyll a (mg/ g of leaf) = $(12.7*A_{663} - 2.69* A_{645})* V/ (1000*W)$

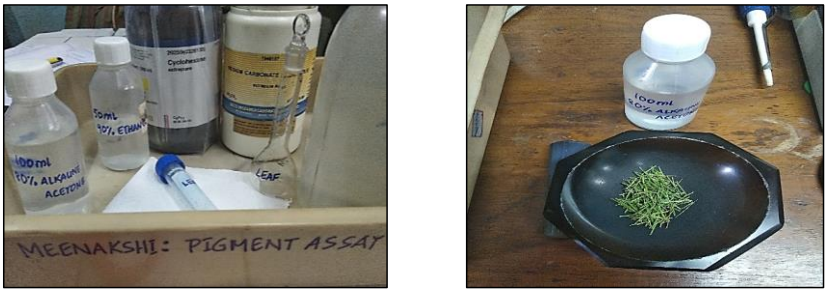
Chlorophyll b (mg/ g of leaf) = $(22.9* A_{645} – 4.68* A_{663})* V/ (1000*W)$

Total Chlorophyll (mg/ g of leaf) = $(20.2* A_{645} + 8.02* A_{663})* V/ (1000*W)$

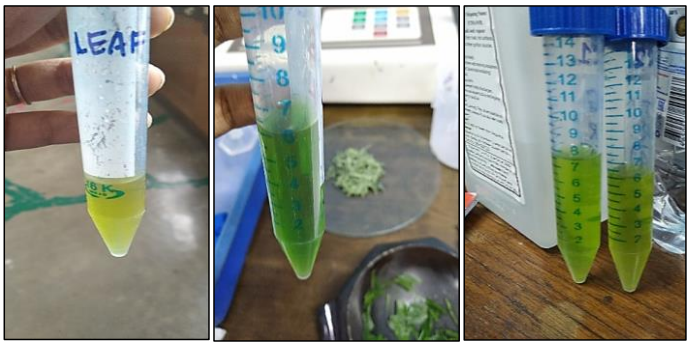
Where, V= final volume of Chlorophyll extracted

W= weight of leaf taken

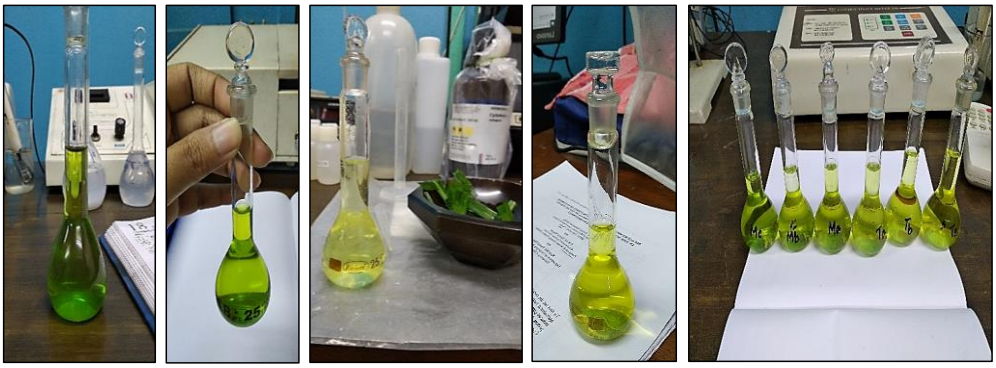
A= absorbance at specific wavelength



(a) (b)



(c)(i) (c)(ii) (c)(iii)



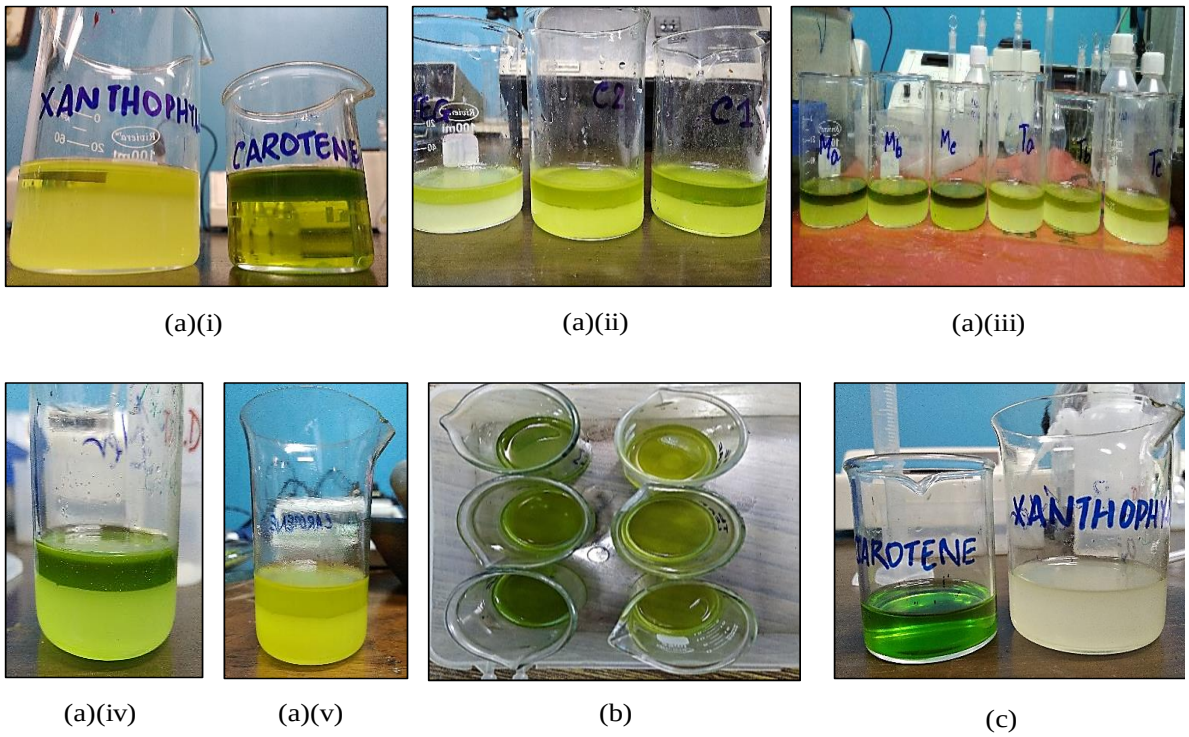
(d)(i) (d)(ii) (d)(iii) (d)(iv) (d)(v)

Fig 3.3: Estimation of Chlorophyll (a) Reagents used (b) Grinding in mortar and pestle (c) Supernatant after centrifugation (d) Chlorophyll sample prepared from different fields

3.3.3.2 Estimation of Carotene and Xanthophyll

Carotene and Xanthophyll contents were estimated according to the method of Davies, 1965. For this, the leaves were first washed three times with double distilled water, dried and kept in a desiccator. The leaves were then cut into small pieces leaving away the midribs. Approximately, 1 g of freshly cut sample was then taken in a mortar and grinded by a pestle with 20 ml of 80% acetone (v/v) (with a pinch of Na_2CO_3 added). 20 ml of cyclohexane was mixed with the pigmented alkaline acetone solution in a separating funnel. The hexane layer was washed with 20 ml of water. Xanthophyll was removed from the upper hexane layer containing carotene by

repeated extraction with 20 ml of 90% methanol (v/v). Carotene and xanthophyll contents were measured by the values of absorbance at 424 nm and 450 nm respectively using Orion Aquamate 8000 UV-VIS spectrophotometer (Thermo Scientific) and data were expressed in terms of optical density g^{-1} fresh weight.



(a)(i) (a)(ii) (a)(iii)

(a)(iv) (a)(v) (b) (c)

Fig 3.4: Estimation of Carotene and Xanthophyll (a) sequential extraction of Carotene and Xanthophyll (b) Carotene extracted from different fields (c) Carotene and Xanthophyll separated

3.4 Results and discussion

3.4.1 Field: Madhusudankathi (Gaighata)

3.4.1.1 Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As

Table 3.1: Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Madhusudankathi

Lot No.	As concentration in leaf (µg/kg)	O.D at 663 nm	O.D at 645 nm	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
1	1567	0.27	0.12	0.3208	0.1533	0.4741
2	1285	0.282	0.124	0.3218	0.1505	0.4722
3	13694	0.112	0.042	0.1627	0.0544	0.2171
4	3762	0.24	0.09	0.2909	0.0972	0.388
5	7167	0.201	0.1	0.2675	0.158	0.424

The Chlorophyll a, Chlorophyll b and total Chlorophyll content (mg/g of leaf) of leaves of paddy plant varying with the As concentration of leaves of 5 lots of samples from the field of Madhusudankathi, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.2.

Table 3.2: Phase wise variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Madhusudankathi

Interval of days	Phase	As concentration in leaf (µg/kg)	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
10-20 days	Vegetative Phase	1426	0.3213	0.1519	0.4731
45-55 days	Reproductive Phase	13694	0.1627	0.0544	0.2171
80-90 days	Ripening Phase	5464	0.2792	0.1276	0.406

From Table 3.2, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1426 µg/kg , 13694 µg/kg and 5464 µg/kg in leaf in the three phases. But, the average Chlorophyll content shows the least value during Reproductive phase with 0.1627 mg/g of leaf, 0.0544 mg/g of leaf and 0.2171 mg/g of leaf of Chlorophyll a, b and total Chlorophyll respectively. The

variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.5, 3.6 and Fig 3.7 respectively.

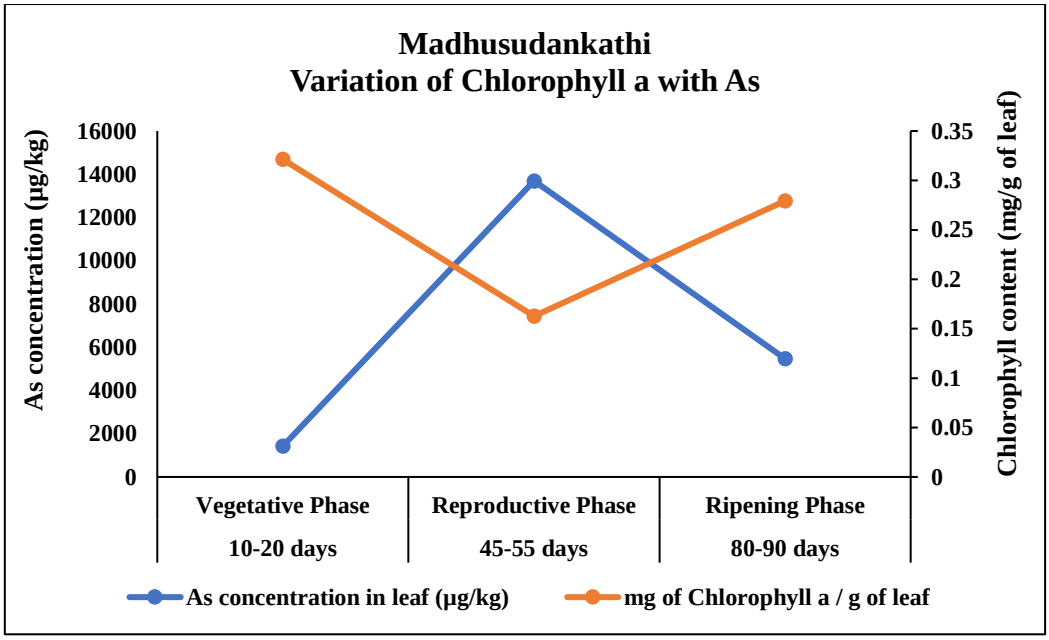


Fig 3.5: Phase wise variation of Chlorophyll a with As accumulation in leaves in Madhusudankathi

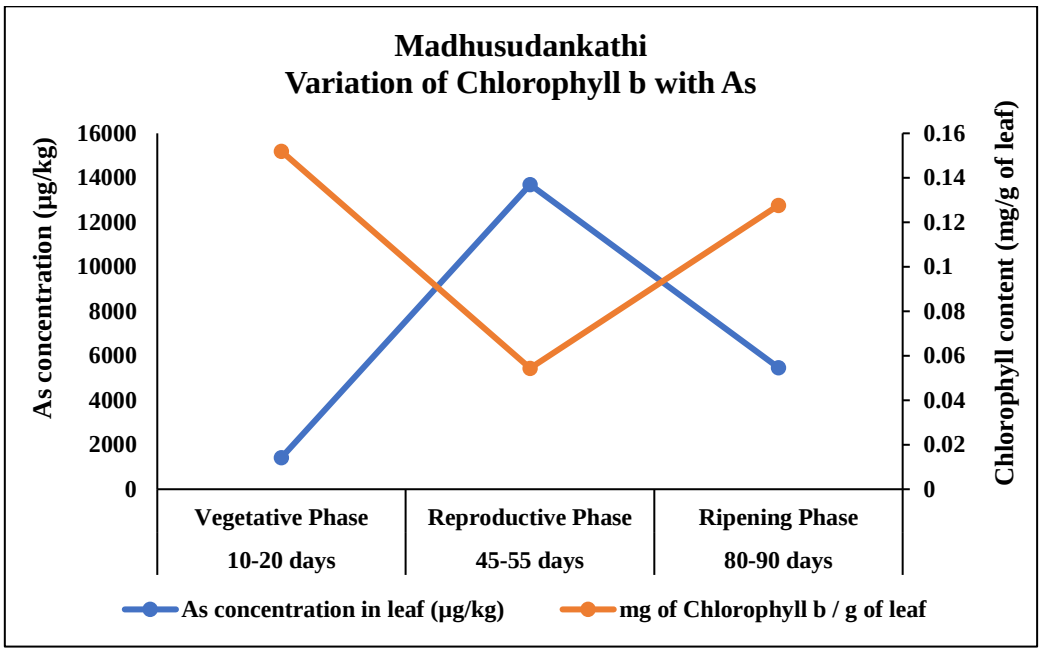


Fig 3.6: Phase wise variation of Chlorophyll b with As accumulation in leaves in Madhusudankathi

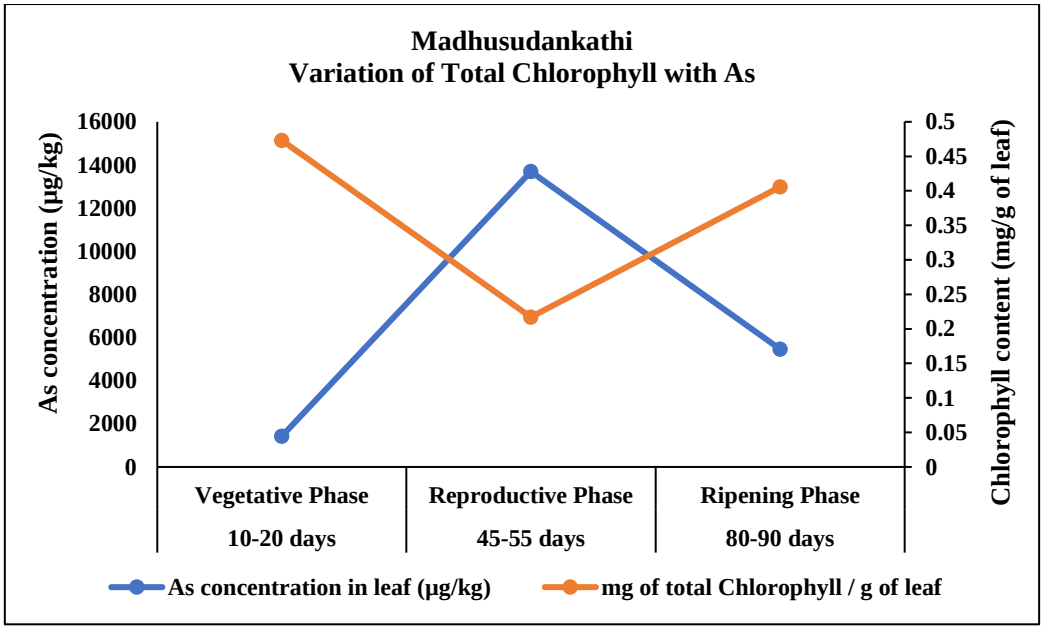


Fig 3.7: Phase wise variation of total Chlorophyll with As accumulation in leaves in Madhusudankathi

Therefore, it is observed that in case of Chlorophyll a, Chlorophyll b and total Chlorophyll, the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Madhusudankathi. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.3, 3.4 and 3.5 shows the correlation between Chlorophyll a, b and total Chlorophyll with the As accumulated in the leaves respectively.

Table 3.3: Correlation between Chlorophyll a (mg/g of leaf) and As accumulated (µg/kg) in Madhusudankathi

	As concentration in leaf (µg/kg)	**mg of Chlorophyll a / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll a / g of leaf	-0.99757	1

Table 3.4: Correlation between Chlorophyll b (mg/g of leaf) and As accumulated (µg/kg) in Madhusudankathi

	As concentration in leaf (µg/kg)	**mg of Chlorophyll b / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll b / g of leaf	-0.99621	1

Table 3.5: Correlation between total Chlorophyll (mg/g of leaf) and As accumulated (µg/kg) in Madhusudankathi

	As concentration in leaf (µg/kg)	**mg of total Chlorophyll / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of total Chlorophyll / g of leaf	-0.99731	1

It is statistically observed from Table 3.3, 3.4 and 3.5, that a correlation of -0.99757, -0.99621 and -0.99731 exists between Chlorophyll a, b and total Chlorophyll (mg/g of leaf) and As accumulated in the leaves (µg/kg) of paddy in Madhusudankathi during post-monsoon cultivation respectively. In all the 3 cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Chlorophyll content decreases.

3.4.1.2 Variation of Carotene and Xanthophyll with As accumulation

Table 3.6: Variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Madhusudankathi

Lot No.	**As concentration in leaf (µg/kg)**	**O.D at 424 nm**	**Carotene (O.D/g)**	**O.D at 450 nm**	**Xanthophyll (O.D/g)**
1	1567	1.022	4.87	0.3318	1.65
2	1285	0.985	4.62	0.3	1.5
3	13694	0.268	1.22	0.016	0.08
4	3762	0.778	3.65	0.245	1.11
5	7167	0.823	3.92	0.2604	1.24

The Carotene and Xanthophyll content (O.D/g) of leaves of paddy plant varying with the As concentration of leaves of 5 lots of samples from the field of Madhusudankathi, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.7.

Table 3.7: Phase wise variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Madhusudankathi

Interval	Phase	As concentration in leaf (µg/kg)	Carotene (O.D/g)	Xanthophyll (O.D/g)
10-20 days	Vegetative Phase	1426	4.75	1.58
45-55 days	Reproductive Phase	13694	1.22	0.08
80-90 days	Ripening Phase	5464	3.79	1.18

From Table 3.7, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1426 µg/kg , 13694 µg/kg and 5464 µg/kg in leaf in the three phases. But, the average Carotene and Xanthophyll content shows the least value of 1.22 (O.D/g) and 0.08 (O.D/g) during Reproductive phase respectively. The variation of Carotene and Xanthophyll content with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.8 and 3.9 respectively.

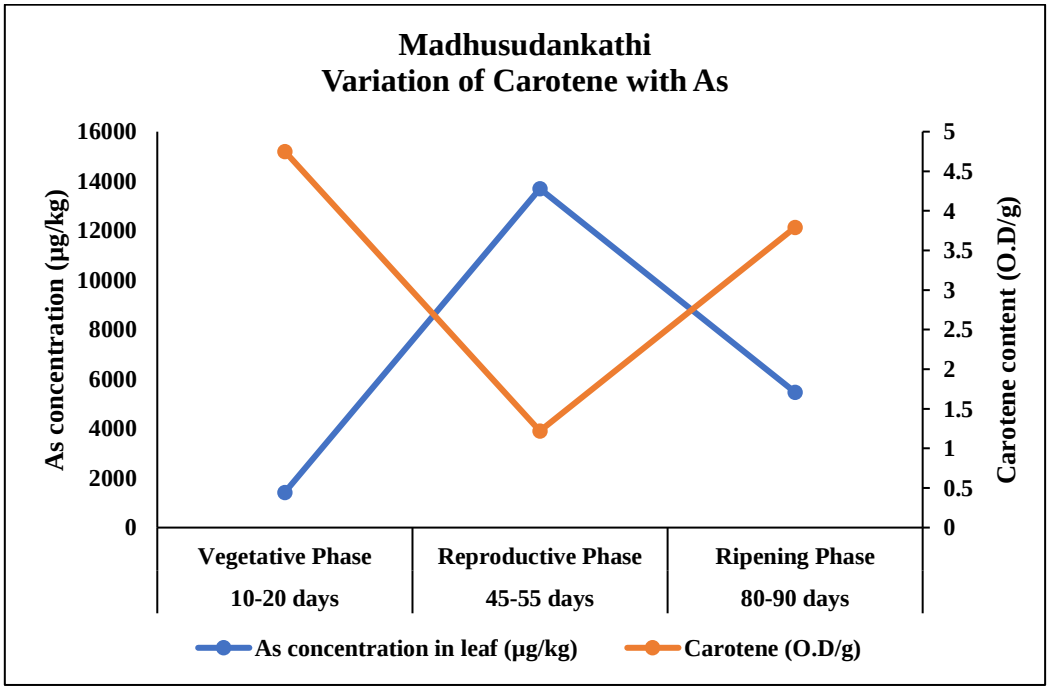


Fig 3.8: Phase wise variation of Carotene with As accumulation in leaves in Madhusudankathi

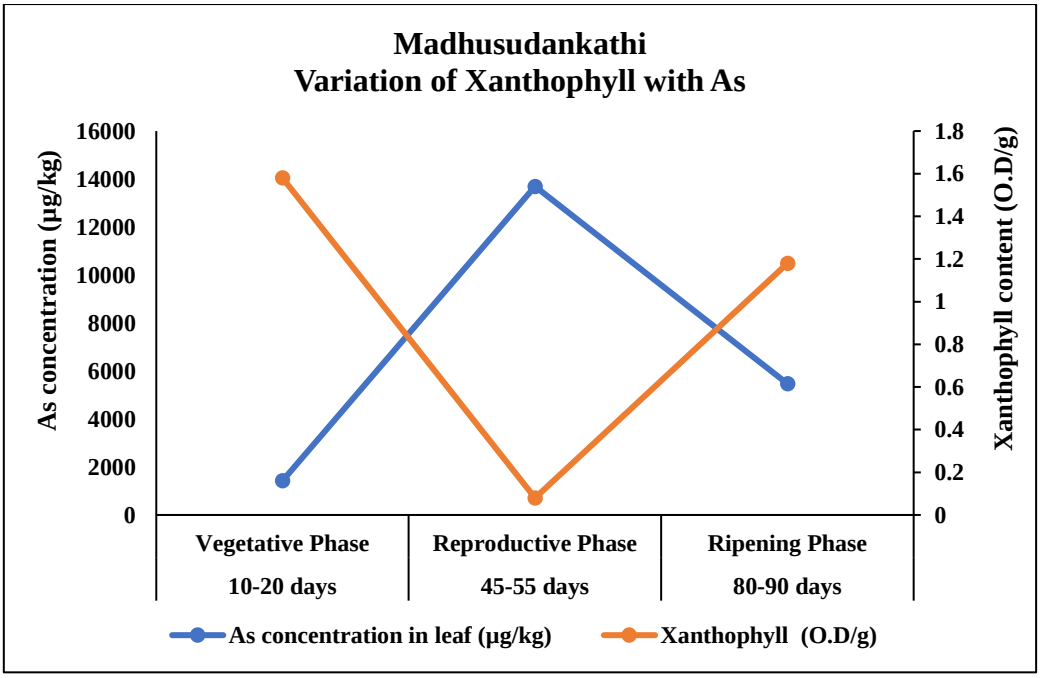


Fig 3.9: Phase wise variation of Xanthophyll with As accumulation in leaves in Madhusudankathi

Therefore, it is observed that in case of Carotene and Xanthophyll the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Madhusudankathi. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.8 and 3.9 shows the correlation between Carotene and Xanthophyll with the As accumulated in the leaves respectively.

Table 3.8: Correlation between Carotene (O.D/g) and As accumulated (µg/kg) in Madhusudankathi

	As concentration in leaf (µg/kg)	**Carotene (O.D/g)**
As concentration in leaf (µg/kg)	1	
Carotene (O.D/g)	-0.99804	1

Table 3.9: Correlation between Xanthophyll (O.D/g) and As accumulated (µg/kg) in Madhusudankathi

	As concentration in leaf (µg/kg)	**Xanthophyll (O.D/g)**
As concentration in leaf (µg/kg)	1	
Xanthophyll (O.D/g)	-0.99766	1

It is statistically observed from Table 3.8 and 3.9, that a correlation of -0.99804 and -0.99766 exists between Carotene and Xanthophyll (O.D/g) and As accumulated in the leaves (µg/kg)

of paddy in Madhusudankathi during post-monsoon cultivation respectively. In both the cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Carotene and Xanthophyll content decreases.

3.4.2 Field: Teghoria (Gaighata)

3.4.2.1 Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As

Table 3.10: Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Teghoria

Lot No.	As concentration in leaf (µg/kg)	O.D at 663 nm	O.D at 645 nm	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
1	3405	0.14	0.09	0.1535	0.1405	0.294
2	3544	0.153	0.073	0.1546	0.0903	0.2533
3	12359	0.091	0.072	0.0807	0.1025	0.1832
4	12564	0.11	0.065	0.1242	0.098	0.223
5	6525	0.12	0.08	0.1362	0.1322	0.268

The Chlorophyll a, Chlorophyll b and total Chlorophyll content (mg/g of leaf) of leaves of paddy plant varying with the As concentration of leaves of 5 lots of samples from the field of Teghoria, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.11.

Table 3.11: Phase wise variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Teghoria

Interval	Phase	As concentration in leaf (µg/kg)	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
10-20 days	Vegetative Phase	3475	0.1540	0.1154	0.2737
55-65 days	Reproductive Phase	12359	0.0807	0.1025	0.1832
110-120 days	Ripening Phase	9545	0.1302	0.1151	0.2455

From Table 3.11, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 3475 µg/kg , 12359 µg/kg and 9545 µg/kg in leaf in the three phases. But, the average Chlorophyll content shows the least value during Reproductive phase with 0.0807 mg/g of leaf, 0.1025 mg/g

of leaf and 0.1832 mg/g of leaf of Chlorophyll a, b and total Chlorophyll respectively. The variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.10, 3.11 and Fig 3.12 respectively.

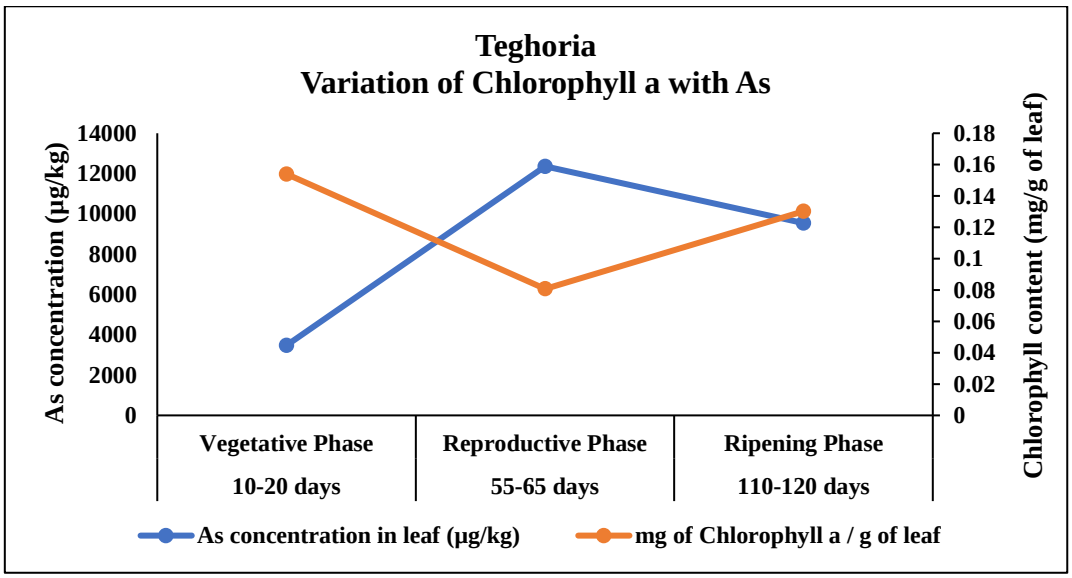


Fig 3.10: Phase wise variation of Chlorophyll a with As accumulation in leaves in Teghoria

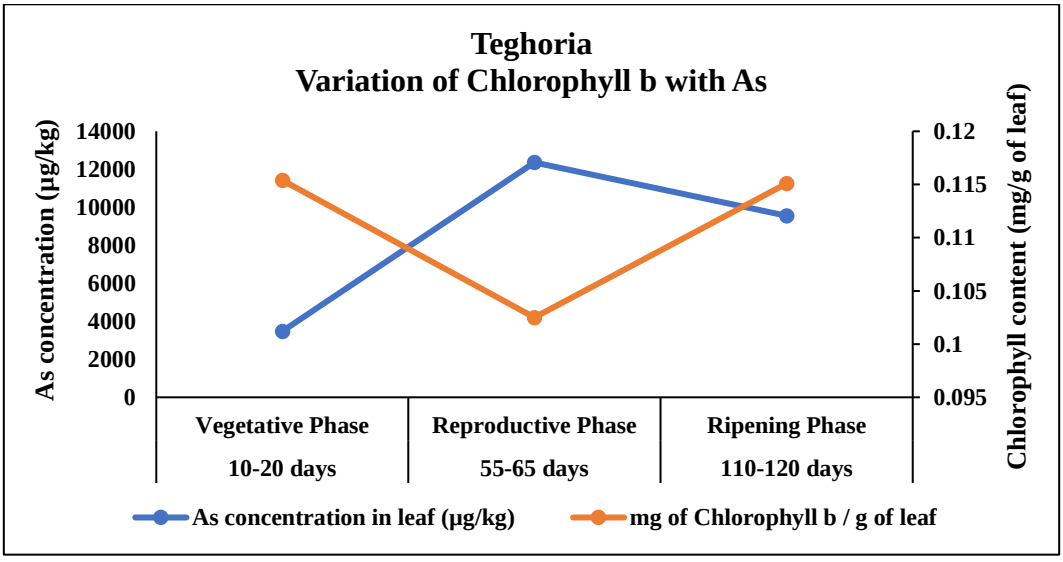


Fig 3.11: Phase wise variation of Chlorophyll b with As accumulation in leaves in Teghoria

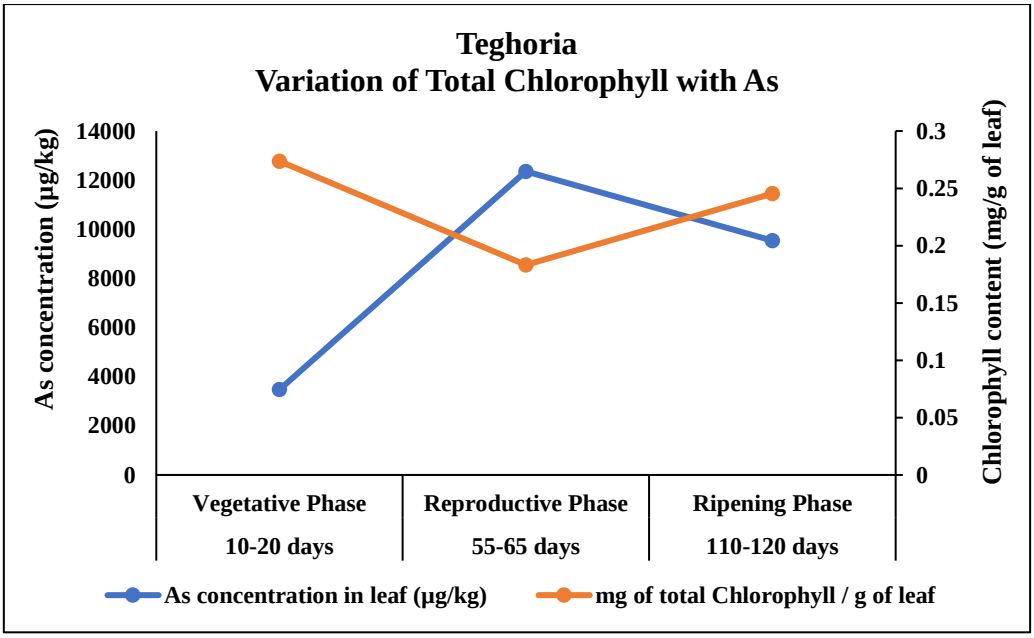


Fig 3.12: Phase wise variation of total Chlorophyll with As accumulation in leaves in Teghoria

Therefore, it is observed that in case of Chlorophyll a, Chlorophyll b and total Chlorophyll, the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Teghoria. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.12, 3.13 and 3.14 shows the correlation between Chlorophyll a, b and total Chlorophyll with the As accumulated in the leaves respectively.

Table 3.12: Correlation between Chlorophyll a (mg/g of leaf) and As accumulated (µg/kg) in Teghoria

	As concentration in leaf (µg/kg)	**mg of Chlorophyll a / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll a / g of leaf	-0.91782	1

Table 3.13: Correlation between Chlorophyll b (mg/g of leaf) and As accumulated (µg/kg) in Teghoria

	As concentration in leaf (µg/kg)	**mg of Chlorophyll b / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll b / g of leaf	-0.75722	1

Table 3.14: Correlation between total Chlorophyll (mg/g of leaf) and As accumulated (µg/kg) in Teghoria

	As concentration in leaf (µg/kg)	**mg of total Chlorophyll / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of total Chlorophyll / g of leaf	-0.91197	1

It is statistically observed from Table 3.12, 3.13 and 3.14, that a correlation of -0.91782, -0.75722 and -0.91197 exists between Chlorophyll a, b and total Chlorophyll (mg/g of leaf) and As accumulated in the leaves (µg/kg) of paddy in Teghoria during post-monsoon cultivation respectively. In the 1st and 3rd cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Chlorophyll content decreases. In the 2nd case however, a negative moderate correlation exists between As accumulated in the leaf and Chlorophyll b in Teghoria.

3.4.2.2 Variation of Carotene and Xanthophyll with As accumulation

Table 3.15: Variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Teghoria

Lot No.	**As concentration in leaf (µg/kg)**	**O.D at 424 nm**	**Carotene (O.D/g)**	**O.D at 450 nm**	**Xanthophyll (O.D/g)**
1	3405	0.57	2.83	0.3139	1.42
2	3544	0.543	2.58	0.258	1.23
3	12359	0.276	1.15	0.21	1.05
4	12564	0.4224	1.92	0.252	1.18
5	6525	0.198	0.98	0.179	0.85

The Carotene and Xanthophyll content (O.D/g) of leaves of paddy plant varying with the As concentration of leaves of 5 lots of samples from the field of Teghoria, Gaighata were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.16.

Table 3.16: Phase wise variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Teghoria

Interval	Phase	As concentration in leaf (µg/kg)	Carotene (O.D/g)	Xanthophyll (O.D/g)
10-20 days	Vegetative Phase	3475	2.71	1.33
55-65 days	Reproductive Phase	12359	1.15	1.05
110-120 days	Ripening Phase	9545	1.45	1.02

From Table 3.16, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 3475 µg/kg , 12359 µg/kg and 9545 µg/kg in leaf in the three phases. But, the average Carotene and Xanthophyll content shows the least value of 1.15 (O.D/g) and 1.05 (O.D/g) during Reproductive phase respectively. The variation of Carotene and Xanthophyll content with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.13 and 3.14 respectively.

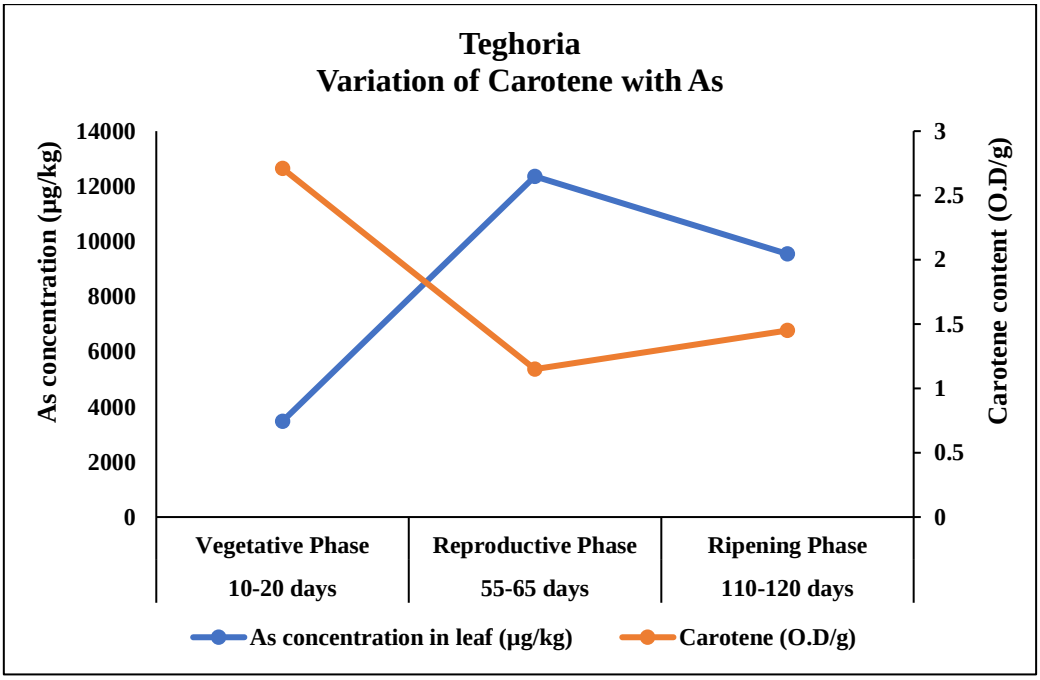


Fig 3.13: Phase wise variation of Carotene with As accumulation in leaves in Teghoria

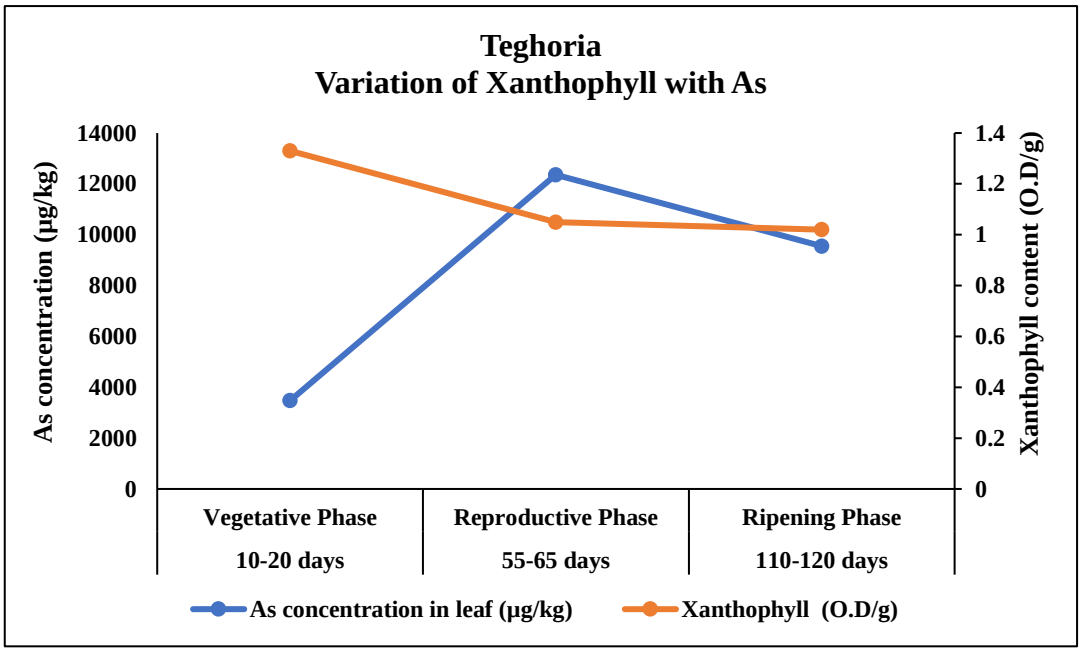


Fig 3.14: Phase wise variation of Xanthophyll with As accumulation in leaves in Teghoria

Therefore, it is observed that in case of Carotene and Xanthophyll the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Teghoria. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.17 and 3.18 shows the correlation between Carotene and Xanthophyll with the As accumulated in the leaves respectively.

Table 3.17: Correlation between Carotene (O.D/g) and As accumulated (µg/kg) in Teghoria

	As concentration in leaf (µg/kg)	**Carotene (O.D/g)**
As concentration in leaf (µg/kg)	1	
Carotene (O.D/g)	-0.99119	1

Table 3.18: Correlation between Xanthophyll (O.D/g) and As accumulated (µg/kg) in Teghoria

	As concentration in leaf (µg/kg)	**Xanthophyll (O.D/g)**
As concentration in leaf (µg/kg)	1	
Xanthophyll (O.D/g)	-0.91992	1

It is statistically observed from Table 3.17 and 3.18, that a correlation of -0.99119 and -0.91992 exists between Carotene and Xanthophyll (O.D/g) and As accumulated in the leaves (µg/kg) of paddy in Teghoria during post-monsoon cultivation respectively. In both the cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Carotene and Xanthophyll content decreases.

3.4.3 Field: Pingla (Medinipur)

3.4.3.1 Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As

Table 3.19: Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Pingla

Lot No.	As concentration in leaf (µg/kg)	O.D at 663 nm	O.D at 645 nm	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
1	1347	0.177	0.081	0.5029	0.2543	0.7571
2	1886	0.27	0.12	0.3694	0.1765	0.5458
3	4078	0.25	0.092	0.288	0.0921	0.3801
4	2490	0.201	0.156	0.216	0.2665	0.4702
5	1441.5	0.31	0.15	0.3679	0.2066	0.5743
6	2032	0.301	0.1	0.4205	0.1049	0.5278

The Chlorophyll a, Chlorophyll b and total Chlorophyll content (mg/g of leaf) of leaves of paddy plant varying with the As concentration of leaves of 6 lots of samples from the field of Pingla, Medinipur were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.20.

Table 3.20: Phase wise variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Pingla

Interval	Phase	As concentration in leaf (µg/kg)	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
10-20 days	Vegetative Phase	1617	0.4362	0.2154	0.6515
55-65 days	Reproductive Phase	4078	0.288	0.0921	0.3801
110-120 days	Ripening Phase	1988	0.3348	0.1927	0.5241

From Table 3.20, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1617

µg/kg , 4078 µg/kg and 1988 µg/kg in leaf in the three phases. But, the average Chlorophyll content shows the least value during Reproductive phase with 0.288 mg/g of leaf, 0.0921 mg/g of leaf and 0.3801 mg/g of leaf of Chlorophyll a, b and total Chlorophyll respectively. The variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.15, 3.16 and Fig 3.17 respectively.

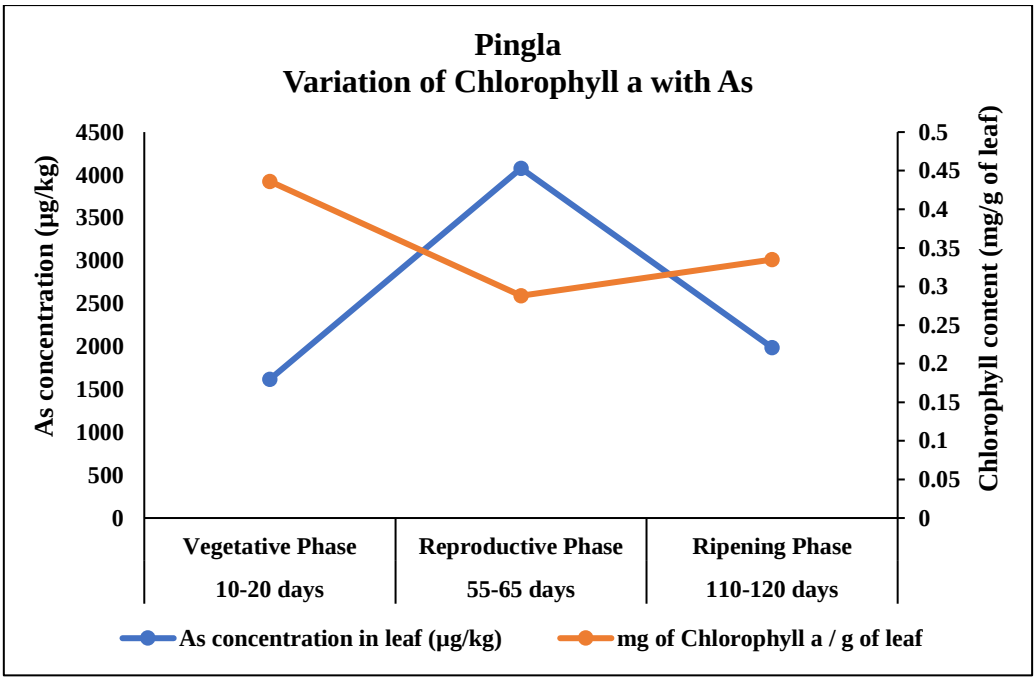


Fig 3.15: Phase wise variation of Chlorophyll a with As accumulation in leaves in Pingla

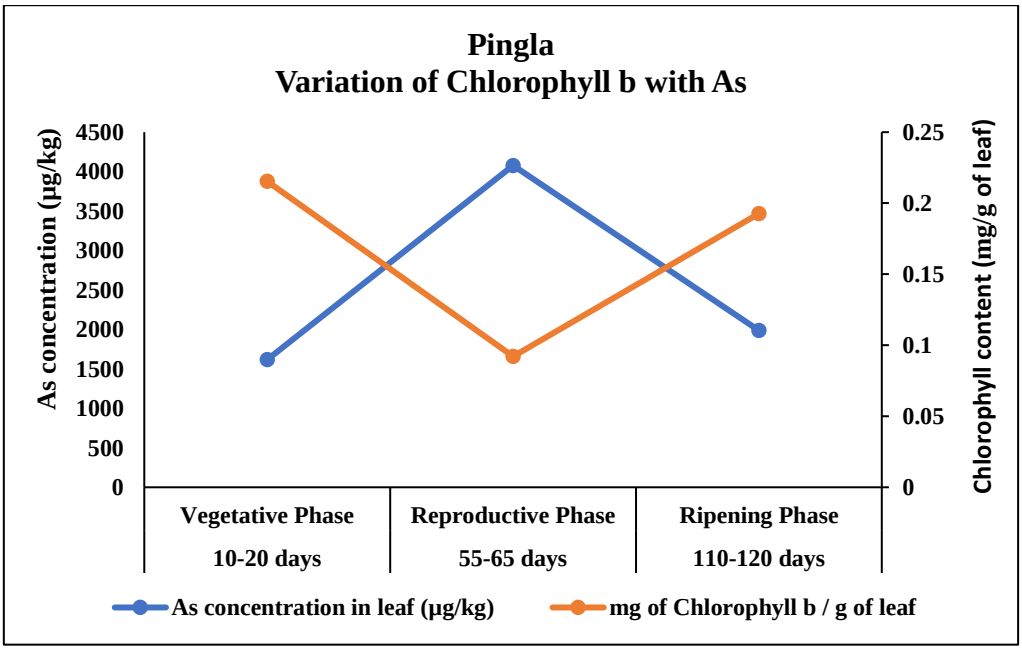


Fig 3.16: Phase wise variation of Chlorophyll b with As accumulation in leaves in Pingla

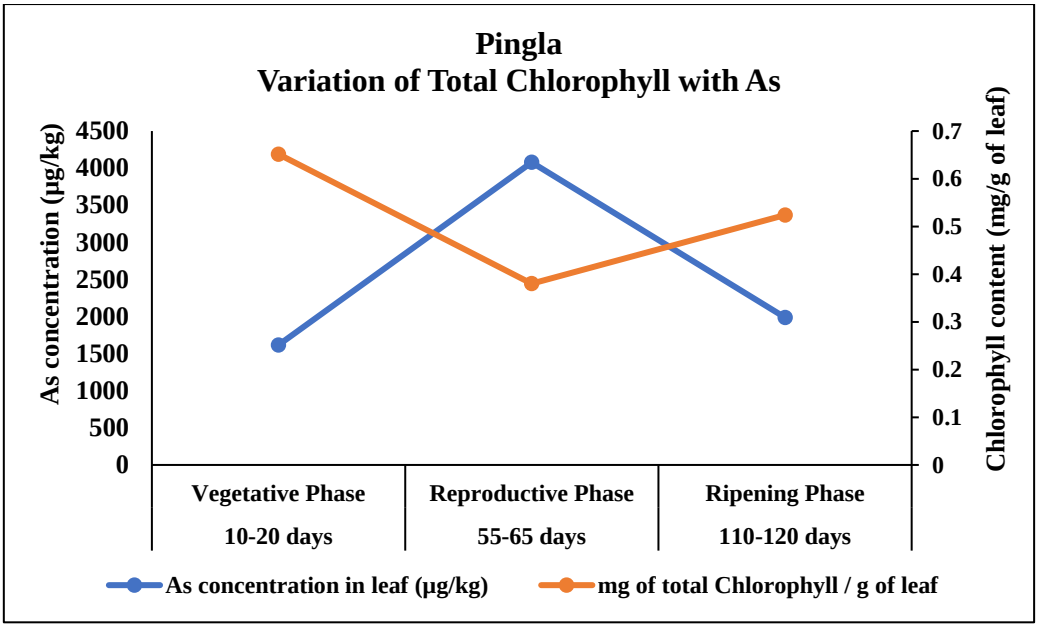


Fig 3.17: Phase wise variation of total Chlorophyll with As accumulation in leaves in Pingla

Therefore, it is observed that in case of Chlorophyll a, Chlorophyll b and total Chlorophyll, the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Pingla. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.21, 3.22 and 3.23 shows the correlation between Chlorophyll a, b and total Chlorophyll with the As accumulated in the leaves respectively.

Table 3.21: Correlation between Chlorophyll a (mg/g of leaf) and As accumulated (µg/kg) in Pingla

	As concentration in leaf (µg/kg)	**mg of Chlorophyll a / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll a / g of leaf	-0.82932	1

Table 3.22: Correlation between Chlorophyll b (mg/g of leaf) and As accumulated (µg/kg) in Pingla

	As concentration in leaf (µg/kg)	**mg of Chlorophyll b / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll b / g of leaf	-0.99944	1

Table 3.23: Correlation between total Chlorophyll (mg/g of leaf) and As accumulated (µg/kg) in Pingla

	As concentration in leaf (µg/kg)	**mg of total Chlorophyll / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of total Chlorophyll / g of leaf	-0.94005	1

It is statistically observed from Table 3.21, 3.22 and 3.23, that a correlation of -0.82932, -0.99944 and -0.94005 exists between Chlorophyll a, b and total Chlorophyll (mg/g of leaf) and As accumulated in the leaves (µg/kg) of paddy in Pingla during post-monsoon cultivation respectively. In all the 3 cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Chlorophyll content decreases.

3.4.3.2 Variation of Carotene and Xanthophyll with As accumulation

Table 3.24: Variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Pingla

Lot No.	**As concentration in leaf (µg/kg)**	**O.D at 424 nm**	**Carotene (O.D/g)**	**O.D at 450 nm**	**Xanthophyll (O.D/g)**
1	1347	1.236	6.146	0.4477	2.025
2	1886	1.116	5.55	0.4217	1.98
3	4078	0.900	3.88	0.2577	1.12
4	2490	0.967	4.32	0.2997	1.42
5	1441.5	1.194	5.972	0.444	2.01
6	2032	1.075	5.12	0.3930	1.76

The Carotene and Xanthophyll content (O.D/g) of leaves of paddy plant varying with the As concentration of leaves of 6 lots of samples from the field of Pingla, Medinipur were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.25.

Table 3.25: Phase wise variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Pingla

Interval	Phase	As concentration in leaf (µg/kg)	Carotene (O.D/g)	Xanthophyll (O.D/g)
10-20 days	Vegetative Phase	1617	5.85	2.01
55-65 days	Reproductive Phase	4078	3.88	1.12
110-120 days	Ripening Phase	1988	5.14	1.73

From Table 3.25, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1617 µg/kg , 4078 µg/kg and 1988 µg/kg in leaf in the three phases. But, the average Carotene and Xanthophyll content shows the least value of 3.88 (O.D/g) and 1.12 (O.D/g) during Reproductive phase respectively. The variation of Carotene and Xanthophyll content with As accumulation in leaves during the 3 phases of monsoon cultivation have been represented in Fig 3.18 and 3.19 respectively.

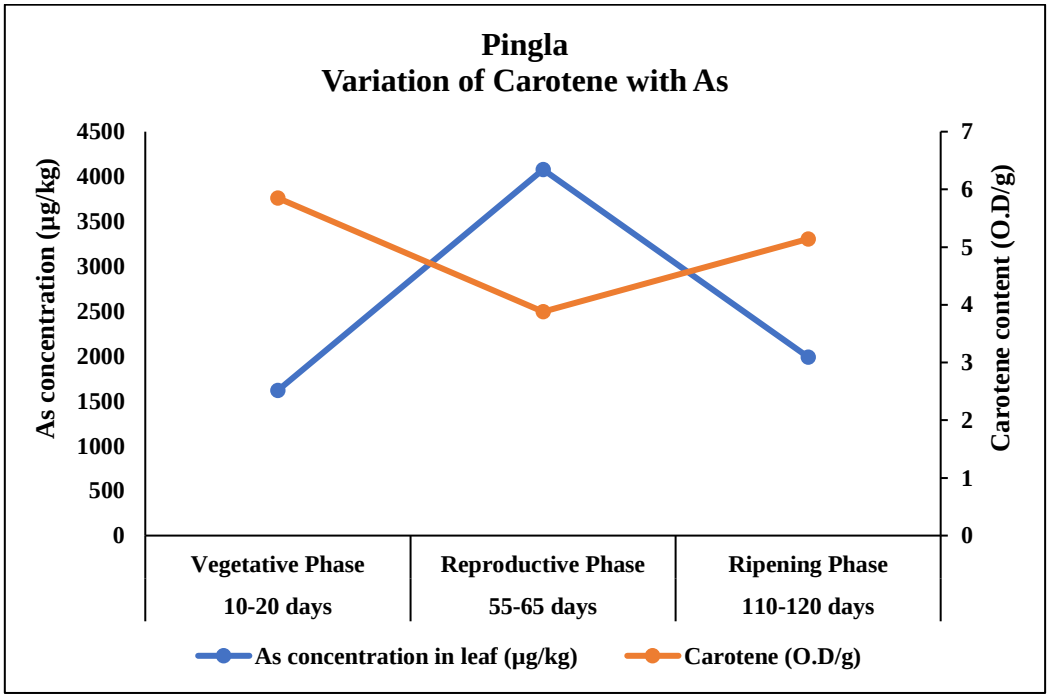


Fig 3.18: Phase wise variation of Carotene with As accumulation in leaves in Pingla

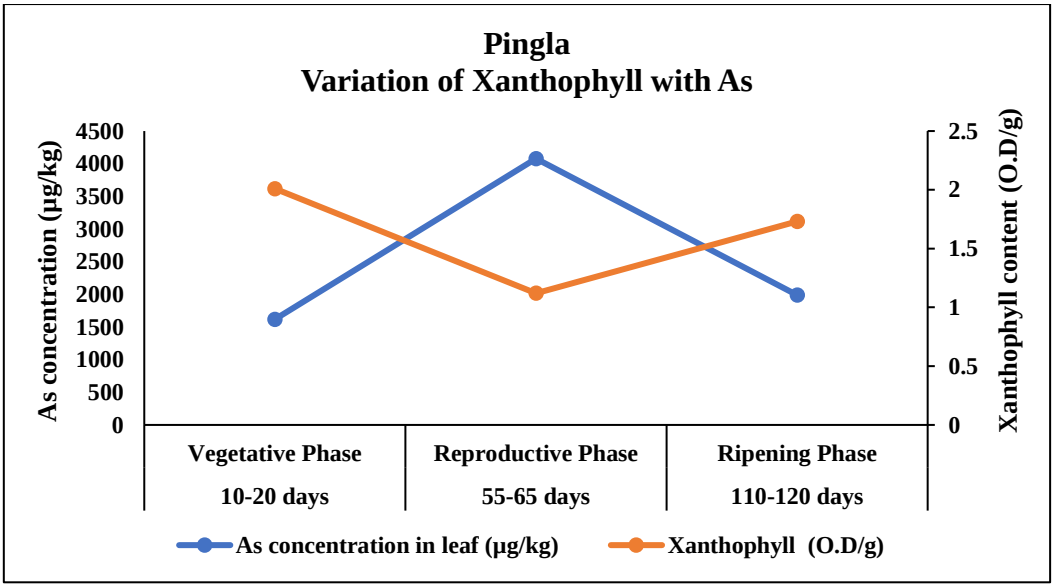


Fig 3.19: Phase wise variation of Xanthophyll with As accumulation in leaves in Pingla

Therefore, it is observed that in case of Carotene and Xanthophyll the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the field of Pingla. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.26 and 3.27 shows the correlation between Carotene and Xanthophyll with the As accumulated in the leaves respectively.

Table 3.26: Correlation between Carotene (O.D/g) and As accumulated (µg/kg) in Pingla

	As concentration in leaf (µg/kg)	**Carotene (O.D/g)**
As concentration in leaf (µg/kg)	1	
Carotene (O.D/g)	-0.97512	1

Table 3.27: Correlation between Xanthophyll (O.D/g) and As accumulated (µg/kg) in Pingla

	As concentration in leaf (µg/kg)	**Xanthophyll (O.D/g)**
As concentration in leaf (µg/kg)	1	
Xanthophyll (O.D/g)	-0.98517	1

It is statistically observed from Table 3.26 and 3.27, that a correlation of -0.97512 and -0.98517 exists between Carotene and Xanthophyll (O.D/g) and As accumulated in the leaves (µg/kg) of paddy in Pingla during post-monsoon cultivation respectively. In both the cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Carotene and Xanthophyll content decreases.

3.4.4 Pot study (Control 1 and Control 2)

3.4.4.1 Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As

Table 3.28: Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Control 1

Lot No.	As concentration in leaf (µg/kg)	O.D at 663 nm	O.D at 645 nm	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
1	1558	0.15	0.06	0.435	0.1676	0.6025
2	761	0.21	0.1	0.5974	0.3242	0.917
3	1684	0.17	0.05	0.5006	0.086	0.5869
4	1136	0.19	0.06	0.5629	0.1212	0.6839

Table 3.29: Variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Control 2

Lot No.	As concentration in leaf (µg/kg)	O.D at 663 nm	O.D at 645 nm	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
1	1639	0.17	0.055	0.5007	0.1155	0.6161
2	1230	0.165	0.09	0.4557	0.3165	0.7714
3	6840	0.09	0.04	0.253	0.1209	0.3738
4	1312	0.149	0.07	0.4234	0.225	0.6483

The Chlorophyll a, Chlorophyll b and total Chlorophyll content (mg/g of leaf) of leaves of paddy plant varying with the As concentration of leaves of 4 lots of samples from the Control pots 1 and 2 were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.30 and 3.31 respectively.

Table 3.30: Phase wise variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Control 1

Interval	Phase	As concentration in leaf (µg/kg)	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
10-20 days	Vegetative Phase	1156	0.5162	0.2459	0.7598
45-55 days	Reproductive Phase	1684	0.5006	0.086	0.5869
80-90 days	Ripening Phase	1136	0.5629	0.1212	0.6839

Table 3.31: Phase wise variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As in Control 2

Interval	Phase	As concentration in leaf (µg/kg)	mg of Chlorophyll a / g of leaf	mg of Chlorophyll b / g of leaf	mg of total Chlorophyll / g of leaf
10-20 days	Vegetative Phase	1435	0.4782	0.216	0.6938
45-55 days	Reproductive Phase	6840	0.253	0.1209	0.3738
80-90 days	Ripening Phase	1312	0.4234	0.225	0.6483

From Table 3.30 and 3.31, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1156 µg/kg, 1684 µg/kg and 1136 µg/kg in leaf in case of Control 1 and 1435 µg/kg, 6840 µg/kg and 1312 µg/kg in case of Control 2 in the three consecutive phases. But, the average Chlorophyll content shows the least value during Reproductive phase with 0.5006 mg/g of leaf, 0.086 mg/g of leaf and 0.5869 mg/g of leaf of Chlorophyll a, b and total Chlorophyll respectively for Control 1 and 0.253 mg/g of leaf, 0.1209 mg/g of leaf and 0.3738 mg/g of leaf of Chlorophyll a, b and total Chlorophyll respectively for Control 2. The variation of Chlorophyll a, Chlorophyll b and total Chlorophyll with As accumulation in leaves during the 3 phases of monsoon cultivation in Control 1 and 2 have been represented in Fig 3.20, 3.21, 3.22 and Fig 3.23, 3.24, 3.25 respectively.

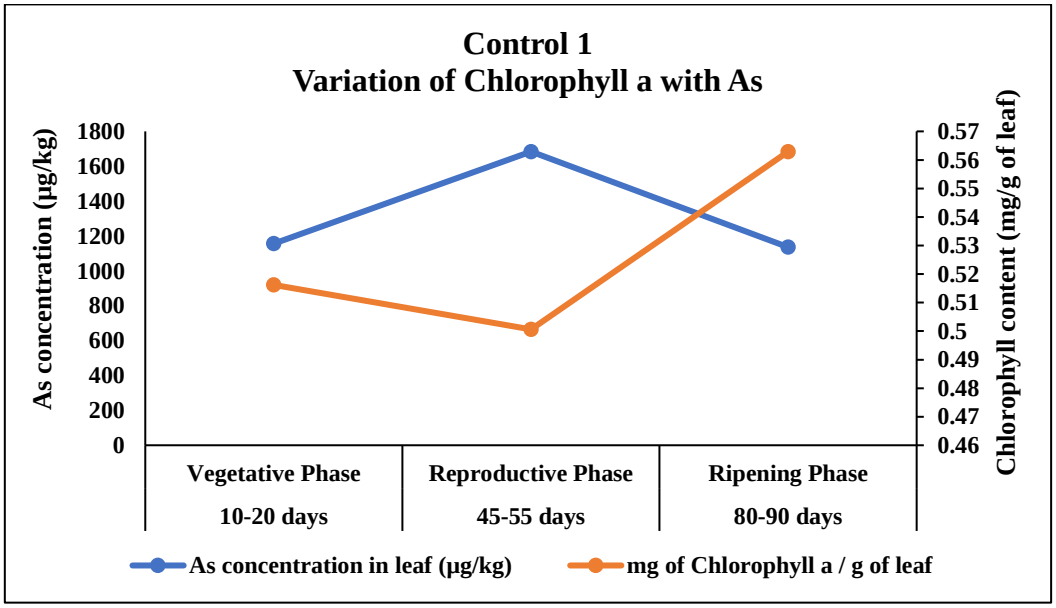


Fig 3.20: Phase wise variation of Chlorophyll a with As accumulation in leaves in Control 1

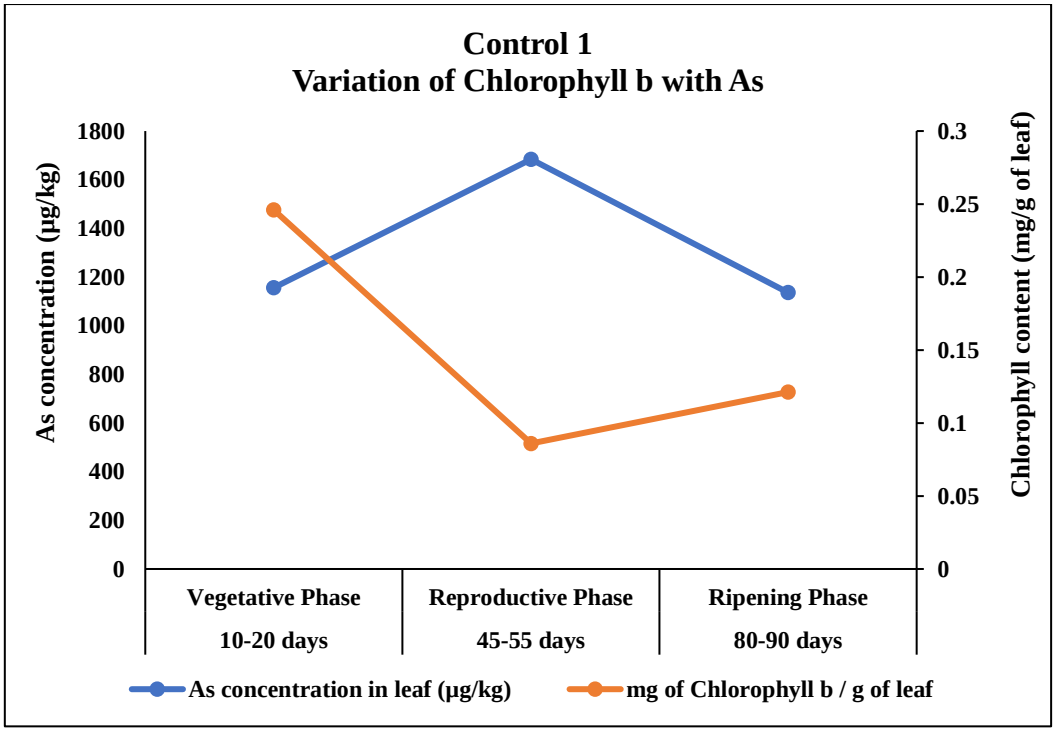


Fig 3.21: Phase wise variation of Chlorophyll b with As accumulation in leaves in Control 1

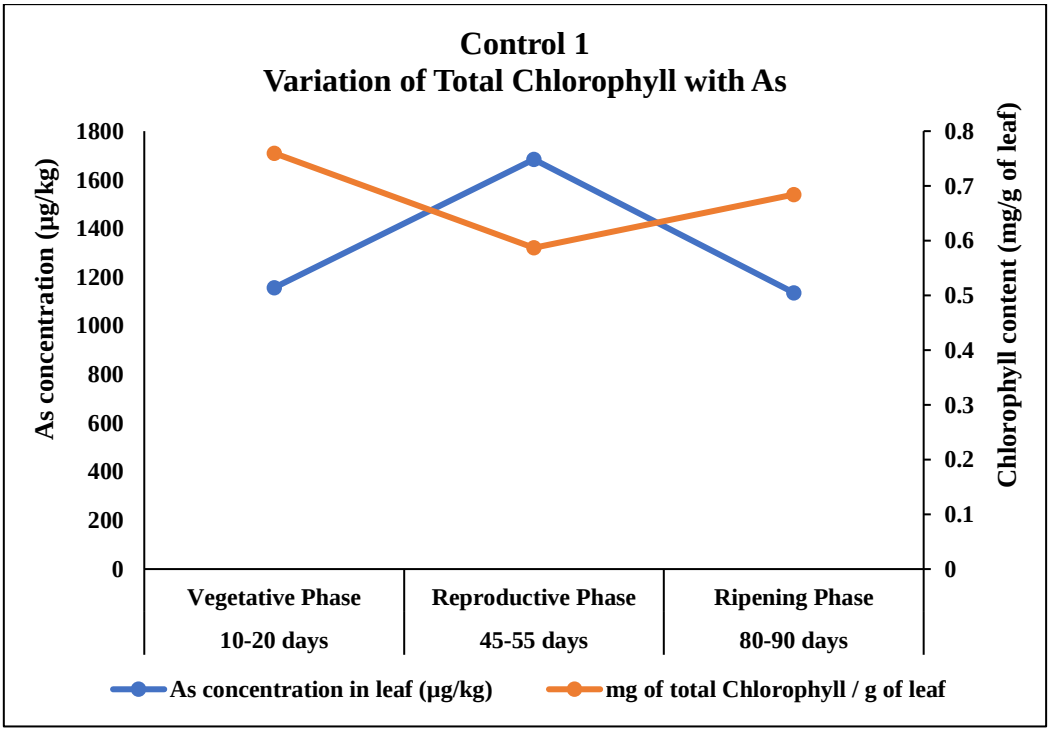


Fig 3.22: Phase wise variation of total Chlorophyll with As accumulation in leaves in Control 1

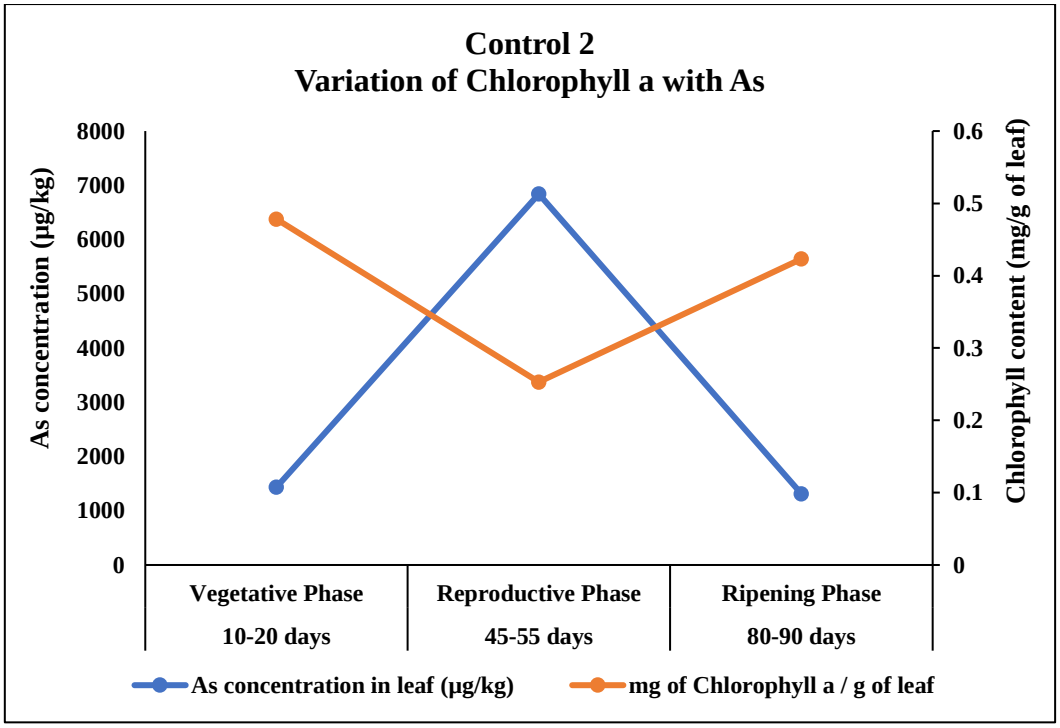


Fig 3.23: Phase wise variation of Chlorophyll a with As accumulation in leaves in Control 2

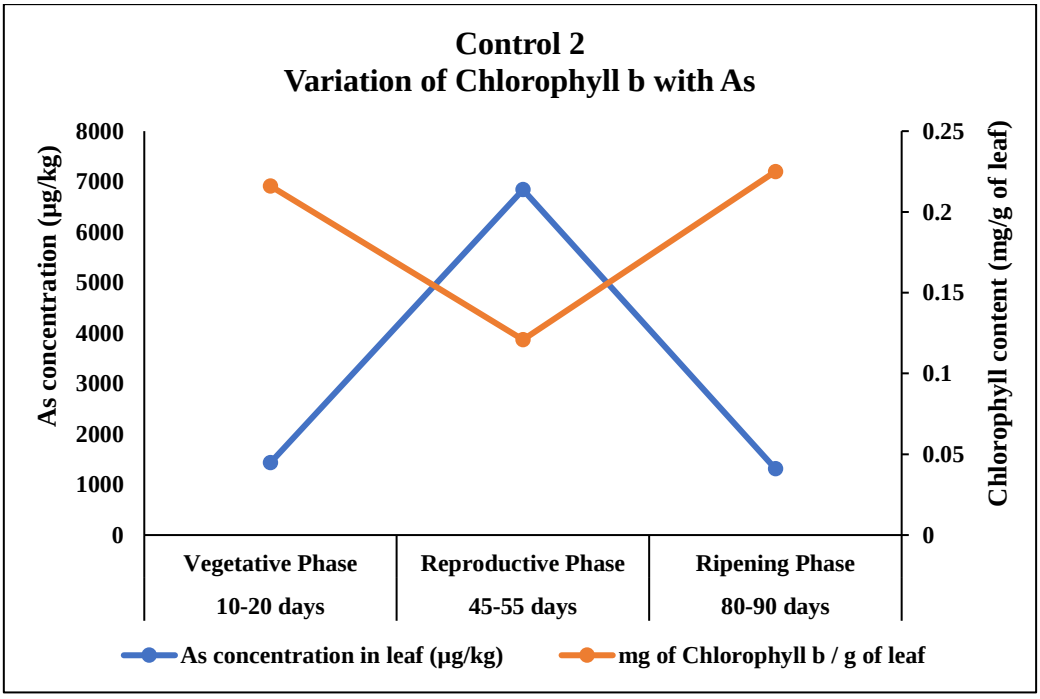


Fig 3.24: Phase wise variation of Chlorophyll b with As accumulation in leaves in Control 2

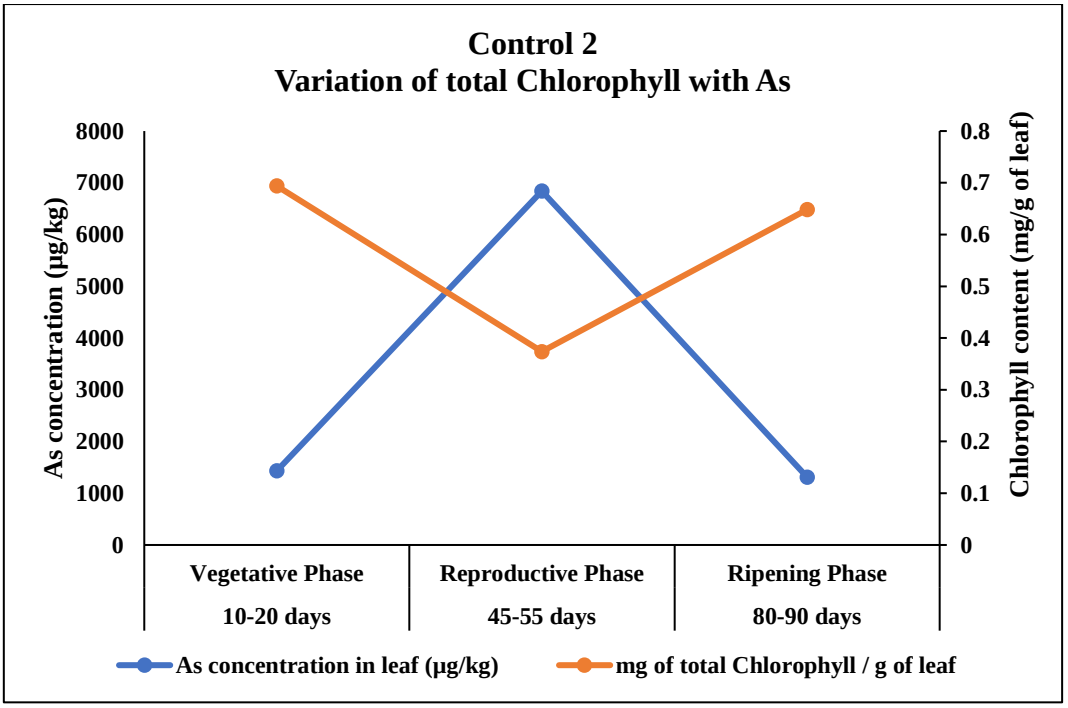


Fig 3.25: Phase wise variation of total Chlorophyll with As accumulation in leaves in Control 2

Therefore, it is observed that in case of Chlorophyll a, Chlorophyll b and total Chlorophyll, the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in case of both Control 1 and Control 2. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.32, 3.33, 3.34 and Table 3.35, 3.36, 3.37 shows the correlation between Chlorophyll a, b and total Chlorophyll with the As accumulated in the leaves of Control 1 and Control 2 respectively.

Table 3.32: Correlation between Chlorophyll a (mg/g of leaf) and As accumulated (µg/kg) in Control 1

	As concentration in leaf (µg/kg)	**mg of Chlorophyll a / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll a / g of leaf	-0.7165	1

Table 3.33:Correlation between Chlorophyll b (mg/g of leaf) and As accumulated (µg/kg) in Control 1

	As concentration in leaf (µg/kg)	**mg of Chlorophyll b / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll b / g of leaf	-0.64609	1

Table 3.34: Correlation between total Chlorophyll (mg/g of leaf) and As accumulated (µg/kg) in Control 1

	As concentration in leaf (µg/kg)	**mg of total Chlorophyll / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of total Chlorophyll / g of leaf	-0.88447	1

Table 3.35: Correlation between Chlorophyll a (mg/g of leaf) and As accumulated (µg/kg) in Control 2

	As concentration in leaf (µg/kg)	**mg of Chlorophyll a / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll a / g of leaf	-0.96767	1

Table 3.36:Correlation between Chlorophyll b (mg/g of leaf) and As accumulated (µg/kg) in Control 2

	As concentration in leaf (µg/kg)	**mg of Chlorophyll b / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of Chlorophyll b / g of leaf	-0.99828	1

Table 3.37: Correlation between total Chlorophyll (mg/g of leaf) and As accumulated (µg/kg) in Control 2

	As concentration in leaf (µg/kg)	**mg of total Chlorophyll / g of leaf**
As concentration in leaf (µg/kg)	1	
mg of total Chlorophyll / g of leaf	-0.98858	1

It is statistically observed from Table 3.32, 3.33, 3.34, 3.35, 3.36 and 3.37, that a correlation of -0.7165, -0.64609, -0.88447, 0.96767, 0.99828 and 0.99858 exists between Chlorophyll a, b and total Chlorophyll (mg/g of leaf) and As accumulated in the leaves (µg/kg) of paddy in Control 1 and Control 2 during post-monsoon cultivation respectively. In case of Control 1, a negative and moderate correlation exists while in case of Control 2, strong and negative

correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Chlorophyll content decreases.

3.4.4.2 Variation of Carotene and Xanthophyll with As accumulation

Table 3.38: Variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Control 1

Lot No.	As concentration in leaf (μg/kg)	O.D at 424 nm	Carotene (O.D/g)	O.D at 450 nm	Xanthophyll (O.D/g)
1	1558	1.308	6.23	0.574	2.73
2	761	1.523	7.21	0.791	3.28
3	1684	1.392	5.97	0.462	2.00
4	1136	1.44	6.52	0.692	3.12

Table 3.39: Variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Control 2

Lot No.	As concentration in leaf (μg/kg)	O.D at 424 nm	Carotene (O.D/g)	O.D at 450 nm	Xanthophyll (O.D/g)
1	1639	0.966	4.83	0.4186	1.99
2	1230	1.117	5.29	0.5027	2.51
3	6840	0.45	2.12	0.1206	0.5
4	1312	1.169	5.08	0.5366	2.32

The Carotene and Xanthophyll content (O.D/g) of leaves of paddy plant varying with the As concentration of leaves of 4 lots of samples from the Control pots 1 and 2, were divided into Vegetative, Reproductive and Ripening phases of monsoon cultivation depending on the day of collection of the paddy samples and their mean concentrations are represented in Table 3.40 and 3.41 respectively.

Table 3.40: Phase wise variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Control 1

Interval	Phase	As concentration in leaf (μg/kg)	Carotene (O.D/g)	Xanthophyll (O.D/g)
10-20 days	Vegetative Phase	1156	6.72	3.01
45-55 days	Reproductive Phase	1684	5.97	2.00
80-90 days	Ripening Phase	1136	6.52	3.12

Table 3.41: Phase wise variation of Carotene and Xanthophyll with As accumulated in the leaves of paddy in Control 2

Interval	Phase	As concentration in leaf (µg/kg)	Carotene (O.D/g)	Xanthophyll (O.D/g)
10-20 days	Vegetative Phase	1435	5.06	2.25
45-55 days	Reproductive Phase	6840	2.12	0.5
80-90 days	Ripening Phase	1312	5.08	2.32

From Table 3.40 and 3.41, it is definite that the As concentration is significantly higher in the Reproductive phase than the Vegetative or Ripening phases showing As concentrations of 1156 µg/kg, 1684 µg/kg and 1136 µg/kg in leaf in case of Control 1 and 1435 µg/kg, 6840 µg/kg and 1312 µg/kg in case of Control 2 in the three consecutive phases respectively. But, the average Carotene and Xanthophyll content shows the least value of 5.97 (O.D/g) and 2.00 (O.D/g) for Control 1 and 2.12 (O.D/g) and 0.5 (O.D/g) for Control 2 during Reproductive phase respectively. The variation of Carotene and Xanthophyll content with As accumulation in leaves in Control 1 and 2 during the 3 phases of monsoon cultivation have been represented in Fig 3.26, 3.27 and 3.28, 3.29 respectively.

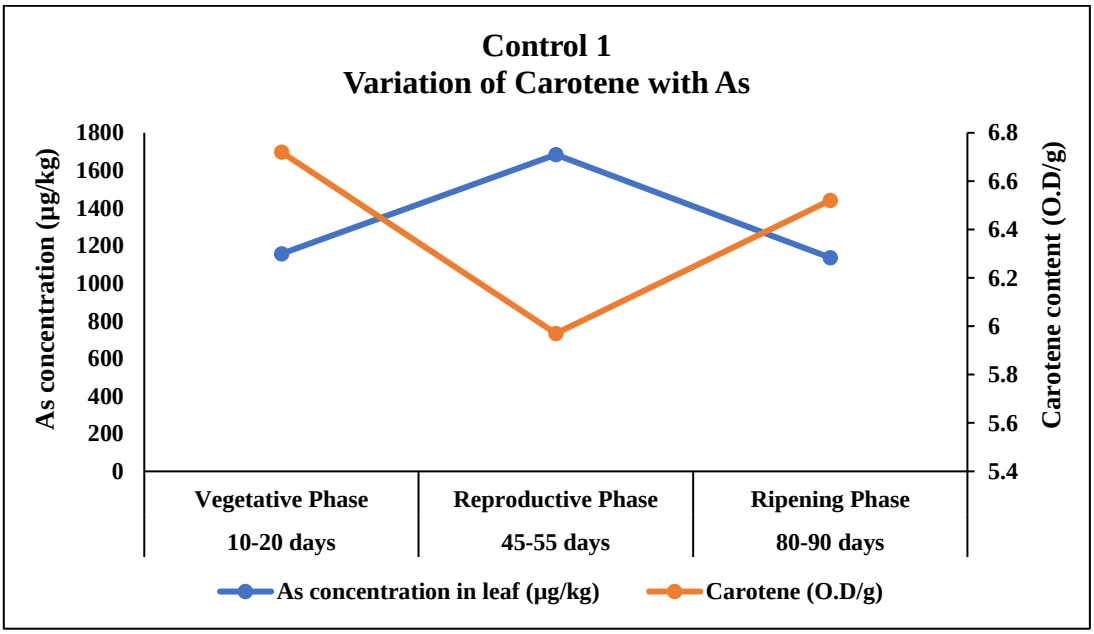


Fig 3.26: Phase wise variation of Carotene with As accumulation in leaves in Control 1

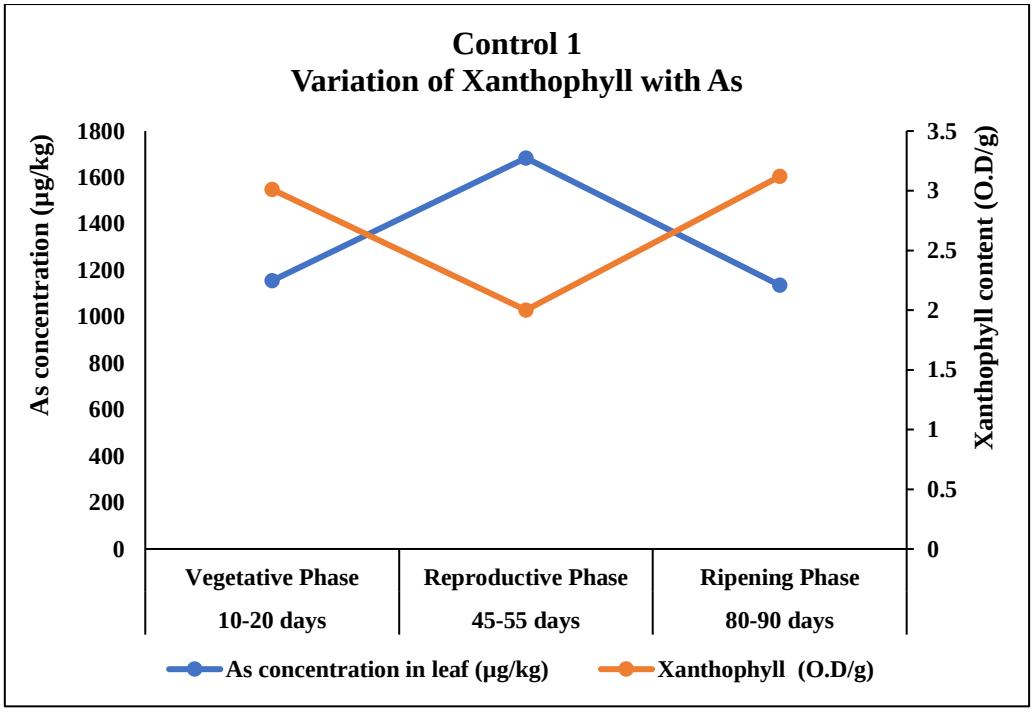


Fig 3.27: Phase wise variation of Xanthophyll with As accumulation in leaves in Control 1

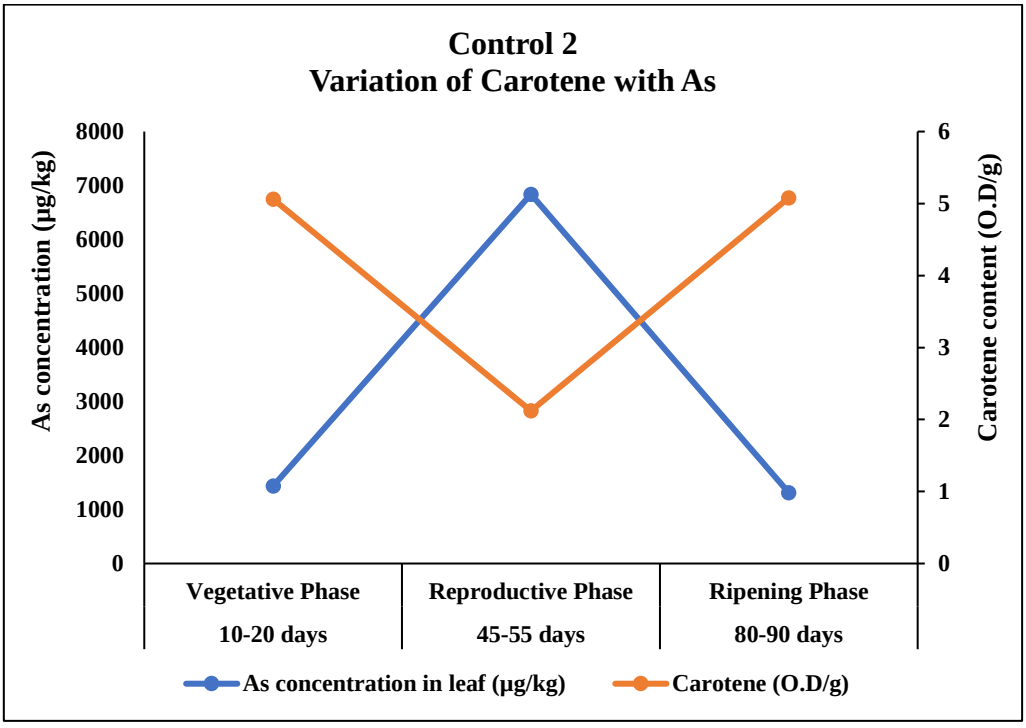


Fig 3.28: Phase wise variation of Carotene with As accumulation in leaves in Control 2

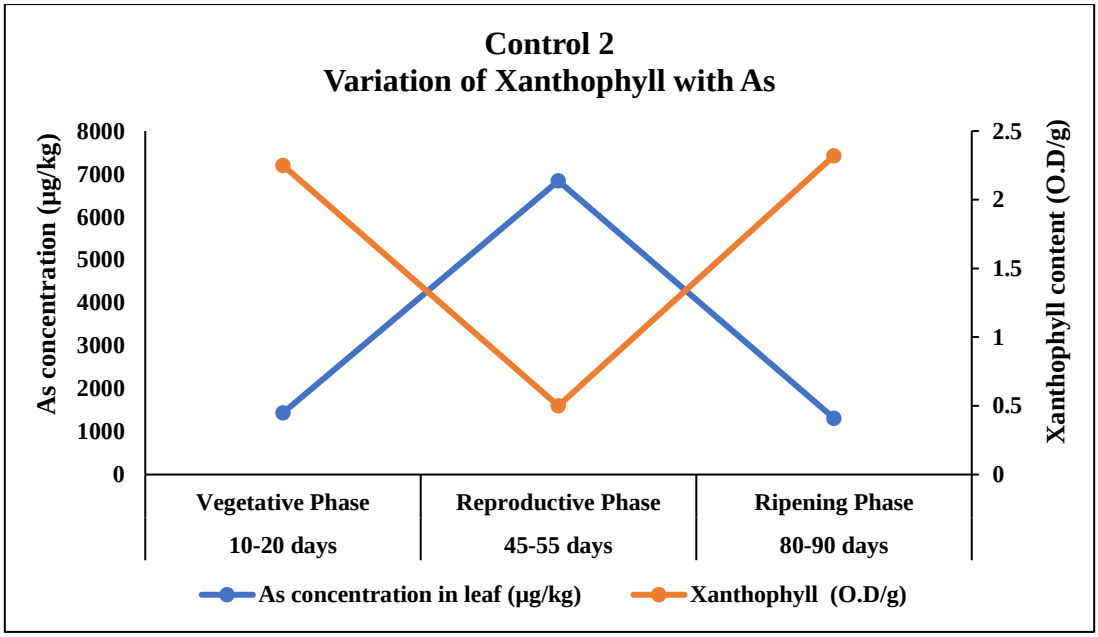


Fig 3.29: Phase wise variation of Xanthophyll with As accumulation in leaves in Control 2

Therefore, it is observed that in case of Carotene and Xanthophyll the lowest content (mg/g of leaf) is observed during the Reproductive phase of post monsoon cultivation period when the As accumulation in leaves is the highest in the pot studies of both Control 1 and Control 2. It can be inferred that As accumulation hinders the pigment production process in the leaves of paddy plant. Table 3.42, 3.43, 3.44 and 3.45 shows the correlation between Carotene and Xanthophyll with the As accumulated in the leaves in Control 1 and Control 2 respectively.

Table 3.42: Correlation between Carotene (O.D/g) and As accumulated (µg/kg) in Control 1

	As concentration in leaf (µg/kg)	**Carotene (O.D/g)**
As concentration in leaf (µg/kg)	1	
Carotene (O.D/g)	-0.9575	1

Table 3.43: Correlation between Xanthophyll (O.D/g) and As accumulated (µg/kg) in Control 1

	As concentration in leaf (µg/kg)	**Xanthophyll (O.D/g)**
As concentration in leaf (µg/kg)	1	
Xanthophyll (O.D/g)	-0.99837	1

Table 3.44: Correlation between Carotene (O.D/g) and As accumulated (µg/kg) in Control 2

	As concentration in leaf (µg/kg)	**Carotene (O.D/g)**
As concentration in leaf (µg/kg)	1	
Carotene (O.D/g)	-0.99991	1

Table 3.45: Correlation between Xanthophyll (O.D/g) and As accumulated (µg/kg) in Control 2

	As concentration in leaf (µg/kg)	**Xanthophyll (O.D/g)**
As concentration in leaf (µg/kg)	1	
Xanthophyll (O.D/g)	-0.9999	1

It is statistically observed from Table 3.42, 3.43, 3.44 and 3.45, that a correlation of -0.9575, -0.99837 and -0.99991, -0.9999 exists between Carotene and Xanthophyll (O.D/g) and As accumulated in the leaves (µg/kg) of paddy in Control 1 and Control 2 during post-monsoon cultivation respectively. In both the cases, a negative and strong correlation exists indicating an inverse relationship between the pigment content and As concentration, i.e. as As accumulation increases, Carotene and Xanthophyll content decreases.

3.5 Conclusion and future scope

3.5.1 Conclusion

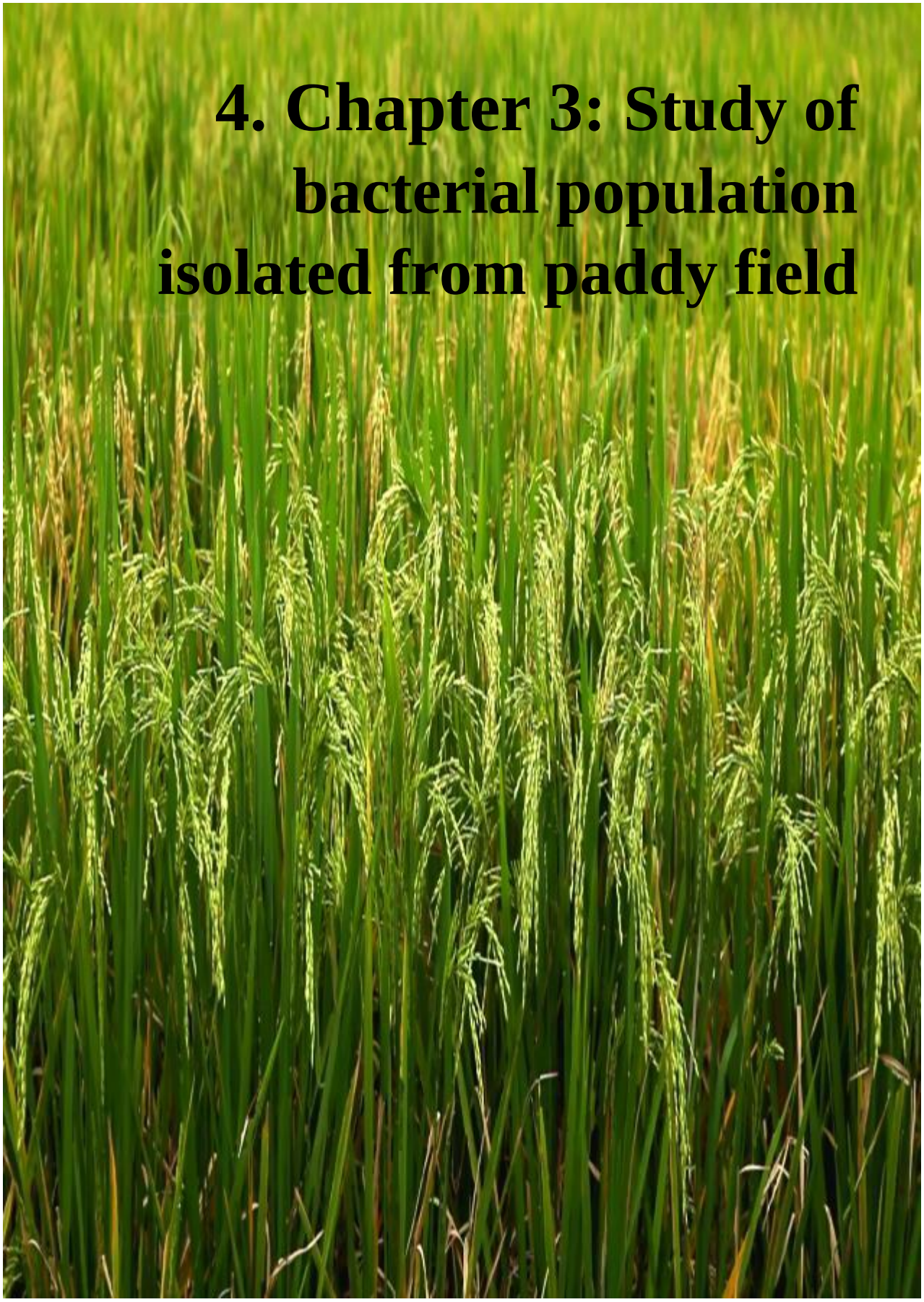
The photosynthetic pigment assay of the leaves of paddy plant samples collected from the fields of Gaighata (contaminated site), Medinipur (control site) as well as from the experimental pot studies (Control 1 and Control 2) during post-monsoon cultivation period showed a significant trend. The maximum As concentration in the leaves has been observed during the Reproductive phase, when the pigment content has been found to be the minimum in case of Chlorophyll a, b, total Chlorophyll as well as Carotene and Xanthophyll. The total Chlorophyll content in the leaves of Teghoria (highly contaminated) was found to be the least, i.e. 0.1832 mg/g of leaf whereas that in the leaves of Madhusudankathi (moderately contaminated) was found to be 0.2171 mg/g of leaf, i.e. higher than Teghoria. The total Chlorophyll content in the leaves of Pingla (control site) was found to be the highest among field samples, i.e. 0.3801 mg/g of leaf. Considering the pot studies, total Chlorophyll content in the leaves of Control 1 was 0.5869 mg/g of leaf and that of Control 2 was 0.3738 mg/g of leaf. The uncontaminated soil used in case of Control 1, explains the higher Chlorophyll content in its leaves compared to Control 2. According to a previous work by Miteva and Merakchiyska 2002, increased As concentrations resulted in an alteration of the chloroplast shape, with concaving membrane bending and partial destruction together with the changes in the accumulation and flow of assimilates leading to the decrease of Chlorophyll contents in paddy leaf. The inverse relationship between As concentration and Chlorophyll content has been well established in this study as well.

Considering the variation in Carotenoid content i.e. Carotene and Xanthophyll in the leaves with As concentration, it is observed that it follows the same inverse trend as that of Chlorophyll. The Carotene content in the leaves of Teghoria (highly contaminated) was found to be the least, i.e. 1.15 O.D/g of leaf whereas that in the leaves of Madhusudankathi (moderately contaminated) was found to be 1.22 O.D/g of leaf, i.e. more than Teghoria. The Carotene content in the leaves of Pingla (control site) was found to be the highest among samples collected from the field, i.e. 3.88 O.D/g of leaf. In case of the pot studies, Carotene content in the leaves of Control 1 was 5.97 O.D/g of leaf and in Control 2 was 2.12 O.D/g of leaf; less than Control 1 due to the use of contaminated soil. Similarly, the Xanthophyll content in the leaves of Teghoria, Madhusudankathi, Pingla, Control 1 and Control 2 were found to be 1.05 O.D/g of leaf, 0.08 O.D/g of leaf, 1.12 O.D/g of leaf, 2.00 O.D/g of leaf and 0.5 O.D/g of leaf respectively which is in the same trend as that of Carotene. According to Choudhury et

al, 2009, Carotene and Xanthophyll contents also declined on As exposure in paddy plant samples. Though the variation of Chlorophyll depends on several other factors like the paddy plant species, location of the field etc., this study is completely in accordance to the trends previously reported in rice plants.

3.5.2 Future scope

a) Besides the photosynthetic pigment assays, the variation of carbohydrate (sugar) content in the rice grain with As exposure can be studied.
b) The effect of As toxicity on the protein content (Proline assay) as well as lipid content (Malondialdehyde assay) in rice plant can be analysed.
c) The variation in the working mechanism of anti-oxidative enzymes like Catalase, Catechol peroxidase, Ascorbic acid oxidase, Gluiacol peroxidase etc. can be estimated in the paddy plant system.



4. Chapter 3: Study of bacterial population isolated from paddy field

4.1 Aim and objective of study

The main objectives of the study of microbial diversity in the soil of paddy fields are as follows:

1. The estimation of Fe and As of the soil collected from different depths (surface and sub-surface) of the paddy field at Madhusudankathi (moderately As contaminated site), located in Gaighata block, in the district of North 24 Parganas, West Bengal during post-monsoon cultivation period.
2. Isolation of pure bacterial colonies from different layers of the soil (surface as well as sub-surface) of the paddy field at Madhusudankathi (moderately As contaminated site), located in Gaighata block, in the district of North 24 Parganas, West Bengal during post-monsoon cultivation period.
3. The estimation of CFU (Colony forming unit) /ml count of bacterial population in different layers of soil of the contaminated paddy field (top soil, root soil, mid soil and bottom soil) in nutrient agar medium under three conditions i.e. i) control ii) amended with As^{3+} and iii) amended with As^{5+}.
4. The evaluation of As tolerance of both As^{+5} and As^{+3} of the selected isolates from the control plates as well the As^{+5} and As^{+3} amended plates with colonies isolated from the four layers of the contaminated soil over a wide range of concentrations.
5. The characterization of selected bacterial isolates from the control plates as well the As^{+5} and As^{+3} amended plates and study of their capabilities to chelate Iron as well as the capability to break down Phosphate (PO_4^{-3}) by Siderophore assay and by Phosphatase assay respectively.
6. The analysis of the capacity of the best performing bacterial isolates selected from the colonies isolated on the control plates as well the As^{+5} and As^{+3} amended plates to absorb and adsorb As^{+5} as well as As^{+3}.

4.2 Literature review

Isolation and characterization of bacterial colonies, from As contaminated soil in different areas of West Bengal, Bangladesh as well as all over the world have been reported. For instance, to identify phylogenetically diverse As resistant bacterial colonies, present in agricultural soil of Bangladesh, enrichment cultures were performed in a minimal medium in the presence of As^{+3} and As^{+5} to isolate As resistant bacteria. Twenty one As resistant bacteria belonging to different genera (Gram- positive and Gram-negative bacteria) were isolated that were capable of reducing As^{+5} to As^{+3} under aerobic conditions (for detoxification purpose). This study provides results on identification, levels of As resistance and reduction of As^{+5} by the bacterial isolates which might play an important role in As cycling in the As contaminated soils in Bangladesh (Bachate et al., 2009). In another study, two rod shaped Gram-positive bacteria isolated from As affected ground water of Purbasthali block of Burdwan, West Bengal, India, have been reported, which can tolerate As^{+5} and As^{+3} concentration up to 4500000 ppb and 550000 ppb respectively (Dey et al., 2016). Nakhro and Dkhar., 2010 used dilution plating method to analyse microbial population count of soil samples, collected from surface and sub-surface of experimental paddy fields of Agronomy Division, Indian Council of Agricultural Research (ICAR) of Meghalaya. According to another study reported, twelve As resistant bacteria were isolated from the agricultural soil of the Chianan Plain in southwestern Taiwan using enrichment techniques. They had a minimum inhibitory concentration (MIC) ranging from 10 to 30 mM and 150 to 320 mM for As^{+3} and As^{+5}, respectively. Among them, eight isolates were capable of oxidizing As^{+3} and exhibiting As^{+3} oxidase enzyme activity. Some of the isolates also responded positively to phosphate solubilization and siderophore test (Das et al., 2014). Another study was performed aiming to isolate As resistant bacteria from terrestrial environment Tamil Nadu, South India for their potential applications in bioremediation strategies. From the isolated fifty As resistant bacteria, two bacterial isolates BC1 and BC2 were taken for further studies due to their higher resistance ability to As. The optimum pH and temperature were found to be at 6.0 and 37 °C respectively (Selvi et al., 2014). According to a study reported, sample was collected from Haringhata in Nadia district, which is a known As contaminated zone. Out of six isolated bacteria, one bacterium revealed as As tolerant bacteria which can tolerate up to 1000 mg/L of As^{+3} and 500 mg/L of As^{+5}. Both the As species has toxic effects on its protein concentration. The strains can tolerate up to a certain limit after that their growth ceased. This strain can accumulate and also transform As^{+3} to As^{+5}. The transformation capacity of strain was assessed qualitatively and quantitatively. The strain can

transform 90% of As^{+3} to As^{+5} (Banerjee et al., 2013). According to Majumder et al, 2013, a few As oxidizing bacterial strains were isolated from the As-contaminated soils of West Bengal, India. They were found to be hyper-resistant to both As^{+5} (167–400 mM) and As^{+3} (16–47 mM). Elevated rates of As^{+3} oxidation (278–1250 μM /hr) and As^{+3} oxidase activity (2.1–12.5 nM min^{-1} mg^{-1} protein) were observed in these isolates. Screening identified four strains as superior As oxidizers of which AMO-10 completely (100%) oxidized 30 mM of As^{+3} within 24 hr. In yet another study, soil samples were collected from an As affected area of West Bengal, India and 10 different bacterial strains were isolated. The minimum inhibitory concentration (MIC) values of the isolates varied widely in the range 50–125 mM (As) as As^{+5} and 10–100 mM (As) as As^{+3}. Apart from the confirmation of intracellular accumulation of As by TEM (Transmission electron microscopy) and EDAX (Energy Dispersive X-ray Analysis), it was found that some of these bacteria could oxidize As^{+3} to As^{+5} and all others could reduce As^{+5} to As^{+3}. The growth pattern of the bacterial strains in presence and absence of As was also observed (Banerjee et al., 2011).

4.3 Materials and methods

4.3.1 Study area

According to Roychowdhury et al, 2010, Gaighata is one of the eminent As contaminated blocks out of 107 blocks located in the district of North 24 Parganas, West Bengal, India. Our study is mainly based on the samples collected from the field of Madhusudankathi, which has previously been reported as moderately As contaminated (Chowdhury et al, 2018) located in the block of Gaighata. Soil samples were collected during post monsoon cultivation period (in the month of August) from different depths of the paddy field (i.e. both surface and sub-surface soil). The sampling site was located in the mid-field area from where the top-soil and root soil were collected aseptically. The mid-soil and deep soil were collected from a depth of approximately 6 inches and 1.5-2 feet respectively and brought to the laboratory in ice box to maintain the aseptic condition. The sampling was done 40 days after sowing of the paddy seedlings.

Fig 4.1: Site of sample collection (Madhusudankathi)

Fig 4.2: Samples collected from the four depths of soil strata

4.3.2 Reagents used

1) Sodium arsenate dibasic heptahydrate ($AsHNa_2O_4$. $7H_2O$) (Merck)
2) Sodium arsenite ($NaAsO_2$) (Merck)
3) Nutrient Broth (SRL)
4) Agar (SRL)
5) 0.9% Saline solution (Sodium chloride (NaCl)) (Merck)
6) Chrome azurol S (CAS) (SRL)
7) Hexa decyl trimethyl ammonium bromide (HDTMA) (Loba Chemie)
8) PIPES (SRL)
9) Tri calcium phosphate (TCP) (SRL)
10) Hydrochloric acid (HCl) (37%) (Merck)
11) Ferric chloride hexahydrate ($FeCl_3.6H_2O$) (SRL)
12) Potassium dihydrogen phosphate (KH_2PO_4) (SRL)
13) Ammonium chloride (NH_4Cl) (SRL)
14) Glucose (SRL)
15) Sodium hydroxide (NaOH) (Merck)
16) Casamino acid (Merck)
17) Potassium chloride (KCl) (SRL)
18) Ammonium sulphate ($(NH_4)_2SO_4$)) (Merck)
19) Magnesium chloride hexahydrate ($MgCl_2$. $6H_2O$) (SRL)
20) Magnesium sulphate heptahydrate ($MgSo_4$. $7H_2O$) (SRL)
21) Concentrated Nitric acid (HNO_3) (69% v/v) (Merck)
22) Hydrogen peroxide (H_2O_2) (30% v/v) (Merck)
23) Potassium iodide (KI) (Merck)
24) Sodium borohydride ($NaBH_4$) (Merck)
25) Sodium hydroxide pellets (NaOH) (Merck)
26) Sodium acetate (CH_3COONa) (Merck)
27) Acetic acid (CH_3COOH) (Merck)
28) Hydroxylamine hydrochloride ($NH_2OH.HCl$) (Merck)
29) Liquor ammonia (NH_4OH) (25%) (Merck)
30) Ortho-phenanthrolin ($C_{12}H_8N_2$) (Merck)
31) Double distilled water

All chemicals used were of analytical grade.

4.3.3 Methodology

4.3.3.1 Estimation of As and Fe interaction in different layers of the soil

The As and Fe estimation of the soil samples collected from the paddy field of Madhusudankathi, during post monsoon cultivation period from different depths, i.e. top soil, root soil, mid soil (6 inches below the surface) and deep soil (2 inches below the surface) were estimated by the protocol mentioned previously in Chapter 2 (Sub-section 2.3.2).

Fig 4.3: Iron estimation of soil of different depths of the paddy field

4.3.3.2 Isolation of pure bacterial colonies from different layers of the soil

Paddy field soil samples during post monsoon cultivation have been collected aseptically from different depths i.e. i) the surface soil or top soil ii) the mid soil from a depth of around 6 inches, iii) the deep soil from a depth of 1.5 to 2 ft and iv) the rhizospheric soil i.e. the soil physically attached with the paddy plant root and brought to the laboratory in an ice box. The bacterial colonies of the four types of soil were isolated by dilution plating on autoclaved Nutrient Agar medium under three conditions i.e i) control ii) amended with As^{3+} (2 mM) prepared from a mother stock of 2 M of $NaAsO_2$ and iii) amended with As^{5+} (25 mM) prepared from a mother stock of 5 M of $AsHNa_2O_4$. $7H_2O$. For dilution plating, 1gm of soil of each type was taken in 1 ml of autoclaved 0.9% saline solution in an Eppendorf and vortexed well for 5 mins followed by serial dilution.

(a)

(b)

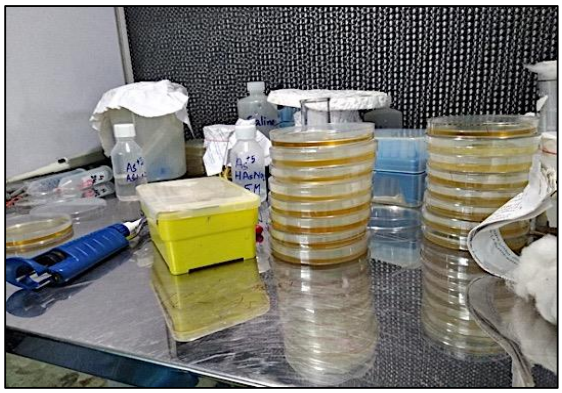
(c)

Fig 4.4: Isolation of pure bacterial colonies from different layers of soil (a) top soil, mid soil and bottom soil inside bore hole pipe under aseptic condition inside laminar (b) Root soil under aseptic condition inside laminar (c) vortexing of the soil sample in saline before serial dilution

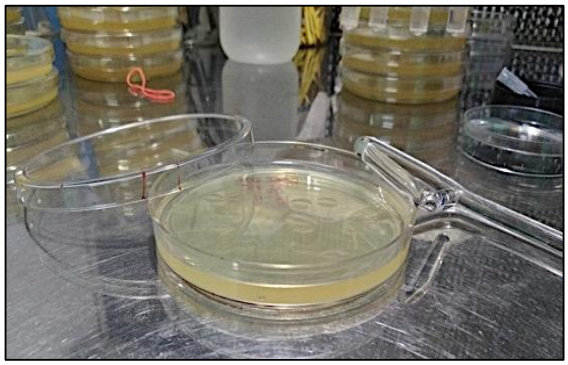
4.3.3.3 The estimation of CFU /ml count of bacterial population in different layers of soil

Dilution plating was done under three conditions i.e. i) control ii) amended with As^{3+} (2 mM) prepared from a mother stock of 2 M of $NaAsO_2$ and iii) amended with As^{5+} (25 mM) prepared from a mother stock of 5 M of $AsHNa_2O_4$. $7H_2O$. Enrichments with As^{3+} and As^{5+} have been done to isolate As resistant bacterial species which may have specific As biotransformation potentials. 0.1 ml of the desired dilution of the soil was plated by spread plate technique on control, As^{+3} amended and As^{+5} amended Nutrient agar plates and incubated overnight at 37 °C. The CFU (colony forming unit)/ml count of the soil collected from the four depths were noted after 48 hours and calculated in case of all the three conditions for the four types of soil.

(a)



(b)



(c)

Fig 4.5: Estimation of CFU/ml of the bacterial population (a) serial dilution of the four types of soil in eppendorfs (b) Nutrient agar plates prepared for plating of serially diluted soil under control, As^{+5} and As^{+3} amended conditions (c) plating of the bacterial isolates by spread plate

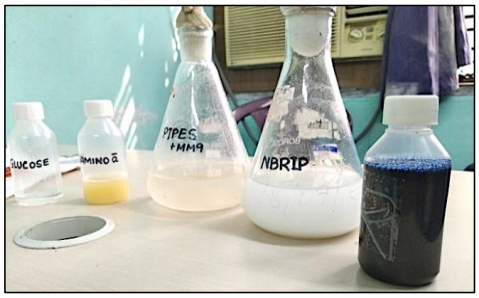
4.3.3.4 Evaluation of As^{+3} and As^{+5} tolerance of the selected bacterial isolates

Out of several colonies grown on control, As^{+5} enriched and As^{+3} enriched Nutrient agar plates,149 colonies were selected and their As tolerance were studied for both As^{+5} and As^{+3} over a wide range of concentrations varying from 50 mM to 600 mM for As^{+5} and 5 mM to 15 mM for As^{+3} . The isolates obtained from top soil grown in control plates, As^{+5} amended plates and As^{+3} amended plates are named as A,B and C respectively followed by Arabic numerals suffixed behind. Similarly, the isolates obtained from root soil, mid soil and deep soil grown in control, As^{+5} and As^{+3} amended plates are named as D, E, F, G, H, I, J, K, and L, respectively followed by Arabic numerals suffixed behind. The tolerance for As^{+5} was checked for the concentrations of 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, 300 mM, 400 mM, 500 mM and 600 mM and for As^{+3} was checked for the concentrations of 5 mM, 10 mM and 15 mM.

4.3.3.5 Characterization of the selected isolates

The 149 selected isolates were checked for their capabilities to chelate Iron as well as the capability to break down Phosphate (PO_4^{-3}) by Siderophore assay and by Phosphatase assay respectively. In case of Siderophore production, CAS (Chrome azurol S) agar media was prepared, autoclaved and plated aseptically. The growth of the 149 selected isolates were checked on the plates after 24 hrs incubation at 37 ℃. In case of Phosphatase production, NBRIP agar media was prepared, autoclaved and plated aseptically. After 24hrs incubation at 37 ℃, the growth of the 149 selected isolates were checked on the plates.

(a)(i)



(a)(ii)

(b)

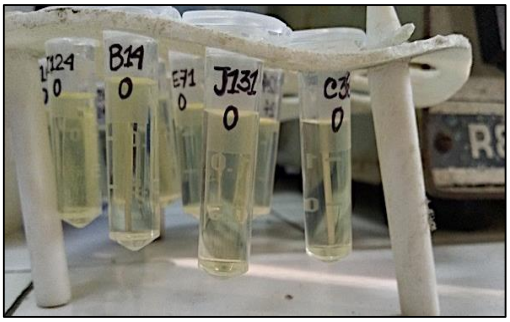
Fig 4.6: Phosphatase and Siderophore assay (a) Reagents used (b) plates prepared for the assay

4.3.3.6 Absorption and adsorption study of the best performing bacterial isolates

Out of the 149 colonies isolated, eight were selected depending on their tolerance to As^{+5} and As^{+3} and their efficiency of absorption or adsorption of As^{+5} as well as As^{+3} were checked. The individual colonies were grown overnight at 37 °C in 200 ml Nutrient broth spiked with 100 ppb of As^{+5} and As^{+3} separately. The initial As content of the broth was determined by AAS (Atomic absorption spectrophotometry). The next day, the broth was centrifuged at 8000 rpm for 10 mins. The As content of the supernatant was directly measured through AAS. The adsorption and absorption capacity of the biomass was determined by first washing the pellet with autoclaved double distilled water and measuring its As content followed by acid digestion

of the same biomass and measuring its As content, respectively. The As content in the initial broth (0 hr), supernatant broth (24 hrs), pellet washed water and acid digested biomass were noted.

(a)



(b)

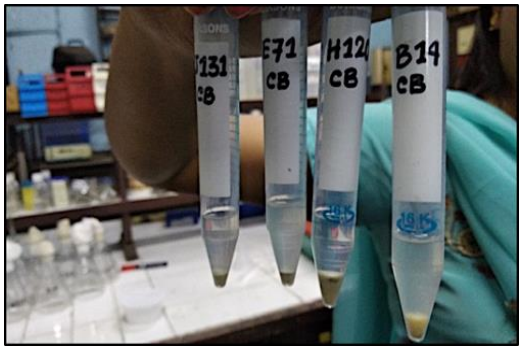


Fig 4.7: Absorption and adsorption study of selected isolates (a) Nutrient broth inoculated with selected isolates (b) 0 hr media stored for As estimation (c) Cell growth in Nutrient broth after 24 hrs incubation (d) cell biomass washed water of the isolates

4.4 Results and discussion

4.4.1 Estimation of As and Fe interaction in different layers of the soil

The As and Fe concentrations (µg/kg) of the soil samples collected from different depths of the paddy field have been tabulated below (Table 4.1) and represented graphically for understanding their interaction in Fig 4.8.

Table 4.1: Interaction of As and Fe in different layers of the soil

Sl No.	Soil type	As concentration (µg/kg)	Fe concentration (µg/kg)
1	Top soil	20030	27381000
2	Root soil	22767	29559000
3	Mid soil	19406	20745000
4	Deep soil	15591	20204000

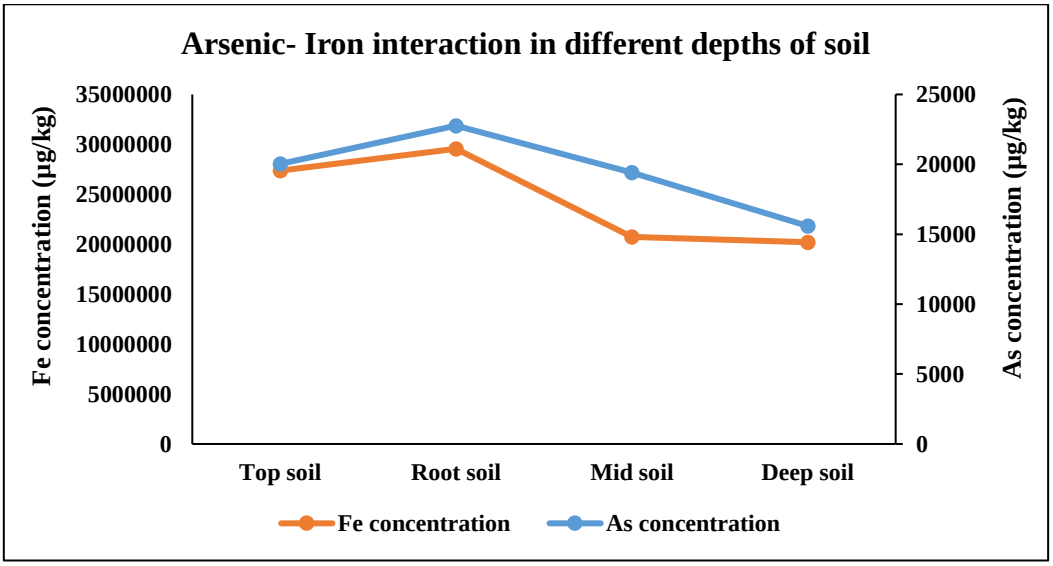


Fig 4.8: Arsenic-Iron interaction in different depths of soil

From Fig 4.8, it is clear that the As and Fe concentrations show a similar trend with maximum values in the rhizospheric soil and minimum values in the deep soil region. In the root soil, As and Fe shows concentrations of 22767 µg/kg and 29559000 µg/kg respectively, somewhat lower concentrations are observed in the top soil, followed by mid soil and finally deep soil.

4.4.2 The estimation of CFU /ml count of bacterial population in different layers of soil

The colony forming units (CFU) obtained under three conditions (control, As^{+5} amended and As^{+3} amended) for all the four soil samples have been tabulated in Table 4.2 and represented through bar graph in Fig 4.9.

Table 4.2: CFU counts of top soil, mid soil, root soil and deep soil obtained in control, As^{+3} and As^{+5} amended plates

Types of soil	Control		Arsenite amended		Arsenate amended	
	cfu X 10^{12}	Average	cfu X 10^{12}	Average	cfu X 10^{12}	Average
Top soil	16	184	10	105	5	113
	352		200		222	
Mid soil	36	225	13	8	12	6
	415		4		0	
Deep soil	37	256	0	89	4	10
	476		178		16	
Root soil	25	193	2	256	2	167
	362		510		332	

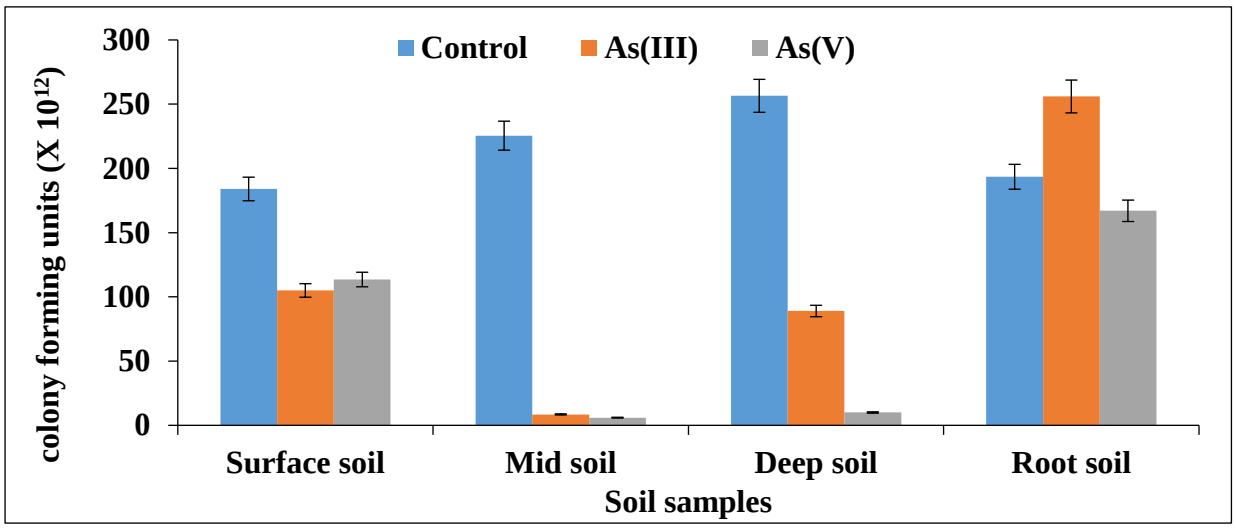
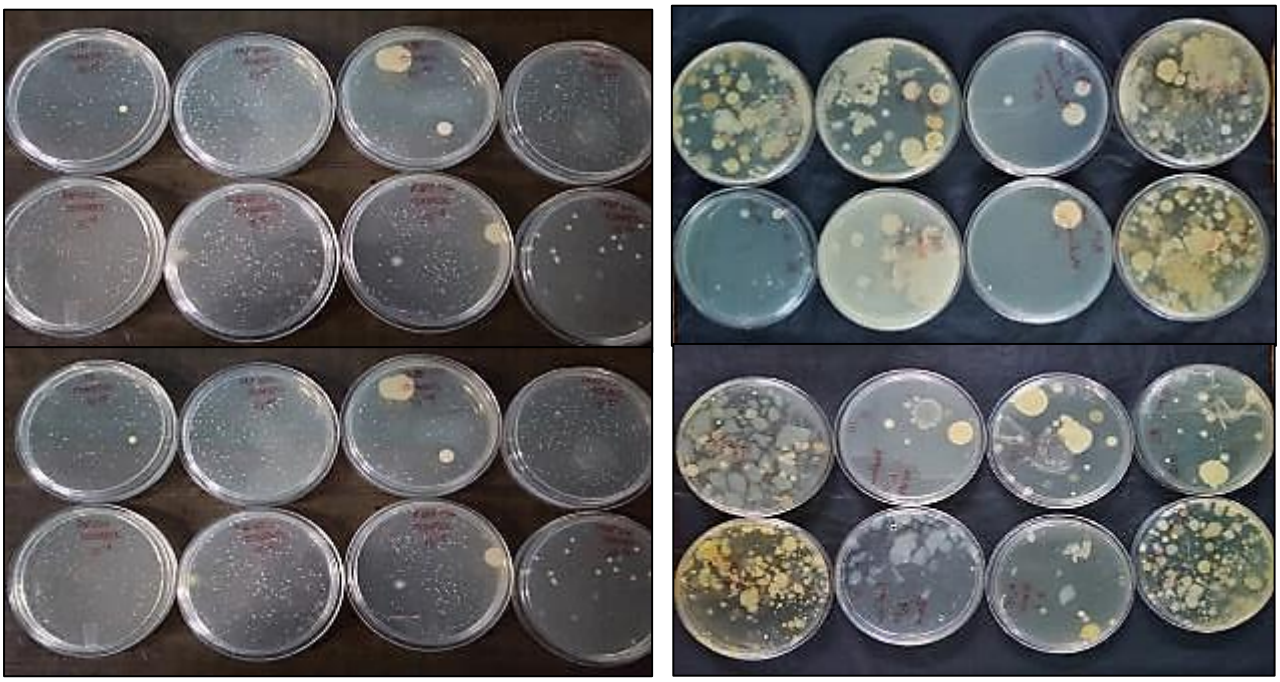


Fig 4.9: Bar diagram indicating the variation of CFU count in different layers of soil in control, As^{+3} and As^{+5} amended plates. Error bars indicate 5% error with respect to the data

The observations suggest that the maximum bacterial count under control condition is found in the deep soil i.e an average maxima of 256 X 10^{12} and a minima of 184 X 10^{12} in surface soil. However, enrichment study suggests that both As^{+3} and As^{+5} resistant bacteria predominate in the root soil followed by in the surface soil. In our previous sub-section (4.5.1), we have reported the maximum concentration of Fe as well as As in the root soil. There might be a correlation in the As and Fe concentration and the bacterial diversity predominating in the rhizospheric zone playing important role in As immobilization.



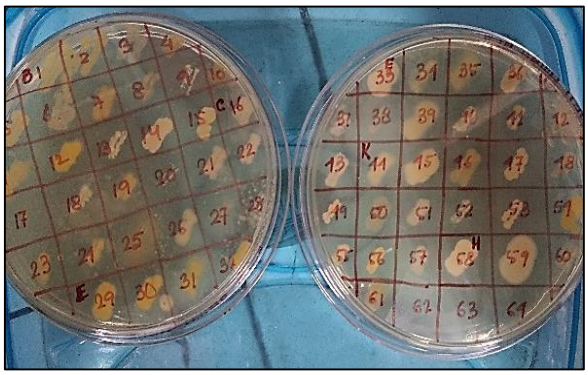
Set 1 Set 2

Fig 4.10: CFU/ml count of four types of soil on control, As^{+3} amended and As^{+5} amended plates

4.4.3 Evaluation of As^{+3} and As^{+5} tolerance of the selected bacterial isolates

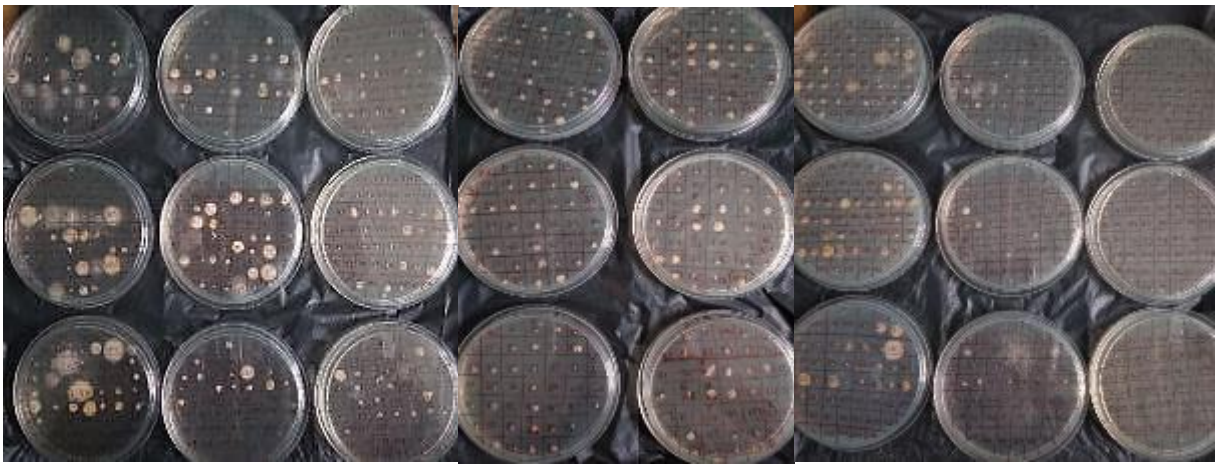
Out of several colonies grown on control, As^{+3} enriched and As^{+5} enriched Nutrient agar plates,149 colonies selected isolates are shown in Fig 4.11 (a). The isolates obtained from top soil grown in control plates, As^{+5} amended plates and As^{+3} amended plates are named as A,B and C respectively followed by Arabic numerals suffixed behind. Similarly, the isolates obtained from root soil, mid soil and deep soil grown in control, As^{+5} and As^{+3} amended plates are named as D,E,F,G,H,I,J,K, and L, respectively followed by Arabic numerals suffixed

behind. The tolerance of the selected isolates to a wide range of As^{+3} and As^{+5} concentrations have been noted and shown in Fig 4.11(b).



(a)

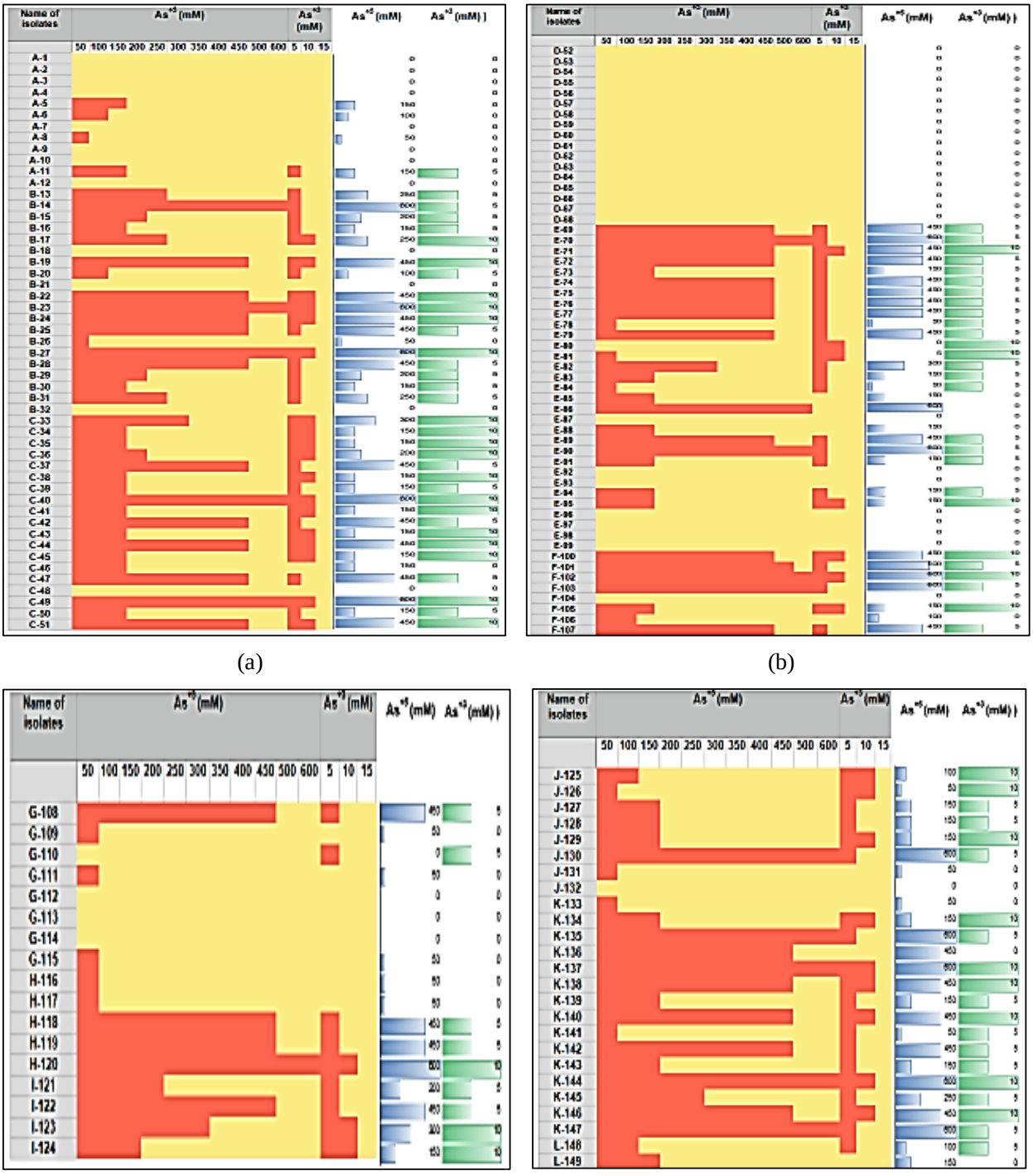
(b)(i)



(b)(ii)

Fig 4.11: Estimation of tolerance of the selected isolates to As^{+3} and As^{+5} (a) colonies selected for further studies from pure colonies isolated from top soil, root soil, mid soil and bottom soil on control and As amended plates (b) Tolerance of the selected colonies to As^{+3} (i) and As^{+5}(ii)

The MTC (Maximum tolerance concentration) of the selected bacterial population from the different types of soil have been represented in Fig 4.12.



(a) (b)

(c) (d)

Fig 4.12: Range of tolerance of As^{+5} and As^{+3} in mM together with MTC (maximum tolerance concentration) of isolated bacterial population from top soil (a), root soil (b), mid soil (c) and deep soil(d), respectively. The area shaded with red denotes positive result. The blue and green bars indicate the MTC values in As^{+5} and As^{+3} respectively

It was observed that 33% of the colonies isolated from top soil grown in control plates were resistant to As^{+5} all of which showed low resistance (50-150 mM) and 8% were resistant to

As^{+3} which showed low resistance. 80% of the colonies isolated from top soil grown in As^{+5} enriched medium were resistant to As^{+5} of which 20% showed low resistance, 25% showed moderate resistance (200-350 mM) and 35% showed high resistance (400-600 mM) while 80% were resistant to As^{+3} of which 50% showed low resistance and 30% showed high resistance (10mM). 95 % colonies isolated from top soil, grown in As^{+3} enriched medium showed As^{+5} resistance of which 47% showed low,11% moderate and 37% showed high resistance whereas of 89% of the population resistant to As^{+3}, 26% showed low resistance and 63% showed high resistance. The colonies isolated from root soil and grown in control plates showed no resistance to either As^{+5} or As^{+3}.Next, 74% of the colonies isolated from root soil grown in As^{+5} enriched medium were resistant to As^{+5} of which 32% showed low resistance, 3% showed moderate resistance and 39% showed high resistance while 68% were resistant to As^{+3} of which 55% showed low resistance and 13% showed high resistance (10mM). 88 % colonies isolated from root soil, grown in As^{+3} enriched medium showed As^{+5} resistance of which 25% showed low and 63% showed high resistance whereas of 74% of the population resistant to As^{+3}, 37% showed low resistance and 37% showed high resistance. 51% of the colonies isolated from mid soil grown in control plates were resistant to As^{+5} of which 38% showed low resistance and 13% showed high resistance and 25% were resistant to As^{+3} all of which showed low resistance. 100% of the colonies isolated from mid soil grown in As^{+5} enriched medium were resistant to As^{+5} of which 40% showed low resistance and 60% showed high resistance while 60% were resistant to As^{+3} of which 40% showed low resistance and 20% showed high resistance. 100 % colonies isolated from mid soil, grown in As^{+3} enriched medium showed As^{+5} resistance of which 25% showed low,50% moderate and 25% showed high resistance whereas out of 100% of the population resistant to As^{+3}, 50% showed low resistance and 50% showed high resistance. 88% of the colonies isolated from deep soil grown in control plates were resistant to As^{+5} of which 75% showed low resistance and 13% showed high resistance and 76% were resistant to As^{+3}, of which 38% showed low resistance and 38%high resistance. 100% of the colonies isolated from deep soil grown in As^{+5} enriched medium were resistant to As^{+5} of which 33% showed low resistance, 7% showed moderate resistance and 60% showed high resistance while 87% were resistant to As^{+3} of which 47% showed low resistance and 40% showed high resistance. 100% colonies isolated from deep soil, grown in As^{+3} enriched medium showed As^{+5} resistance all of which showed low resistance whereas of 50% of the population were resistant to As^{+3} all of which showed low resistance. Therefore, we can infer that the colonies isolated from root soil grown in control plates, without As^{+5} or As^{+3} stress showed no tolerance towards the two species of As. Moreover, we can find a trend in case of all the four layers of

soil where % of population resistant to As^{+5} as well as As^{+3} is the maximum in case to As^{+3} enriched medium compared to control plates or As^{+5} enrichment.

4.4.4 Characterization of the selected isolates

The bacterial population that is capable of chelating Iron, after 24 hrs of incubation undergoes color change and turns into orange colonies while in case of Phosphatase assay, those bacteria capable of producing Phosphatase and utilizing Phosphate, after incubation produces a translucent ring like structure around their colony.

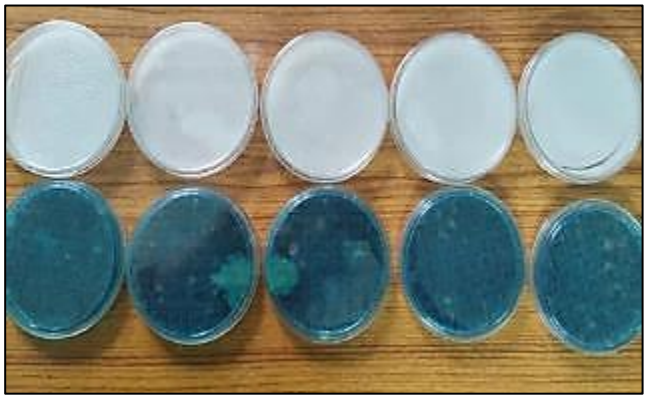


Fig 4.13: Results of Siderophore and Phosphatase test

As shown in Fig 4.13, none of the isolates responded to Siderophore assay or Phosphatase assay indicating their lack of capacity to chelate Iron or break down Phosphate (PO_4^{-3}).

4.4.5 Absorption and adsorption study of the best performing bacterial isolates

The As content in the initial broth (0 hr), supernatant broth (24 hrs), pellet washed water and acid digested biomass are given in the following table (Table 4.3).

Table 4.3: As content in initial broth (0 hr), supernatant broth (24 hrs), pellet washed water and acid digested cell biomass of eight isolates for As^{+3} and As^{+5} spiked set ups

Name of isolates	Sample analyzed	As $^{+3}$ (µg)	S.D	As $^{+5}$ (µg)	S.D
K 144	0 hr broth	15.8	0.08825	19.16	0.62826
	24 hr supernatant broth	15.38		14.77	
	Pellet washed water	0.0482		0.0765	
	Acid digested cell biomass	0.247		3.425	
H 120	0 hr broth	16.375	0.27096	25.83	1.36684
	24 hr supernatant broth	14.595		13.33	
	Pellet washed water	0.0498		0.078	
	Acid digested cell biomass	1.347		14.355	
B 14	0 hr broth	18.032	0.22507	19.9	1.1878
	24 hr supernatant broth	16.006		10.11	
	Pellet washed water	0.1157		0.1148	
	Acid digested cell biomass	1.592		11.355	
C 38	0 hr broth	20.37	0.09327	19.08	1.63865
	24 hr supernatant broth	19.86		13.4	
	Pellet washed water	0.0656		0.0715	
	Acid digested cell biomass	0.3125		3.2911	
E 71	0 hr broth	24.29	1.85616	17.99	0.62911
	24 hr supernatant broth	11.15		8.66	
	Pellet washed water	0.08		0.0747	
	Acid digested cell biomass	10.435		10.145	
F 104	0 hr broth	19.91	0.25258	23.45	0.49462
	24 hr supernatant broth	16.65		13.44	
	Pellet washed water	0.0228		0.0645	
	Acid digested cell biomass	2.88		10.645	
J 131	0 hr broth	20.29	0.02072	25.5	1.52191
	24 hr supernatant broth	18.21		15.34	
	Pellet washed water	0.0637		0.1273	
	Acid digested cell biomass	1.987		12.185	
I 124	0 hr broth	15.82	0.06718	25.82	2.45868
	24 hr supernatant broth	14.81		16.14	
	Pellet washed water	0.127		0.1321	
	Acid digested cell biomass	0.788		13.025	

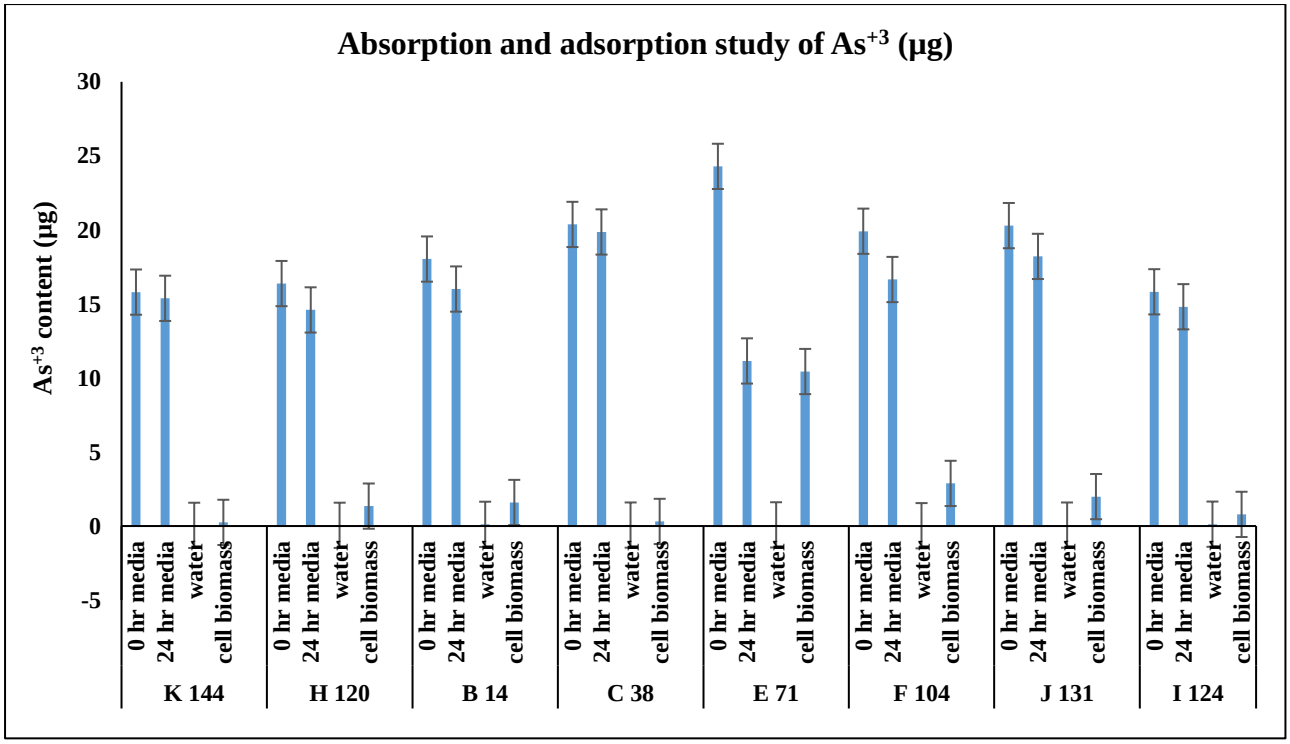


Fig 4.14: Mass balance of As^{+3} content in different parts of the experimental set-up. Error bars indicate 5% error with respect to the data

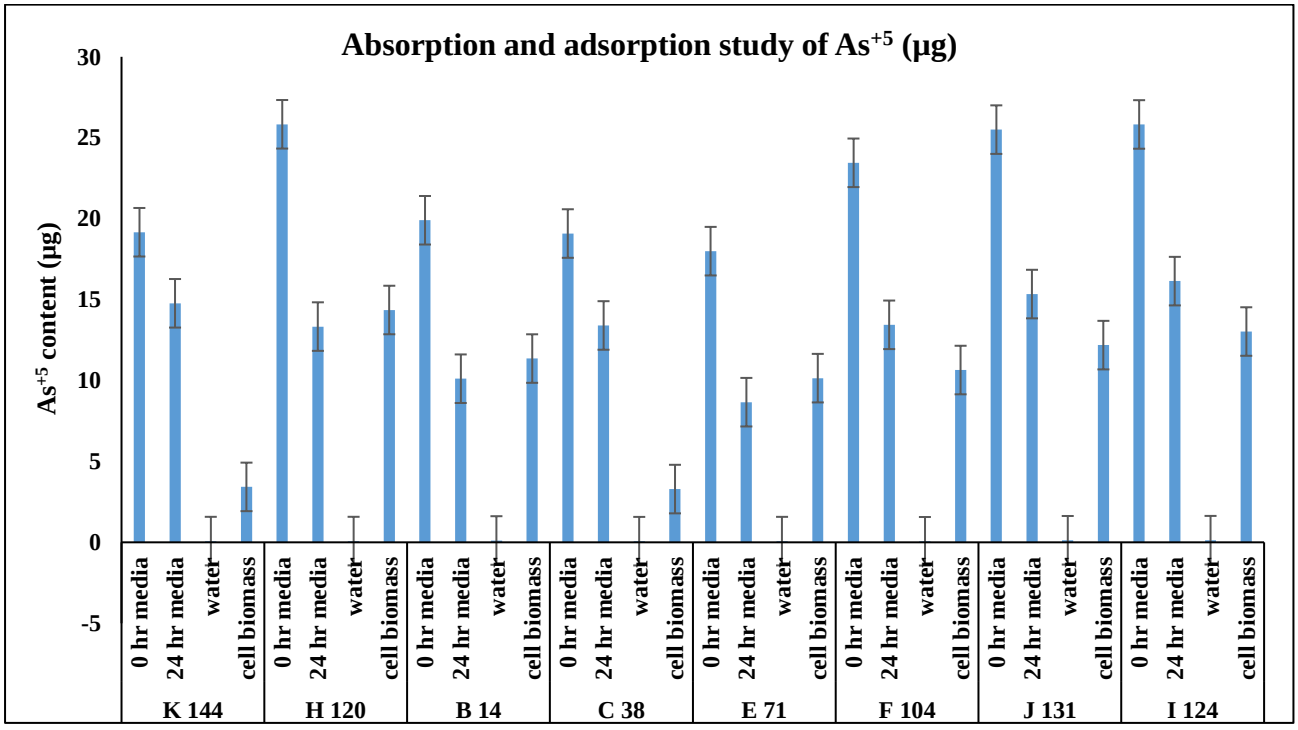


Fig 4.15: Mass balance of As^{+5} content in different parts of the experimental set-up. Error bars indicate 5% error with respect to the data

From Fig 4.14 and 4.15 it is evident that maximum absorption of 10µg of As^{+3} was shown by E71 and 14 µg of As^{+5} was shown by H120, respectively while maximum adsorption of 0.127 µg of As^{+3} and 0.1321 µg of As^{+5} was shown by I124, respectively. Mass balance of As^{+3} and As^{+5} content in different parts of the experimental set-up have been represented by bar-diagrams in Fig 4.14 and 4.15 respectively. Hence it can be concluded that the selected strains are capable of remediating As from As^{+3} and As^{+5} spiked media effectively by absorption as well as adsorption mechanisms.

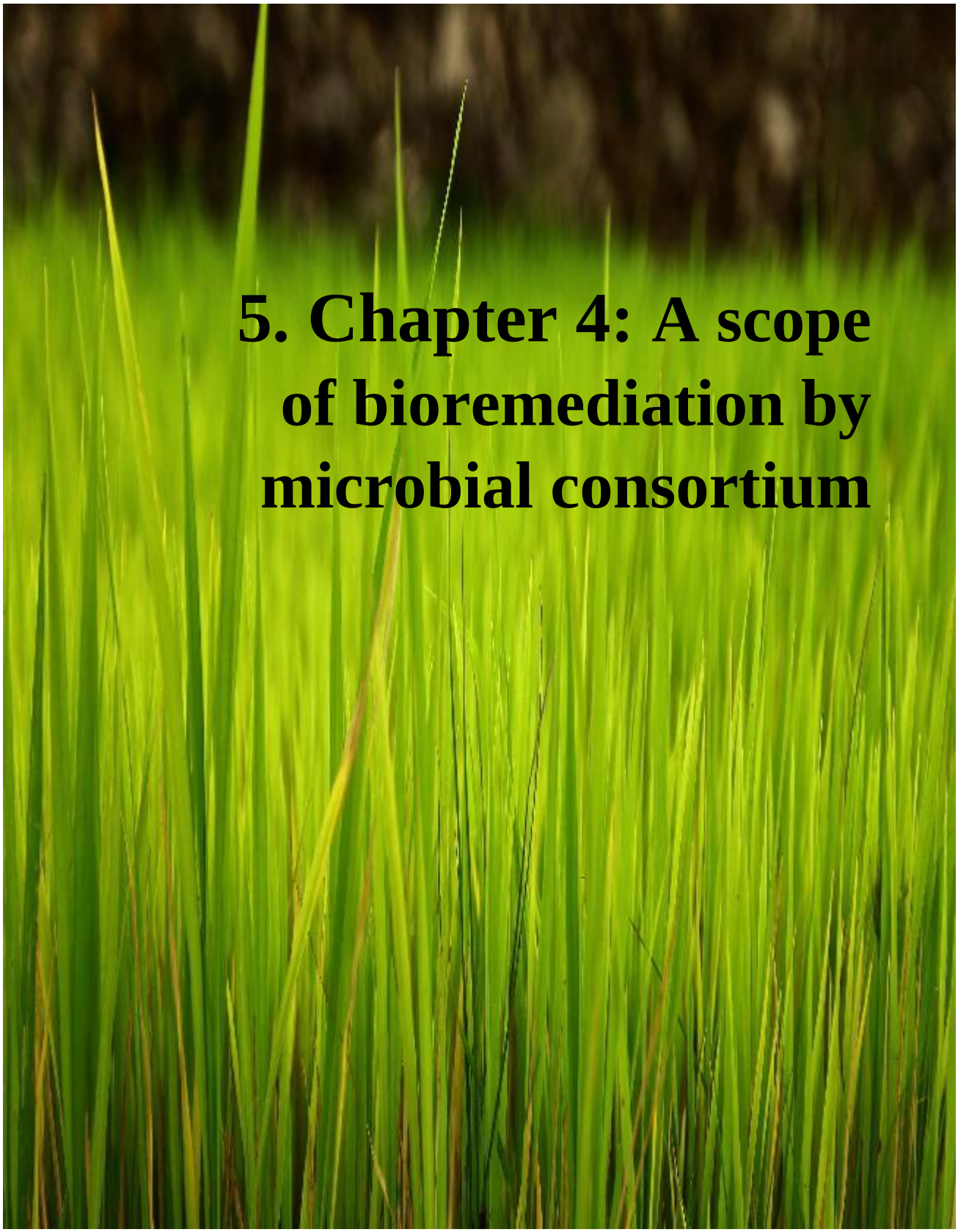
4.5 Conclusion and future scope

4.5.1 Conclusion

The summary of the study begins with 149 bacterial colonies, which were isolated on Nutrient agar plates under 3 conditions of control, As^{+3} enriched and As^{+5} enriched from top soil, root soil, mid soil and bottom soil of moderately As contaminated field of Madhusudankathi of Gaighata block, North 24 Parganas, West Bengal. The enrichment study suggests that both As^{+3} and As^{+5} resistant bacteria predominate in the root soil followed by the surface soil. Their tolerance was checked for a concentration range of 5 mM to 15 mM for As^{+3} and 50 mM to 600 mM for As^{+5}. From here we can infer that the colonies isolated from root soil grown in control plates, without As^{+5} or As^{+3} stress showed no tolerance towards the two species of As. Moreover, we can find a trend in case of all the four layers of soil where % of population resistant to As^{+5} as well as As^{+3} is the maximum in case to As^{+3} enriched medium compared to control plates or As^{+5} enrichment. Out of the 149 colonies, eight best performing colonies were selected and were grown overnight in Nutrient Broth spiked with 100 ppb of As^{+3} and As^{+5} respectively at 37 °C to check their absorption and adsorption capacity. This can be followed by a discussion that maximum absorption of 10 µg was shown by E71 and 14 µg by H120, for As^{+3} and As^{+5} respectively while maximum adsorption of 0.127 µg and 0.1321 µg was shown by I124 for As^{+3} and As^{+5} respectively. From table 4.3 it is also evident that the standard deviation in case of mass balance for As^{+5} varies from 0.49 to 2.45 and in case of As^{+3} varies from 0.088 to 1.85; which indicates sufficiently significant data. Hence it can be concluded that the selected strains are capable of remediating As effectively by absorption as well as adsorption mechanisms.

4.5.2 Future scope

a) These observations together with previous studies trigger the interest to further characterize these bacterial isolates to understand their phylogenetic affiliations as well as their metabolic capabilities to arrest As preventing it to further being translocated in the paddy plant.
b) A bacterial consortium with the eight potent bacteria can be prepared and further applied to high as well as moderately contaminated soil under different conditions and their remediation capacities by absorption and adsorption mechanisms can be checked. This can be further used as an alternate mitigation strategy.



5. Chapter 4: A scope of bioremediation by microbial consortium

5.1 Aim and objective of study

In the previous study, eight best performing bacteria had been isolated which were capable of showing significant tolerance to As^{+3} as well as As^{+5}. Some of them showed brilliant potency in removal of As from As^{+3} and As^{+5} spiked medium by absorption and adsorption mechanisms. The main objectives of the study are discussed as follows:

1. To check the chances of survival and rate of multiplication of the bacterial isolates in real life scenario, i.e. when applied to As contaminated soil samples under different circumstances.
2. To study the potency of the eight selected bacterial isolates of removing As from contaminated soil samples under various conditions.
3. To compare the activity of the bacterial consortium in removing As from highly contaminated soil (sample collected from Teghoria, North 24 Parganas, West Bengal) and that in case of moderately contaminated soil (sample collected from Madhusudankathi, North 24 Parganas, West Bengal).
4. To analyse whether the bacterial consortium worked more efficiently when applied under aerobic condition or under anaerobic condition.
5. To evaluate whether the bacterial consortium worked better when applied as control, or when applied together with the indigenous bacterial population already present in the soil samples.
6. To draw an overall conclusion regarding the success in the application of the prepared bacterial consortia in remediating As from contaminated soil.

5.2 Literature review

As biological agents represent an affordable alternative to the costly metal decontamination technologies, As oxidising bacteria from the As contaminated soils of many fields of West Bengal have been isolated. A new As oxidizing bacterium *Stenotrophomonus* sp., isolated from less As contaminated soil (8800 µg/kg) was capable of bioremediation of As (Bahar et al., 2012). According to another study aimed to isolate As resistant bacteria from terrestrial environment Tamil Nadu, South India for their potential applications in bioremediation strategies, 2 bacterial isolates, belonging to the genera *Enterobacter asburiae* and *Enterobacter cloacae* out of 50 As resistant colonies were taken for further studies due to their higher resistance ability to As. The optimum pH and temperature were found to be at 6 and 37 °C respectively (Selvi et al., 2014). In this context, bio-mineralization based remediation of As^{+3} contaminated soil by *Sporosarcina ginsengisoli* CR5, isolated from Urumqi, China has already been reported by Achal et al., 2011. The role of microbial calcite precipitated by this bacterium to remediate soil contaminated with As^{+3} was investigated. The bacterium was able to grow at high As^{+3} concentration of 50 mM. In order to obtain As distribution pattern, five stage soil sequential extraction was carried out. Arsenic mobility was found to significantly decrease in the exchangeable fraction of soil and subsequently, after bioremediation the As concentration was markedly increased in the carbonated fraction. The results from this study have implications that bioremediation by *S. ginsengisoli* is a viable, environmental friendly technology for remediation of the As contaminated sites. (Achal et al., 2012). Among the various methods so far reported for reclamation of As contaminated rhizosphere soil, bioremediation using bacteria has been found to be most promising. An As resistant bacterial isolate *Brevibacillus* sp. KUMAs2 was obtained from As contaminated soil of Nadia, West Bengal, India, which could resist a maximum of 265 mM and 17 mM As^{+5} and As^{+3} respectively. The strain could remove 40 % As under aerobic culture conditions. The strain could promote plant growth under As contaminated soil environment by decreasing As accumulation in plant upon successful colonization in the rhizosphere, which suggests the possibility of using this isolate for successful bioremediation of As in the crop field. (Mallick et al., 2014). Dey et al., 2016 in a study, isolated two rod shaped Gram-positive bacteria from As affected ground water of Purbasthali block of Burdwan, West Bengal, India, which can tolerate As^{+5} concentration up to 4500,000 ppb and 550,000 ppb of As^{+3} concentration. The isolates *Bacillus* sp. and *Aneurinibacillus aneurinilyticus* can remove 51.45% and 51.99% of

As^{+3} and 53.29% and 50.37% of As^{+5}, respectively from As containing culture media. These two As resistant bacteria can be used as a novel pathway for the bioremediation of As (Dey et al., 2016). Banerjee et al., 2013 chose one bacterium showing molecular similarity with *Brevibacillus brevis* out of six isolated bacteria as it can tolerate upto 1000,000 ppb of As^{+3} and 500,000 ppb of As^{+5}. This strain can accumulate and also transform 90% of As^{+3} to As^{+5}. As As^{+5} is absorbed into iron oxyhydroxides and get immobilize thus a remediation mechanism can be designed with this strain. Banerjee et al, 2011 isolated 10 bacterial isolates, of which seven isolates belonged to γ-*proteobacterium,* two isolates belonged to *Firmicutes* and one was identified as *Kocuria* genera and all of these bacterial strains could be conveniently used for bioremediation.

5.3 Materials and methods

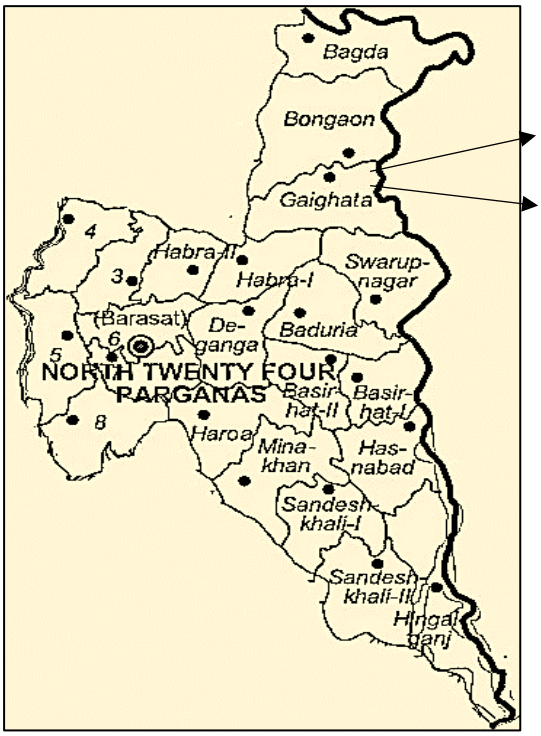


Fig 5.1: Sites of sample collection for Bioremediation Pot study

5.3.1 Study area

For this study, soil samples were collected from highly contaminated fields of Teghoria and moderately contaminated fields of Madhusudankathi, both situated in Gaighata block in North 24 Parganas, West Bengal. These two villages have been chosen based on the background study as reported by Roychowdhury et al, 2010 which establishes the block of Gaighata located in North 24 Parganas as As contaminated. Soil samples were collected during the ripening phase of post monsoon cultivation (in the month of October).

5.3.2 Reagents used

32) Nutrient Broth (SRL)
33) Agar (SRL)
34) 0.9% Saline solution (Sodium chloride (NaCl)) (Merck)
35) Hydrochloric acid (HCl) (37%) (Merck)
36) Concentrated Nitric acid (HNO_3) (69% v/v) (Merck)
37) Hydrogen peroxide (H_2O_2) (30% v/v) (Merck)

38) Potassium iodide (KI) (Merck)

39) Sodium borohydride ($NaBH_4$) (Merck)

40) Sodium hydroxide pellets (NaOH) (Merck)

41) Double distilled water

All chemicals used were of analytical grade.

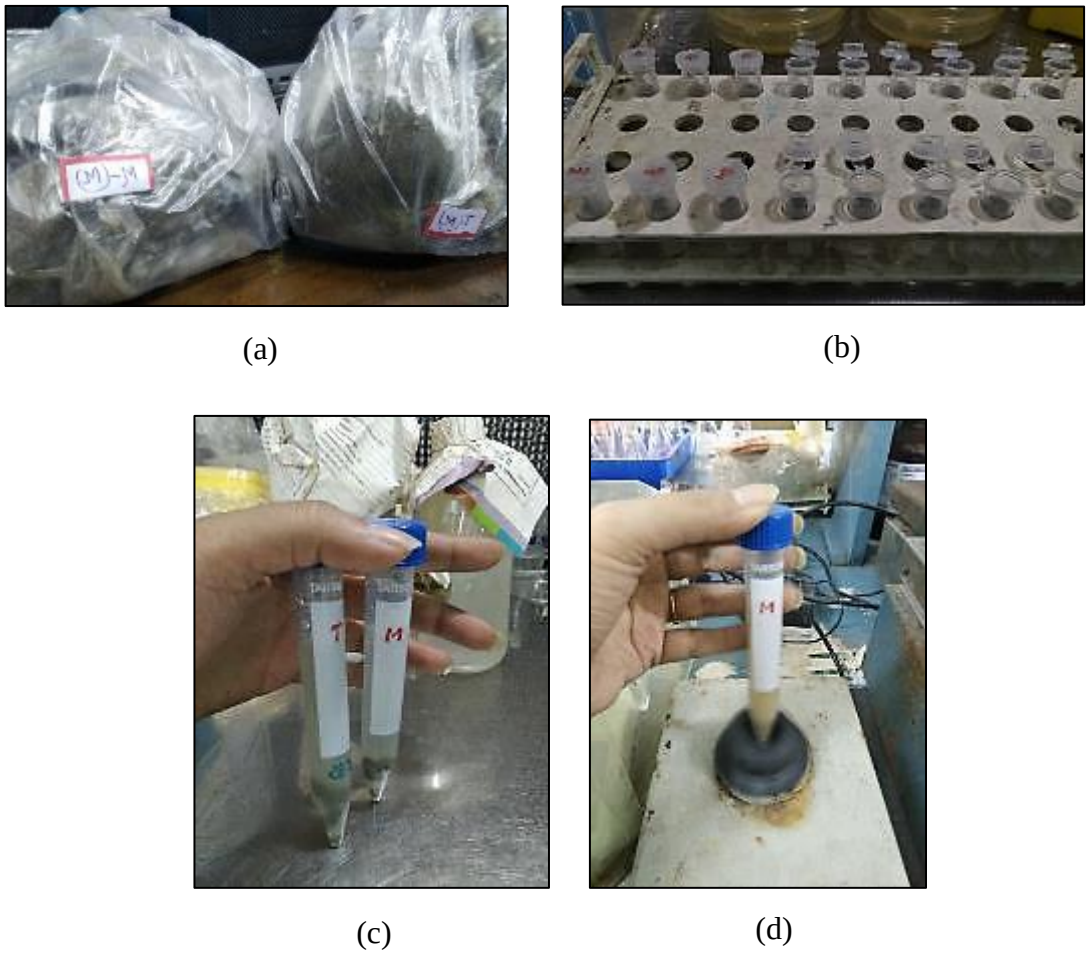
5.3.3 Methodology

5.3.3.1 Preparation of bacterial consortium with the selected isolates

Initially, eight autoclaved test tubes containing 5 ml of Nutrient broth each, were inoculated with eight selected isolates (i.e. K I44, H 120, J 131, F 104, E 71, C 38, B 14 and I 124) each and incubated overnight at 37 °C. The following day, CFU/ml of each of the isolates was estimated by taking 100 µl of each of the culture and serially diluting it, followed by spreading on Nutrient agar plates. Based on the CFU values, a microbial consortium was prepared in an autoclaved 50 ml Falcon tube by mixing 3.5 ml of bacterial cultures from each of the eight culture tubes and making up the volume up to 30 ml by adding 2 ml of 0.9% saline solution. After proper mixing of the consortium, the CFU count of the consortium was taken by taking 100 µl of the consortium and serially diluting it and then spreading it on Nutrient agar plates.

Fig 5.2 Preparation of bacterial consortium

5.3.3.2 Sample collection and estimation of CFU/ml of soil samples



(a) (b)

(c) (d)

Fig 5.3 Sample collection and data interpretation (a) samples collected from the field (b) serial dilution (c) water washing of the soil samples (d) vortexing of the soil samples

Soil samples of 1 kg each were collected from the highly As affected fields of Teghoria and moderately affected fields of Madhusudankathi, both situated in Gaighata block of North 24 Parganas, West Bengal aseptically. Approximately 0.1 gm of both the soil samples were serially diluted upto 10^8 dilution and plated on Nutrient agar plates for estimation of the CFU count/ml. Besides, the As estimation of the initial soil samples was also done by Hot plate digestion as mentioned earlier (Sub-section 2.3). Approximately 1gm of both the soil samples were washed with 10 ml of double distilled water and As content of the soil washed water was measured.

5.3.3.3 Setting up of the pot experiment

First of all, eight conical flasks were taken. Four conical flasks (1, 2, 3, 4) were first autoclaved and then filled with 200 gm of soil each aseptically (two conicals filled with soil from Teghoria and the other two with that of Madhusudankathi); whereas the other four flasks (5, 6, 7, 8) were first filled with 100gm of soil each (two with that of Teghoria and the other two with Madhusudankathi) and then autoclaved. In the first case, all the four conicals contained soil along with the indigenous microbes and in the second case, all the microbes present in the soil were killed. Next, 5 ml of the prepared consortium was added aseptically to the first four conicals (1, 2, 3, 4) containing 200 gm of soil each and 2.5 ml of the consortium was added to the other four conicals (5, 6, 7, 8) containing 100 gm of soil each so that the consortium: soil ratio remains constant in all the eight pots. Therefore in pots 1, 2, 3 and 4 the consortia added will grow under control conditions whereas in pots 5, 6, 7, and 8 the bacterial population in the consortia will grow together with the indigenous microbes present in the soil itself. In conicals 2 and 3, 100 ml and in conicals 5 and 6, 50 ml of autoclaved double distilled water was added maintaining the soil: water ratio to mimic anaerobic conditions similar to that of the paddy fields. The pot no. together with the varied parameters are tabulated in Table 5.1.

Table 5.1: Setting up of the pots containing soil from the two fields with added consortium and varied aerobic and anaerobic conditions

Pot No.	Conditions prevailing
1	Teghoria aerobic control
2	Madhusudankathi anaerobic control
3	Teghoria anaerobic control
4	Madhusudankathi aerobic control
5	Teghoria anaerobic indigenous
6	Madhusudankathi anaerobic indigenous
7	Teghoria aerobic indigenous
8	Madhusudankathi aerobic indigenous

The pots were then sealed and left undisturbed and readings of total As content were taken at an interval of 15 days to check the efficiency of the prepared consortium.


Fig 5.4: Setting up of the pot experiment

5.3.3.4 Estimation of total CFU/ml of the soil and total As content in the pots

Initially, approximately 0.1 gm of the soil samples from the eight pots were serially diluted upto 10^5 dilution and plated on Nutrient agar plates for estimation of the CFU count/ml. Moreover, approximately 1gm of the eight soil samples were washed with 5 ml of double distilled water and As content of the soil washed water was estimated. From this 1gm of soil, 0.1 gm was taken for As estimation by total digestion as mentioned earlier (Section 2.3). The As concentration of the water of the four anaerobic pots were analysed directly. After an interval of 15 days, the same set of parameters were evaluated for the eight pots. After the conditions were stabilized and the CFU was seen to increase, indicating the adaptation of the bacterial population in the consortium to the environment, the biomass harvesting was started. On the 30^{th} day, besides the CFU count of the soil, As concentration of the total digestion of the soil, As concentration of the soil washed water and As concentration of the supernatant water of anaerobic pots, 3 more parameters were evaluated. Approximately 1ml of supernatant water taken from the anaerobic pots for As estimation was centrifuged twice at 8000 rpm in a mini-centrifuge to harvest the biomass present in the water (2. 3, 5 and 6). The As concentration accumulated inside the biomass was then measured by hot plate digestion of the harvested biomass. The supernatant was used for estimated of As of the water of the anaerobic pots. Similarly, approximately 1 gm of soil taken from each of the eight pots (for water washing followed by total digestion) was first vortexed for 15 mins by adding 5 ml of autoclaved double distilled water so that the biomass present in the soil is suspended in the water, followed by centrifugation at 1000 rpm (at which the soil settles down but the biomass remains suspended in the water). The pellet was used for estimation of As concentration of the soil by total digestion. The supernatant was extracted and subjected to centrifugation at 8000 rpm (at which the biomass settles down) in a mini-centrifuge to harvest the biomass. The supernatant was

then analysed to estimate the As concentration of the soil washed water and the As concentration accumulated inside the biomass was then measured by hot plate digestion of the harvested biomass (pellet). Readings of the above mentioned parameters were taken at an interval of every 15 days up to the 90th day.

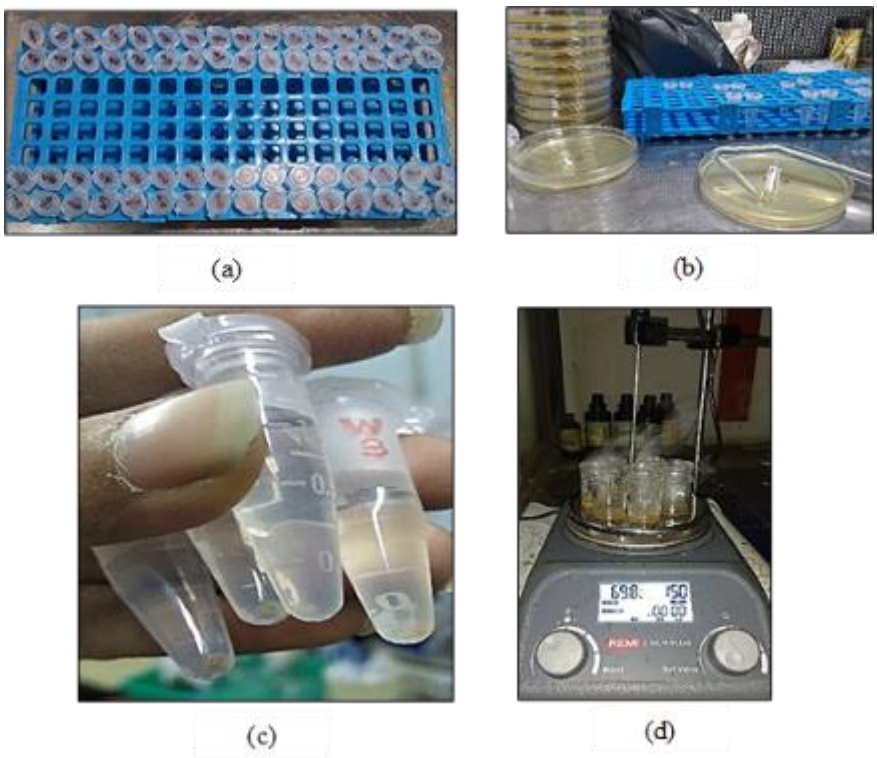


Fig 5.5: Estimation of total CFU/ml of the soil and total As content in the pots (a) serial dilution (b) spread plate technique (c) biomass harvested (d) hot plate digestion of the biomass

5.4 Results and discussion

5.4.1 Initial CFU counts of the colonies, consortium and indigenous soil samples

Before preparation of the bacterial consortium the CFU count/ml of the eight individual colonies are tabulated in Table 5.2. The CFU count of the prepared bacterial consortium was noted to be $23X10^9$. The CFU/ml of the soil samples collected from Madhusudankathi and Teghoria were found to be $267X10^6$ and $8X10^9$ respectively.

Table 5.2: CFU/ml of individual colonies before preparation of the consortium

Name of the colonies	CFU/ml ($X10^7$)
K 144	300
H 120	1700
J 131	20
F 104	10
E 71	110
C 38	170
B 14	100
I 124	200

5.4.2 CFU count of the soil of the pots and the water of the anaerobic pots

The average CFU count/ml of the soil from the eight pots and water from the four anaerobic pots during the 90 days study period are tabulated in Table 5.3 and 5.4 respectively.

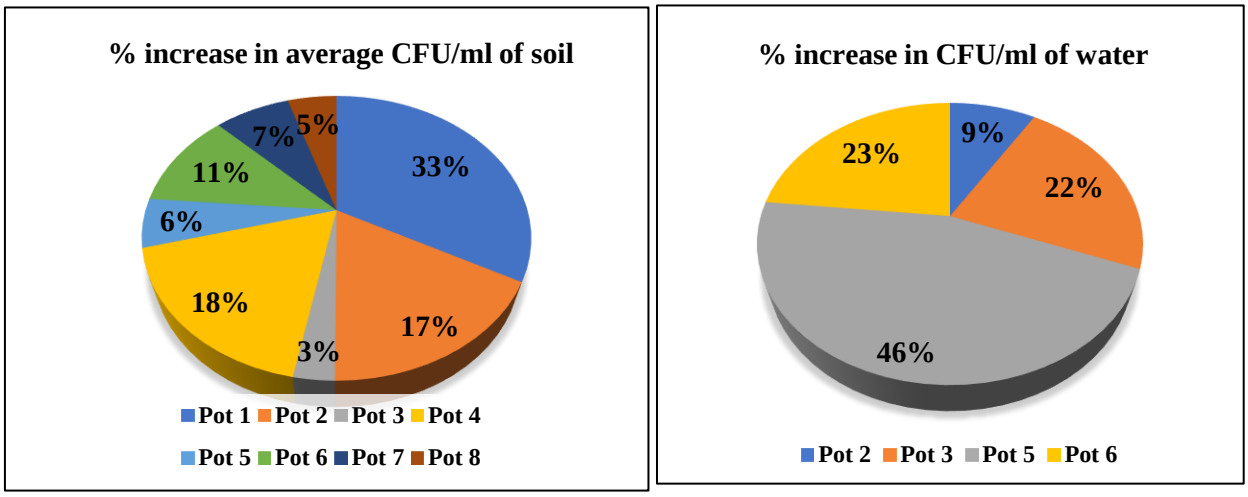
Table 5.3: Average CFU/ml of the soil of eight pots during 90 days study period

Pot No.	Average CFU/ml ($X10^5$)						
	0 day	15 days	30 days	45 days	60 days	75 days	90 days
Pot 1	247	555	1165	1995	4240	5530	6545
Pot 2	473	885	1090	4550	6145	6460	6775
Pot 3	537	360	805	1575	1430	1500	1725
Pot 4	395	815	1460	2610	4555	4890	5845
Pot 5	160	340	300	685	640	635	850
Pot 6	515	690	1355	2265	3865	4540	4980
Pot 7	451	525	1830	2275	2310	2710	3090
Pot 8	493	545	905	1375	2120	2190	2370

Table 5.4: CFU/ml of the water of 4 anaerobic pots during 90 days study period

Pot No.	CFU/ml (X10^6)				
	30 days	45 days	60 days	75 days	90 days
Pot 2	620	630	710	780	810
Pot 3	570	820	920	950	1020
Pot 5	40	70	90	80	105
Pot 6	60	80	90	70	110

Therefore, the steady increase in average CFU count of the soil of the eight pots signifies the adaptation of the applied consortium in control environment as well as along with the indigenous microbes in both aerobic and anaerobic conditions. The percentage increase in CFU/ml in the soil (represented in Fig 5.6a) of control pots 1, 2, 3 and 4 was found to be greater than pots 5, 6, 7 and 8 (containing indigenous microbes). Therefore the microbial consortium grows faster in control conditions than when applied together with the indigenous microbes of the soil. The percentage increase in CFU/ml in the water of anaerobic pots 2, 3, 5 and 6 (represented in Fig 5.6b) was noted and it was found that the water in anaerobic indigenous pots i.e. 5 and 6 had greater CFU count/ml than that of control anaerobic pots 2 and 3.



(a) (b)

Fig 5.6: % increase in CFU/ml of soil and water from the pots over 90 days study period

5.4.3 Estimation of the As concentration in the soil, soil washed water, water of the pots, biomass of the soil and water

5.4.3.1 Estimation of As concentration in the soil of the pots

The As concentration of the acid digested soil samples of the eight pots during the period of 90 days are tabulated in Table 5.5.

Table 5.5: Estimation of As concentration in the acid digested soil samples of the eight pots over 90 days study period

Pot No.	Arsenic concentration (µg/kg)						
	0 Day	15 Days	30 Days	45 Days	60 Days	75 Days	90 Days
Pot 1	18069	17270	15593	15517	14612	13998	13669
Pot 2	6070	5912	5865	5296	5079	5058	4858
Pot 3	19328	18922	17022	16024	15184	14658	13737
Pot 4	6490	6217	5612	5397	4640	4595	4317
Pot 5	20332	19145	18000	17384	17285	16052	15968
Pot 6	6271	5774	5625	5484	5430	5170	4606
Pot 7	18452	16720	14542	14466	14084	13101	13197
Pot 8	5654	5426	5013	4857	4521	4509	4276

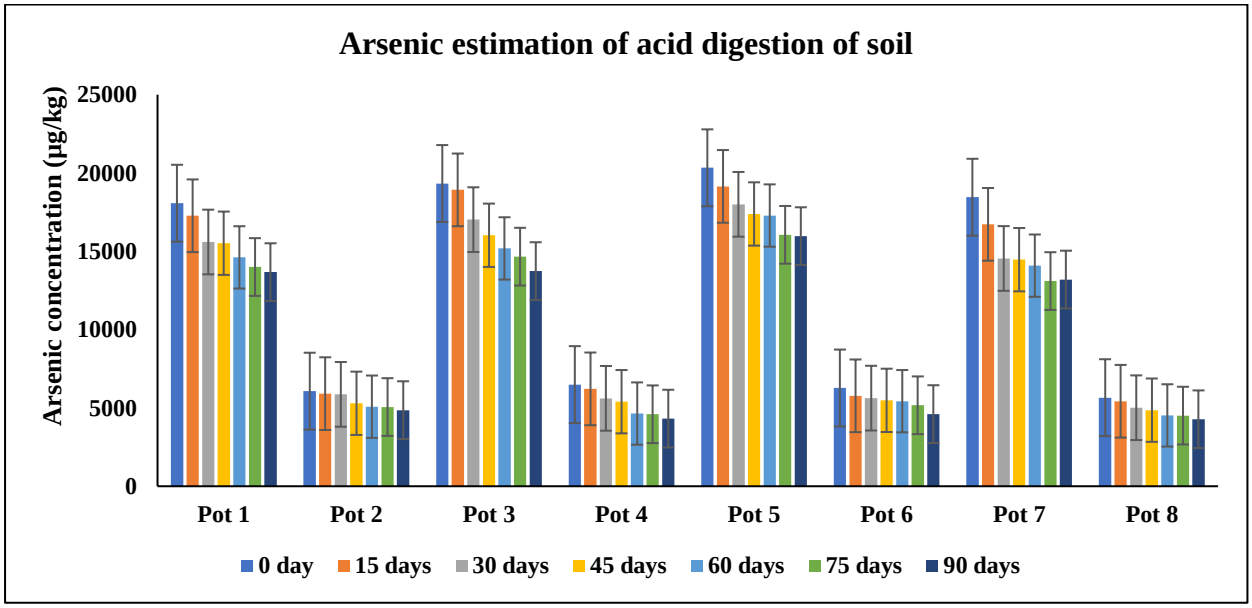


Fig 5.7: Bar diagram of the As concentration in acid digested soil samples of the eight pots over 90 days study period, error bars indicate 5% error

From Fig 5.7, it is seen that the As concentration in the acid digested soil samples of the eight pots are reducing gradually over a period of 90 days. In pots 1, 3, 5 and 7, i.e. soil samples of

Teghoria, the gradual reduction is more prominent than that of pots 2, 4, 6 and 8, i.e. soil samples of Madhusudankathi. The decrease in As concentration in the soil samples with the increase in CFU/ml of the bacterial population in the eight pots is represented by the scatter plot in Fig 5.8.

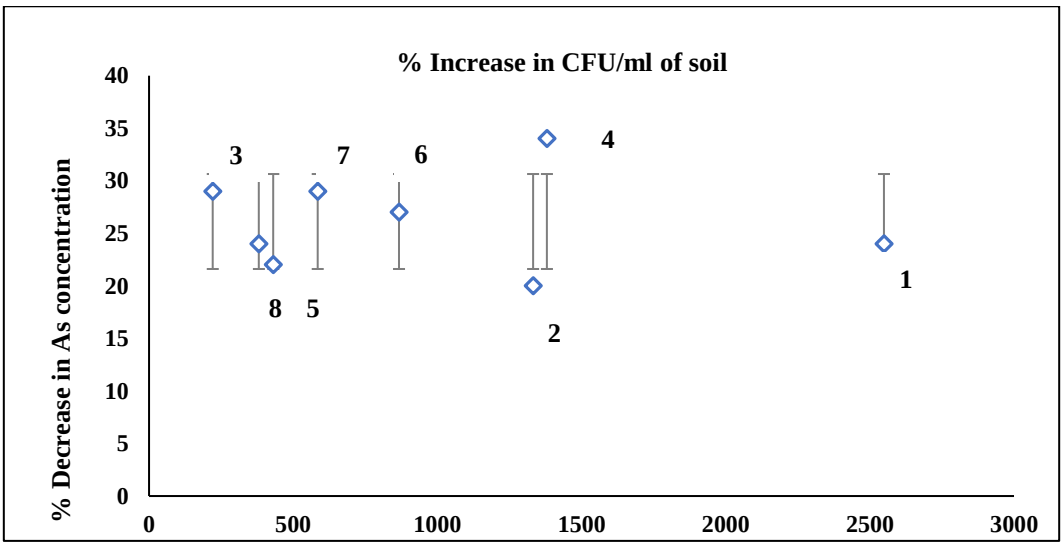


Fig 5.8: Scatter plot of % increase in CFU/ml with % decrease of As concentration of soil, the numbers indicate Pot numbers, error bars indicate standard deviation

5.4.3.2 Estimation of As concentration in the soil washed water

The As concentration of soil washed water of the eight pots during the period of 90 days are tabulated in Table 5.6.

Table 5.6: Estimation of As concentration in the acid digested soil samples of the eight pots over 90 days study period

Pot No.	Arsenic concentration (µg/L)						
	0 Day	15 Days	30 Days	45 Days	60 Days	75 Days	90 Days
Pot 1	22.51	26.55	35.95	37.75	39	41.01	53.13
Pot 2	6.31	6.625	8.55	15.25	19.2	47.13	57.33
Pot 3	12.31	15.52	18.85	31.65	33.22	50	59.01
Pot 4	8.87	19.37	24.75	25.81	27.27	32.4	37.26
Pot 5	8.68	10.38	14.58	19.812	23.95	24.83	29.56
Pot 6	4.05	5.4	6.77	8.32	9.075	12.76	11.01
Pot 7	8.88	8.5	35.95	36.43	37.25	37.75	38.125
Pot 8	2.98	2.75	6.65	11.12	12.81	15.95	17.81

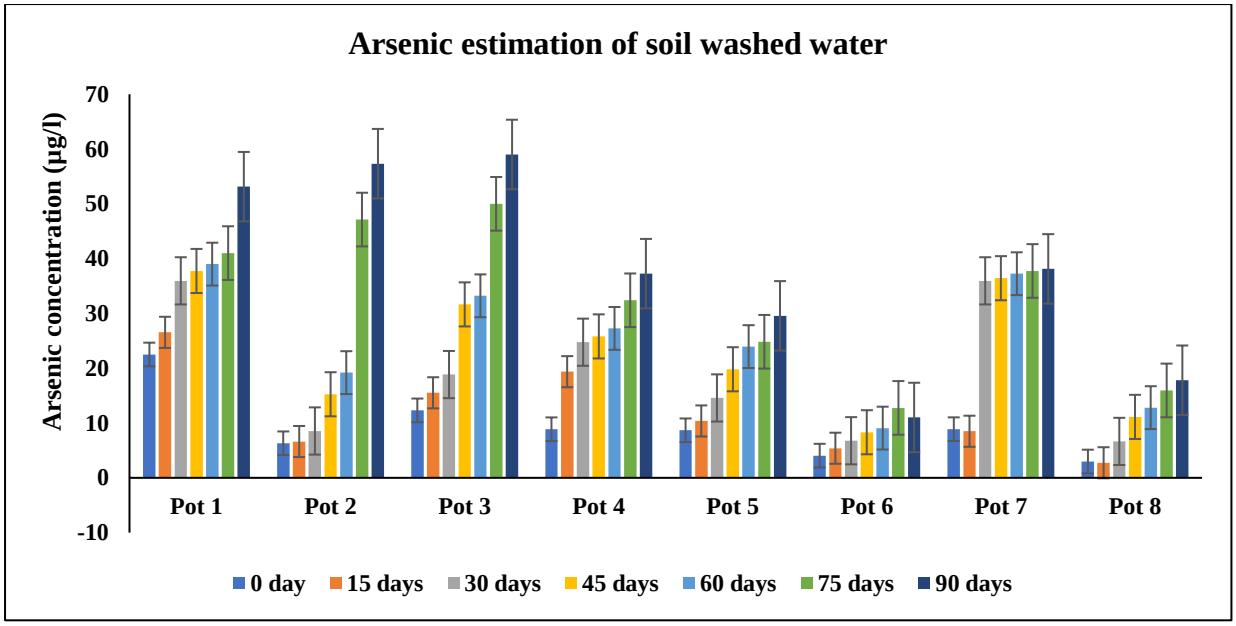


Fig 5.9: Bar diagram of the As concentration in soil washed water of the eight pots over 90 days study period, error bars indicate 5% error

From Fig 5.9, it is seen that the As concentration in soil washed water of the eight pots are increasing gradually over a period of 90 days. With passing days, the rate of leaching out of the As from the soil increases and shows maximum effect in case of control pots 1, 2, 3 and 4 compared to indigenous pots 5, 6 and 8 with the exception of Pot 7 . Hence it can be concluded that the bacterial consortium is capable of loosening the soil bound As and suspending them into water in control pots (1, 2, 3 and 4) but is not as much effective when growing with indigenous microbes as in pots 5, 6, 7 and 8.

5.4.3.3 Estimation of As concentration in the biomass of soil of the pots

The As concentration of the acid digested biomass of the soil samples of the eight pots during the period of 90 days are tabulated in Table 5.7.

Table 5.7: Estimation of As concentration in the acid digested biomass of the soil samples of the eight pots over 90 days study period

Pot No.	Arsenic concentration (µg/g of soil)				
	30 Days	45 Days	60 Days	75 Days	90 Days
Pot 1	250	652	1021	1765	1796
Pot 2	157.5	235	385	582.5	1181
Pot 3	412.5	485	515	782.5	950
Pot 4	152.5	258	387	617.5	742
Pot 5	202.5	292	357	402.5	586
Pot 6	130	184	225	440	476
Pot 7	157.5	432	677	997.5	1303
Pot 8	150	253	378	515	715

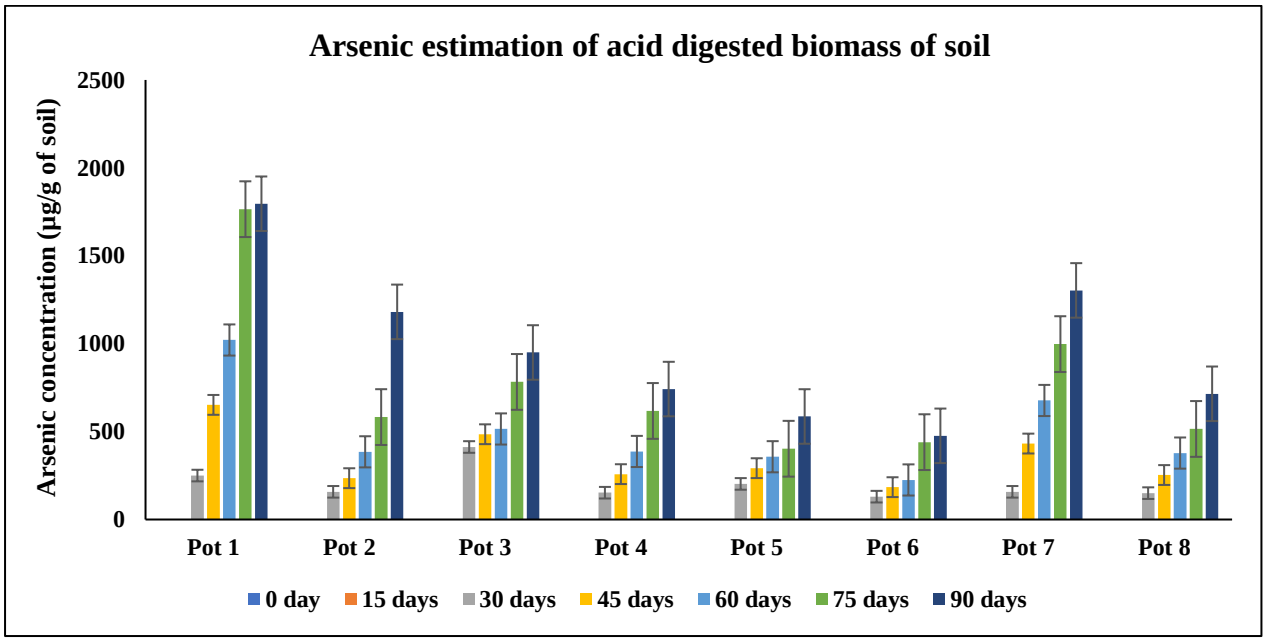


Fig 5.10: Bar diagram of the As concentration in acid digested biomass of the soil samples of the eight pots over 90 days study period, error bars indicate 5% error

From Fig 5.10, it is seen that the As concentration in the acid digested biomass of soil samples of the eight pots are increasing gradually over a period of 90 days. Accumulation of As in bacterial biomass was found to be greater in Pots 1, 3, 5 and 7 containing highly contaminated soil samples from Teghoria than Pots 2, 4, 6 and 8 containing moderately contaminated soil

samples from Madhusudankathi. The microbial consortia was seen to accumulate As more effectively in control pots (1, 2, 3 and 4) than indigenous pots (5, 6 and 8 with the exception of Pot 7).

5.4.3.4 Estimation of As concentration in the supernatant water of anaerobic pots

The As concentration of the supernatant water of the four anaerobic pots during the period of 90 days are tabulated in Table 5.8.

Table 5.8: Estimation of As concentration in the water of four anaerobic pots over 90 days study period

Pot No.	Arsenic concentration (µg/L)						
	0 Day	15 Days	30 Days	45 Days	60 Days	75 Days	90 Days
Pot 2	11.51	12.21	13.92	12.36	12.12	18.24	30.87
Pot 3	24.21	26.5	26.1	23.1	19.98	20.85	32.13
Pot 5	2.02	2.78	3.27	3.69	9.05	12.72	21.84
Pot 6	4.28	5.27	5.28	4.95	5.13	15.63	25.44

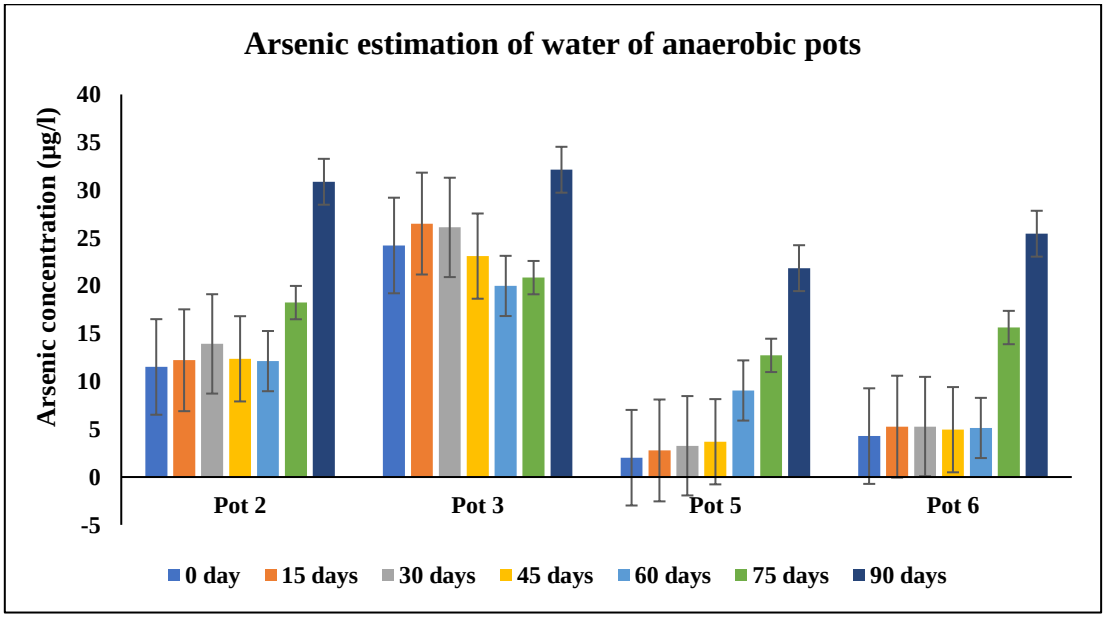


Fig 5.11: Bar diagram of the As concentration in water of the four anaerobic pots over 90 days study period, error bars indicate 5% error

From Fig 5.11, it is seen that the As concentration in the supernatant water of the four anaerobic pots are increasing gradually over a period of 90 days. The As concentration in the water of anaerobic pots 2 and 3 was found to be greater than the indigenous anaerobic pots 5 and 6. The reason behind this is the same as explained in Sub-section 5.4.3.2 i.e. the bacterial consortium

is capable of loosening the soil bound As and suspending them into water in control pots (2, 3) but is not as much effective when growing with indigenous microbes as in pots 5 and 6.

5.4.3.5 Estimation of As concentration in the biomass of water of anaerobic pots

The As concentration of the biomass of water of the four anaerobic pots during the period of 90 days are tabulated in Table 5.9.

Table 5.9: Estimation of As concentration in the biomass of water of four anaerobic pots over 90 days study period

Pot No.	Arsenic concentration (µg/ml of water)				
	30 Days	45 Days	60 Days	75 Days	90 Days
Pot 2	33.5	49.2	62	88.5	92.6
Pot 3	29.5	79.8	105	190	254.8
Pot 5	11.5	24.6	77.4	115	91.6
Pot 6	13	27.2	59.6	89	92.6

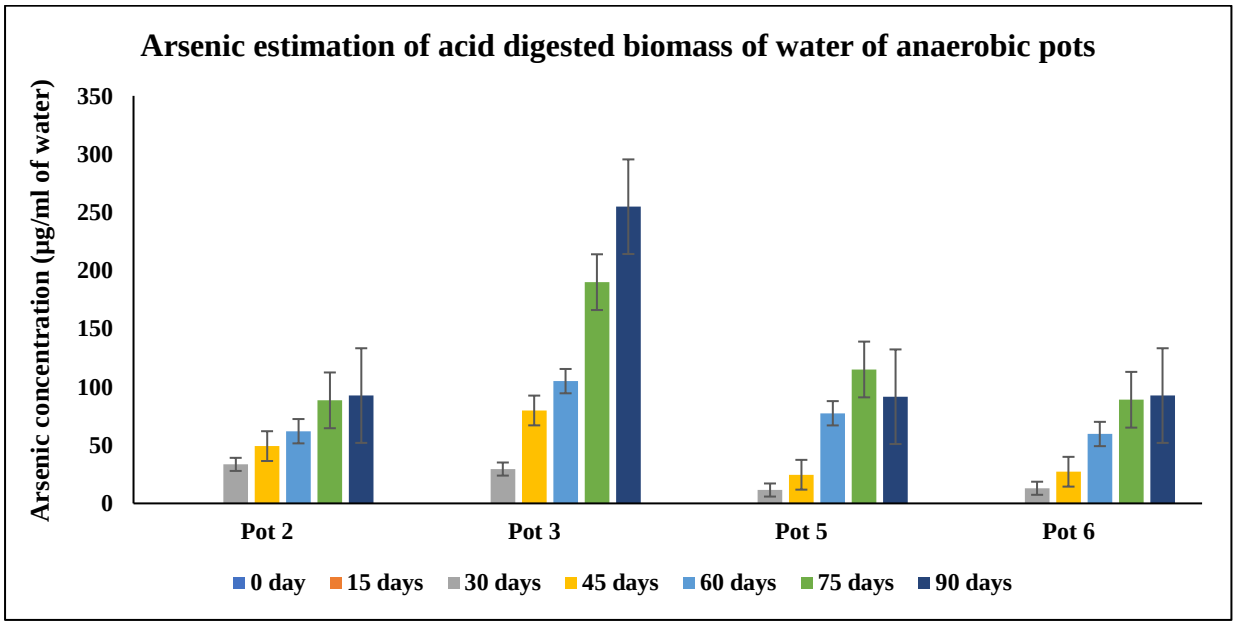


Fig 5.12: Bar diagram of the As concentration in the biomass of water of the four anaerobic pots over 90 days study period, error bars indicate 5% error

From Fig 5.12, it is seen that the As concentration in the biomass of water of the four anaerobic pots are increasing gradually over a period of 90 days. Maximum As concentration in the acid digested biomass of water was found in control Pot 3 which contains highly contaminated soil of Teghoria compared to control Pot 2 containing moderately contaminated soil of Madhusudankathi as well as the indigenous pots of 5 and 6.

5.4.4 Mass balance of the As content in different parts of the experimental set up

Mass balance of the different parts of the experimental set-up day at an interval of 15days from the 0^{th} day till the 90^{th} day was evaluated. In case of the aerobic pots (1, 4, 7 and 8) the As content of the soil, soil washed water and bacterial biomass of the soil was taken into account and for the anaerobic pots (2, 3, 5 and 6), the As content of the soil, soil washed water, bacterial biomass of the soil, the water and the bacterial biomass of the water was taken into account. The mass balance of the study period is tabulated in Table 5.10.

Table 5.10: Mass balance of As content of the eight experimental set ups over 90 days study period

Pot No.	Arsenic content (µg)						
	0 day	**15 days**	**30 days**	**45 days**	**60 days**	**75 days**	**90 days**
Pot 1	3614 + 23 =**3637**	3454 + 27 =**3481**	3119 + 29 + 44 =**3192**	3103 + 39 + 130 =**3272**	2923 + 39 +204=**3166**	2800 + 42 +353=**3196**	2740 + 53 + 360 =**3154**
Pot 2	1214 + 6 +1=**1221**	1183 + 7 + 1=**1191**	1173 + 9 + 32 + 1 + 3 =**1218**	1059 + 15 + 47 + 1 + 5 =**1127**	1016 + 19 + 77 + 1 + 6 =**1119**	1012 + 47 + 117 + 2 + 9 =**1187**	972 + 57 + 236 + 3 + 9 =**1277**
Pot 3	3866 + 12 + 2=**3880**	3784 + 16 + 3=**3803**	3404 + 19 + 83 + 3 +3=**3512**	3205 + 32 + 97 + 2 + 8=**3344**	3037 + 33 + 103 + 2 + 11=**3186**	2932 + 50 + 157 + 2 + 19=**3160**	2747 + 59 + 190 + 3 + 25 =**3025**
Pot 4	1298 + 9 =**1306**	1244 + 20 =**1264**	1123 + 25 + 31=**1179**	1080 + 26 + 52 =**1158**	928 + 28 + 78=**1034**	919 + 33 + 124=**1076**	864 + 38 + 150 =**1052**
Pot 5	2033 + 4 + 0.1 =**2037**	1915 + 5 + 0.1 =**1920**	1800 + 7 + 20 + 0.2 + 0.6 =**1828**	1738 + 10 + 29 + 0.1 + 1=**1778**	1729 + 12 + 36 + 0.5 + 4=**1782**	1605 + 12 + 40 + 0.6 + 6=**1664**	1597 + 15 + 59 + 1 + 5=**1677**
Pot 6	627 + 2 + 0.2=**629**	577 + 3 + 0.3=**580**	563 + 3 + 13 + 0.3 + 0.7=**580**	548 + 4 + 18 + 0.2 + 1= **571**	543 + 5 + 23 + 0.2 + 3=**574**	517 + 6 + 44 + 0.8 + 4 =**571**	461 + 6 + 48 + 1 + 5 =**521**
Pot 7	1845 + 4 =**1849**	1672 + 4 =**1676**	1454 + 18 + 16 =**1488**	1446 + 18 + 43 =**1507**	1408 + 19 + 68=**1495**	1310 + 19 + 100=**1429**	1320 + 19 + 130 =**1469**
Pot 8	564 + 1 =**565**	543 + 1 =**544**	501 + 3 + 15=**519**	486 + 6 + 25 =**517**	452 + 6 + 38=**496**	451 + 8 + 52=**511**	428 + 9 + 72=**509**

For the aerobic pots (1, 4, 7 and 8), the mass balance is given as As content in µg in the form of (acid digestion of soil + soil washed water + acid digestion of biomass in soil) and for anaerobic pots (2, 3, 5 and 6), in the form of (acid digestion of soil + soil washed water + acid digestion of biomass in soil + water + acid digestion of biomass of water). The mean As content of control Pots 1, 2, 3 and 4 are 3300±187 µg, 1191±55 µg, 3416±329 µg and 1153±105 µg respectively and that of the indigenous pots 5, 6, 7 and 8 are 1812±132 µg, 575±31 µg, 1559±150 µg and 523±24µg respectively.

5.5 Conclusion and future scope

5.5.1 Conclusion

The study of the addition of the consortium prepared with eight best performing bacterial isolates to contaminated soil samples under varying conditions indicate that the consortium had started working effectively in the experimental set-up. The bacterial consortium worked better individually than when applied together with the indigenous microbes. The activity of the consortium was better when applied to highly contaminated soil of Teghoria than moderately contaminated soil of Madhusudankathi. The percentage increase in CFU/ml of the bacterial population signifies the adaptation of the consortium in control as well as indigenous pots under aerobic and anaerobic conditions. The As concentration in the acid digested soil was found to decrease with time whereas the concentration of As leaching out in the soil washed water was found to increase over the span of study period. The As concentration in the supernatant water of anaerobic pots was seen to increase with time indicating the capability of the bacterial isolates in the consortia of loosening and thereby mobilizing the As which is otherwise bound tightly to the soil. The concentration of As accumulated in the biomass of soil as well as that of water was seen to increase indicating the capability of the applied consortium in accumulating As from the highly contaminated as well as moderately contaminated soil by the mechanisms of absorption and adsorption. Lastly, the mass balance is seen to be almost constant for the 0^{th} day, 15^{th} day, 30^{th} day, 45^{th} day, 60^{th} day, 75^{th} day as well the 90^{th} day. Maximum standard deviation of ± 329 μg was found in Pot 3. The high values of standard deviation can be explained by the fact that some of the microbes present in the consortium as well as the indigenous microbes might be capable of biomethylation by which they can convert the As present in soil into gaseous Arsine gas, the content of which could not be measured or taken into account in the present experimental set-up.

5.5.2 Future scope

a) The study could be continued for a period of another 90 days more to prove the effectiveness of the bacterial consortium.
b) The working principle of the consortium could be checked when added with some external carbon source like acetate, glucose and other organic compounds.
c) Moreover the consortium could be applied to paddy field conditions to check its applicability in real life scenario.

REFERENCES

1. Abedin, M. J., & Meharg, A. A. (2002). Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (Oryza sativa L.). *Plant and soil*, *243*(1), 57-66.
2. Abedin, M. J., Cotter-Howells, J., & Meharg, A. A. (2002). Arsenic uptake and accumulation in rice (Oryza sativa L.) irrigated with contaminated water. *Plant and soil*, *240*(2), 311-319.
3. Abedin, M. J., Feldmann, J., & Meharg, A. A. (2002). Uptake kinetics of arsenic species in rice plants. *Plant physiology*, 128(3), 1120-1128.
4. Achal, V., Pan, X., Fu, Q., & Zhang, D. (2012). Biomineralization based remediation of As (III) contaminated soil by Sporosarcina ginsengisoli. *Journal of hazardous materials*, *201*, 178-184.
5. Adriano, D. C. (2001). Arsenic. In *Trace elements in terrestrial environments* Springer, New York, NY, 219-261.
6. Akhtar, M. S., Chali, B., & Azam, T. (2013). Bioremediation of arsenic and lead by plants and microbes from contaminated soil. *Research in plant sciences*, *1*(3), 68-73.
7. Alvarado, S., Guédez, M., Lué-Merú, M. P., Nelson, G., Alvaro, A., Jesús, A. C., & Gyula, Z. (2008). Arsenic removal from waters by bioremediation with the aquatic plants Water Hyacinth (Eichhornia crassipes) and Lesser Duckweed (Lemna minor). *Bioresource technology*, *99*(17), 8436-8440.
8. Arnon, D. I. (1949). Copper enzyme in isolated chloroplast: Polyphenol oxidase in Beta vulgaris. *Plant phyisol*. 24: 1-15.
9. Arora, N., Gulati, K., Tripathi, S., Pruthi, V., & Poluri, K. M. (2018). Algae as a Budding Tool for Mitigation of Arsenic from Aquatic Systems. *In Mechanisms of arsenic toxicity and tolerance in plants*, Springer, Singapore, 269-297.
10. Artus, N. N. (2006). Arsenic and cadmium phytoextraction potential of crambe compared with Indian mustard. *Journal of plant nutrition*, *29*(4), 667-679.
11. Atagana, H. I. (2008). Compost bioremediation of hydrocarbon-contaminated soil inoculated with organic manure. *African journal of biotechnology*, *7*(10).
12. Atlas, R. M., & Raymond, R. L. (1977). Stimulated petroleum biodegradation. *CRC critical reviews in microbiology*, *5*(4), 371-386.

13. Bachate, S. P., Cavalca, L., & Andreoni, V. (2009). Arsenic-resistant bacteria isolated from agricultural soils of Bangladesh and characterization of arsenate-reducing strains. *Journal of applied microbiology*, *107*(1), 145-156.
14. Bagga, D. K., & Peterson, S. (2001). Phytoremediation of arsenic-contaminated soil as affected by the chelating agent CDTA and different levels of soil pH. *Remediation journal: The journal of environmental cleanup costs, technologies & techniques*, *12*(1), 77-85.
15. Bahar, M. M., Megharaj, M., & Naidu, R. (2012). Arsenic bioremediation potential of a new arsenite-oxidizing bacterium Stenotrophomonas sp. MM-7 isolated from soil. *Biodegradation*, *23*(6), 803-812.
16. Baker, R. S., Barrentine, W. L., Bowman, D. H., Hawthorne, W. L., & Pettiet, J. V. (1976). Crop response and arsenic uptake following soil incorporation of MSMA. *Weed science*, 322-326.
17. Banerjee, S., Datta, S., Chattyopadhyay, D., & Sarkar, P. (2011). Arsenic accumulating and transforming bacteria isolated from contaminated soil for potential use in bioremediation. *Journal of environmental science and health, Part A*, *46*(14), 1736-1747.
18. Banerjee, S., Majumdar, J., Samal, A. C., Bhattachariya, P., & Santra, S. C. (2013). Biotransformation and bioaccumulation of arsenic by Brevibacillus brevis isolated from arsenic contaminated region of West Bengal. *IOSR J Environmental science toxicology and food technology*, *3*(1), 1-10.
19. Barrachina, A. C., Carbonell, F. B., & Beneyto, J. M. (1995). Arsenic uptake, distribution, and accumulation in tomato plants: effect of arsenite on plant growth and yield. *Journal of plant nutrition*, *18*(6), 1237-1250.
20. Bhattacharya, A. K. (2019). An Analysis of Arsenic Contamination in the Groundwater of India, Bangladesh and Nepal with a Special Focus on the Stabilisation of Arsenic Laden Sludge from Arsenic Filters, EJGE.
21. Bhattacharya, P., Frisbie, S. H., Smith, E., Naidu, R., Jacks, G., & Sarkar, B. (2002). Arsenic in the environment: a global perspective. *Handbook of heavy metals in the environment. Marcell Dekker Inc., New York*, 147-215.
22. Bhattacharya, P., Samal, A. C., Majumdar, J., & Santra, S. C. (2009). Transfer of arsenic from groundwater and paddy soil to rice plant (Oryza sativa L.): a micro level study in West Bengal, India. *World journal of agricultural sciences*, *5*(4), 425-431.

23. Bissen, M., & Frimmel, F. H. (2003). Arsenic—a review. Part I: occurrence, toxicity, speciation, mobility. *Acta hydrochimica et hydrobiologica*, *31*(1), 9-18.
24. Bleeker, P. M., Assunção, A. G., Teiga, P. M., de Koe, T., & Verkleij, J. A. (2002). Revegetation of the acidic, As contaminated Jales mine spoil tips using a combination of spoil amendments and tolerant grasses. *Science of the total environment*, *300*(1-3), 1-13.
25. Bogdan, K., & Schenk, M. K. (2008). Arsenic in rice (Oryza sativa L.) related to dynamics of arsenic and silicic acid in paddy soils. *Environmental science & technology*, *42*(21), 7885-7890.
26. Bose, P., & Sharma, A. (2002). Role of iron in controlling speciation and mobilization of arsenic in subsurface environment. *Water research*, *36*(19), 4916-4926.
27. Bundy, J. G., Paton, G. I., & Campbell, C. D. (2002). Microbial communities in different soil types do not converge after diesel contamination. *Journal of applied microbiology*, *92*(2), 276-288.
28. Cao, X., Ma, L. Q., & Shiralipour, A. (2003). Effects of compost and phosphate amendments on arsenic mobility in soils and arsenic uptake by the hyperaccumulator, Pteris vittata L. *Environmental pollution*, *126*(2), 157-167.
29. Čerňanský, S., Kolenčík, M., Ševc, J., Urík, M., & Hiller, E. (2009). Fungal volatilization of trivalent and pentavalent arsenic under laboratory conditions. *Bioresource technology*, *100*(2), 1037-1040.
30. Chakraborti, D., Das, B., Rahman, M. M., Chowdhury, U. K., Biswas, B., Goswami, A. & Hossain, A. (2009). Status of groundwater arsenic contamination in the state of West Bengal, India: A 20-year study report. *Molecular nutrition & food research*, *53*(5), 542-551.
31. Chakraborti, D., Sengupta, M. K., Rahman, M. M., Ahamed, S., Chowdhury, U. K., Hossain, A. & Quamruzzaman, Q. (2004). Groundwater arsenic contamination and its health effects in the Ganga-Meghna-Brahmaputra plain. *Journal of environmental monitoring*, *6*(6), 74.
32. Choudhury, B., Chowdhury, S., & Biswas, A. K. (2011). Regulation of growth and metabolism in rice (Oryza sativa L.) by arsenic and its possible reversal by phosphate. *Journal of plant interactions*, *6*(1), 15-24.
33. Chowdhury, N. R., Das, R., Joardar, M., Ghosh, S., Bhowmick, S., & Roychowdhury, T. (2018). Arsenic accumulation in paddy plants at different phases of pre-monsoon cultivation. *Chemosphere*, *210*, 987-997.

34. Chowdhury, N. R., Ghosh, S., Joardar, M., Kar, D., & Roychowdhury, T. (2018). Impact of arsenic contaminated groundwater used during domestic scale post harvesting of paddy crop in West Bengal: Arsenic partitioning in raw and parboiled whole grain. *Chemosphere*, *211*, 173-184.
35. Chowdhury, N. R., Joardar, M., Ghosh, S., Bhowmick, S., & Roychowdhury, T. (2019). Variation of Arsenic Accumulation in Paddy Plant with Special Reference to Rice Grain and Its Additional Entry During Post-harvesting Technology. In *Ground water development-issues and sustainable solutions* (pp. 289-303). Springer, Singapore.
36. Cox, M. S., Bell, P. F., & Kovar, J. L. (1996). Differential tolerance of canola to arsenic when grown hydroponically or in soil. *Journal of plant nutrition*, *19*(12), 1599-1610.
37. Cullen, W. R., & Reimer, K. J. (1989). Arsenic speciation in the environment. *Chemical reviews*, *89*(4), 713-764.
38. Das, D., Chatterjee, A., Mandal, B. K., Samanta, G., Chakraborti, D., & Chanda, B. (1995). Arsenic in ground water in six districts of West Bengal, India: the biggest arsenic calamity in the world. Part 2. Arsenic concentration in drinking water, hair, nails, urine, skin-scale and liver tissue (biopsy) of the affected people. *Analyst*, *120*(3), 917-924.
39. Das, D., Chatterjee, A., Samanta, G., Mandal, B., Chowdhury, T. R., Samanta, G., & Lodh, D. (1994). Arsenic contamination in groundwater in six districts of West Bengal, India: the biggest arsenic calamity in the world. *The analyst*, *119*(12), 168N.
40. Das, S., Jean, J. S., Kar, S., Chou, M. L., & Chen, C. Y. (2014). Screening of plant growth-promoting traits in arsenic-resistant bacteria isolated from agricultural soil and their potential implication for arsenic bioremediation. *Journal of hazardous materials*, *272*, 112-120.
41. Davies, B. H. (1965). Analysis of carotinoid pigments. InTW Goodwin, ed, *Chemistry and biochemistry of plant pigments.*
42. Dey, U., Chatterjee, S., & Mondal, N. K. (2016). Isolation and characterization of arsenic-resistant bacteria and possible application in bioremediation. *Biotechnology reports*, *10*, 1-7.
43. Dheeraj Pandey & Amit Kumar Singh (2018). Cancer caused by ground water Arsenic (As) contamination. *Scientific India (Health)*

44. Eisler, R. (2004). Arsenic hazards to humans, plants, and animals from gold mining. In *Reviews of environmental contamination and toxicology* (pp. 133-165). Springer, New York, NY.
45. Elektorowicz, M. (1994). Bioeemediation of petroleum-contaminated clayey soil with pretreatment. *Environmental technology*, *15*(4), 373-380.
46. Farnese, F. S., Oliveira, J. A., Lima, F. S., Leão, G. A., Gusman, G. S., & Silva, L. C. (2014). Evaluation of the potential of Pistia stratiotes L.(water lettuce) for bioindication and phytoremediation of aquatic environments contaminated with arsenic. *Brazilian journal of biology*, *74*(3), S108-S112.
47. Favas, P. J., Pratas, J., & Prasad, M. N. V. (2012). Accumulation of arsenic by aquatic plants in large-scale field conditions: opportunities for phytoremediation and bioindication. *Science of the total environment*, *433*, 390-397.
48. Fayiga, A. O. (2005). *Phytoremediation of arsenic-contaminated soil and groundwater* (Doctoral dissertation, University of Florida).
49. Finnegan, P., & Chen, W. (2012). Arsenic toxicity: the effects on plant metabolism. *Frontiers in physiology*, *3*, 182.
50. Fitz, W. J., & Wenzel, W. W. (2002). Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. *Journal of biotechnology*, *99*(3), 259-278.
51. Forsyth, J. V., Tsao, Y. M., & Bleam, R. D. (1995). *Bioremediation: when is augmentation needed?* (No. CONF-950483-). Battelle Press, Columbus, OH (United States).
52. Fries, J., Getrost, H., & Merck, D. E. (1977). *Organic reagents trace analysis*. E. Merck.
53. Ghosh, N. C., & Singh, R. D. (2009). Groundwater arsenic contamination in India: Vulnerability and scope for remedy.
54. Hossain, M. F. (2006). Arsenic contamination in Bangladesh—an overview. *Agriculture, ecosystems & environment*, *113*(1-4), 1-16.
55. Islam, S., Rahman, M. M., Islam, M. R., & Naidu, R. (2016). Arsenic accumulation in rice: consequences of rice genotypes and management practices to reduce human health risk. *Environment international*, *96*, 139-155.
56. Jahan, I., Hoque, S., Ullah, S. M., & Kibria, M. G. (2003). Effects of arsenic on some growth parameters of rice plant. *Dhaka University Journal of biological science*, *12*(1), 71-77.

57. Kang, L., Li, X., Liu, J., & Zhang, X. (1996). Effect of arsenic on growth development of rice and residual rules in the loam paddy soil. *Acta agriculturae universitatis jilinensis*, *18*(3), 58-61.
58. Kapaj, S., Peterson, H., Liber, K., & Bhattacharya, P. (2006). Human health effects from chronic arsenic poisoning–a review. *Journal of environmental science and health, Part A*, *41*(10), 2399-2428.
59. Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiology and molecular biology reviews*, *54*(3), 305-315.
60. Leung, M. (2004). Bioremediation: techniques for cleaning up a mess. *Bioteach journal*, *2*, 18-22.
61. Lievremont, D., Bertin, P. N., & Lett, M. C. (2009). Arsenic in contaminated waters: biogeochemical cycle, microbial metabolism and biotreatment processes. *Biochimie*, *91*(10), 1229-1237.
62. Lim, K. T., Shukor, M. Y., & Wasoh, H. (2014). Physical, chemical, and biological methods for the removal of arsenic compounds. *BioMed research international*, *2014*.
63. Lin, S. C., Chang, T. K., Huang, W. D., Lur, H. S., & Shyu, G. S. (2015). Accumulation of arsenic in rice plant: a study of an arsenic-contaminated site in Taiwan. *Paddy and water environment*, *13*(1), 11-18.
64. Liu, C. W., Lin, C. C., Jang, C. S., Sheu, G. R., & Tsui, L. (2009). Arsenic accumulation by rice grown in soil treated with roxarsone. *Journal of plant nutrition and soil science*, *172*(4), 550-556.
65. Liu, W. J., Zhu, Y. G., Hu, Y., Williams, P. N., Gault, A. G., Meharg, A. A., ... & Smith, F. A. (2006). Arsenic sequestration in iron plaque, its accumulation and speciation in mature rice plants (Oryza sativa L.). *Environmental science & technology*, *40*(18), 5730-5736.
66. Liu, W. J., Zhu, Y. G., Smith, F. A., & Smith, S. E. (2004). Do iron plaque and genotypes affect arsenate uptake and translocation by rice seedlings (Oryza sativa L.) grown in solution culture? *Journal of experimental botany*, *55*(403), 1707-1713.
67. Ma, L., Wang, L., Jia, Y., & Yang, Z. (2017). Accumulation, translocation and conversion of six arsenic species in rice plants grown near a mine impacted city. *Chemosphere*, *183*, 44-52.

68. Mahimairaja, S., Bolan, N. S., Adriano, D. C., & Robinson, B. (2005). Arsenic contamination and its risk management in complex environmental settings. *Advances in agronomy*, *86*, 1-82.
69. Majumder, A., Bhattacharyya, K., Bhattacharyya, S., & Kole, S. C. (2013). Arsenic-tolerant, arsenite-oxidising bacterial strains in the contaminated soils of West Bengal, India. *Science of the total environment*, *463*, 1006-1014.
70. Mallick, I., Hossain, S. T., Sinha, S., & Mukherjee, S. K. (2014). Brevibacillus sp. KUMAs2, a bacterial isolate for possible bioremediation of arsenic in rhizosphere. *Ecotoxicology and environmental safety*, *107*, 236-244.
71. Mandal, B. K., Chowdhury, T. R., Samanta, G., Mukherjee, D. P., Chanda, C. R., Saha, K. C., & Chakraborti, D. (1998). Impact of safe water for drinking and cooking on five arsenic-affected families for 2 years in West Bengal, India. *Science of the total environment*, *218*(2-3), 185-201.
72. Marin, A. R., Masscheleyn, P. H., & Patrick, W. H. (1992). The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration. *Plant and soil*, *139*(2), 175-183.
73. Marin, A. R., Pezeshki, S. R., Masschelen, P. H., & Choi, H. S. (1993). Effect of dimethylarsenic acid (DMAA) on growth, tissue arsenic, and photosynthesis of rice plants. *Journal of plant nutrition*, *16*(5), 865-880.
74. Masscheleyn, P. H., Delaune, R. D., & Patrick Jr, W. H. (1991). Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environmental science & technology*, *25*(8), 1414-1419.
75. McGrath, S. P., & Zhao, F. J. (2003). Phytoextraction of metals and metalloids from contaminated soils. *Current opinion in biotechnology*, *14*(3), 277-282.
76. Miah, M. M. A., Rahman, M. S., Islam, A., Paul, D. N. R., Farid, A. T. M., & Jahiruddin, M. (2005). Nationwide survey of arsenic in soils, water and crops in Bangladesh. Behaviour of arsenic in aquifers, soils and plants: implications for management. Arsenic Symposium, Dhaka, January 2005. *Abstracts). CIMMYT, Dhaka*.
77. Miteva, E., & Merakchiyska, M. (2002). Response of chloroplasts and photosynthetic mechanism of bean plants to excess arsenic in soil. *Bulgarian journal of agricultural science*.

78. Mkandawire, M., Taubert, B., & Dudel, E. G. (2004). Capacity of Lemna gibba L.(Duckweed) for uranium and arsenic phytoremediation in mine tailing waters. *International journal of phytoremediation*, *6*(4), 347-362.
79. Nakhro, N., & Dkhar, M. S. (2010). Populations and biomass carbon in paddy field soil. *Agron J*, *9*, 102-110.
80. Nickson, R., McArthur, J., Burgess, W., Ahmed, K. M., Ravenscroft, P., & Rahmanñ, M. (1998). Arsenic poisoning of Bangladesh groundwater. *Nature*, *395*(6700), 338.
81. Ohno, K., Matsuo, Y., Kimura, T., Yanase, T., Rahman, M. H., Magara, Y., & Matsui, Y. (2009). Effect of rice-cooking water to the daily arsenic intake in Bangladesh: results of field surveys and rice-cooking experiments. *Water science and technology*, *59*(2), 195-201.
82. Oremland, R. S., & Stolz, J. F. (2003). The ecology of arsenic. *Science*, *300*(5621), 939-944.
83. Patel, K. S., Sahu, B. L., Ramteke, S., & Bontempi, E. (2016). Contamination of paddy soil and rice with arsenic. *Journal of environmental protection*, *7*(05), 689.
84. Paul, S., Majumdar, S., & Giri, A. K. (2015). Genetic susceptibility to arsenic-induced skin lesions and health effects: a review. *Genes and environment*, *37*(1), 23.
85. Piehler, M. F., Swistak, J. G., Pinckney, J. L., & Paerl, H. W. (1999). Stimulation of diesel fuel biodegradation by indigenous nitrogen fixing bacterial consortia. *Microbial ecology*, *38*(1), 69-78.
86. Purakayastha, T. J. (2011). Microbial remediation of arsenic contaminated soil. In *Detoxification of heavy metals*, Springer, Berlin, Heidelberg, 221-260.
87. Rahman, M. A. (2004). Influence of soil arsenic concentrations on rice (Oryza sativa L.). *Subtropical agricultural resource and development*, *2*(3), 24-31.
88. Rahman, M. A., & Hasegawa, H. (2011). Aquatic arsenic: phytoremediation using floating macrophytes. *Chemosphere*, *83*(5), 633-646.
89. Rahman, M. A., Hasegawa, H., Rahman, M. M., Islam, M. N., Miah, M. M., & Tasmen, A. (2007). Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (Oryza sativa L.) varieties in Bangladesh. *Chemosphere*, *67*(6), 1072-1079.
90. Rahman, M. A., Hasegawa, H., Rahman, M. M., Miah, M. M., & Tasmin, A. (2008). Arsenic accumulation in rice (Oryza sativa L.): human exposure through food chain. *Ecotoxicology and environmental safety*, *69*(2), 317-324.

91. Rahman, M. A., Hasegawa, H., Rahman, M. M., Rahman, M. A., & Miah, M. A. M. (2007). Accumulation of arsenic in tissues of rice plant (Oryza sativa L.) and its distribution in fractions of rice grain. *Chemosphere*, *69*(6), 942-948.
92. Raskin, I., & Ensley, B. D. (2000). *Phytoremediation of toxic metals*. John Wiley and Sons.
93. Ravenscroft, P., Brammer, H., & Richards, K. (2009). *Arsenic pollution: a global synthesis* (Vol. 28). John Wiley & Sons.
94. Rhykerd, R. L., Crews, B., McInnes, K. J., & Weaver, R. W. (1999). Impact of bulking agents, forced aeration, and tillage on remediation of oil-contaminated soil. *Bioresource technology*, *67*(3), 279-285.
95. Roberts, L. C., Hug, S. J., Dittmar, J., Voegelin, A., Kretzschmar, R., Wehrli, B., & Badruzzaman, A. B. M. (2010). Arsenic release from paddy soils during monsoon flooding. *Nature geoscience*, *3*(1), 53.
96. Rosen, B. P., Ajees, A. A., & McDermott, T. R. (2011). Life and death with arsenic: Arsenic life: An analysis of the recent report "A bacterium that can grow by using arsenic instead of phosphorus". *Bioessays*, *33*(5), 350-357.
97. Roychowdhury, T. (2008). Impact of sedimentary arsenic through irrigated groundwater on soil, plant, crops and human continuum from Bengal delta: special reference to raw and cooked rice. *Food and chemical toxicology*, *46*(8), 2856-2864.
98. Roychowdhury, T. (2008). Influence of several factors during collection and preservation prior to analysis of arsenic in groundwater: A case study from West Bengal, India. *Journal of international environment and applied science*, *3*(1).
99. Roychowdhury, T. (2010). Groundwater arsenic contamination in one of the 107 arsenic-affected blocks in West Bengal, India: Status, distribution, health effects and factors responsible for arsenic poisoning. *International journal of hygiene and environmental health*, *213*(6), 414-427.
100. Roychowdhury, T., Tokunaga, H., Uchino, T., & Ando, M. (2005). Effect of arsenic-contaminated irrigation water on agricultural land soil and plants in West Bengal, India. *Chemosphere*, *58*(6), 799-810.
101. Roychowdhury, T., Uchino, T., Tokunaga, H., & Ando, M. (2002). Arsenic and other heavy metals in soils from an arsenic-affected area of West Bengal, India. *Chemosphere*, *49*(6), 605-618.

102. Roychowdhury, T., Uchino, T., Tokunaga, H., & Ando, M. (2002). Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. *Food and chemical toxicology*, *40*(11), 1611-1621.
103. Samanta, G., Chowdhury, T. R., Mandal, B. K., Biswas, B. K., Chowdhury, U. K., Basu, G. K., & Chakraborti, D. (1999). Flow injection hydride generation atomic absorption spectrometry for determination of arsenic in water and biological samples from arsenic-affected districts of West Bengal, India, and Bangladesh. *Microchemical journal*, *62*(1), 174-191.
104. Samanta, G., Das, D., Mandal, B. K., Chowdhury, T. R., Chakraborti, D., Pal, A., & Ahamed, S. (2007). Arsenic in the breast milk of lactating women in arsenic-affected areas of West Bengal, India and its effect on infants. *Journal of environmental science and health, Part A*, *42*(12), 1815-1825.
105. Sandhi, A., Greger, M., Landberg, T., Jacks, G., & Bhattacharya, P. (2017). Arsenic concentrations in local aromatic and high-yielding hybrid rice cultivars and the potential health risk: a study in an arsenic hotspot. *Environmental monitoring and assessment*, *189*(4), 184.
106. Selvi, M. S., Sasikumar, S., Gomathi, S., Rajkumar, P., Sasikumar, P., & Govindan, S. (2014). Isolation and characterization of arsenic resistant bacteria from agricultural soil, and their potential for arsenic bioremediation. *International journal of agricultural policy and research*, *2*(11), 393-405.
107. Shaibur, M. R., & Kawai, S. (2009). Effect of arsenic on visible symptom and arsenic concentration in hydroponic Japanese mustard spinach. *Environmental and experimental botany*, *67*(1), 65-70.
108. Shankar, S., & Shanker, U. (2014). Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. *The scientific world journal*, *2014*.
109. Sharma, V. K., & Sohn, M. (2009). Aquatic arsenic: toxicity, speciation, transformations, and remediation. *Environment international*, *35*(4), 743-759.
110. Shrivastava, A., Barla, A., Singh, S., Mandraha, S., & Bose, S. (2017). Arsenic contamination in agricultural soils of Bengal deltaic region of West Bengal and its higher assimilation in monsoon rice. *Journal of hazardous materials*, *324*, 526-534.
111. Sikdar, P. K., & Chakraborty, S. (2008). Genesis of arsenic in groundwater of North Bengal Plain using PCA: a case study of English Bazar Block, Malda district, West Bengal, India. *Hydrological processes: An international journal*, *22*(12), 1796-1809.

112. Silver, S., & Phung, L. T. (2005). Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Applied environmental Microbiology*, *71*(2), 599-608.
113. Smedley, P. L., & Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied geochemistry*, *17*(5), 517-568.
114. Smith, A. H., Lingas, E. O., & Rahman, M. (2000). Contamination of drinking-water by arsenic in Bangladesh: a public health emergency Bull WHO 78 (9): 1093–1103.
115. Takahashi, Y., Minamikawa, R., Hattori, K. H., Kurishima, K., Kihou, N., & Yuita, K. (2004). Arsenic behavior in paddy fields during the cycle of flooded and non-flooded periods. *Environmental science & technology*, 38(4), 1038-1044.
116. Tsuji, J. S., Garry, M. R., Perez, V., & Chang, E. T. (2015). Low-level arsenic exposure and developmental neurotoxicity in children: A systematic review and risk assessment. *Toxicology*, 337, 91-107.
117. Yadav, K. K., Gupta, N., Kumar, A., Reece, L. M., Singh, N., Rezania, S., & Khan, S. A. (2018). Mechanistic understanding and holistic approach of phytoremediation: A review on application and future prospects. *Ecological engineering*, *120*, 274-298.
118. Yamaguchi, N., Nakamura, T., Dong, D., Takahashi, Y., Amachi, S., & Makino, T. (2011). Arsenic release from flooded paddy soils is influenced by speciation, Eh, pH, and iron dissolution. *Chemosphere*, 83(7), 925-932.
119. Yu, M. Q., Liu, G. Q., & Jin, Q. (1983). Determination of trace arsenic, antimony, selenium and tellurium in various oxidation states in water by hydride generation and atomic-absorption spectrophotometry after enrichment and separation with thiol cotton. *Talanta*, *30*(4), 265-270.
120. Zobrist, J., Dowdle, P. R., Davis, J. A., & Oremland, R. S. (2000). Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environmental science & technology*, 34(22), 4747-4753.

APPENDIX I: MEDIA COMPOSITIONS & PREPARATIONS

1. Nutrient agar media:

Table 7.1: Compositions of Nutrient agar media

Ingredient	gm/100 ml
Peptone	1
Beef extract	0.5
Sodium chloride	0.5
Agar	1.5
pH	7.2-7.4

2. NBRIP agar media:

Table 7.2: Compositions of NBRIP agar media

Ingredient	gm/L
Glucose	10
Tri calcium phosphate (TCP)	5
Magnesium chloride hexahydrate	5
Magnesium sulphate heptahydrate	0.25
Potassium chloride	0.2
Ammonium sulphate	0.1
Agar	1.5

3. Chrome azurol S agar media:

A. Blue Dye:

a. Solution 1:
   i. 0.06 g of CAS was dissolved in 50 ml of double distilled water.

b. Solution 2:
   i. 0.0027 g of $FeCl_3$-6 H_2O was dissolved in 10 ml of 10 mM HCl.

c. Solution 3:
   i. 0.073 g of HDTMA was dissolved in 40 ml of double distilled water.

d. Solution 1 was mixed with 9 ml of Solution 2 and then with Solution 3. The solution had turned blue in color. It was then autoclaved and stored in a plastic bottle.

B. Mixture solution:

a. Minimal Media 9 (MM9) Salt Solution Stock
    i. 15 g of KH_2PO_4, 25 g of NaCl, and 50 g NH_4Cl was dissolved in 500 ml of double distilled water.
b. 20% Glucose Stock
    i. 20 g of glucose was dissolved in 100 ml of double distilled water.
c. NaOH Stock
    i. 25 g of NaOH was dissolved in 150 ml of double distilled water; pH ~12.
d. Casamino Acid Solution
    i. 3 g of Casamino acid was dissolved in 27 ml of double distilled water.

C. CAS agar Preparation:

a. 100 ml of MM9 salt solution was added to 750 ml of double distilled water.
b. 32.24 g of piperazine-N,N'-bis(2-ethanesulfonic acid) PIPES.
    i. As PIPES does not dissolve below pH 5, the pH was brought up to 6 and slowly PIPES was added while stirring. The pH was maintained at 6.8 because beyond that the solution turns green.
c. 15 g of Bacto agar was added and the solution was autoclaved.
d. 30 ml of sterile Casamino acid solution and 10 ml of sterile 20% glucose solution was added to MM9/PIPES mixture.
e. 100 ml of Blue Dye solution was added slowly along the glass wall with enough agitation to mix thoroughly. The CAS agar media was ready to be plated asceptically.

APPENDIX II: INSTRUMENTS AND EQUIPMENT USED

Table 8.1: List of instruments and equipment used

Sl No.	Instruments used	Company
1	UV VIS Spectrophotometer	Orion AquaMate 8000 Thermo scientific
2	Atomic absorption spectrophotometer (VGA)	Varian
3	Weighing balance (fine)	Metler
4	Hot plate	Tarson Hot-top
5	Laboratory centrifuge	Remi
6	Vortex	Remi
7	Hot air oven	Labotech
8	Autoclave	GB Enterprises (Kolkata)
9	Mini spin centrifuge	Eppendorf
10	Laminar airflow chamber	Neo equipments (Kalyani)
11	Desiccator	In-Chem
12	Vaccum filtration unit	In-Chem
13	Separation funnel	In-Chem

PUBLICATIONS

1. **BOOK CHAPTERS:**
    a) Ghosh.S, **Mukherjee.M**, Sar.P, "Impact of Arsenic on structural and functional composition of dominant bacterial populations associated with various natural ecosystems" in *Environmental Pollution, Biodiversity, and Sustainable Development: Issues and Remediation*; Apple academic press, USA (In press)
    b) Das.A, Das.A, **Mukherjee.M**, Das.B, Mukherjee.S.C, Pati.S, Dutta.R.N, Quamruzzaman.Q, Saha.K.C, Rahman.M, Chakraborti.D, RoyChowdhury.T, "Groundwater arsenic contamination in the GMB plain and its health, environment and socio-economic implications" in *Encyclopedia* (In communication)
2. **PAPERS:**
    a) Paul.M, Goswami.C, **Mukherjee.M**, Roychowdhury.T, "Phyto-remedial detoxification of Arsenic by Pistia stratiotes and assessment of its anti-oxidative enzymatic changes", *Bioremediation*; (In communication)
    b) Biswas.A, Swain.S, Chowdhury.N.R, Joardar.M, Das.A, **Mukherjee.M**, Roychowdhury.T, "Arsenic contamination in Kolkata metropolitan city: Perspective of transportation of agricultural products from arsenic endemic areas", *Environmental Science and Pollution Research*; (In communication)

3. **SEMINAR PUBLICATIONS:**
    a) **Mukherjee.M**, Ghosh.S, Roychowdhury.T, "Sustainable solution towards Arsenic mitigation by microbial bioremediation", ", *Groundwater Arsenic Contamination Problem In Gmb Plain: Its Health Effects, Socio-Economic Implications And Mitigation Strategies*; (Published)
    b) Roychowdhury.T, Chowdhury.N.R, Joardar.M, Swain.S, Das.A, **Mukherjee.M**, "Groundwater Arsenic contamination with special reference to its entry in rice grain post-harvest in Bengal delta", *Challenges In Energy Resource Management & Climate Change (NSE 20, 2018)*; (Published)
    c) Chowdhury.N.R, Joardar.M, Swain.S, Das.A, De.A, **Mukherjee.M**, Roychowdhury.T, "Arsenic toxicity through drinking water and rice grain with special reference to health effects: A village level study from West Bengal,

India", *Challenges In Energy Resource Management & Climate Change (NSE 20, 2018)*; (Published)

d) Joardar.M, Chowdhury.N.R, Swain.S, Das.A, De.A, **Mukherjee.M**, Roychowdhury.T, "Groundwater Arsenic contamination in West Bengal, India: Special reference to status, distribution, food chain contamination, health effects and mitigation strategies*", Environmental Issues: Current Scenario From The View Point Of Scientific Studies*; (Published)

e) Chowdhury.N.R, Joardar.M, Swain.S, Das.A, De.A, **Mukherjee.M**, Das.S, Ghosh.B, Roychowdhury.T, "State water scenario and its mitigation options: with special reference to Arsenic and Fluoride", *National Conclave On Water Resources Management;* (Published)

f) Swain.S, Chowdhury.N.R, Joardar.M, Das.A, **Mukherjee.M**, Roychowdhury.T, "Arsenic accumulation in food chain West Bengal, India: Special reference to rice grain and its potential health hazard to population", *Groundwater Arsenic Contamination Problem In Gmb Plain: Its Health Effects, Socio-Economic Implications And Mitigation Strategies*; (Published)

g) Das.A, Joardar.M, Chowdhury.N.R, Swain.S, De.A, **Mukherjee.M**, Ghosh.B, Das.S, Majumder.S, Roychowdhury.T, "An insight of Arsenic contamination in groundwater and foodchain with special reference to health efects on domestic animals", *Groundwater Arsenic Contamination Problem In Gmb Plain: Its Health Effects, Socio-Economic Implications And Mitigation Strategies*; (Published)