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Sustainability in food production: the effects and the risks of the arsenic contamination in soil and irrigation water

Ph. D. dissertation by

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Abstract

Arsenic (As) is ubiquitous in many environments and highly toxic to all forms of life. Sources of As contamination are both natural and anthropogenic and the scale of contamination ranges from local to regional. Arsenic has been detected in groundwater in several countries of the world with concentration levels exceeding the WHO drinking water guideline value of 10 μg/L. For instance, in the Bengal Delta Plain of Bangladesh and West Bengal, India, As in groundwater has emerged as the largest environmental health disaster putting at least 100 million people at risk of cancer and other As-related diseases. Food and drinking water are the major routes of As exposure for humans.
It occurs predominantly in inorganic form as arsenate (AsV) and arsenite (AsIII), whereas the organic species most common are monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA). The inorganic species are more acutely toxic than organic species. Arsenate is an analogue of phosphate and thus interferes with essential cellular processes such as oxidative phosphorylation and ATP synthesis, whereas the toxicity of As(III) is due to its propensity to bind to sulfhydryl groups, with consequent effects on protein functions.
Due to the toxicity and potential environmental risks related to the mobility of As, it is of quite importance in agriculture, investigate for minimize the transfer from contaminated agricultural soils and/or contaminated water to the crop plants. It is necessary to limit entering in food chain and reduce the economic damage linked to productivity. There are many areas of research that are being actively pursued to address the As contamination problem. My Ph.D.: project was focus on the possible strategies to reduce the translocation of this element to the plant using of amendments and / or microorganisms and investigations on As exposure in food.

During the first year of my Ph.D., I conducted a study on influence of phosphatic fertilization in tomato plants (*Lycopersicum esculentum* L. cv piennolo), grown on uncontaminated soil and irrigated with solutions containing increasing concentrations of arsenic (0, 0.5, 2.4 mg L^{-1}). It was found that the total biomass of the plants decreases significantly with increasing concentration of arsenic. Plants grown without phosphorus (P-) showed a decrease in biomass of 17% , 42% and 58% with increasing concentration of As compared to the control, while plants that have had phosphorus (P +) showed a significantly lower equal to 13%, 30% , and 42% more than the control. The concentration of arsenic in tomato plants increases as the concentration of arsenic in irrigation water. This fact is particularly evident in the roots. The concentration of arsenic in fruit is very low in both P- and P + (P- 0.18 to 0.28 mg kg^{-1}, P + 0.15 to 0.22 mg kg^{-1}).

During the second year, my main research was carried out at Aberdeen University (Scotland, UK), and had as its objective is the study of the presence arsenic and trace elements in samples of rice purchased in Italy, with particular attention to the quantification and speciation of arsenic.

The total concentration of As in the samples analyzed according to the variety, includes a range of mean values from 0.18 (Ribe) and 0.28 mg kg $^{-1}$ (Vialone Nano). The results analyzed according to the region of provenience showed that the highest concentration of total As was recorded in Emilia Romagna (0.28 mg kg $^{-1}$), the lowest in Calabria (0.11 mg kg $^{-1}$). The data indicate that the speciation of inorganic arsenic concentration varies between 0.08 and mg kg $^{-1}$ (Ribe, Rome) 0.11 mg kg $^{-1}$ (Vialone Nano) and between 12.06 (Calabria) mg kg^{-1} and 0.10 (Lombardia) based on the origin. For organic species on the other hand, the concentration varies between 0.02 to 12.08 mg kg^{-1} depending on variety, and between 0.01 to 0.08 mg kg^{-1} on the basis of geographical origin. We also had interesting results for chromium (individual values range between 0.11 to 1.51 mg kg^{-1}, and for cadmium (individual values range 0.001-0.16 mg kg^{-1}).

In the third year of my Ph.D., research had as objective to evaluate the effectiveness of *Trichoderma* in alleviating the toxic effects of arsenic in plants of lettuce (*Lactuga sativa* L.). Were tested two different strains of *Trichoderma: T. harzianum* (T22) and *T. atroviride* (P1). The plants are grown in pots with uncontaminated soil but irrigated with solutions containing 0-5-10 mg As L^{-1}. In plants not inolculate, the toxic effect of arsenic was revealed especially in reduced root growth and leaf. Inoculated plants, however, with both strains of *Trichoderma* have shown a significant increase in total biomass compared to the control. In particular, the inoculum with the T22, has increased biomass production compared to the control total of 30.1% (As0), 39% (As5) and 52.5% (As10). Lettuce has a great sensitivity to As, in fact during our studies we have it detected a high concentration both in the roots than in leaves, compared to the control. The highest concentration was found in the roots. The control -T where has not been used or contaminated water or inoculum of *Trichoderma*, has a concentration of As equal to 0.160 mg kg^{-1} in the roots, instead of in plants irrigated with contaminated water but not inoculated, we have a concentration equal to 3.84 (As5) and of 7.30 (1 As) mg kg^{-1}. In plants inoculated instead of As concentration measured is lower compared to the control: 2.95 (As5) and 6.13 (As10) mg kg^{-1} for those inoculated with T22 and 3.14 (As5) and 6.25 (As10) mg kg^{-1} with P1. Treatment with T22 and P1 in the leaves also showed a lower absorption of As compared to the control (1.24 mg kg^{-1} for As5 and 2.66 mg kg^{-1} for As10). In fact, for plants inoculated with T22 we found a concentration of 2.95 (5As), 6.13 (As10) mg kg^{-1}, for those inoculated with P1 3.14 (5As) and 6.24 (As10) mg kg^{-1}.

CHAPTER I

Introduction

1.1. General characteristics of arsenic

Arsenic (As) is an element whose name derived from the Greek word *arsenikon*, meaning potent (Cutter, 1992). It belongs to group 15 (old group 5) of the periodic table together with nitrogen, phosphorus, antimony and bismunt (Henke, 2009). Its atomic weight is equivalent to 75 and commonly occurs naturally in two oxidation state +5, +3, and more rarely in the 0 or -3 state (Cutter, 1992). Arsenic is classified as a metalloid, for its chemical nature intermediate between that of metals and nonmetals (Phillips, 1990). Arsenic is a toxic element, widely distributed in the environment, found in rocks, soil, water, sediments, and air. Its ranks 20^{th} in abundance in the earth's crust, 14^{th} in the sea waters, and 12^{th} in the human body (Mandal and Suzuk, 2002). Mobility and toxicity of As is strongly depend on the oxidation state and structure (Ng et al., 2003). The most common forms of As in the environment are the inorganic oxyanions of As(III) and As(V). Arsenite [As(III)] is more toxic and relatively mobile in contaminated soils, whereas arsenate [As(V)] is relatively less toxic. Different forms of organic As, are present in environment, the most common as monomethylarsonic acid [MMA, $CH_3AsO(OH)_2$], dimethylarsenic acid [DMA, $(CH_3)_2AsO(OH)$] (Stolz et al., 2006).

The inorganic species are more acutely toxic than organic species (Gao and Burau, 1997). ***Inorganic Arsenic*** Arsenite [As(III); $H_xAsO_3^{x-3}$, x=0 to 3] and arsenate [As(V); $H_xAsO_4^{x-3}$, x=0 to 3] are the two most relevant inorganic forms of arsenic in environments (Fig. 1). Arsenate is an anion at the pH of most natural waters ($H_2AsO_4^-$ and $HAsO_4^{2-}$), while arsenite is a neutral species. The structure and chemistry of arsenate are similar to those of phosphate; this similarity has significant implications for sorption behavior and microbial metabolism (NRC, 1999; 2001). Arsenate is thermodynamically stable under oxic conditions, while As(III) is stable under more reducing conditions. Interconversion between these two As species is driven by both biotic and abiotic processes and strongly influenced by the redox potential and pH. Both arsenate-reducing and arsenite oxidizing bacteria are present in soil and in water environments. (Inskeep and al., 2002; Stolz et al., 2006).

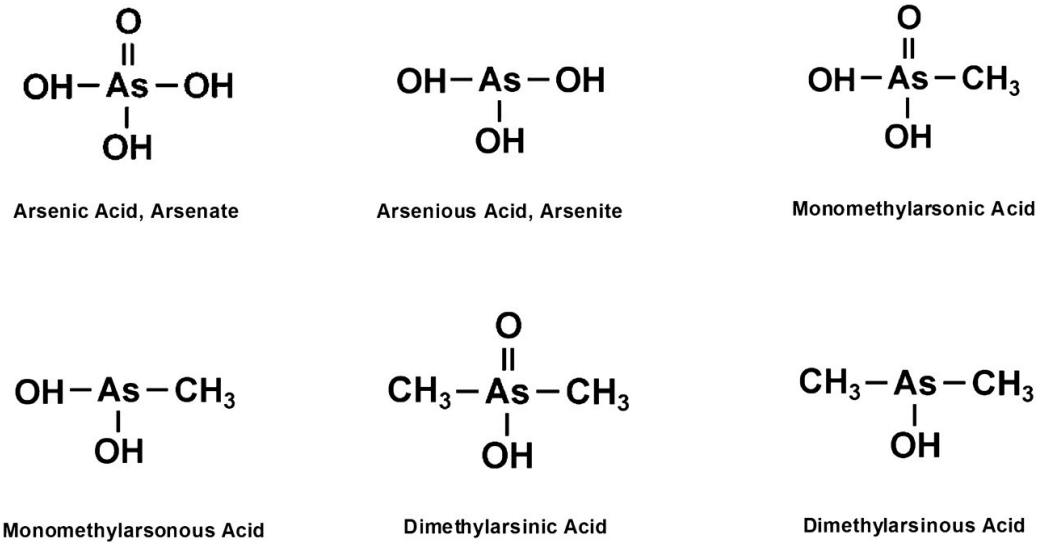

Figure 1. Arsenic compounds.

Organic Arsenic Methylated As compounds (Fig. 1), such as monomethylarsonic acid [MMA:$CH_3AsO(OH)_2$], dimethylarsinic acid [DMA: $(CH_3)_2AsOOH$] and trimethylarsine oxide [TMAO: $(CH_3)_3AsO$] are found in some soils, sometimes as a minor component (Huang et al., 2007), but can reach high concentrations. MMA and DMA can be produced from inorganic As through biomethylation by some soil microorganisms or algae. Arsenic speciation is determined by both biotic and abiotic variables and it is important not only for understanding the biogeochemical cycling of As in different ecosystems and mechanisms of As accumulation and detoxification, but also for designing safe disposal options of As-rich biomass (Tu et al., 2003; Watt and Le, 2003).

The fate of As accumulated in the surface environment depends essentially on its retention and mobility in the host medium, soil and groundwater. The mobilization of As into the terrestrial and aquatic ecosystems occurs through a complex combination of natural biogeochemical reactions and anthropogenic activities (Garelik et al., 2008). It has been used in medicine (as an ingredient of drugs for the treatment of some diseases) and in various other fields; some of these activities include industrial processes that contribute to both atmospheric and terrestrial depositions, such as mining and metallurgy, wood preservation, urban and industrial wastes, and applications of sewage sludge and fertilizer (Jacks and Bhattacharya, 1998). This metalloid is also used in hardening of alloys and in production of semiconductors, pigments, glass manufacturing, pesticides, rodenticides and fungicides (Hathaway et al., 1991). Because of its usefulness and exploitation, arsenic contamination is now widespread in the environment. As a toxic compound, arsenic could occur

naturally or anthropogenically in the environment, and its toxic effects is warranted (Mandal and Suzuk, 2002). Arsenic cannot be easily destroyed but could only be converted into different forms or transformed into insoluble compounds in combination with other elements (Choong et al., 2007). In terrestrial and aquatic ecosystems, it attracts worldwide attention primarily because of its adverse impact on human health. Arsenic contamination of surface and groundwater occurs worldwide and has become a sociopolitical issue in several parts of the globe (Mahimairaja et al., 2005). Wide distribution of As in natural environments, its geochemical characteristics, and an increased dependence on groundwater for drinking have derived in severe As toxicity for millions of people worldwide. For instance, high risk from drinking As-contaminated water occurs in West Bengal (India) (Chakraborti et al., 2002; Chatterjee et al., 1995) and Bangladesh (Smith et al., 2000). Milions of people from China (Wang, 1984), Vietnam (Berg et al., 2001), Taiwan (Lu, 1990), Chile (Smith et al., 1998), Argentina (Hopenhayn-Rich et al., 1998) and Mexico (Del Razo et al., 1990) are likely at risk as well. Following the accumulation of evidence for the chronic toxicological effects of As in drinking water, recommended and regulatory limits of many authorities are being reduced. The World Health Organization (WHO) guideline value for As in drinking water was provisionally reduced in 1993 from 50 to 10 $\mu g\ L^{-1}$. The new recommended value was based on the increasing awareness of the toxicity of As, particularly its carcinogenicity, and on the ability to measure it quantitatively (WHO, 1993). In recent years, it has become apparent that both WHO guideline value and current national standards are quite frequently exceeded in drinking water sources. Awareness of the problems associated with arsenic in drinking water and in environment has grown significantly over the last two decades and today an enormous literature exists documenting its occurrence behavior and impacts in many places across the globe. Human exposure to arsenic occurs through a number of pathways including air, food, water and soil. The relative impacts of these depend on local circumstances. Although trace levels of As have been shown to be beneficial in plant and animal nutrition (Smith et al., 1998; USEPA, 1993), no comparable data are available for humans and elevated, concentrations of As in the biosphere pose a significant threat to mankind (Adriano, 2001). Indeed, As is now recognized as the most serious inorganic contaminants in drinking water on a worldwide basis (Smedly, 2002).

1.2. Sources of As

Arsenic is not a rare element in the earth's crust. The average As content in continental crust varies between 1 and 2 mg As kg^{-1} (Taylor and McLennan, 1995). Two principal pathways of As emission in the environment, are natural processes and industrial activities. Industrial activity is, however, the more important source of As emission and the major cause of widespread As contamination.
The cycling of As is caused by the interactions of natural water with bedrock, sediments, and soils as well as the influence of local atmospheric deposition. Weathering and leaching of geological formations and mine wastes result in elevated concentrations of As in natural waters in several areas (Bhattacharya et al., 2002b).

1.2.1.Natural Source

Arsenic is widely distributed in all geological materials at varying concentrations. It is released in the natural environment through natural processes such as windblown dust from weathered continental crust, forest fires, volcanoes eruptions, sea spray, hot springs, geysers, (Bhattacharya et al., 2002) and may be transported over long distances as suspended particulates through water or air. The mean concentrations of As in igneous rocks range from 1.5 to 3.0 mg kg^{-1}, whereas in sedimentary rocks range from 1.7 to 400 mg kg^{-1}(Smith et al., 1998).
Arsenic occurs as a major constituent in more than 200 minerals including elemental arsenic, arsenides, sulphides, oxides, arsenates. Most are minerals or their alteration products (O'Neill, 1995). These are mostly sulfide-containing ores of copper (Cu), nickel (Ni), lead (Pb), cobalt (Co), zinc (Zn), gold (Au). The most important ores of As include pyrites, realgar, and orpiment (Yan-Chu, 1994). Though not a major component, As is also often present in varying concentrations in other common rock-forming minerals. The chemistry of As follows closely that of S, the greatest concentrations of the element tend to occur in sulphide minerals, of which pyrite is the most abundant. High As concentrations are also found in many oxide minerals and hydrous metal oxides, either as part of the mineral structure or as sorbed species (Smedly, 2002). Arsenic is introduced into soil and water during the weathering of rocks and minerals followed by subsequent leaching and runoff. Therefore, the primary source of As in soil is the parent (or rock) materials from which it is derived (Yan-Chu, 1994). Natural occurrence of As is widely reported in groundwater in several parts of the world, and the concentrations vary significantly depending on the redox characteristics of the groundwater and the lithological characteristics of the bedrock (Bhattacharya

et al., 2002b). One typical example is the extensive As contamination of groundwater in Bangladesh and West Bengal in India (Bose and Sharma, 2002).

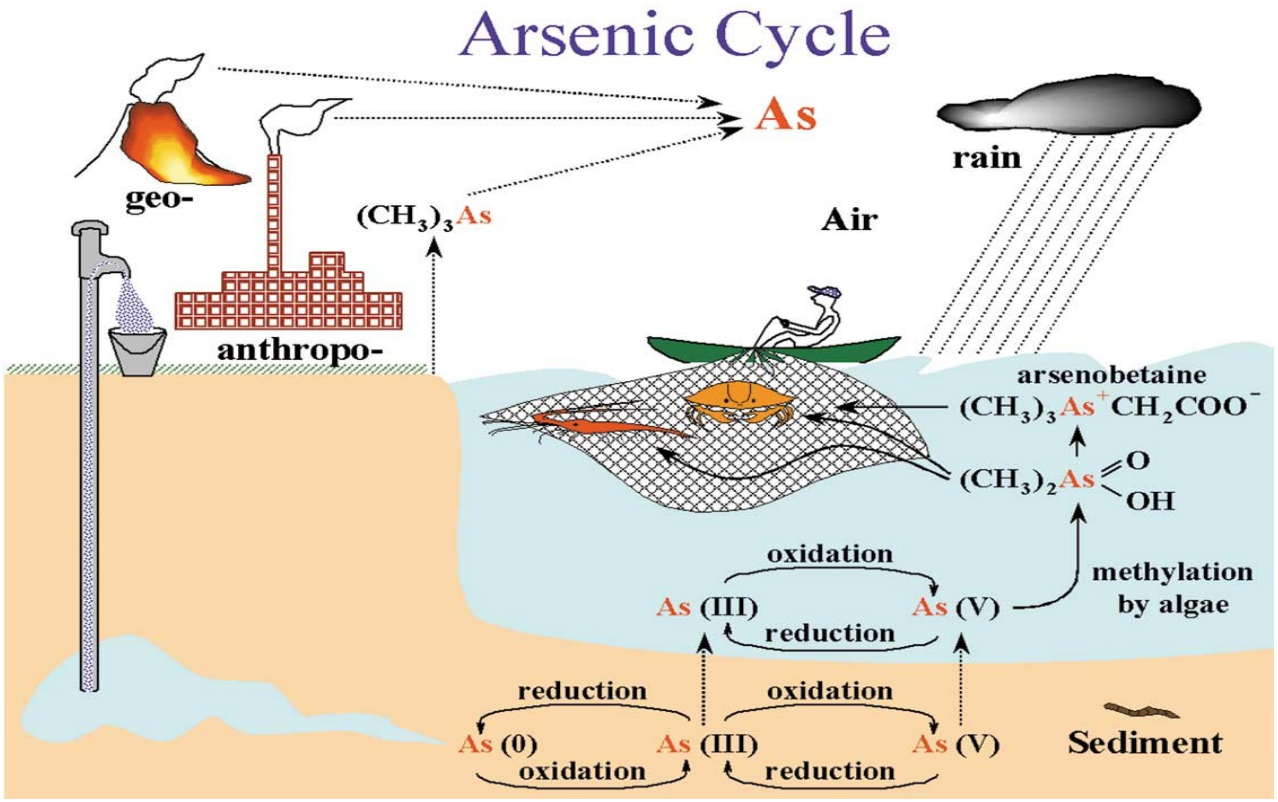

Figure 2. The arsenic's cycle.

Arsenic concentration is usually higher in soil and shales than in earth crust because of its continuous accumulation during weathering and translocation in colloidal fractions. Arsenic may also be coprecipitated with Fe hydroxides and sulfides in sedimentary rocks. Therefore, Fe deposits and sedimentary Fe ores are rich in As, and the soils derived from such sedimentary rocks may contain as high as 20 to 30 mg As kg^{-1} (Zou, 1986). About 50% of the Fe in freshwater sediments is in the form of Fe oxides and about 20% of the Fe is 'reactive' Fe. Clays also adsorb As because of the oxide-like character of their edges (Millero et al., 2001). Of these mineral components, Fe oxides are probably the most important adsorbents in sandy aquifers because of their greater abundance and the strong binding affinity. Concentrations in Fe oxides can also reach weight percent values, particularly where they form as the oxidation products of primary Fe sulphide minerals, which have an abundant supply of As (Bhattacharya et al., 2002a).

Adsorption of arsenate to hydrous Fe oxides is particularly strong and sorbed loadings can be appreciable even at very low As concentrations in solution (Goldberg, 1986; Manning and

Goldberg, 1996). Adsorption to hydrous Al and Mn oxides may also be important if these oxides are present in quantity (Brannon and Patrick, 1987). Arsenic may also be sorbed to the edges of clays and on the surface of calcite, a common mineral in many sediments (Goldberg and Glaubig, 1988). However, these loadings are much smaller on a weight basis than for the Fe oxides. Adsorption reactions are responsible for the relatively low concentrations of As found in most natural waters. Arsenic concentrations in phosphate minerals are variable but can also reach high values, for example up to 1000 mg kg^{-1} in apatite However, phosphate minerals are much less abundant than oxide minerals and so make a correspondingly small contribution to the As concentration in most sediments. Arsenic can also substitute for Si^{4+}, Al^{3+}, Fe^{3+} and Ti^{4+} in many mineral structures and is therefore present in many other rock-forming minerals, albeit at much lower concentrations. Most common silicate minerals contain around 1mg As kg^{-1} or less. Carbonate minerals usually contain less than 10 mg kg^{-1} As (Smedly, 2002).

Natural emission of As in the atmosphere is estimated to be around 2.8 gigagrams/year as dust and 21 gigagrams/year as volatile phases. Among the natural sources, windblown dust from crustal weathering, forest fires, vegetation emissions, volcanoes, and sea spray are significant. Volcanoes are also considered as a geogenic source of As to the environment with the total atmospheric annual emissions from volcanoes being estimated at 31.000 mg (Smith et al., 1998).

1.2.2. Anthropogenic Sources

Arsenic is also being introduced into the environment through various anthropogenic activities. These sources release As compounds that differ greatly in chemical nature (speciation) and bioavailability. Major sources of As was originated from commercial wastes (40%), coal ash (22%), mining industry (16%), and the atmospheric fallout from the steel industry (13%) (Eisler, 2004). Anthropogenic emissions of As account for as high as 78 gigagrams/year and are thus significantly higher compared to the natural inputs. The concentration of As can therefore be appreciably high in the areas affected by anthropogenic activities. A considerable amount of As is released by the combustion of fossil fuels, especially coal, from wood preservation industries as well as the use of the preserved wood products. Arsenic trioxide (As_2O_3) is used extensively in the manufacturing of ceramic and glass, electronics, pigments and antifouling agents, cosmetics, fireworks, and Cu based alloys (Leonard, 1991). Arsenic compounds such as monosodium methylarsonate ($NaCH_3HAsO_3$), disodium methylarsonate ($Na_2CH_3AsO_3$), and diethylarsenic acid [$(CH_3)_2AsO(OH)$] are widely used as agricultural insecticides, larvicides, and herbicides. Sodium arsenite (NaH_2AsO_4) is used for

aquatic weed control and for sheep and cattle dips. Arsenic acid (H_3AsO_4) is used to defoliate cotton bolls prior to harvesting and as a wood preservative. Elemental As is mainly used in Pb, Cu, Sb, Sn, Al, and Ga alloys (Greenwood and Earnshaw, 1989).

Wood preservation In the past, arsenic was used for wood preservation in conjunction with Cu and chromium (Cr), forming the Chromated Copper Arsenate (CCA). The use of CCA and other As-based chemicals in wood preservation industries has caused widespread contamination of soils and aquatic environments (Abernathy et al., 1997). CCA had attained wide-scale industrial application as a wood preservative owing to biocidic characteristics of Cu(II) and As(V). The preservative chemical used for pressure impregnation comprises a waterbased mixture of dichromic acid ($H_2Cr_2O_7$), arsenic acid (H_3AsO_4), and Cu(II) as divalent cation at variable proportions. Chromium is used to bind As and Cu into the cellular structure of the wood. Fixation of CCA is dependent on the transformation of Cr(VI) to Cr(III), a reaction that is dependent on the temperature and water content of the wood. Cr(III) forms insoluble complexes with both As and Cu (Bhattacharya et al., 2002a). Further stabilization of these complexes takes place after complete fixation of the As and Cu in the wood tissues and minimizes the risk of leaching of the CCA components from the processed wood. Among the active ingredients of CCA wood preservatives, As is most mobile and toxic to a broad range of organisms, including human beings. Studies around an abandoned wood preservation site at Konsterud, Kristinehamns Community in Central Sweden revealed soil As concentrations between 10 and 1067 mg kg^{-1}, and the order of abundance for metal contaminants (Bhattacharya et al., 1996; 2002a). Incineration of CCA impregnated wood from a sawmill was found to be a source of As contamination to the environment. The content of As in air particulates from open fires was found to exceed the German air quality standards by 100-fold. The ashes, spread on lawns or vegetable cultivations, pose further risk to human health (Bhattacharya et al., 2002a).

Agricultural sources Over hundreds of years, inorganic arsenicals (arsenic trioxide, arsenic acid, arsenates of calcium, copper, lead, and sodium, and arsenates of sodium and potassium) have been widely used in pigments, pesticides, insecticides, herbicides, and fungicides. At present, As is no longer used in agriculture, but persistence of the inorganic arsenic residues in soils is an issue of environmental concern. Continuous application of fertilizers that contain trace levels of As also results in As contamination of soil, thereby reaching the food chain through plant uptake (McLaughlin et al., 1996).

Industries that manufacture As-containing pesticides and herbicides release As-laden liquid and solid wastes that, upon disposal, are likely to contaminate soil and water bodies. Arsenic is also present in many pesticides, herbicides, and fertilizers. The use of horticultural pesticides, lead

arsenate ($PbAsO_4$), calcium arsenate ($CaAsO_4$), magnesium arsenate ($MgAsO_4$), zinc arsenate ($ZnAsO_4$), zinc arsenite [$Zn(AsO_2)_2$], and Paris Green [$Cu(CH_3COO)_2$* $3Cu(AsO_2)_2$] in orchards has contributed to soil As contamination in many parts of the world (Merry et al., 1983; Peryea and Creger, 1994). For instance, indiscriminate discharge of industrial effluents from the manufacturing of Paris Green (copper acetoarsenite, an arsenical pesticide) resulted in the contamination of soil and groundwater in residential area of Calcutta, India (Chatterjee and Mukherjee, 1999). Studies by Kenyon et al. (1979) and Aten et al. (1980) have indicated elevated concentrations of As in vegetables grown in soils contaminated by lead arsenate used as an insecticide in apple orchards. The recalcitrant nature of arsenical herbicides has, however, been observed in agricultural soils particularly around old orchards. Soil contamination due to the use of organoarsenical herbicides such as monosodium methanearsonate (MSMA) and disodium methanearsonate (DSMA) was also reported (Smith et al., 1998). The use of sodium arsenite ($NaAsO_2$) to control aquatic weeds has contaminated small fish ponds and lakes in several parts of United States with As (Adriano, 2001). Arsenic contamination in soil was also reported due to the arsenical pesticides used in sheep and cattle dips to control ticks, fleas, and lice (McLaren et al., 1998). A study of 11 dip sites in New South Wales indicated considerable surface soil (0 –10 cm) contamination with As (37–3542 mg kg^{-1}) and significant movement of As (57–2282 mg kg^{-1}) down the soil profile at 20–40 cm depth (McLaren et al., 1998).

Mining and smelting Elevated concentrations of As, as well as other metals such as cadmium, copper, iron, lead, nickel, and zinc, are commonly encountered in the acid mine effluents (Rimstidt et al., 1994). Mining and smelting of ore minerals including sulfides of copper, lead, and zinc, as well as gold processing, have contributed to significant environmental As emissions in the past, but changes in smelting processes during the last decade have significantly reduced the emission of As from these sources (USEPA, 1994). However, according to an estimate made by the USEPA, nearly 6.000.000 people living within 12 miles of these copper, zinc, and lead smelters may be exposed to 10 times the average atmospheric levels of As in the United States. In another study it has been shown that nearly 40.000 people were at risk of exposure to As levels exceeding the national atmospheric levels by 100 times in the vicinity of some copper smelters. Significant bioaccumulation of As occurs in crops grown in contaminated soils around lead smelters (USEPA, 1994). The principal source of As in mine tailings is the oxidation of arsenopyrite (FeAsS). Arsenopyrite can be oxidized by both O_2 and Fe(III), but the rate of oxidation by Fe(III) is faster than for pyrite. Under acidic conditions (pH 3.0), As(V) may substitute SO_4 in the structure of jarosite [$KFe_3(SO_4)_2(OH)_6$] in different mine wastes. The flue gases and particulate from smelters can contaminate nearby ecosystems from the operation with a range of toxic metalloids, including

As (Adriano, 2001). Coal combustion not only releases gaseous As into the atmosphere, but also generates fly and bottom ash containing varied amounts of As. Disposal of these materials often leads to As contamination of soil and water (Beretka and Nelson, 1994).

Feed additives Many arsenic compounds are used for feed additives, such as H_3AsO_4, 3-nitro-4-hydroxy phenylarsonic acid, 4-nitrophenylarsonic acid etc. All substituted phenylarsonic acids were used for feed additives under Food Additives Law of 1958 (Feed Additive Compendium, 1975).

Drugs The medicinal virtues of arsenic are acclaimed for nearly 2500 years. In Austria, the peasants consumed a large quantity of arsenic for softness and cleanliness of the skin, to give the plumpness to the figure, beauty and freshness to the complexion and also to improve the breathing problem (Sollman, 1957).

Common medicinal preparations, which contained arsenic, include Fowler's solution (potassium arsenite), Donovan's solution (arsenic and mercuric iodides), Asiatic pills (arsenic trioxide and black pepper), de Valagin's solution (liquor arsenii chloridi), sodium cacodylate, arsphenamine (Salvarsan), neoarsphenamine, oxophenarsine hydrochloride (Mapharsen), arsthinol (Balarsen), acetarsone, tryparsamide and carbarsone (Arnold et al., 1990).

Others sources In addition, tobacco smoke is another source of As emission in the indoor environment. It is interesting to note that mainstream cigarette smoke contains 40–120 ng As per cigarette (USEPA, 1994).

1.3. Arsenic episodes round the word

Chronic exposure of As due to drinking of contaminated groundwater is a global catastrophe affecting several millions of people particularly in the developing world. Chronic As poisoning has been reported from Argentina, Bangladesh, Chile, China, Ghana, Hungary, India, Mexico, Taiwan, Thailand, the United Kingdom, and the United States (BGS/MMI, 1999; Bhattacharya and Mukherjee, 2001), where groundwater has been used primarily for drinking. The situation in the Bengal Delta Plain (BDP) in Bangladesh and in West Bengal, India, one of the densely populated regions of the world, is still critical where several millions are suffering from chronic As-related health effects (Bhattacharyaet al., 1997; Chatterjee et al., 1995) due to wide-scale dependence on groundwater for drinking. The occurrence, origin, and mobility of As in groundwater of sedimentary aquifers is primarily influenced by the local geology, hydrogeology, and geochemistry of the sediments as well as several other anthropogenic factors such as the land use pattern (Bhattacharya et. al, 1997).

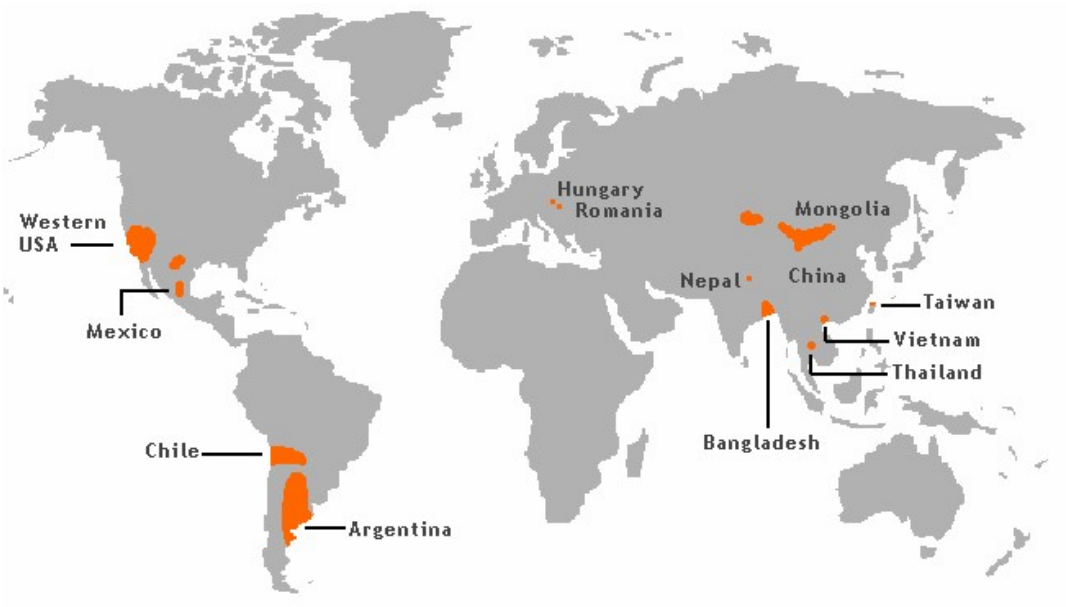

Figure 3. Arsenic contamination in world.

Arsenic poisoning episodes have been reported all over the world. Exposure to arsenic may come from natural sources, from industrial sources, or from food and beverages. So, arsenic episodes all over the world are divided into three categories:

1. Natural groundwater arsenic contamination.
2. Arsenic contamination from industrial source.
3. Arsenic contamination from food and beverage.

Here some episodes around the world are described.

Argentina. A population of nearly 1.200.000 in rural Argentina depends on groundwater with As concentrations exceeding 10 μg L^{-1} and the local Argentinian permissible limit of 50 μg L^{-1}. The most affected areas are extended parts of the Pampean plain, some parts of the Chaco plain, and some small areas of the Andean range where drinking-water wells contain 50–2000 μg As L^{-1} (Smedley et al., 1998; Bundschuh et al., 2000). ‘‘Bell Ville Disease,’’ is a local term describing the As-induced skin cancer, and other cancers of the kidney and liver are associated with As exposure through groundwater (Hopenhayn-Rich et al., 1998).

Bengal Delta Plain (BDP)**.** The natural incidence of high-As groundwater in the vast tract of alluvial aquifers within the BDP in Bangladesh and West Bengal, eastern India, has caused a health crisis for a population of over 75 million in the region. Nearly 50 million in Bangladesh are drinking well water with As levels above the acceptable limits. Manifestations of chronic As-related diseases such as arsenical dermatosis, hyperkeratosis, and hyperpigmentation and cancers of the skin have been identified by several epidemiological studies (Chatterjee et al., 1995). Large-scale exploitation of groundwater resources to meet the rising demand of safe water for drinking and agriculture has resulted in this largest As calamity in the world. In addition, As exposure from the diet (Naidu, 2000) and the synergetic effects of As and other toxic metals in groundwater and air and their impact on human health also need to be studied in detail. Arsenic was first identified in Bangladesh’s well water by the Department of Public Health Engineering in 1993 (BGS/MMI, 1999). Of 64 districts in Bangladesh, in 60 districts covering approximately 118.000 km^2 (nearly 80% of the country), groundwaters in a majority of wells have As concentrations exceeding the WHO limit and 30% of the groundwater contains As at levels 50 μg L^{-1}, the Bangladesh drinking water standard (Karim, 2000). Arsenic concentrations exceeding 1000 μg L^{-1} and as high as 14 mg L^{-1} in shallow tube wells are reported from 17 districts in Bangladesh (Karim, 2000).

Chile. Approximately 400.000 residents of northern Chile drink water from public supplies that are diverted from rivers in the Andes Mountains to the arid regions. However, many of these rivers have high levels of natural As that often ends up in northern Chile's drinking water (Borogoñ̃o et al., 1972). High As in drinking water has been associated with increased mortality from bladder, lung, kidney, and skin cancers. Epidemiological studies in Chile indicated that exposure to As concentrations through drinking water containing 50 μg L^{-1} had had severe toxic effects on human health. The public water system of Antofagasta (the largest city in this region) had approximately 800–1000 μg L^{-1} of As (Sancha, 1995).

China. Large areas in the Xinjiang and Inner Mongolia provinces of China have drinking water with high As concentrations (around 50–1860 μg L^{-1}) (Lianfang et al., 1994). The source of As in both provinces is geogenic. In the Kuitun area of Xinjiang province of China, was found several people with endemic arsenicosis, fluorosis, and combined As and fluoride poisoning. The Huhhot Alluvial Basin (HAB) of Inner Mongolia is predominantly an agricultural area with wheat, rice, millet, corn, green beets, potatoes, and sunflowers as the primary cultivated crops. There are no sources of anthropogenic emissions of As from industries and mines into the atmosphere, water, or soil and no arsenical pesticides have been used. Analyses of the surface soils, air, fish, and crops, however, do not show levels of As above the regulatory limits. Approximately 5.3% of the people from this basin had visible hyperkeratosis with hyperpigmentation or hypopigmentation (Luo et al., 1997). The concentration of As in groundwater from the HAB exceeds the provisional limits of the WHO (10 μg L^{-1}) for safe drinking water by a factor of more than 5–100 as well as the Chinese national drinking water standard by factors 1–20 (Luo et al., 1997).

Mexico. Mining has been an important economic activity in the Zimapàn Valley in Mexico and several towns were developed around these mines. Oxidation of arsenopyrite and solubilization of scorodite in the mine wastes, generated during centuries of silver, zinc, and lead mining (Armienta and Rodriguez, 1995), leach As into the aquifers and cause natural As contamination in the drinking water wells of the region. Groundwater is the only drinking water source for the community of nearly 10.000 inhabitants in Zimapàn. The highest levels of As found in groundwater range up to 1100 μg L^{-1}. The shallow wells are contaminated from the mine tailings and fumes emanated by the smelters and have As concentration up to 530 μg L^{-1}. Chronic As poisoning, which includes skin cancer, and kidney and liver diseases are common among the residents in the Zimapàn area (Armieta et al., 1997).

UK. The old Cornwall and Devon mining and smelting regions currently have agricultural soils and household dusts with up to 1000 mg kg^{-1} or more of As (Smith and Lloyd, 1986). Treated surface water is currently used for drinking owing to extensive groundwater contamination. The concentrations of As in untreated and treated surface waters are 10–50 μg L^{-1} and typically less than 10 μg L^{-1}, respectively.

In Armadale, a town in central Scotland having population 7000, the standardized mortality ratio (SMR) for respiratory cancer are high and high sex-ratios of births were observed during 1969–1973 which was due to arsenic contamination from a steel foundry located in that area. The arsenic concentration in soil samples in Armadale was around 52–64 μg g^{-1} (Smith and Lloyd, 1986).

USA. Nearly 10% of groundwater resources in the United States indicate As concentrations exceeding the drinking water guideline of 10 μg L^{-1}(Welch, 2000). In general, highest As concentrations are encountered in the western part and large areas of the midwest and northeast, exceeding the national and WHO drinking water guideline value of 10 μg L^{-1}. The most prevalent mechanisms of widespread concentrations of As are desorption and reductive dissolution of iron oxides and oxidation of sulfide minerals, in addition to up flow of geothermal water and evaporative concentrations (Schreiber et al., 2000). An independent survey of Alaska, Arizona, California, Idaho, Indiana, Nevada, Oregon, and Washington evaluated the effect of geological environment on groundwater As concentration. Aquifers made from basins that were filled with alluvial (windblown) or lacustrine (lake) deposits had As concentrations that ranged from 50 to 2750 μg L^{-1}. Aquifers in volcanic terrain, adjacent to geothermal systems, and in uranium and gold mining areas had As concentrations that ranged from 170 to 3400.800 to 15.000, and 130 to 48.000 μg L^{-1}, respectively (Welch et al., 1988). In addition, five public drinking water supply systems in Nevada had As concentration above 50 μg L^{-1} standard (Fontaine, 1994).

Taiwan. In Taiwan, a population of approximately 20.000 were exposed to As due to drinking groundwater from artesian wells containing up to 1820 μg L^{-1} As. Nearly 1141 cases of chronic As poisoning were diagnosed in 1975. The term ‘‘Blackfoot disease’’ was coined to describe arsenicosis of the lower legs (Hsu et al. 1997; Chen et al., 1994). In addition, As exposure is also associated with skin, bladder, kidney, ureter, urethral, liver, and lung cancers. However, recent improvements to the drinking water supply have reduced As exposure, which in turn has reduced the incidence of cancer (Guo et al., 1997).

Japan. Several instances of accidental arsenic poisoning through contaminated food-stuffs are reported in Japan. Soyasauce, which contained arsenic at 5.6–71.6 mg L^{-1}, is implicated in a toxicity outbreak. The arsenic is in the amino acids (260–275 mg L^{-1}) used in making the sauce; hydrochloric acid may have been the source of arsenic in the preparation of amino acid (Nose, 1957). After that were examined 220 out of 417 patients, who had been poisoned by soyasauce contaminated with inorganic arsenic at a concentration of 0.1 mg L^{-1}. The average estimated injection of arsenic per person was 3 mg, daily for 2–3 weeks (Mandal and Suzuk, 2002).
Contaminated powdered milk was implicated in a similar outbreak in Japan. It contains arsenic at 13.5–21 mg kg^{-1}. Contamination of the milk is from sodium phosphate (7.11% arsenic) used in its manufacture (Mandal and Suzuk, 2002).

1.3.1. Arsenic diffusion in Italy

In Italy, the largest arsenic concentration is found in the Lazio and Campania regions, predominantly in fluvial and sea sediment (Beni et al., 2011). High arsenic concentrations have been detected in topsoil and subsoil in southern Campania, in eastern Lombardia, in the Roman-Neapolitan volcanic province, all along Puglia, and in central Calabria (De Vivo et al., 2009). Furthermore, arsenic concentrations above 50 μg L^{-1} have been found in groundwater of several regions, including Campania, Lazio, Toscana, Emilia-Romagna, Lombardia, Veneto, and Sardegna (Aiuppa et al., 2003), which is of concern because long-term use of arsenic contaminated water for irrigation can lead to elevated arsenic levels in soils. This geochemical background, as modified by agricultural soil management practices and impacts from other human activities, including industrial processes and other anthropogenic sources of arsenic contamination, determines the distribution of arsenic in agricultural soils. The value concentration of As in surface water has been estimated to be 2 μg L^{-1} (Ivanov, 1996), a value too high compared with the value for European surface water (0.63 μg L^{-1}, Salminen et al., 2005). For Italian surface waters FOREGS database found a median value around 0.47 μg L^{-1} (De Vivo et al., 2008; 2009); for European tap water (0.19 μg L^{-1}) (Reimann and Birke, 2010); whereas Italian tap waters range from 0.016 to 27.2 μg L^{-1}. The geographic distribution of As concentrations, at regional scale, is not homogeneous with the highest median value in Lombardia and Campania. Lazio, Campania and southern Toscana belong to the Roman Comagmatic volcanic Province which is well known for its high As background values, recorded in soils and stream waters (De Vivo et al., 2008; 2009; 2010) related to the alkaline volcanic products (Dall'Aglio et al., 2001; Giuliano et al., 2005) and to the hydrothermal activity (Mantelli et al.,

2005) Other areas where high As concentrations are known in groundwater are Lombardia (Castelli et al., 2005) and the central Po River Plain (Castelli et al., 2005; Farina et al., 2005) where relatively high As concentrations in tap waters have also been found.

The highest value (27.2 μg L^{-1}) has been registered in a tap water from Viterbo (Lazio) exceeding the EU and Italian drinking water limit of 10 μg L^{-1}. For Lazio, because of its elevated As background values, registered in different environmental media (De Vivo et al., 2009), as already pointed out, the threshold has been increased to 50 μg L^{-1} by regional authorities. This poses some questions because chronic exposure to As increases the risk of cancer and skin pigmentation (WHO, 1996).

In Toscana, there is an extensive soil contamination with As due both to natural sources and to mining and processing activities (Costagliola et al., 2004). Highly As-polluted soils have been found in Scarlino, close to Grosseto, Toscana (ARPAT-UNIFI, 2003). Arsenic contamination in soils, gravitational and clean water, diet and milk in bovine milk chain have already been considered (Beni et al., 2007) results showed that the geological presence of As could justify the high concentrations found in drinking water (Beni et al., 2008).

1.4. Toxicity

Arsenic is toxic to nearly all forms of life. The chemical forms and oxidation states of arsenic are more important as regards to toxicity. It is generally recognized that the soluble inorganic arsenicals are more toxic than the organic ones. In fact inorganic arsenic (Asi) has been evaluated by the International Agency for Research on Cancer (IARC) as group 1 carcinogen (IARC, 2004).

Arsenite is known to inhibit more than 200 enzymes in the body (Abernathy et al., 1997) because it can interfere with sulfhydryl groups of proteins and enzymes (Gebel, 2000) increasing of reactive oxygen species in the cells, consequently causing cell damage (Chen et al., 1998; Ahmad et al., 2000).

Although As(III) is regarded the more toxic form of the element, As(V) as arsenate can be disruptive by competing with phosphate. Arsenate has a similar structure as phosphate, it can substitute for phosphorus in the body, (NRC, 1995). As(V) is hydrolyzed easily, it prevents subsequent transfer of phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) (Winship, 1984). Arsenate may also replace the phosphorous in DNA and this appears to inhibit the DNA repair mechanism. Hence the energy metabolism is inhibited and glucose-6-arsenate is produced rather than glucose-6-phosphate.

Trivalent methylated arsenicals can induce oxidative stress for instance inhibiting glutathione (GSH) reductase (Styblo et al., 1997) and thioredoxin reductase (Lin et al., 1999) with subsequent impairment of cellular protective mechanism against oxidants. While depletion of cellular GSH sensitizes cells to arsenicals and may also contribute to cell transformation (Shimizu et al., 1998), thioredoxin depletion affects gene expression due to the fact that it modulates DNA binding activity of some transcriptional factors (Powis et al., 2000).

1.4.1. Toxicity to plant

Arsenic contamination of soil and water poses a serious threat to plants and animals. Plants and microorganisms are known to accumulate As in their tissues and exhibit a certain degree of tolerance. Biotoxicity is mostly determined by the nature and bioavailability of As species present in the contaminated habitat. An average toxicity threshold of 40 mg kg^{-1} has been established for crop plants (Sheppard, 1992). At high concentrations, As in plants inhibits their metabolic processes, such as photosynthesis through interference of the pentose–phosphate pathway, thereby inhibiting growth and often leading plant to death (Tu and Ma, 2002). Arsenic can also be the cause

of direct and indirect damages in plants. Direct damages in roots reduce uptake and transport of the nutrients. The decrease of these compounds, can causes severe anomalies in plant growth, development and reproduction (Epstein and Bloom, 2005). Biomass production and yields of a variety of crops have been shown to reduce significantly at high concentrations of As in soils (Carbonell- Barrachina et al., 1997). For instance, significant yield reductions of barley (*Hordeum vulgare L.*) and ryegrass (*Lolium perenne L.*) have been reported with the application of only 50 mg As kg^{-1} soil (Jiang and Singh, 1994). Plant uptake of As is greatly influenced by its species in soil. As discussed before, different As species have different solubility and mobility, thereby differing in their bioavailability to plants. Marin et al. (1992) reported that the order of As availability to rice (*Oryza sativa L.*) is as follows: As(III) > MMA > As(V) > DMA. They observed that upon absorption, DMA is readily translocated to the plant shoot, whereas As(III), As(V), and MMA accumulate primarily in the roots. While the application of As(V) and DMA did not affect rice growth, both As(III) and MMA were found to be phytotoxic to rice. Burlo et al. (1999) noted that both MMA and DMA in tomato plants (*Lycopersicon esculentum Mill*.) had a greater upward translocation than As(III) and As(V). In general, the accumulation of As in the edible parts of most plants is low (O'Neill, 1995), which is attributed to a number of reasons, including (Wang et al., 2002) low bioavailability of As in soil; restricted uptake by plant roots; limited translocation of As from roots to shoots; and phytotoxicity and subsequent premature plant death at relatively low As concentrations in plant tissues. Apart from chemical forms, it has been shown that the phytotoxicity of As varies with the soil conditions (Wang et al., 2002). Arsenic phytotoxicity is expected to be greater in sandy soils than in other soil types, as the former soils generally contain low amounts of Fe and Al oxides and silicate clays, which have been implicated in the adsorption of As from soil solution (Sheppard, 1992; Smith et al., 1998). The antagonistic and synergistic effects of various nutrient anions also determine the phytotoxicity of As to some extent. Arsenite penetrates the plant cuticle to a greater degree than As(V) and generally results in the loss of turgor (Adriano, 2001).

Arsenate is taken up by plant roots via phosphate transporters. Evidence for this comes from physiological and electrophysiological studies showing a potent inhibition of phosphate on arsenate uptake (Ullrich et al., 1989; Abedin et al., 2002). Different phosphate transporters may vary in their affinity for arsenate. For example, the As hyperaccumulator *P. vittata* appears to have a higher affinity for arsenate than nonhyperaccumulator plants (Wang et al., 2002). Therefore, plant roots take up arsenite mainly as the neutral molecule $As(OH)_3$. As in microorganisms and mammalian tissues (Bhattacharjee and Rosen, 2007), arsenite enters plant root cells via some aquaglyceroporin channels (Wallace et al., 2006).

Biochemical responses to toxic metals are complex and several defense strategies have been suggested such as the activation of the antioxidant enzymatic system (SOD, CAT, POX, GR and APX) (Gunes, 2009). SOD is induced primarily and it is responsible for the detoxification of O_2; CAT promotes H_2O_2 conversion to water in peroxisomes while in cytosol and chloroplasts this reaction occurs through ascorbate -glutathione cycle, which involves APX and GR (Lee et al., 2007; Shri et al., 2009). The evaluation of antioxidant enzymes activity and lipid peroxidation has been commonly used as oxidative stress indicators in plants (Lee et al., 2007).

1.4.2. Toxicity to humans and animals

Drinking water is the most important source of dietary intake of As by animals and humans (Fitz and Wenzel, 2002). However, food also forms a source of As exposure (Adriano, 2001). The occurrence of inorganic As in drinking water has been identified as a source of risk for human health even at relatively low concentrations. As a consequence, more stringent safer limits for As in drinking water have been proposed (Wenzel et al., 2001). Early symptoms of As poisoning in humans include abdominal pain, vomiting, diarrhea, muscular pain, and weakness, with flushing of the skin (Cullen, 1989). These symptoms are often followed by numbness and tingling of the extremities, muscular cramping, and the appearance of an erythematous rash. Further symptoms may appear within a month, including burning paraesthesias of the extremities, hyper/hypopigmentation, Mee'slines on fingernails, and progressive deterioration in motor and sensory responses (Murphy et al., 1981). Acute oral As poisoning at doses of 8 mg As kg^{-1} and above have been reported to affect the respiratory system (Civantos et al., 1995). Soluble As compounds can be rapidly absorbed from the gastrointestinal tract (Hindmarsh and McCurdy, 1986).

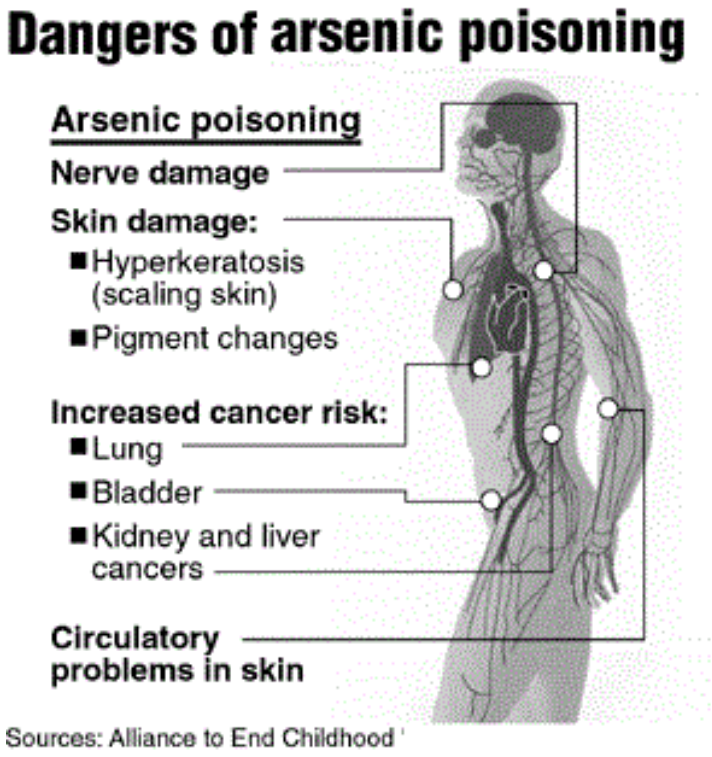

Figure 4. Arsenic poisoning in human.

Several studies in humans indicate that both inorganic form As(III) and As(V) and organic form MMA and DMA are well absorbed across the gastrointestinal tract (Buchet et al., 1981; USDHHS, 2000). Once absorbed, simultaneous partial oxidation of As(III) to As(V) and partial reduction of As(V) to As(III) occur, yielding a mixture of As(III) and As(V) in the blood. The As(III) may undergo enzymatic methylation primarily in the liver to form MMA and DMA, but the rate and relative proportion of methylation production vary among animal species. Most As is promptly excreted in the urine as a mixture of As(III), As(V), MMA, and DMA, and relatively smaller amounts are excreted in the feces. Arsenic may accumulate in skin, bone, and muscle and its half-life in humans is between 2 and 40 days (USDHHS, 2000). Some As may remain bound to tissues, depending on the rate and extent of methylation. Chronic exposure to inorganic arsenic may give rise to several health effects including effects on the gastrointestinal tract, respiratory tract, skin, liver, cardiovascular system, hematopoietic system, nervous system etc. (Fig. 4) The earliest reports date back to the latter part of the 19th century when the onset of skin effects (including pigmentation changes, hyperkerotosis and skin cancers, (Fig. 5) were linked to the consumption of arsenic in medicines and drinking water (Mandal and Suzuki, 2002).

Ingested elemental arsenic is considered less toxic, poorly absorbed and largely eliminated unchanged from the human body. Signs of chronic arsenic toxicity include dermal lesions (Zaloga

et al., 1985), peripheral neuropathy, skin cancer and peripheral vascular disease (Fig. 4). These signs have been observed mostly in populations whose drinking water contains arsenic (Cebrian et al., 1983; Smith et al., 2000). Among these symptoms, dermal lesions were most dominant, and were also known to occur within a period of about five years. The skin is known to localize and store arsenic because of its high keratin content, which contains several sulfhydryl groups to which As(III) may bind (Kitchin, 2001) and may be the reason for its sensitivity to arsenic toxic effect. Studies from Taiwan was clearly demonstrated that exposure to arsenic via drinking water is associated with Blackfoot disease (BFD), with significant exposure–response relationships relating both the duration and level of exposure to observed effects, which is characterized by a progressive loss of circulation in the hands and feet, which ultimately leads to severely painful gangrene formation of the extremities (particularly the toes and feet), often necessitating amputation of the limb (Duker, 2005). Skin cancers, including in situ cell carcinoma (or Bowen's disease), invasive cell carcinoma and multiple basal cell carcinomas, are all known to be associated with chronic arsenic exposure (Shneidman and Belizaire, 1986). Chen et al. (1995) observed that hypertension was linked to long-term arsenic ingestion as well as cerebrovascular disease. Other effects are hematopoietic depression, anhydremia, liver damage characterized by jaundice, portal cirrhosis and ascites, sensory disturbance and peripheral neuritis, anorexia and loss of weight (Webb, 1966). Although the effects of arsenic, as recounted above, result in several kinds of diseases, it certainly may also impact adversely on the immune system, which may predispose to viral/bacterial infections.

a) b)

Figure 5. some effects (a, b) of arsenic poisoning in human.

Arsenic does not appear to be mutagenic in bacterial and mammalian assays, although it can induce chromosomal breakage, chromosomal aberration, and chromatid exchange. The acute toxicity of As compounds in humans is a function of their rate of removal from the body. Lethal doses in humans

range from 1.5 mg kg^{-1} to 500 mg kg^{-1} of body weight (DMA). Studies have also revealed hepatic effects of As poisoning (USDHHS, 2000), as indicated by swollen and tender liver with elevated levels of hepatic enzymes in blood. Arsenic is perhaps the only human carcinogen for which there is adequate evidence of carcinogenic risk by both inhalation and ingestion (Chen et al., 1945).

1.5. Distribution in the air

Arsenic enters in the atmosphere through inputs from wind erosion, volcanic emissions, low temperature volatilisation from soils, marine aerosols and pollution and is returned to the earth's surface by wet and dry deposition. The most important anthropogenic inputs are from smelter operations and fossil-fuel combustion. The As appears to consist of mainly $As(III)_2O_3$ dust particles (Cullen and Reimer, 1989).

In air, arsenic exists predominantly absorbed on particulate matters, and is usually present as a mixture of arsenite and arsenate, with the organic species being of negligible importance except in areas of arsenic pesticide application or biotic activity. The human exposure of arsenic through air is generally very low and normally arsenic concentrations in air ranges from 0.4 to 30 ng m^{-3} (WHO, 1996). According to United States Environmental Protection Agency **(**USEPA) the estimated average national exposure in the U.S. is at 6 ng As m^{-3}. Absorption of inhaled arsenic ranges between 30 and 85%, depending on the relative portions of vapour and particulate matters. USEPA estimates that the general public will be exposed to a range of approximately 40–90 ng per day by inhalation. The As amount inhaled per day is about 50 ng or less (assuming that about 20 m^{-3} of air is inhaled per day) in unpolluted areas. The daily respiratory intake of As is approximately 120 ng of which 30 ng would be absorbed. Typical As levels for the European region are currently quoted as being between 0.2 and 1.5 ng m^{-3} in rural areas, 0.5 and 3 ng m^{-3} in urban areas and no more than 50 ng m^{-3} in industrial areas (WHO, 1981). Microorganisms can form volatile methylated derivatives of arsenic under both aerobic and anaerobic conditions, and can reduce As compounds to release arsine gas (AsH_3) (Tamaki and Frankenberger, 1992).

1.6. Distribution in water

Arsenic is found at low concentration in natural water (Frankenberger, 2002). In aquatic environment it is distributed in both the aqueous solution and sediments. At high Eh values, arsenic acid species (H_3AsO_4, $H_2AsO_4^-$, $HAsO_2^{4-}$, and AsO_3^{4-}) are stable. At mildly reducing conditions, arsenious acid species (H_3AsO_3, H_2AsO^{3-}, and $HAsO_2^{3-}$) become stable (Smith, 1986). The speciation of As in aquatic environment is critical in controlling the adsorption/desorption reactions with sediments. As well as in soil, in aquatic environments redox reactions not only determine the nature of chemical species, but also the solubility and mobility of As (Adriano, 2001). Concentrations and relative proportions of As(V) and As(III) vary according to changes in input sources, redox conditions and biological activity. (Cullen and Reimer, 1989).

Background concentrations of As in groundwater are in most countries less than 10 mg L^{-1} (Welch et al., 2000). However, values quoted in the literature show a very large range from 0.5 to 5000 mg L^{-1}. Elevated concentrations of As in natural waters are usually associated with As-rich sedimentary rocks of marine origin, weathered volcanic rocks, fossil fuels, geothermal areas, mineral deposits, mining wastes, agricultural use, and irrigation practices (Korte and Fernando, 1991).

In groundwater As is mobilized through complex geochemical processes in natural environments. Anoxic conditions in the subsurface environments enhance As mobility, which renders groundwater more vulnerable for As contamination as compared to surface water. In groundwater, inorganic As commonly exists as As(V) and As(III), the latter being considered to be more mobile and toxic for living organisms. In aqueous environments prokaryotes and eukaryotes reductively biomethylate inorganic As to DMAA and MMAA (Smedley and Kinniburgh, 2002).

The seawater ordinarily contains 1–8 μg L^{-1} of arsenic. In oxic seawater, the As is typically dominated by As(V), though some As(III) is invariably present and becomes of increasing importance in anoxic bottom waters. As (V) should exist mainly as $HAsO_4^{2-}$ and $H_2AsO_4^-$ in the pH range of seawater (pH around 8.2) and As (III) mainly as the neutral species H_3AsO_3. Increases in organic As species have also been recorded in these zones as a result of methylation reactions by phytoplankton (Cullen and Reimer, 1989).

Baseline concentrations of As in river waters are also low (in the region of 0.1–0.8 mg L^{-1} but can range up to 2 mg L^{-1}). They vary according to the composition of the surface recharge, the contribution from base flow and the bedrock lithology. Relatively high concentrations of naturally-occurring As can occur in some areas as a result of inputs from geothermal sources or high-As groundwater (McLaren and Kim, 1995).

Arsenic concentrations in river waters from geothermal areas have been reported typically at around 10–70 mg L^{-1} (Nimick et al., 1998). In lake waters As concentrations are typically close to or lower than those found in river water. Baseline concentrations have been found at < 1 mg L^{-1} (Azcue and Nriagu, 1995; Azcue et al., 1995). In lake waters, increased concentrations are found in lake waters affected by geothermal water and by mining activity. Ranges of typically 100–500 mg L^{-1} have been reported in some mining areas and up to 1000 mg L^{-1} in geothermal areas (Azcue and Nriagu, 1995). In lake and river waters, As(V) is also generally the dominant species (Pettine et al., 1992), though significant seasonal variations in speciation as well as absolute concentration have been found. The presence of As(III) may be maintained in oxic waters by biological reduction of As(V), particularly during summer months.

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1.7. Distribution of arsenic in the soil

Arsenic can exist in soil in different oxidation states but mostly as inorganic species, As(V) or As(III) (Adriano, 2001). In addition to inorganic species, microbial methylation of As in soil results in the release of organic methylarsenic compounds, such as MMA and DMA, and ultimately arsine gas (Smith et al., 1998). Both inorganic and organic species of As undergo various biological and chemical transformations in soils, including adsorption, desorption, precipitation, complexation, volatilization, and methylation (Maeda, 1994). The most thermodynamically stable species of As(III) (H_3AsO_3 and $H_2AsO_4^-$) and As(V) ($HAsO_2^{4-}$) occur over the normal soil pH range of 4 to 8 (Irgolic et al., 1995). Although the dominant source of As in soils is geological, and hence dependent to some extent on the concentration in the parent rock material, additional inputs may be derived locally from industrial sources such as smelting and fossil-fuel combustion products and agricultural sources such as pesticides and phosphate fertilisers (Bhattacharya et al., 2002b). Generally, baseline concentrations of As in soils are generally of the order 5-10 mg kg^{-1}. However, anthropogenic sources of As have elevated the background concentration of As in soils (Adriano, 2001). In areas near As mineral deposits, As levels in soils may reach up to 9300 mg kg^{-1} (Ashley and Lottermoser, 1999). Depending on the nature of the geogenic and anthropogenic sources, As concentration in soils can range from 1 to 250.000 mg kg^{-1}. Arsenic forms solid precipitates with Fe, aluminium (Al), calcium (Ca), magnesium (Mg), and Ni. A number of studies involving solid phase speciation have shown that As is prevalent mostly in the oxalate fractions associated with amorphous and crystalline Fe and Al oxides, indicating the strong affinity of As for these soil components (Wenzel et al., 2001). The soluble As concentration in soil is largely determined by redox conditions, pH, biological activity, and adsorption reactions. The adsorption and mobility of As in soil are affected more strongly by the presence of $H_2PO_4^-$ ion than any other anions. Arsenic is subject to both chemical and biological transformations in soils, resulting in the formation of various species (Mahimairaja, 2005).

Arsenates of Fe and Al ($AlAsO_4$, $FeAsO_4$) are the dominant phases in acid soils and are less soluble than calcium arsenate (Ca_3AsO_4), which is the main chemical form in any alkaline and calcareous soils (Fordyce et al., 1995).

Biotransformation of As, involving the oxidation of As(III) to As(V) and the reduction of As(V) to As(III) by a variety of microorganisms, may occur in contaminated soil. For example, *Alcaligenes faecalis* was found to oxidize As(III) to As(V) (Phillips and Taylor, 1976). Bacteria, fungi, and algae are also able to reduce As(V) to As(III) and subsequently to arsine (Frankenberger and Losi,

1995). However, the effect of microbial activity on the transformation and movement of As in soil is difficult to quantify (Smith et al., 1998).

1. 8. Controls on As mobilization in soil

In soil environments, redox reactions not only determine the nature of chemical species, but also the solubility and mobility of As and thus its environmental significance. Arsenic in soils is subject to both abiotic and biotic redox. The reaction of As compounds in soils is affected by sorption/desorption on/from soil components or co-precipitation with metal ions. The importance of oxides (mainly Fe-oxides) in controlling the mobility and concentration of arsenic in natural environments has been studied for a long time (Frankenberger and Losi, 1995; Smedley and Kinniburgh, 2002). Arsenic is known to have high affinity for oxide surfaces, and several biogeochemical factors are found to play a major role in adsorption. Soil particle size, organic matter, type and nature of constituent minerals, pH, redox potential, and competing ions have all been shown to influence As adsorption (Chiu and Hering, 2000; Jones et al., 2000; Smith et al., 1998). In general, adsorption of As(V) decreases with increasing pH. In contrast, adsorption of As(III) increases with increasing pH. The effect of pH on As adsorption varies considerably among soils and is dependent on the nature of mineral surface. In soils containing low oxidic minerals, increasing the pH has little effect on the amount of As(V) adsorbed, whereas in highly oxidic soils, adsorption of As(V) decreases with increasing pH (Smith et al., 1998). The Fe(III) oxides, Mn(III) oxides, and organic compounds in soils play a major role in catalyzing the abiotic oxidation of As(III) through an electron transfer mechanism (Adriano, 2001). Fe oxides are probably the most important adsorbents in sandy aquifers because of their greater abundance and the strong binding affinity. Nevertheless, Al oxides can also be expected to play a significant role when present in quantity (Manning and Goldberg, 1997). Experience from water treatment suggests that below pH 7.5, Al hydroxides are about as effective as Fe hydroxides for adsorbing As(V) but that Fe salts are more efficient at higher pH and for adsorbing As(III) (Edwards, 1994). Ferrous oxides/hydroxides are involved most commonly in the adsorption of As in both acidic and alkaline soils. Ferric hydroxide generally plays a much more important role in controlling the concentration of As in soils as well as in aqueous media. The precipitation of ferric hydroxide can be expressed by the reaction:

$$Fe^{3+} + 3H_2O \Leftrightarrow Fe(OH)_3 + 3H^+$$

This reaction has critical importance for retention and mobilization of As in soils. Both As(V) and As(III) are adsorbed on $Fe(OH)_3$, but affinity for adsorption is higher for As(V) as compared to As(III). The adsorption optimum for As(III) is around pH 7.0, while As(V) adsorbs optimally at pH 4.0. Carbonate minerals adsorb As in calcareous soils. In acidic soils, Mn oxides and biogenic particles play a dominant role in the adsorption of As (Arai et al., 2003).

Brookins (1988) observed that amorphous Al and Fe hydroxides adsorbed more As(V) than As(III). The surface charge properties of variable charge soil components are strongly influenced by pH. At acid pH these soil components contain large amounts of positive charges, and adsorption of As(V) may become important. Arsenate ions are attracted to positively charged colloidal surfaces either at broken clay lattice edges where charged Al^{3+} groups are exposed or on surfaces of Fe and Al oxides and hydroxide films. Arsenate is strongly adsorbed at acidic pH values on amorphous $Al(OH)_3$, a-Al_2O_3, ferrihydrite, and hematite (Arai et al., 2001; Raven et al., 1998 ; Xu et al., 1988). Several spectroscopic, macroscopic and thermodynamic modeling, have revealed innersphere bidentate binuclear and/or monodentate As(V) complexes on ferrihydrite, goethite, amorphous Fe and Al oxides, and the bayerite polymorph (Arai et al., 2001; Fendorf et al., 1997) and on both inner sphere and outer sphere As(III) complexes on Al oxides (Arai et al., 2001). The adsorption of As(V) and As(III) is strongly affected by changes in ionic strength, which is indicative of inner-sphere complexation (Krauskopf and Bird, 1995). Monodentate, bidentate-binuclear and bidentate-mononuclear complex in different proportions depending on pH and surface coverage (Fendorf et al., 1997; O'Reilly et al., 2001).

There are two types of surfaces complexes, *inner-sphere* and *outer-sphere*. An *inner-sphere* surface complex has no water molecules interposed between the surface functional group and the small ion or molecule it binds, whereas an *outer-sphere* surface complex has at least one interposed water molecule (Sposito, 2000). In outer sphere complex, the absorbent is indirectly attached to the adsorbent surface through one or more water molecules. The inner-sphere adsorption complexes are further divided into monodentate, bidentate-mononuclear and bidentate binuclear types. In monodentate complexes each adsorbed species attaches onto only one atom on the adsorbent surface. Bidentate-mononuclear complexes consist of two atoms bridging between an atom and the adsorbed species and adsorbent metal atom. Bidentate- binuclear complexes consist of one adsorbed atom bonding to two separate metal oxides (Krauskopf and Bird, 1995).

Soil organic matter content also affects the adsorption of As and thus its bioavailability as organic molecules compete with As for sorption to surface sites. Thanabalasingam and Pickering (1986) showed that the maximum adsorption of As(V) on humic acids occurred around pH 5.5, whereas adsorption of As(III) increased up to pH 8. Due to its strong adsorption onto organic and clay

colloids, As(V) is likely to persist in soils for a long time, especially in fine-textured soils with high Fe contents (Woolson, 1983). In these soils, leaching of As(V) is low and therefore As contamination of groundwater is considered unlikely (Woolson, 1983). However, under certain environmental conditions (low pH and low Eh), As would leach in the soil profile, thereby contaminating the surface and groundwaters (Hingston et al., 2001; Ruokolainen et al., 2000). Considerable amounts of solubilized As could move downward in the soil profile with leaching water, especially in coarse-textured soils.

Arsenic in soil is also subject to biological transformation resulting in the formation of organo-arsenicals and other compounds (Maeda, 1994). The pathway of As(V) methylation initially involves the reduction of As(V) to As(III), with the subsequent methylation of As(III) to dimethylarsine by coenzyme S-adenosylmethionine (Frankenberger and Losi, 1995). Methylation is often enhanced by sulfate-reducing bacteria. In addition to bacteria, several fungal species also have shown their ability to reduce As. Inorganic As is incorporated by autotrophic organisms such as algae and is then transported through the food chain. Arsenic becomes progressively methylated during this transfer. Therefore, methylation of As is considered a major detoxifying processes for these microorganisms (Adriano, 2001). The methylated As species is also subject to volatilization and photochemical reactions that may eliminate As from soil. Demethylation of methylarsenicals can occur under both aerobic and anaerobic conditions. Anaerobic demethylation reactions may result in the formation of toxic and reactive AsH_3 from less toxic DMA, whereas aerobic demethylation of DMA is likely to yield As(V), thereby retaining As in the system. Although AsH_3 undergoes rapid chemical oxidation under oxic conditions, it can exist for long periods in an aerobic environment (Adriano, 2001).

1.9. Controls on mobilization in aquatic environment

The speciation of As in aquatic environment is critical in controlling the adsorption/desorption reactions with sediments. Adsorption to sediment particles may remove As(V) from contaminated water, as well as inhibiting the precipitation of As minerals such as scorodite ($FeAsO_4 \cdot 2H_2O$) that control the equilibrium aqueous concentration (Foster et al., 1997). Under the aerobic and acidic to near-neutral conditions, As(V) is adsorbed very strongly by oxide minerals in sediments. Adsorption occurring in natural environments protects water bodies from widespread As toxicity problems. As species are adsorbed by sediments are as follows: As (V) > As(III) > As (II) > DMA (Smedley and Kinniburgh, 2002). In As-contaminated sediments, Clement and Faust (1981) found that a significant portion of the As was bound in organo-complex forms and indicated that adsorption–desorption equilibrium must be considered as well as the redox effects in examining the dynamics of As in aquatic environment. As pH increases, especially above pH 8.5, As desorbs from the oxide surfaces, thereby increasing the concentration of As in solution. Desorption of As from As-contaminated sediments at high pH is the most likely mechanism for the development of groundwater As problems under the oxidizing conditions (Smedley et al., 2002).

Arsenic undergoes a series of biological transformations in the aquatic environment, yielding a large number of compounds, especially organoarsenicals. Certain reactions, such as oxidation of As(III) to As(V), may occur both in the presence and in the absence of microorganisms, whereas other reactions, such as methylation, are not thermodynamically favorable in water and can occur only in the presence of organisms. Some bacteria and marine phytoplankton are capable of reducing As(V) to As(III) or oxidizing As(III) to As(V) (Andreae, 1977). Biological reduction of As(V) to As(III) reportedly occurs most easily at a pH between 6 and 6.7 (Korte and Fernando, 1991). Methylation may play a significant role in the mobilization of As by releasing it from the sediments to aqueous environment. Benthic microbes are capable of methylating As under both aerobic and anaerobic conditions to produce methylarsines and methyl-arsenic compounds.

1.10. Influence of Phosphate and other ions on Arsenic adsorption and desorption in soil.

In soil environments, several cations, anions, and molecules are present together, and then mutual interactions occur among them for sorption on soil components (phyllosilicates, humic substances, variable charge minerals, carbonate, and microorganisms). The soil components responsible for cation and anion (nutrients and pollutants) sorption include phyllosilicates, soil organic matter, carbonates, variable charge minerals (crystalline and short-range ordered Fe-, Al-, Mn-oxides, phyllosilicates coated by OH–Al and OH–Fe species, allophanes, imogolite), and microorganisms. Soil components differ greatly in their sorption capacities, their cation and anion exchange capacities, and the binding energies of their sorption sites (Violante, 2013).

Dynamics and mobility of cations, anions, and uncharged species in soil environments are significantly influenced by sorption/desorption reactions. All soil components are able to sorb cations, anions, and uncharged species, but they greatly differ in their sorption capacities and binding energies. In fact, even a single soil component (phyllosilicate or metal oxide), typically has different kinds of sorption sites that include a range of binding energies (Jackson, 1998). Many factors such as pH, surface coverage, ionic strength, nature, physicochemical properties of the surfaces of the sorbent, nature and oxidation state of the sorbate, and residence time affect the type of the complex formed by a single metal cation or anion. Numerous studies have been carried out on the sorption of As(III) and As(V) onto Fe or Al oxides. The presence of inorganic and organic ligands affects the sorption of As onto soil minerals and soils by competing for available binding sites and/or reducing the surface charge of the sorbents. Many studies on the competition between two or more anions for the surfaces of clay minerals and soils have assumed that the only mechanism implied was competition for sites. The competition in sorption is affected by the affinity of the competing anions for the surfaces of the sorbents, the nature and surface properties of the minerals and soils, the surface coverage and the reaction time (Zhu et al., 2011).

Most attention has been given to PO_4, which certainly affects the behavior of As. Despite their opposed toxic and life-supporting characteristics, the chemistry of AsO_4 and PO_4 have much in common (Fig. 6). Arsenic and P belong to the same chemical group and both have comparable dissociation constants for their acids and solubility products for their salts. Therefore, $H_2AsO_4^-$ and $H_2PO_4^-$ ions compete for the same sorption sites in soils, although some sites are preferentially available for the sorption of either $H_2PO_4^-$ or $H_2AsO_4^-$ ions. Both of these oxyanions are specifically

adsorbed on soil minerals, mainly on variable charge minerals (Al, Fe, and Mn oxides; allophanes, imogolite), forming inner-sphere complexes. These oxyanions may form three different surface complexes on inorganic soil components: a monodentate complex, a bidentate-binuclear complex, and a bidentate-mononuclear complex in different proportions, depending on surface coverages (Hsia et al., 1994; Fendorf et al., 1997; Smith et al., 1998; O'Reilly et al., 2001). The effect of Pi on the sorption/desorption of As in soil environments has received quite attention. A number of studies have shown that arsenate may be partially removed from soil colloids by Pi, but even large amounts of Pi cannot desorb all the applied arsenate (Frankenberger 2002; Violante and Pigna, 2002).

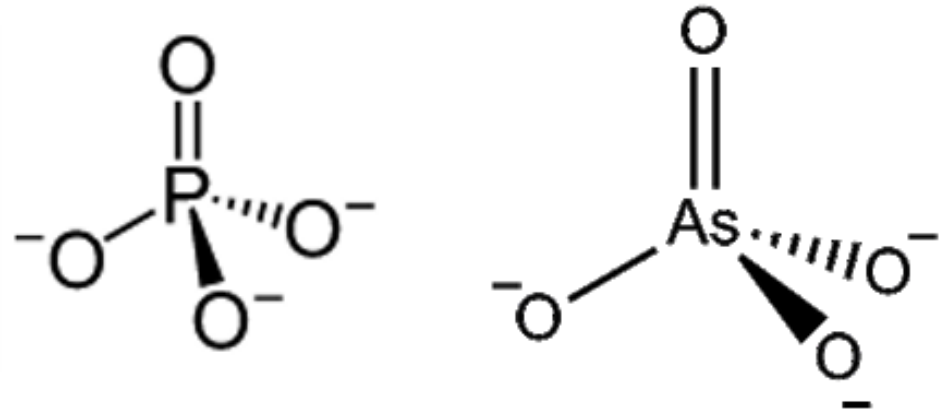

Figure 6. The Phosphate and Arsenate structure.

Soil pH and phosphate addition are the most important factors that control the desorption As. Phosphate has been reported to suppress the sorption of AsO_4 and to displace sorbed AsO_4 from soils (Smith et al., 1998). Woolson et al. (1973) observed that phosphate addition to an As-contaminated soil displaced about 77% of the total As in the soil. Although phosphate addition increases As solubility, Peryea (1991) reported that desorption of As was dependent on the soil type, as no increase in As concentration in soil solution from a volcanic soil (with high anion-fixing and pH-buffering capacity) was observed. This suggests that only large additions of P (>400 mg kg^{-1}) would affect the As solubility in these soils (Chen et al., 2002). One of the most important factor affecting the adsorption/desorption characteristics of As is the residence time in soils and sediments. For instance, Arai and Sparks (2002) reported that longer was the residence time, greater was the decrease in As(V) desorption at pH 4.5 and 7.8, suggesting non singular reactions. The surface transformation processes, such as rearrangement of surface complexes and conversion of surface complexes into aluminum arsenate-like precipitates, might be responsible for the decrease in As(V) reversibility with aging. Thus, the fate and transport of the contaminants must be

predicted/modeled not only on short-term adsorption and desorption studies, but also on long-term reactions. Jain and Loeppert (2000) found that PO_4 reduces As(III) adsorption onto ferrihydrite at low pH, but that the effect becomes insignificant at pH 9.0. At pH 7.0-8.0, conditions typical of waters in which arsenite is dominant, the sorbed load could be reduced by 20%. The effect of PO_4 on As(V) adsorption follows the opposite trend where adsorption decreases rapidly above about pH 6.0, and by 60% at pH 9.0. However these experiments were conducted at much higher concentrations than are normal in nature, and so the effect will be small in most practical situations. Dixit and Hering (2003) also showed that, in the presence of PO_4, As(III) is sorbed preferentially to As(V) over the pH range of 4-10. PO_4 has similar effects on adsorption by both goethite and ferrihydrite (Manning and Golgberg, 1996). Violante e Pigna (2002) showed how PO_4. Variably reduces the adsorption of As(V) (but not AsIII) on a variety of oxides, clay and soils in the range pH 4-8. Tested alone, most minerals adsorbed similar quantities of arsenate and PO_4; however, Fe, Mn and tioxides and iron-rich clay minerals (such as smectite) retained arsenate more strongly than PO_4. On the other hand, Al-rich minerals allophane, kaolinite and halloysite retained PO_4 more stronger than As(V).

In addition to phosphate, other ions can influence As sorbtion/desorbtion on soil components. Silicate decreases the adsorption of As(III) on ferryhydrite between pH 4-10 by as much a 35% and also decreases the adsorption of As(V) above pH 6 by as much as 60% (Swedlund and Webster, 1999). However, in natural waters these effects may be partly counteracted by Mg^{2+} and Ca^{2+} which increases As(V) adsorption on ferryhydrite at pH 9 (Wilkie and Hering, 1996) Ca^{2+}, also increases the adsorption of As(V) on Al oxides. Sulfate reduce the adsorption of As(III) between pH 2 and 6. Therefore SO_4 will not normally be a significant control on As sorption in sedimentary aquifers. Zhu et al. (2010) studied the sorption of As(III) and As(V) onto ferrihydrite as affected by pH. The capability of organic and inorganic ligands in preventing As sorption follows the sequence: $SeO_4 \approx SO_4 < OX < MAL \approx TAR < CIT < SeO_3 \ll PO_4$. The efficiency of most of the competing ligands preventing As(III) and As(V) sorption increased by decreasing pH, but whose efficiency increased by increasing pH. In acidic systems all the competing ligands inhibited the sorption of As(III) more than As(V), but in alkaline environments As(III) and As(V) seem to be retained with the same strength on the Fe-oxide.

1.11. Influence of Phosphate on Arsenic uptake by plant

Arsenate is a phosphate analogue as reported before, which enters in plant cells via the phosphate transporters (Ullrich-Eberius et al., 1989; Meharg and Macnair, 1990), and can interfere with phosphate metabolism. Use of phosphorus fertilizers can decrease As accumulation in plants, but has not always been successful, because phosphate competes with arsenate in both root uptake and adsorption on Fe oxides/hydroxides (Zhao et al., 2010). In fact in some cases, application of PO_4 fertilizers can enhance arsenic's phytoavailability in soil. (Peryea, 1991; Melamed et al., 1995). The bioavailable fraction of As in soils to crop plants depends on different physical and chemical properties of soils. In fact soils rich in variable charge minerals (Al or Fe oxides) do not release As easily. Arsenic toxicity in crops can be more prevalent in situation where As contamination is found coexisting with low available P. Plant uptake of As has been shown to increase upon P application in pot experiments (Jiang and Singh, 1994) and at field scale (Small and McCants, 1962). Arsenate can being transported across root plasma membrane, and there, Pi can compete much more effectively for transport sites (Meharg et al., 1994). In fact, arsenate resistance has been identified in a range of plant species, which is generally achieved through a decreased uptake of arsenate because of suppression of the high-affinity phosphate uptake system (Meharg and Macnair, 1991, 1992; Hartley-Whitaker et al., 2001). Nonresistant plants can be made more resistant to arsenate by raising their phosphorus status, which in turn leads to lower levels of arsenate accumulation through suppression of phosphate/arsenate uptake (Meharg et al., 1994). Arsenic tolerant varieties of Holcus lanatus down-regulate phosphate transporters which also accumulate arsenate from the rhizosphere (Meharg and Macnair, 1992). Bleeker et al. (2003) found a similar mechanism for the woody legume Cytisus striatus. The phosphate/arsenate transporter has a higher affinity for phosphate, and if external phosphate status is high, phosphate will be taken up more effectively compared to arsenate (Meharg and Macnair, 1994). However, in most arsenate-resistant plants the high-affinity uptake system is always suppressed and is insensitive to plant phosphorous status (Meharg and Macnair, 1992). Increasing cellular P status alleviates As toxicity. Thus decreased sensitivity in resistant plants with high phosphate status is not due to a difference in arsenate influx, but is presumably a result of higher cytoplasmic phosphate status. This may enable phosphate to compete more effectively with arsenate for ATP, decreasing arsenate toxicity within the cell (Meharg, 1994). In fact Lee et al. (2003) suggest for a tolerant Arabidopsis thaliana that increase phosphorus concentration in the cell which would allow phosphorus to out-compete arsenic in metabolic processes. Pigna et al. 2009, showed the role of Phosphorus application ameliorating the toxic

effects of As in wheat grown in contaminated soil. Peryea (1991) reported increased As solubility and, thus, the phytoavailability on P-fertilizer application to soils.

1.12. Remediation

Several technologies are currently available for As removal, ranging from simple and effective coagulation– flocculation, to sophisticated technologies such as ion exchange and reverse osmosis (Naidu and Bhattacharya, 2006). In addition, low-cost remediation methods, such as auto-attenuation and the use of geological material as natural sorbents for As (laterite, bauxsols, natural red earth or Fe-rich oxisols) have emerged as possible alternatives for the removal of As from groundwater in the developing world (Genç-Fuhrman et al., 2004; 2005; Naidu and Bhattacharya, 2006; Vithanage et al., 2006), but there is a pressing need to develop these methods further and in a cost-effective way. A large variety of methods have been developed to remediate metalloid-contaminated sites. The selection and adoption of these technologies depend on the extent and nature of As contamination, type of soil, characteristics of the contaminated site, cost of operation, availability of materials, and relevant regulations. The natural background concentration of As in soils is an important factor in assessing the environmental quality and strategies for subsequent remediation. Remediation of As in contaminated soil systems is much more complicated and often involves designing economically feasible and effective techniques that are site-specific (Mahimairaja et al., 2005). Both in situ and ex situ remediation technologies have been developed, although neither of these technologies have gained popularity because of the inconsistencies in results as well as involvement of high costs in the remediation process. In situ processes reflect all technologies directed to an unexcavated soil that remains relatively undisturbed. Ex situ processes treat soils that are disturbed either on- or off-site. In addition to these remediation technologies, As contaminated soils may be managed through chemical fixation techniques that reduce the bioavailability of As.

1.12.1. Soil remediation

Chemical fixation. Among the in situ remediation technologies, chemical fixation technique is often used to reduce the mobility of contaminants. Such a process either minimizes the potential for groundwater contamination by reducing contaminant leaching or environmental and human health risks through reduced contaminant availability. Chemical fixation involves addition of additives to the soil that immobilize hazardous elements. A number of methods have been developed mainly involving adsorption, immobilization, precipitation, and complexation reactions (Connor, 1990). However, such methods are often expensive for the remediation of large areas. Chemical fixation processes have been applied both in situ and ex situ, the latter being both on- and off-site. Four different approaches, usually, are often used in the chemical remediation of As- contaminated soils:

- Immobilization
- Adsorption
- Coprecipitation/precipitation
- Liming

Chemical immobilization is achieved mainly through adsorption/precipitation of As in contaminated sites through the addition of soil amendments. The mobilization of metalloids in soils for plant uptake and leaching to groundwater can be minimized by reducing their bioavailability through chemical and biological immobilization (Bolan et al., 2004). There has been interest in the immobilization of metalloids using a range of inorganic compounds such as lime, P fertilizers and alkaline waste materials, and organic compounds such as biosolids (Basta et al., 2001; Knox et al., 2000). Depending on the source, the application of P compounds can cause direct adsorption of As onto these materials, promote As complex formation, or induce desorption of As through competition. This method is considered more economical and less disruptive than the conventional remediation option of soil removal (Bolan et al., 2003). A number of organic and inorganic amendments are known to immobilize a range of metalloids including As by chemical adsorption. These include ion-exchange resin, ferrous sulfate, silica gel, gypsum, clay minerals such as bentonite, kaolin, and zeolite, green sand, and liming materials. These materials are naturally occurring and nontoxic with a large specific surface area and a significant amount of surface charge. Ferrous salt was used to generate in situ mineral phases to immobilize As. This reaction requires oxygen to be available to the soil and also generates considerable amounts of acid, which may be

counterproductive to As immobilization in poorly buffered soils. The increased acidity could be neutralized by the amendment (Faust, 1983).

A possible process of controlling As sorption was investigated by van der Hoek and Comans (1996). Using controlled leaching experiments they studied the sorption characteristics of As and Se on crystalline and amorphous Fe (hydr)oxide. They found that virtually all As and Se at the fly ash surface was associated with amorphous iron (hydr)oxides in the fly ash matrix. Using isotopic exchange experiments they concluded that at pH 10 the oxyanions were partly coprecipitated with secondarily formed amorphous iron (hydr)oxide, a process that reduced their availability (van der Hoek and Comans, 1996).

Liming is increasingly being used as an important soil management practice in reducing the toxicity of certain metalloid in soils. In addition to the traditional agricultural lime, a large number of studies have examined the potential value of other liming materials as immobilizing agents in reducing the bioavailability of a range of metalloid in soils (Bolan et al., 2003).

Physical remedation. Major physical in situ treatment technologies to remediate metalloid-contaminated sites include soil mixing, soil washing (Mahimairaja et al., 2005). The simplest technique for reducing the toxic concentration of As in soils is mixing the contaminated soil with uncontaminated soil. This results in the dilution of As to acceptable levels. This can be achieved by importing clean soil and mixing it with As-contaminated soil or redistributing clean materials already available in the contaminated site. (Mahimairaja et al., 2005).

Soil washing or extraction has also been used widely for the remediation of metal(loid)-contaminated soils in Europe (Tuin and Tels, 1991) and this method may be applicable for As-contaminated soils to some extent. The success of soil washing largely depends on speciation of As present in the contaminated soils, as it is based on the desorption or dissolution of As from the soil inorganic and organic matrix during washing with acids and chelating agents (Mahimairaja et al., 2005).

Bioremedetion- Heavy metals are among the most difficult contaminants to treat as these cannot be destroyed. Bioremediation of soils contaminated with organic compounds such as pesticides and hydrocarbons is widely accepted in which native or introduced microorganisms and/or biological materials, such as compost, animal manures, and plant residues, are used to detoxify or transform contaminants. There has been increasing interest in the application of this technology for the remediation of metal(loid)-contaminated soils, that undergo biological transformation. Although it

has several limitations, this technology holds continuing interest because of its cost effectiveness. The unique aspect in bioremediation is that it relies mainly on natural processes and does not necessarily require the addition of chemical amendments other than microbial cultures and biological wastes.

Four approaches could be used in the bioremediation of As-contaminated soils:

(a) bioaccumulation;

(b) biotrasformation;

(c) Volatilization;

(d) Phytobial remedation.

Bioaccumulation. Microorganisms exhibit a strong ability to accumulate As from a substrate containing very low concentrations of this element. Bioaccumulation is activated by two processes, namely biosorption of As by microbial biomass and its by products and physiological uptake of As by microorganisms through metabolically mediated or physico-chemical pathways of uptake. It consists of a metabolism-independent binding to negatively charged free groups in several biopolymers that form the microbial cell wall (Errasquin and Vazquez, 2003; Cernansky et al., 2007). Factors such as soil pH, moisture and aeration, temperature, concentration and speciation of As, soil amendments, and rhizosphere are known to influence the process of bioaccumulation of As in microbial cells (Zeng et al., 2010).

Biotrasformation. Heterotrophic bacteria have been found to oxidize toxic As(III) in soils and sediments to less toxic As(V) and thus could play an important role in the remediation of contaminated environment (Wakao et al., 1988). Because As(V) is strongly adsorbed onto inorganic soil components, microbial oxidation could result in the immobilization of As. Strains of *Bacillus* and *Pseudomonas spp.* (Frankenberger and Losi, 1995) and *Alcaligenes faecalis* (Phillips and Taylor, 1976) and *Alcaligenes spp*. were found capable of oxidizing As(III) to As(V) (Newman et al., 1997).

Biovolatization. It is an enzymatic conversion of organic and inorganic compounds of metalloids into their volatile derivatives by an intracellular biochemical reaction, which is well known as biomethylation (Mukhopadhyay et al., 2002). Biomethylation of As in soils and aquatic systems is well documented, as it is important in controlling the mobilization and subsequent distribution of

arsenicals in the environment (Frankenberger and Losi, 1995; Gao and Burau, 1997). The methylation of As(III) and As(V) has been observed in studies with fungi, bacteria, and algae in defined growth media amended with As. This process has been observed under both aerobic and anaerobic conditions. The earlier works of Challenger et al. (1954) demonstrated that two fungi, *Scopulariopsis brevicaulis* and *Aspergillus niger*, were able to produce trimethylarsine from various As compounds. In most cases, these organisms were tested in laboratory conditions; however, their performance should be assessed under field conditions in contaminated sites (Mahimairaja et al., 2005).

Phytobial remediation. Phytobial remediation aims to combine phytoremediation and bioremediation using microbes (Harman et al., 2004a). Plants are grown whose roots are colonised by symbiotic microbes that efficiently create stable microbial communities that degrade toxicants and assist plants in taking up toxic materials. It thus combines the best of both traditional bio- and phyto-remediation. One of the potential reasons why bioremediation fails is that the microbes bringing about the remediation do not have an energy source to promote their growth other than the toxicant which in itself may not be an energy source but instead be a transformation substrate. The whole concept of the phytobial process is that it utilises the rhizodeposition products of the plant root as the energy source for the microorganisms to function. In quantitative terms this can account for up to 40% of the photosynthate produced or the dry matter production by the plant (Lynch and Whipps, 1990).

Phytoremediation. Phytoremediation technology uses plants to absorb contaminants from the soil and translocate them to the shoots. Contaminants may then be removed by harvesting the aboveground tissue for subsequent volume reduction (ashing) and storage (Brooks et al., 1977). It involves soil–plant systems in which metal(loid)s-accumulating plants are grown in contaminated sites. It is considered an economically feasible and environmentally viable technology for remediating metal(loid)-contaminated systems. The effectiveness of this technology is, however, variable and highly site dependent. In phytoremediation, plants are exploited as a biopump that use the energy of the sun to remove water and contaminants from the soil to the aboveground portion and return some of the products of photosynthesis back into the root zone in the form of root exudates involved in the immobilization of contaminants. Certain plants, termed "hyperaccumulators" (Brooks et al., 1977), accumulate an enormous concentration of metal(loid)s in their aboveground biomass. The minimum concentration of As required for a plant to be classified as a hyperaccumulator of As was set at 1000 mg kg^{-1} (0.1%) on a dry weight basis (Ma et al., 2001). The hyperaccumulation of metalloids involves uptake of the soluble metal(loid)species by the root system, translocation to the aerial parts, and storage in a nontoxic form in the aerial

portions. There are about 400 species of known terrestrial plants that hyperaccumulate one or more of several metal(loid)s. Following the first report of brake fern (*Pteris vittata*) as an As hyperaccumulator (Ma et al. 2001; Zhao et al. 2009), 12 species of As hyperaccumulators have so far been identified, all fern species within the Pteridaceae family (mostly within the *Pteris* genus) (Ma et al., 2001). Phytoremediation technologies have been grouped into various categories that include phytostabilization, rhizofiltration, and phytoextraction (Cunningham et al., 1995). In phytostabilization (Fig. 7), transpiration and root growth are used to immobilize contaminants, including As by reducing leaching, controlling erosion, creating an aerobic environment in the root zone, and adding organic matter to the substrate that binds As. It involves the establishment of metalloid-tolerant vegetation on the contaminated site that is left in perpetuity.

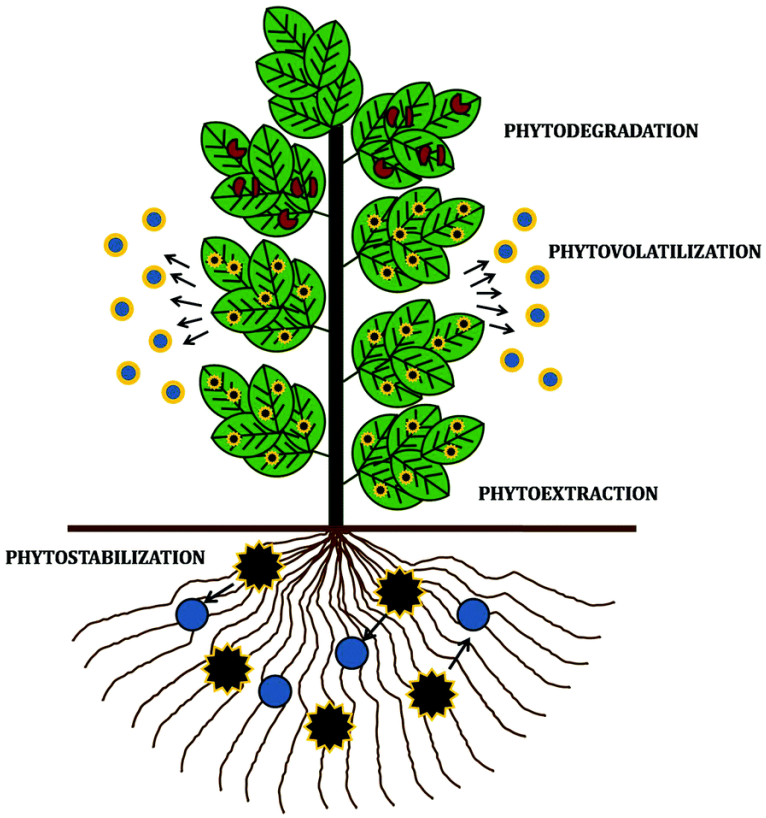

Figure 7. Different phytoremediation technonologies.

The stabilization of As in the root zone could be achieved through the addition of organic matter as well as soil amendments. In rhizofiltration, the roots can be used to adsorb or absorb metalloids, which are subsequently removed by harvesting the whole plant. In this case, metalloid tolerance and translocation of the metal(loid)s to aerial parts are largely irrelevant. In phytoextraction, plants can be grown on contaminated soil and the aerial parts harvested. In this case, plants need to be tolerant only if the soil metalloid content is very high, but they need to accumulate very high concentrations

in their aerial parts. Phytoextraction involves repeated cropping of plants until the metalloid concentration in the soil has reached the acceptable level. The cost involved in phytoremediation is much lower than other technologies, such as soil removal and ex situ cleansing. Other advantages include the ultimate fertility of the cleaned site, the high public appeal of "green" technology, and the possibility of producing secondary products that offset the cost of the operation or even produce a small profit. (Mahimairaja et al., 2005).

1.12.2. Water remediation

As discussed earlier, because most cases of As toxicity in humans have resulted from the consumption of As-contaminated water, there have been intensive research efforts in developing technologies aimed to decontaminated water from As. A plenty of methods suitable for the removal of As from water at both household and community levels are currently available. These methods are most all based on: (1) removal of solid-phase As through coagulation, sedimentation, or filtration; 2) removal of solution-phase As through ion exchange, osmosis, or electrodialysis; oxidation of As(III) to As(V) and its subsequent removal through adsorption and/or precipitation; biosorption using microorganisms; and rhizofiltration using aquatic plants (Mahimairaja et al., 2005).

Physiochemical methods. Filtration, adsorption, and chemical precipitation are the most common physicochemical methods used for stripping As from water. While the particulate As in water can be removed by simple filtration, the aqueous As can be removed through adsorption or precipitation followed by filtration. The majority of the contaminated water remediation techniques are based on mechanisms that involve an initial oxidation of As (III) to As (V) and subsequent precipitation using chemicals (Montgomery, 1985). Successes in the water treatment for As in the past have generally relied on the relatively poor solubility of arsenate As (V). Usually As (III) is oxidized to As(V) by an oxidant such as chlorine (Cl_2), potassium permanganate ($KMnO_4$), or oxygen (O_2) prior to As removal. Coagulation, absorption to activated alumina, ion exchange with strong-base anion-exchange resins, and reverse osmosis are conventional technologies that have been used to treat As-contaminated water (Montgomery, 1985). Most of the domestic drinking water treatment systems for As removal involve filtration. The "Pitcher filter" involving porous ceramics (Neku and Tandukar, 2003) and sand filters (Yokota et al., 2001) have been found to be effective in stripping As from water. Seidel et al. (2001) noticed that the porous nanofiltration anion-exchange membrane removed about 90% of As (V) present in water at a concentration of 316 µg L^{-1}. Although this technology could achieve a high degree of As removal, it involves a high initial investment and high operation and maintenance costs.

A number of compounds, including activated alumina, Fe-coated sand, and ion-exchange resins are used to adsorb As. In most geologic environments, Fe_2O_3 carries a positive surface charge that preferentially adsorbs As. Similarly, $Al(OH)_3$ and silicate clays also adsorb large amounts of As. (Yoshida et al., 1976).

In recent decades, a class of anionic clays known as layered double hydroxides (LDHs) has attracted attention from both industry and academia (Goh et al., 2008). These clays can use to remove environmental contaminants such as oxyanions (arsenite, arsenate, chromate, phosphate, selenite, selenate, borate, nitrate, etc.) and monoatomic anions (fluoride, chloride, bromide, and iodide) from aqueous solutions by the process of adsorption. These syntetic clay exist with a similar layered structure to that exhibited by natural $Mg(OH)_2$. Although LDHs exist as naturally occurring minerals, they are also relatively simple and economical to synthesize. The structure of LDHs is based on positively charged brucite-like sheets and the positive charges are balanced by intercalation of anions in the hydrated interlayer regions. LDHs have relatively weak interlayer bonding and, as a consequence, exhibit excellent ability to capture organic and inorganic anions arsenic included (Cavani et al., 1991).

Phytoremediation- Phytoremediation of As contaminated waters may be readily achieved by the use of aquatic plants because unlike soil, most of the As in water is available for plant uptake. In the case of soils, the plant must first solubilize the metalloid in the rhizosphere and then should have the ability to transport it to the aerial tissue (Brooks and Robinson, 1998). The difference between aquatic and terrestrial plants As accumulation, was noticed by Outridge and Noller (1991). For instance, in terrestrial systems, the solubilization of As in the rhizosphere is necessary to allow the plant roots to take up and transport this element to the aerial parts of the plant. This is not the case when the plant grows in an aqueous medium, where As is already present in a bioavailable form (Brooks and Robinson, 1998).

Biosorption and biomethylation. Biosorption and biomethylation are the two important processes by which As can be removed from water using microorganisms. The biosorption process generally lacks specificity in metalloid binding and is sensitive to ambient environmental conditions, such as pH, solution composition, and the presence of chelators. Genetically engineered microorganisms (*Escherichia coli*) that express a metal binding protein and a metal-specific transport system have been found to be successful in their selectivity for accumulation of a specific metal in the presence of a high concentration of other metals and chelating agents in solution (Chen and Wilson, 1997). Methylation is the most reliable biological process through which As can be removed from aquatic medium. This process was described in previous section.

1.13. Source of human exposure

1.14.1. Exposure from water and drinking water criteria

Most large-scale episodes of chronic arsenic poisoning have resulted from arsenic contamination of drinking water. The problem associated with establishing a threshold for arsenic is that the current epidemiology studies are ecological in nature and, as such, are poorly suited for this task. Studies examining the effects of arsenic in drinking water at concentrations of 10–200 μg L^{-1} or acceptance of mechanisms of action for arsenic are necessary to resolve this question (Smith et al., 1992). Thus the risks of ingesting water with a content that provides an arsenic intake of less than 400 μg/day are unresolved and have been the target of extensive study by the U.S. Environmental Protection Agency (EPA) and the U.S. National Research Council (NRC), who have used the above data in an attempt to determine the upper limit of acceptable arsenic content for drinking water for the United States (NRC, 1999). The purpose for recommending or instituting regulatory limits for a chemical in drinking water is ideally to prevent or at least decrease the occurrence of adverse health effects after consumption of water containing that chemical. One of the first considerations is whether the chemical causes adverse human health effects. The World Health Organization (WHO), Canada, and the U.S. EPA have all classified arsenic as a human carcinogen (WHO, 1981). In the 1942 US Public Health Service (USPHS) sets a drinking water standard of 50 μg As L-1. In 1975 Environmental Protection Agency (EPA) set to 50 μg L^{-1} the limit for As in drinking water, based on a originally Public Health Service standard established (USEPA, 1996). In 1993 World Health Organization (WHO) recommends lowering arsenic in drinking water to 10 μg As L^{-1}, which is based on a 6 $x10^{-4}$ excess skin cancer risk, which is 60 times higher than the factor that is typically used to protect public health. WHO states that the health-based drinking water guideline for As should be 0.17 μg L^{-1}. However, the detection limit for most laboratories is 10 μg L^{-1}, which is why the less protective guideline was adopted (WHO, 1993; 1999). On the basis of the investigations initiated by National Academy of Sciences, it was concluded that the previous standard did not eliminate the risks of long-term exposure from low As concentrations in drinking water causing skin, bladder, lung, and prostate cancer (NRC, 1999).

At the present time, WHO and Canada have published recommendations for arsenic in drinking water, while the United States is in the process of proposing a regulatory level. To achieve the EPA's goal of protecting public health, recommendations were made to lower the safe drinking

water limit to 5µg L^{-1}, which is higher than the technically feasible level of 3 µg L^{-1} (NRC, 1999). However, the current drinking water guideline for As, 10 µg L^{-1}, adopted by WHO and the USEPA is higher than the Canadian and Australian maximum permissible concentrations of 5 and 7 µg As L^{-1}, respectively (USEPA, 2001).

1.13.2. Exposure from food: arsenic in food crops and implication for human health

For populations not exposed to elevate As in drinking water, foods represent the main sources of As intake for humans. During the past years, researchers have mainly focused on ingestion of arsenic through contaminated drinking-water, but the incidence of arsenicosis in the population was not consistent with the concentration of arsenic in drinking-water obtained from groundwater. In fact, in most countries, As contamination of groundwater is insignificant, however. This inconsistency has raised questions on potential pathways of ingestion of arsenic. The observed clinical symptoms of arsenic toxicity vary greatly, which poses a considerable challenge in relating the potential pathways of transfer of arsenic from groundwater to human metabolic system through food-chain (Duxbury and Zavala, 2005). This issue has only been recognized in recent years (Meharg and Rahman, 2003; Meharg et al., 2009).

In addition to potential human health impacts caused by ingestion of food containing As, the potential for reduced crop yield due to its build-up in the soil is an active area of research. Recently the fate of As in agricultural soils is often less well studied compared to groundwater, and in general has been studied in the context of As uptake by different plants (Das et al., 2004). Crop quality and the effect of As on crop quality and yield is becoming a major worldwide concern, particularly for rice which forms the staple for many South-Asian countries where groundwater is widely used for irrigation (Meharg and Rahman, 2003). Dietary intake of total As ranges from 10 to 200 µg per person per day in various countries (Schoof et al., 1999; WHO, 1989). Seafood accounts for the majority (60%–90%) of the total dietary intake of As in countries such as the United States, Canada, and Japan. Most of the As in seafood is present in organic forms that are relatively non toxic (Schoof et al., 1999).There are, however, some notable exceptions. For instance, the edible marine alga hijiki (*Hizikia fusiforme,* also called hiziki), can contain inorganic arsenic (present as arsenate) at concentrations of >60 mg kg^{-1} (EFSA, 2009a), and blue mussel (*Mytilus edulis*) has shown inorganic arsenic concentrations up to 30 mg kg^{-1} dry mass (Sloth and Julshamn, 2008). Food

basket surveys (Yost et al., 1998) in North America have shown that inorganic arsenic is present in food stuffs constituting 21–40% of the total diet. The most common sources were rice, grape juice, and cooked spinach (Schoof et al., 1999). It is As*i* species that are of particular concern because they are chronic human carcinogens. Inorganic arsenic has been classified by the International Agency for Research on Cancer (IARC, 1987) in group 1 as carcinogenic to humans. This was based on the induction of primary skin cancer, as well as the induction of lung and urinary bladder cancer. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a Provisional Tolerable Weekly Intake (PTWI) for inorganic arsenic of 0.015 mg kg^{-1} bodyweight/week equivalent to 130 μg day^{-1} for a 60-kg person in 1988 (WHO, 1989). In addition to "fish and seafood" the following food categories from the EFSA (2009) food classification need to be considered as possible significant contributors of inorganic arsenic (even though they have relatively low total arsenic levels):

1. cereal and cereal products;
2. all vegetables, nuts and pulses;
3. fruit and vegetable juices, soft drinks and bottled water;
4. coffee, tea and cocoa;
5. alcoholic beverages.

Cereal and cereal products and vegetables have been reported to contain on average from 30 to 100 % inorganic arsenic (Muñoz et al., 2002, Diaz et al., 2004; Schoof et al., 1999). No value has been set for organic As due to the lack of toxicological data (EFSA, 2009a). Arsenic in terrestrial food plants is dominated by As*i* (Zhao et al., 2010). Dietary intake of As*i* from terrestrial food plants is generally low except for populations with rice as the staple diet (Meharg et al., 2009). Most data reported for arsenic in food describe the content of total arsenic. Analyses that provide information about the type of arsenic are much more difficult to perform, and relatively few laboratories are able to provide these data. Such data, however, are becoming increasingly important because different foods can contain different types of arsenic species, and because these species have very different degrees of toxicity. The analytes are usually arsenite and arsenate, and hence data are often recorded as these two species. Similarly, in food samples inorganic arsenic is often reported as

arsenite and arsenate even though it is likely bound to thio groups in peptides or proteins in the food itself (EFSA, 2009a).

Arsenobetaine is the major form of arsenic in marine fish and most other seafoods. Arsenobetaine has also been found in some terrestrial foods, in particular in some mushroom species, although generally as a minor compound (Francesconi and Kuehnelt, 2002). There have also been several reports of arsenobetaine in freshwater organisms (Schaeffer et al., 2006), although the levels are generally low (<0.1 mg arsenic kg^{-1} dry mass), much lower than those found in marine samples.

Arsenobetaine (Fig. 8) is structurally similar to glycine betaine [$(CH_3)_3N+CH_2COO-$], a nitrogen betaine which is used as an osmolyte by aquatic organisms to maintain osmotic balance under conditions of changing salinity. The coincidental structural similarity between arsenobetaine and glycine betaine might explain why arsenobetaine levels are much higher in marine animals than they are in freshwater animals. Although arsenic forms species under reducing conditions with the arsenic atom in oxidation state -3 and +3, the most stable arsenic species found under normal environmental conditions contain the arsenic atom in oxidation state +5. Consequently, the vast majority of arsenic species found in organisms and in foods also contain arsenic in oxidation state +5 (arsenate, dimethylarsinate, arsenobetaine, arsenosugars) (Edmonds and Francesconi, 1993; Sloth et al., 2005).

Arsenosugars (Fig. 8) are usually the major arsenical constituents of marine algae (typically 2-50 mg arsenic/kg dry mass), and they also are found at significant concentrations in animals feeding on algae (mussels and oysters; typically 0.5-5 mg kg^{-1} dry mass) (Francesconi and Kuehnelt, 2002). They occur in many other marine organisms as well, albeit at lower concentrations. In terrestrial organisms, arsenosugars occur generally at trace levels only, although interesting exceptions have been reported (Geiszinger et al., 1998). More than 20 naturally occurring arsenosugars have been identified, most of which are dimethylarsinoylribosides.

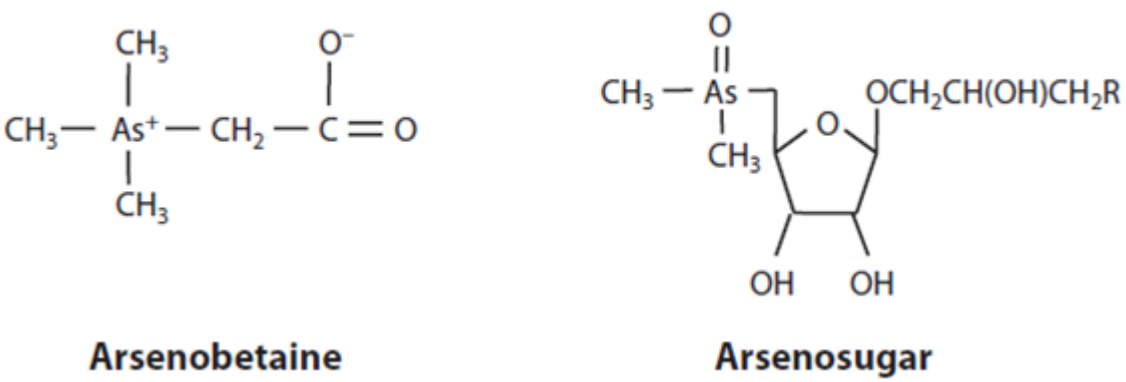

Figure 8. Arsenobetaine and Arsenosugar structure

The term lipids is a broad term encompassing all fat-soluble naturally occurring compounds; those lipids that contain arsenic are referred to as arsenolipids. Although the presence of fat-soluble arsenic compounds in fish was first reported in the late 1960s, the structures of some of these arsenolipids have only recently been elucidated.

Other organoarsenic species Trimethylarsoniopropionate, a compound similar to arsenobetaine, was first identified in 2000 in a fish species (Francesconi et al., 2000), and is now known to be a common minor constituent of marine organisms (typically at concentrations of 0.2-2 mg arsenic kg^{-1} dry mass; Kirby et al., 2002). Arsenocholine also occurs commonly, but generally at modest levels in marine organisms (typically <0.2 mg arsenic kg^{-1} dry mass) (Francesconi et al., 1989). The simple methylated arsenic species (those without other alkyl substituents), namely methylarsonate, dimethylarsinate, trimethylarsine oxide, and tetramethylarsonium ion are also often found in organisms (and hence in foods) but generally at low concentrations (<0.5 mg arsenic kg^{-1} drymass) (Francesconi and Kuehnelt, 2002). Methylarsonate and dimethylarsinate are also important human metabolites of ingested arsenic species (EFSA, 2009a).

Arsenic accumulation has been observed on some species of fruits and vegetables grown on contaminated soils and/or those from plants irrigated with contaminated water (Khattak et al. 1991; NAS, 1977). The amount of accumulated arsenic varies from 0.01 to 5 mg kg^{-1} of the plant's dry material (NAS, 1977).

Meharg and Hartley-Whitaker (2002), reported that accumulation of arsenic varies from place to place and crop to crop, the reason may be due to variation in soil properties and variation in crop physiology and morphology. The bioavailability of inorganic arsenic in various vegetables and plants was studies under experimental conditions. It has been observed that a variety of vegetable crops accumulate arsenic by root uptake from the soil or by absorption of airborne arsenic deposited on the leaves. Thus, Larsen et al. (1992) found that leafy plants like kale accumulated arsenic by atmospheric deposition whereas tuberous plant such as potatoes and carrots accumulated arsenic by both root uptake and atmospheric deposition. Further studies by Helgesen and Larsen (1998), speciating the arsenic compounds from carrots grown on contaminated soils, showed that the bioavailability of arsenic was dependent on the concentration of the arsenic in the soil. However, a study by Bunzl et al. (2001) on a range of vegetables, established that the observed bioavailability was considerably smaller than the bioaccessible fractions, but this ratio varied with the type of vegetable. Arsenic concentrations in vegetables and crops have been reported to increase in the following order; grain, leaf, stem, root (Marin et al., 1992; Liu et al., 2004 in rice studies, Cobb et al., 2000 and Queirolo et al., 2000 in bean studies; Burlo et al., 1999 and Cobb et al., 2000 in

tomato). Except in hyper-accumulators, the translocation of inorganic arsenic from the roots to the above ground parts appear limited. Organic arsenic is more readily translocated, but the uptake is much lower than the uptake of inorganic arsenic (Dembitsky and Rezanka, 2003; Carbonell et al., 1998; Carbonell-Barrachina et al., 1998). It is unclear whether organic arsenic in plants is taken up from the soil or is formed naturally by the plants (Meharg and Hartley-Whitaker 2002; Sneller et al., 1999).

In a recent study it was reported that irrigation has increased in Bangladesh since 1970, while since 1980, the area under groundwater irrigation for the cultivation of Boro rice has increased by almost an order of magnitude (Harvey et al., 2005). Based on available information on the distribution of As concentration in groundwater (BGS and DPHE, 2001) and the area under shallow tube well irrigation (BADC, 2005), Saha (2007) estimated that approximately 1000 metric tons of As is cycled with irrigation water during the dry season of each year. Rice yield has been reported to decrease by 10% at a concentration of 25 mg kg^{-1} As in soil (Xiong et al., 1987). A greenhouse study by Abedin et al. (2002b) revealed reduced yield of a local variety of rice (BR-11) irrigated with water having As concentrations in the range of 0.2 to 8 mg L^{-1}. The accumulation of As in rice field soils and its introduction into the food chain through uptake by the rice plant is of major concern (Duxbury et al., 2003). Hence, arsenic accumulates in soil, contaminates both surface and groundwater, is taken up by plants and is then entrenched in mammalian /insectivore food chain (Green et al., 2001). Irrigation, especially with wastewaters, can cause a problem of build-up of mobile and potentially toxic metals in soils and in surface runoff (Siegel, 2002).

No standards for As in food currently exist in the EU or US, a major gap in food health policy (Francesconi, 2007). China only had established a limit for the maximum Asi concentration in rice 0.15 mg kg^{-1} (Heikens, 2006).

1.13.3. Arsenic in rice

Rice (Oryza sativa) is the dominant staple food for over half of the world's population, especially in developing Asian countries, contributing 70% of the energy provided by their daily food intake (Phuong et al., 1999; Torres-Escribano et al., 2008). Unfortunately, rice is much more efficient at assimilating arsenic into the grain than other staple cereal crops like barley or wheat (Meharg et al., 2009; Williams et al., 2007). Rice production is heavily concentrated in Asia, with just four countries, China, India, Indonesia, and Bangladesh, accounting for nearly 70% of global production (FAO, 2011). In the European Union, Italy is the leading rice producer with approximately 50% of the total EU harvest (Italian Grain and Feed Report, 2012).

In the Bengal Delta region, where As-contaminated water has been used for irrigation, relatively high concentrations of As have been reported in some vegetables and spices (Williams et al., 2006), with As present only in inorganic forms (Williams et al., 2006). However, among all food categories, consumption of rice makes the largest contribution to the dietary intake of As*i* (Meharg et al., 2009). In a global survey of 901 samples of polished rice, total As concentration varied from 10 to 820 μg kg^{-1} with a mean of 150 μg kg^{-1} (Meharg et al., 2009).

A global "normal range," not from an As contaminated environment, of 80–200 μg kg^{-1} has been suggested (Zavala et al., 2008). Arsenic contamination due to irrigation with As-tainted groundwater in South Asia or mining activities in China has resulted in further elevation of As levels in rice (Meharg, 2004; Zhu et al., 2008). For comparison, As concentrations in wheat grain or flour are generally <50 μg kg^{-1}, with a mean value approximately tenfold lower than that of rice (Williams et al., 2007b).

The As levels in the rice grain are influenced by cultivation method, genetic variation, soil biogeochemistry and cooking (Meharg and Zhao, 2012). Most foods with elevated As content are of marine origin where speciation is dominated by organic As species (Aso) (Mass et al., 2001; Styblo et al., 2000) as reported before. Rice, however, appears to be an exception because it contains high Asi concentrations, typically between 0.05 and 0.4 mg arsenic kg^{-1} (Sun et al., 2008a; Meharg et al., 2009). In addition to Asi, MMA and DMA have been detected in samples of rice (Heitkemper et al., 2001; Williams et al., 2005; Nardi et al., 2009; D'Amato et al., 2004). Previous investigations have determined that rice is the primary food source of As exposure in non-seafood diets in EU (Robberecht et al., 2002) and US (Tao and Bolger, 1999), and that rice is a dominant source of Asi to humans, with exception of those regions with elevated Asi in drinking water (Sun et al., 2008 b).

In a global survey of 901 samples of polished rice, total As concentration varied from 10 to 820 μg

kg^{-1} with a mean of 150 µg kg^{-1} (Meharg et al., 2009). Few data are available on this but two studies from Taiwan report rice arsenic contents of 0.15 and 0.7 mg kg^{-1}, the former diet providing a calculated daily intake of approximately 19 mg/ (plus 31 mg from yams). This intake would be the equivalent of ingesting 1 L of drinking water containing arsenic at the widely accepted standard of 50 mg L^{-1}. Speciation of arsenic in food has repeatedly shown it to be predominantly inorganic species (Meharg and Zhao, 2012).

The relatively high As accumulation in rice is due to two reasons: (*a*) enhanced As bioavailability under the anaerobic conditions of submerged paddy soils and (*b*) the inadvertent uptake and transport of arsenite through the Si pathway, which is highly efficient in rice (Zhao et al., 2010). Rice is the only staple crop grown under flooded soil conditions. Under anaerobic conditions, arsenic in soil is converted readily to arsenite which is mobile, leading to arsenic in rice grain being around 10-fold higher than for other crops (William, 2007b).

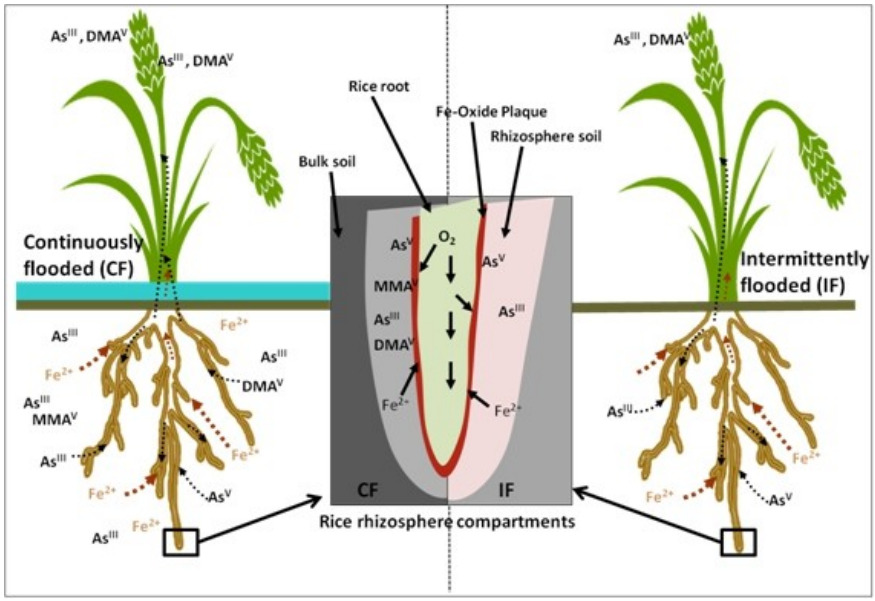

Figure 9. Arsenic mobilization and transformation in flooded paddy soil and interactions with rice.

Inorganic arsenic, a class 1 non-threshold carcinogen, and dimethyl arsinic acid (DMA) constitute the dominant arsenic species present in rice while traces of monomethyl arsonic acid (MMA) are sometimes reported, as well as a residual fraction. In aerobic soils, arsenate is usually present at very low concentrations (<1 µM) in soil solution because of the strong adsorption by oxides/hydroxides of iron and aluminum (Fendorf et al.2008; Fitz and Wenzel, 2002); therefore, the bioavailability of arsenate is generally low. When soils are submerged, as in flooded paddy fields, As is mobilized into the soil solution mainly as arsenite (Marin et al., 1993b; Xu et al., 2008). This

is a result of two processes (Fig. 9): (*a*) the reductive dissolution of iron oxides/ hydroxides and release of the associated As, (*b*) reduction of strongly adsorbed arsenate to more weakly adsorbed arsenite leading to an enhanced partition of As from the solid to the solution phase (Fendorf et al., 2008) Consequently, the bioavailability of As is more enhanced to rice plants grown under submerged conditions than to those grown under aerobic conditions (Li et al., 2009; Xu et al., 2008).

Raab et al. (2009) systematically investigated total arsenic and inorganic arsenic in different rice types (basmati, long-grain, polished (white) and whole grain (brown) that had undergone various forms of cooking in non-contaminated waterLow volume water cooking did not remove arsenic. High volume water cooking did effectively remove both total and inorganic arsenic for the long-grain and basmati rice by 35% and 45% for total and inorganic arsenic content, respectively, compared to uncooked (raw) rice. (Raab et al., 2009). The situation for vegetables seems to be similar to that for rice. Cooking vegetables in water with high levels of inorganic arsenic leads to an increase in the arsenic concentration in the vegetables compared to the raw product. On the other hand, cooking in distilled water resulted in lower levels compared to those detected in the products prior to cooking (Diaz et al., 2004). Some further studies with uncontaminated water substantiate the decrease of arsenic after cooking. She and Kheng, (1992) reported arsenic losses of up to 60% after subjecting various kinds of vegetables to boiling. Cubadda et al. (2003) also showed a significant decrease in arsenic (about 60%) in all pasta samples after a cooking process (EFSA, 2009a). Even rice, which typically contains between 0.1 to 0.4 mg total arsenic kg^{-1}, has a relatively high percentage of inorganic arsenic (30-90%). Those observations from literature data are supported by the results reported for rice grain samples and rice-based products in the current data set (around 200 samples) showing that the inorganic arsenic content varied between 50 and 60% of the total arsenic content (EFSA, 2009a).

1.14. The role of microorganism in arsenic cycle

Throughout evolution, microorganisms have developed the ability to survive in almost every environmental condition on Earth. Their metabolism depends on the availability of metal ions to catalyze energy-yielding and synthetic reactions on their aptitude to protect themselves from toxic amount of metals by detoxification process. As is called as "essential toxin", because it is required in trace amounts for growth and metabolism of certain microbes but is toxic at high concentrations (Stolz et al., 2006). However, it is now evident that various types of microorganism gain energy from this toxic element and these reactions have important environmental implications (Satinder, 2008). Microbes in the soil are important in providing nutrients to plant roots. Soil bacteria degrade organic compounds and modify the inorganic products. Soil fungi provide a large surface area for scavenging soil bound nutrients such as inorganic phosphate and, through ectomycorrhizal and endomycorrhizal associations with roots, transport these to the plant. Toxic compounds in soils are often modified by microbes (Van Zwieten et al., 2003), but many such toxins also may hinder growth of soil microbes and impair their ability to promote plant growth. Certain microbes can adapt to arsenic toxicity (Cervantes et al., 1994) and a wide range of microorganism can thrive in toxic arsenic-enriched environments (Ahmann et al., 1994; Laverman et al., 1995). In order for these microorganisms to thrive and function in this environment, several have developed resistance to arsenic toxicity. In addition to being resistant to arsenic toxicity, certain microorganisms are able to reduce the less toxic arsenate form to the more toxic arsenite (Nies and Silver, 1995; Rensing et al., 1999). The reduction is an energy generating process for the microorganism, however, the impact of such arsenic geochemistry in anoxic systems cannot be underestimated, especially with respect to arsenic mobilization (Cummings et al., 1999). Sharples et al. (2000) presented evidence that the ericoid mycorrhizal fungus *Hymenoscyphus ericae* acts as a filter to maintain low arsenic uptake rates by roots of the plant *Calluna vulgaris* when growing in arsenic contaminated soil. In a study of evolved arsenate resistance in cultivars of the grass *Holcus lanatus*, Gonzalez-Chavez et al. (2002) found that colonization by the arbuscular-mycorrhizal fungus *Glomus* suppressed high-affinity arsenate and phosphate transport into the roots. Conversely, mycorrhizal association with the fern *Pteris vittata* has been reported to stimulate arsenic accumulation by the host (Liu et al., 2005). Arsenic contamination of soil and water has a direct impact on microbial community and structure. At high concentrations, a reduction in the soil microbial population has been reported by a number of researchers (Bisessar, 1982; Van Zwieten et al., 2003). In general, as in the case of higher plants, As (III) is more toxic to microorganisms than As (V) (Maliszewska et al., 1985).

Hiroki (1993) has shown that As (III) is more toxic to bacteria and actinomycetes than As (V) and that fungi not only display a higher tolerance to As (III) than bacteria and actinomycetes, but also show the same tolerance to both As (V) and As (III). Arsenite also inhibits enzyme activities in soil.

1.14.1. Trichoderma

Trichoderma is a genus of soil inhabiting, teleomorph-bearing filamentous fungi belonging to Hypocreales order of the Ascomycota division. The genus *Trichoderma* is genetically very diverse with a number of capabilities among different strains with agricultural and industrial significance (Azevedo et al., 2000; Harman et al., 2004a; Lorito et al., 2010). It has attained a special position in the field of agriculture as a potent biocontrol agent besides being a plant growth promoter and improves soil fertility due to its disease suppressiveness and composting ability (Harman et al., 2004a; Lorito et al., 2010). Some strains, for example, have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo and Horwitz, 2010). *Trichoderma* has found application in industrial production of enzymes, paper and pulp treatment, and food industry (Nguyen et al., 2008; Singh and Singh, 2009). Some *Trichoderma* rhizosphere- competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage (Shoresh et al., 2010). In recent years, an increasing number of studies have contributed to unravelling the molecular basis of the plant–*Trichoderma* dialogue and the beneficial effects of *Trichoderma* to plants. Reports are available for its potential application to remediate soil and water pollution (Harman et al., 2004a; Ezzi and Lynch, 2005). Certain *Trichoderma spp*. has beneficial effects on plant growth and enhance resistance to both biotic and abiotic stresses. Early work revealed that *Trichoderma* promotes growth responses in radish, pepper, cucumber and tomato (Chang et al., 1986). Moreover, has been shown that *T. harzianum* can solubilize several plant nutrients (Altomare et al., 1999), and the colonization of cucumber roots by *T. asperellum* to enhance the availability of P and Fe to plants, with significant increases in dry weight, shoot length and leaf area (Yedidia et al., 2001).

Mechanisms employed by *Trichoderma* in facilitating metal stress tolerance in plants are attributed to enhanced production of root biomass (Harman et al., 2004b), hyper accumulation of toxicants in plant tissues (Tu et al., 2004; Arriagada et al., 2009). For instance, it was reported that different strains *of Trichoderma* influence uptake and translocation of Ni, Zn, As and Cd in *Brassica juncea*

(Cao et al., 2008). Some strains of this fungus can also defend the plant against oxidative damage and enhanced efficiency and availability in nutrient use (Harman et al., 2004b).

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CHAPTER II

The aim of project

Arsenic is a toxic metalloid of global concern. In drinking water and the food industry, As is a worldwide problem. Today an enormous literature exists documenting its occurrence, behavior and impacts in many places across the globe.

The relative impacts of As vary depending on local circumstances but the biggest As sources for human contamination are through the ingestion of water and food crops products that accumulated As during irrigation or cultivation (Williams et al., 2006; Ahsan et al., 2010).

Several epidemiological studies have demonstrated a relation between food habits and diseases risk (Mulabagal et al., 2010). The inadvertent exposure through the consumption of food crops grown in As-contaminated soils or irrigated with contaminated water, may represent a potential exposure pathway. The uptake of As by vegetables and other commonly consumed plants has been of considerable interest, with a number of reports appearing in the scientific literature (Cobb et al., 2000; Queirolo et al. 2000; Bunzl et al., 2001). Arsenic absorption by plants is influenced by many factors including plant species (Walsh and Keeney, 1975), the concentration of As in the soil (NAS, 1977), soil properties such as pH and clay content and the presence of other ions (Khattak et al., 1991). No standards for As in food currently exist in the EU or US and limited information is currently available regarding the As uptake by commonly consumed vegetables. In addition to health problem, the presence of As in irrigation water or in soil at an elevated level could modify the normal growth of plants with the toxicity symptoms such as biomass reduction in root and shoot (Tang and Miller, 1991; Carbonell-Barrachina et al., 1998), wilting and necrosis of leaf blades (Odanaka et al., 1987), reduction in leaf area and photosynthesis (Marin et al., 1993a; Knauer et al., 1999), lower fruit and grain yield (Tsutsumi, 1980; Carbonell-Barrachina et al., 1995) and consequently decreasing in crop production.

In the last decade, the research group of Prof. Violante (Department of Agraria, Portici), have conducted several study on the uptake and distribution of arsenic (As) in different crop plant using contaminated water or soils. Particular, they have studied the influence of phosphorus (P) fertilization/compost fertilization and microorganism in preventing As uptake and translocation in wheat, bean and lettuce plants grown in As-polluted soils and in an unpolluted soil or/and irrigated

with As-contaminated water (Pigna et al. 2009; Cozzolino et al. 2010; Caporale et al., 2013). On account of these considerations, my Ph.D. had the aim to give more detailed information on effects of this metalloid on the plant growth and technique for alleviate or reduce the arsenic accumulation by the plants using As contaminated water.

In particular, my research project has been articulated on the study of: i) alternative techniques for limiting the mobility and the phyto-availability of As in the soil-plant system (I-III year); ii) market survey on exposure by arsenic in food (II year). The purpose of the first part of the project was to study the influence of the phosphate fertilizer on uptake of arsenic by plants tomato irrigated with contaminated water grown on uncontaminated soil. A second study was carried out in collaboration with working group of Prof. Matteo Lorito, using *Trichoderma* to restrict the uptake of As by lettuce plants.

The second part, made mostly at the University of Aberdeen, had as its purpose the monitoring quality and quantity of As and other elements of nutritional and toxicological interest in rice marketed in Italy.

During my 1st year, we have designed an experiment using As contaminated water for irrigation on the tomato (*Solanum lycopersicum* L. cv piennolo). These plants are grown in an uncontaminated soil, irrigated with As solutions at four concentrations (0, 0.5, 2 and 4 mg L^{-1}). In this study P fertilization was used as a factor to facilitate As stress tolerance (Pigna et al., 2012). In fact, as reported before, P is one of such competing ions, which is used as a fertilizer in irrigated field, which may compete with As for sorption site and accumulated in vegetable for nutrition purpose. Tomato plant was selected because is very sensitive to arsenic toxicity, so this plant may accumulate arsenic and this may enter in the human food chain through its fruits.

Therefore, a greenhouse experiment was designed to evaluate: 1) the As uptake, 2) its phytotoxicity, 3) partitioning among different plant tissues (tomato berries, shoots and roots) 4) eventually beneficial effects of P fertilization.

In 2009, the European Food Safety Authority (EFSA, 2009a) reviewed the diet of the European Union (EU) population and stated that inorganic As level should be reduced, It was determined that the cereal based products were the predominant route of exposure to Asi in the EU; rice is the main contributor due to its high content of total arsenic. Several market survey was reported in literature about arsenic in food, in particular in rice.

So I have investigated during my 2nd year, on total As (As_t) and As species present in samples of Italian commercial rice. Also, other elements of nutritional and toxicological interest Cr, Cd Se,

Mg, K, Zn, Ni were analysed. The principal aim of this study was to quantify and qualify how Italian rice varied in As content dependent on regional origin and variety. This project was conducted in collaboration with work group of Professor Meharg at the Aberdeen University (Scotland, UK). Rice cultivation in Italy is mostly located in the northern regions and extends over about 240,000 ha which represent only 1.4% of the total arable area (16.800.000 ha). Rice cultivation is primarily based in the Po Valley. About 52% of the rice area is in Piemonte, mostly in Vercelli and Novara provinces, and about 41% in Lombardia, for the most part in Pavia and Milano (Istat, 2012). So it was considered of interest to evaluate the concentration and the species of Arsenic in commercial Italian rice.

Exploring novel microorganisms is important in agriculture to promote soil nutrient cycling and to reduce dependence on inorganic fertilizers. Soil fungi associated with roots have the potential to either increase or ameliorate the uptake of inorganic contaminants by plants. It has been demonstrated that mycorrhizal fungi in As contaminated soils alleviate the toxicity of excessive As by improving P nutrition without increasing As concentrations in the lettuce plants (Cozzolino et al., 2010), while *T. harzianum* promote growth of crack willow *(Salix fragilis)* in metal-contaminated soil (Adams et al., 2007) and increase root growth of arsenic hyperaccumulating fern *Pteris Vittata*. *Trichoderma asperellum* also, showed superior abilities for the absorption of extracellular As and accumulation of intracellular As, which accounted for 82.2 and 63.4% of the total accumulated As, respectively (Arriagada et al., 2009a).

Little is known about the efficiency of metal (loid)-tolerant remediating microbes on plant growth promotion. Recently, some metal (loid)-tolerant microbial strains were isolated from metal (loid)-contaminated soils and evaluated for their role in promoting plant growth.

During the 3th year of my Ph.D., I investigated on the As uptake by lettuce (*Lactuca sativa* L.) and the role of *Trichoderma* to facilitate its As stress tolerance. This plant was selected as a tester because is a leafy vegetable widely consumed due to its characteristics and therapeutical properties (Mulabagal et al., 2010). Lettuce belonging to the Asteraceae family, is the most popular vegetal in salads which is consumed in increasing amounts due to their perception as being ‘‘healthier” foods (Dupont et al., 2000). Therefore, it is of great interest to understand and evaluate the ability of lettuce plant (*Lactuca sativa* L.) to accumulate and/or to be sensitive to arsenic in presence and in absence of trichoderma. In this experiment we have used two strains of *Trichoderma*, *T. harzianum* (T22) and *T. atroviride* (P1) These plants grown in uncontaminated soil irrigated with three different solutions containing 0-0.5 or 10 mg As L^{-1} . These studies were conducted in collaboration with plant pathology group of Professor Lorito (Faculty of Agriculture, Portici, Italy).

CHAPTER III

Materials and Methods

3.1. Experimental work to study the influence on the arsenic uptake by tomato irrigated with contaminated water

3.1.1. Soil preparation and characterization

The As uncontaminated soil used in the experiments was collected from the sub-surface layer (0-30 cm) of a natural grassland in Portici, Italy. The physical and chemical properties of the soil are reported in Table 1. After air-drying, the soil samples for cultivation and chemical analysis were passed through 5 and 2 mm mesh sieves, respectively. Soil fractions were separated by pipette and sieving following pre-treatment with H_2O_2 to oxidize organic matter. Soil pH was measured by potentiometry in distilled water (1:2.5 soil: water ratio). Organic C content of soil was determined by wet digestion with Walkley-Black procedure (Nelson and Sommers, 1982). For determination of CEC the soil was extracted with NH_4OAc (1 M) at pH 7.0. Total soil nitrogen was determined using a NCS auto-analizer (NA 1500 Series 2). Available P concentration was determined by colorimetric method as described in a published methodology (Olsen, 1954), and $NaHCO_3$ (0.5 *M)* was used as the extractant. The As concentration in digested soil was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP – AES, Varian, Liberty 150).

Soil proprieties	Amount
Coarse sand g kg^{-1}	131
Fine sand g kg^{-1}	388
Silt g kg^{-1}	275
Clay g kg^{-1}	206
Soil pH (in H_2O)	6.3
Organic carbon (%)	1.6
CEC (mequiv/100g)	18.5
Available P (P_2O_5) mg kg^{-1}	13.5
Total nitrogen %	0.12
Total As mg kg^{-1}	6.8

Table 1. The chemical and physical propeties of soil used**.**

3.1.2. Experimental conditions

Experiment was conducted from April 2010 to July 2011 in an unheated greenhouse. Tomato plants (*Lycopersicum esculentum* L. cv Piennolo) were grown in pots filled with 12 kg of the uncontaminated soil; they were planted at a density of 3 seeds per pot, sown directly in the pots and irrigated with water during the first 2 weeks. After this period the seedlings were transplanted to 1 per pot and irrigated with water containing sodium arsenite (Na_2HAsO_3) at four different concentrations: 0 (control treatment), 0.5, 2, and 4 mg As L^{-1}. The range of As concentrations was chosen to encompass the concentrations occurring in underground waters of the As-affected areas

of world. Contaminated water was added as required to maintain 60% water holding capacity. All the pots were fertilized every 2 weeks with 80 mL of nutrient solution containing 30.0 mM of NH_4NO_3 and 25 mM of K_2SO_4. In addiction, half of the pots were treated with 6 mM of K_2HPO_4 in order to evaluate the influence of P on As uptake by tomato plants so, the plants were separated in two different classes with P fertilization (P^+) and without P fertilization (P^-). The design was completely randomized and re-arranged every day, and each treatment was replicated 4 times to give a total of 32 pots. Arsenite irrigation was stopped 1 week before harvest.

3.1.3. Harvest and sample collection

Tomato plants were harvested by cutting at 3-4 cm above the soil (to avoid basal tissue contaminated by applied As solution). Tomato berries were collected, dried at 70 °C and finally weighed. Shoots and roots were washed with tap water and then rinsed twice with deionized water. The dry weight of the roots and shoots were determined after oven drying at 70 °C for 48 h. Roots, shoots and tomato berries were analyzed for total concentration of As and P. All samples were ground using a PM 200 ball mill (Retsch).

3.1.4. Sample preparatation for total As and P

A sample of about 0.5 g was accurately weighed into a PTFE pressure vessel and 7 mL of HNO_3 (65%), 0.5 mL of HF (50%), and 2 mL of H_2O_2 were added. All glassware and plastic ware were previously acid-washed in HCl (3M), and rinsed in deionized water. After that, the samples were digested in a microwave (Milestone, Digestor/Dring Ethos 900).

Total concentrations of As and P in root, shoots and tomato berries were determined by Inductively Coupled Plasma (ICP – AES, Varian, Liberty 150).

3.1.5. CRM recovery

Arsenic and P detection limits provided by this method were 8 and 12 mg L^{-1}, respectively. All analysis was carried out in triplicate. In each analytical batch at least, one reagent blank, one internationally certified reference material was included to assess precision and accuracy of the chemical analysis. Certified reference material (Oriental tobacco leaves CTA-OTL-1) was used. Repeated analyses of certified reference material gave 0.588 ± 0.013 µg $g^{-1}$for As (certified value 0.539 ± 0.060 µg g^{-1}) and 3006 ± 85 µg g^{-1} for P (certified value 2892 ± 134 µg g^{-1}).

3.1.6. Statistical analysis

Data analyses were performed with Kaleidagraph 3.6. Treatment effects were determined by analysis of variance. Where necessary, data were transformed logarithmically to stabilize the variance. Differences were considered as statistically significant at $p < 0.05$ (Tukey's test).

3.2. Experimental work on total arsenic, inorganic arsenic, and other elements concentrations in Italian rice

3.2.1.Market basket design and sample collection

Rice was collected from a range of geographic market basket throughout three Italian cities. Napoli, Milano and Cosenza. All the samples (101) were collected by sourcing rice at different markets (Table 2). All the market survey samples were collected as white (milled/polished) rice. The information about type and provenience of rice were taken only from each label on rice packaging. All the analysis was conducted at Institute of Biological and Environmental Sciences, University of Aberdeen, Scotland (UK).

Variety	n. samples	Region of provenience
Arborio (japonica, very fine)	29	19 Lombardia 8Piemonte 1Emilia Romagna 1Calabria
Carnaroli (japonica, very fine)	17	10 Lombardia 6 Piemonte 1Calabria
Ribe (japonica, fine)	21	11Lombardia 8Piemonte 1 Emilia Romagna 1 Calabria
Ribe/Roma parboiled (japonica)	10	6 Lombardia 4Piemonte
Roma (japonica, very fine)	8	4 Lombardia 3 Piemonte 1Emilia Romagna
Vialone Nano (japonica, semi fine)	5	3 Lombardia 2 Piemonte
Originario (japonica, common)	3	2 Piemonte 1 Lombardia
Others (mixture of these varieties)	8	5 Lombardia 2 Calabria 1 Piemonte

Table 2. The origin and varieties of rice samples analysed.

3.2.2. Samples preparation to total elemental quantification

All samples were dried overnight in an oven at 70 °C and ground by a mill ball. Subsequently, samples were processed as described in a published methodology (Williams et al., 2007b). Briefly, summarizing this analysis, approximately 0.2 g of the milled samples were accurately weighed into 50 mL polypropylene digest tube (Corning, NY) to which 2.5 ml of concentrated nitric acid (HNO_3) was added. The mixture was left overnight. After that 2.50 mL of hydrogen peroxide (H_2O_2) was added and the samples were digested in a microwave oven (CEM Mars 5, CEM Corp., Matthews, NC). The temperature for digest was increased first to 55 °C for 15 minutes, then to 75 °C for 15 min. and finally to 95 °C for 30 min.

Microwaved samples were cooled to room temperature and then diluted to 50 mL with ultrapure deionized water obtained from a Milli-Q system (Millipore, Billerica, MA), after rhodium ($10\mu g^{-1}$) was added as internal standard. In each analytical batch one reagent blank and one certified reference material (CRM) (rice flour NIST SRM 1568a) were included. A quadrople ICP-MS 7500 (Agilent Technologies) was used to determine metal and trace element (As, Cd, Cr, Zn, Se, Ni). Samples were randomized before the analysis. Standards were run after every 30 samples. The concentration of samples was determined using a seven-point calibration (from 0.1 to 100.0 mg L^{-1}) calculated from a multi-element standard solution (Claritas PPT). Atomic Absorption Spectroscopy (AAS) was used to analyze Mg and K, using a Perkin Elmer AAnalyst 100.

3.2.3. Samples preparation for As speciation

The procedure for speciation extraction followed that described in Zhu et al., 2008 (a, b). Around 0.2 g of rice was accurately weighed for each milled samples into a 50 mL polypropylene digest tube and10 mL of 1% HNO_3 were added and was left overnight. Then, the samples were extracted using a microwave oven with the same conditions as previously described for samples preparation for ICP-MS and atomic adsorption analysis. When the samples were cooled to room temperature, 1.5 mL samples were centrifuged at 10,000 g for 10 min and 900 µL of supernatant was mixed with 100 µL of H_2O_2. The samples were left overnight at 4 °C before analysis. Quality controls of CRM and blanks were run with each extract batch. Arsenic speciation was quantified by HPLC (HP1100, Agilent Technologies) coupled to the ICP-MS (Sun et al., 2008 a,b). Chromatographic separation consisted of a precolumn (11.2 mm, 12-20 µm Hamilton, Reno, NV, USA) and a PRP-X100 10-µm anion-exchange column (150×4.1 mm, Hamilton). The mobile phase consisted of 6.66 mM ammonium hydrophosphate ($NH_4H_2PO_4$) and 6.66 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using ammonia 3%. Retention time for the As species was determined using a species standards mix of 10 µg L^{-1} containing arsenite, arsenate, DMA and MMA, and DMA standards (0-0.5-2.5-5.0-10.0-25.0 µg L^{-1}), used to calibrate the instrument. The species standards mix was run after every 20 samples.

3.2.4.CRM recovery

The CRM mean and standard error Ast recovery was 0.27±0.02 mg kg^{-1} compared to a certified value of 0.29 mg kg^{-1} (Raab et al., 2009). Limit of detection (LOD) was 0.016 mg kg^{-1}. No rice CRM certified speciation is available; so rice flour CRM speciation data obtained here was compared with that previously reported by Raab et al. (2009) for As_i 0.09, which was 0.09±0.007 here and Aso 0.18mg kg^{-1}, which was 0.17±0.02mg kg^{-1} here.

3.2.5. Statistical analysis

All statistics were performed using Minitab v.14 (State College, PA) and Sigma Plot. Total arsenic levels in rice data were ranked before analysis to normalize distribution. The differences between type and provenience of rice were assessed using one-way ANOVA.

3.3. Experimental work on*Trichoderma* spp. interacting with lettuce (*Lactuca sativa* L.) roots can alleviate arsenic toxicity

3.3.1. In vitro As removal by Trichoderma harzianum (T22) and Trichoderma atroviride (P1)

In vitro experiment was prepared for testing trichoderma ability to grow in As contaminated media by adding different concentrations of As (0; 3; 5; 10; 15; 20 mg L^{-1}) into flasks containing 50 ml of Potato Dextrose Broth (PDB). After sterilization of the media, 50 µl of a 10^7 spore ml^{-1} of T22 or P1 were used for the inoculum. The flasks were let to grow for 7 days on the orbital shaker at 150 rpm at 25 °C, with 3 replicates for each treatment. After this time the mycelia were separated from the culture liquid through a vacuum filter and collected separately. The lyophilized mycelia and the culture filtrates were then used to detect the quantity of residual As. The ability of two fungal strains to volatilize and bioaccumulate As was calculated, following the protocol by Srivastava et al. (2012), by comparing the As content in the liquid medium before cultivation and that in the fungal biomass plus the medium at the end of cultivation.

3.3.2. Soil preparation and characterization

The soil used in this experiment was collected from the sub-surface layer (10-30 cm) of a grassland of the University of Naples, Italy. Its principal physical and chemical properties are reported in Table 3. According to the soil classification of FAO World Reference Base for Soil Resources, we can considerate this soil as a Calcari-Vitric Cambisol. After air-drying, the soil samples for lettuce cultivation and chemical analysis were passed through 5 and 2 mm sieves respectively.

Soil Properties	Amount
Sand ($g\,kg^{-1}$)	467 ± 26
Silt ($g\,kg^{-1}$)	348 ± 19
Clay ($g\,kg^{-1}$)	185 ± 8
Soil pH (in H_20)	7.23 ± 0.20
Organic Carbon ($g\,kg^{-1}$)	10.42 ± 0.31
CEC (mequiv $100\,g^{-1}$)	18.65 ± 0.77
Total N ($g\,kg^{-1}$)	0.97 ± 0.05
C/N	10.74
Available P ($mg\,P_2O_5\,kg^{-1}$)	11.09 ± 0.62
Total As ($mg\,kg^{-1}$)	5.83 ± 0.25

Table 3. The chemical and physical propeties of soil used.

Soil fractions (sand, silt and clay) were separated using the pipette and sieving method following pretreatment with H_2O_2 to oxidize organic matter, and dispersion was aided by sodium hexametaphosphate (Indorante et al., 1990). Soil pH was measured by potentiometry in distilled water (1:2.5 soil/water ratio). The soil organic C content was determined by wet digestion using the modified Walkley-Black procedure (Nelson and Sommers, 1982). For determination of CEC, the soil was extracted with 1 M NH_4OAc at pH 7.0. The total soil N was determined using a NCS Elemental Analyzer (NA 1500 Series 2). Available P concentration was determined by the colorimetric method using 0.5 M $NaHCO_3$ as the extractant (Olsen et al., 1954). Available As concentration was extracted with 0.05 M $(NH_4)_2SO_4$ for 4 h at 20 °C using the first step sequential extraction procedure (SEP) (Wenzel et al., 2001). The As background in the soil was determined by extracting the soil with concentrated HNO_3 and HF at 5:1 ratio.

3.3.3. Experimental design

Lettuce plants (*Lactuca sativa* L., cv. Marvel), were seeded into a polystyrene alveolar seedbed consisting of 90 holes (25 mm diameter, 40 mm depth) containing soil. After 10 days when plant emerging, the seedlings were transplanted one plant per pot into pots containing 6 kg of the soil. The roots of the obtained seedlings were sprayed with a spore suspension of Trichoderma as follows: 1) 1/3 were sprayed with a *T. harzianum* (T22) spore suspension (10^7 spores/ml); 2) 1/3 were sprayed with a *T. atroviride* (P1) spore suspension (10^7 spores/ml); 3) 1/3 were used as a control and sprayed with pure water only (–T). For the first 5 days after transplanting, all the lettuce plants were initially irrigated with As-uncontaminated water, and thereafter irrigated for 30 days with water containing sodium arsenite ($NaAs^{III}O_2$) at two different As water concentrations: 5 (As 5) and 10 (As 10) mg As L^{-1} and there was also control set of plants (control) irrigated with As-uncontaminated water. Constant volumes of irrigation waters were frequently added to each pot, in order to maintain the soil moisture at 65 % of the field capacity, avoiding any phenomenon of leaching. A basal fertilizer, consisting of 1.87 g N pot^{-1} (as NH_4NO_3 and KNO_3), 0.95 g P pot^{-1} (as KH_2PO_4) and 2.31 g pot^{-1} K (as K_2SO_4 and KNO_3) was supplied to the lettuce plants as nutrient solution. The experimental design to be composed of 4 replicates for each treatment. The pots were arranged in a completely randomized design and rearranged every 5 days. The population of both species of *Trichoderma* in the rhizosphere of the inoculated plants was kept constant by water applications (10 and 20 days after transplanting) of T22 and P1 spores suspension (10^7 spores/ml). The watering of the lettuce plants was stopped 3 days before harvesting. This experiment was conducted in an unheated greenhouse.

3.3.4. Measurement of net photosynthesis rate

The measurement of net photosynthesis rate was made with a portable leaf gas exchange analyzer (ADC Bioscientific Ltd. LCA4, UK). Measurements were taken on 2-3 leaves per plant (only mature, fully expanded, symptom-free to As toxicity, well-exposed leaves were chosen), in three different days, from 11:00 am to 1:00 pm, under conditions of fully saturating radiation (when photosynthetically active radiation (PAR) was in excess of 1500 mmol m^{-2} s^{-1}).

3.3.5. Harvest and plant samples collection

The lettuce plants were harvested at the market size 30 days after transplanting. Roots and leaves were sampled separately at harvest time. Above-ground biomass was removed by cutting the base of the plant 1 cm above the soil surface. The fresh tissues of the lettuce plants were weighed and washed with deionized water to remove soil residues; then dried in an oven for 2 days at 70°C. Portions of fresh root were cut and weighed and used for the determination of the rate of root colonization by the two *Trichoderma* strains (T22 and P1). The remaining parts of the root were dried and weighed and then ground using a PM 200 ball mill (Retsch).

3.3.6. Determination of the rate of root colonization by two Trichoderma strains (T22 and P1)

At the end of each test, portions of fresh root from plant of each treatment were analyzed to evaluating the presence or absence of the root-colonizing fungus. The roots were washed three times in distilled sterile water and cut into small pieces. Five pieces from each plant root were placed in a petri dish containing PDA with 1% lactic acid and checked for up to 10 days for the appearance of *Trichoderma* (T22 or P1) colonies.

3.3.7. Valutation of As and P concentrations in plant tissues, PDB liquid medium and mycelium

Approximately 0.5 g of dried tissues were weighed into PTFE vessels and was added 5 mL of HNO_3 (65%), 0.5 mL of HF (50%) and 2 mL of H_2O_2. Then all samples were digested in a microwave (Milestone, Digestor/Dring Ethos 900), filtered through 0.22 mm filters and diluted to 50 mL with deionized water. While the mycelium samples were weighed around 0.1 g and digested with the same protocol described before for plant tissue. The measurement of As and P concentrations, both in the filtrates and PDB liquid medium was performed by a flow-injection hydride generation atomic absorption spectrometer (Perkine Elmer AAnalist 700 interfaced with the FIAS 100 hydride generator), and a inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian, Liberty 150), respectively.

3.3.8. CRM

The As and P detection limits of these methods were $1.5 \cdot 10^{-3}$ mg L^{-1} and $1.2 \cdot 10^{-2}$ mg L^{-1}, respectively. All analyses were carried out in triplicate. In each analytical batch at least one reagent blank and one internationally certified reference material (CRM), oriental tobacco leaves CTA-OTL-1 (Dybczyfiski et al., 1993).

3.3.9. Statistical analysis

Mean values were compared by ANOVA and the Tukey's test at the significance level $p < 0.05$. Data on plant biomass, As concentration and content in plant tissues, and available As and P in the soils were treated by two-way ANOVA with factors As and inoculation, and tested for normality using the Shapiro-Wilk test. All the statistical analysis was performed with SPSS (V 20, IBM).

CHAPTER IV

Results

4.1. Experimental work to study the influence on the arsenic uptake by tomato irrigated with contaminated water

4.1.1. Plant growth and As toxicity

Tomato plants growth was significantly influenced by As presence in irrigation water (Fig. 10) (Pigna et al, 2012). The totally plant biomass decreased with increasing As concentration in irrigation water (Fig. 10, d) like observed by Carbonell-Barrachina et al. (1997) in tomato and bean plants and Requeio and Tena (2006) in maize. Plants non-fertilized with P (P-) and irrigated with solutions containing 0.5, 2 and 4 mg As L^{-1} showed a decrease in their biomass of 17%, 42%, and 58%, respectively, compared to their own As control treatment. In plants fertilized with P (P+), this reduction was less severe (13%, 30%, and 42%, respectively) (Fig. 10) (Pigna et al., 2012). Similar results were also obtained on rice (Abedin et al., 2002), wheat (Liu et al., 2005; Pigna et al., 2009) and *P. Vittata* (Tu and Ma, 2004). The As exposure was clearly visible in the roots system (Fig.10, a). In fact P- plants irrigated with the 0.5, 2 and 4 mg As L^{-1} solutions showed a quite reduction of roots biomass of 2%, 65%, 74%, respectively, as referred to control treatment; whereas, in P+ plants, were significantly lower (4.1%, 18%, 34%, respectively) (Pigna et al., 2012). These data indicated that the addition of P to the system has significantly increased root biomass, regardless As treatment. Quaghebeur and Rengel (2003) found that by increasing AsO_4 concentration in nutrient solution there was a decrease in the roots and shoots dry weight of *Holcus lanatus*, accentuated when plants were non-fertilized with P. These results were comparable to those obtained by Pigna et al., (2009) studying the influence of phosphatic fertilizer on wheat plants irrigated with AsO_4-contaminated solutions.

We also analyzed the shoots (stem plus leaves) and berries tomato. The shoot dry weight was significantly influenced by the interaction between As and P treatments ($p<0.05$). In fact, the shoots biomass decreased with increasing concentration of As in irrigation water, especially in P- plants.

The same trend we have noticed in the tomato berries yield (mass of tomatoes per pot), which decreased significantly with higher As exposure. P- plants non-irrigated with As produced 12.60 g of tomato berries for pot; whereas, when irrigated with the most As-contaminated solution (4 mg L^{-1}) realized only 5.30 g pot^{-1}, with a percentage drop of dry weight of 58%. This reduction of yield was less severe in plants fertilized with P where it was from 17.40 to 8.40 g pot^{-1}, with percentage drop of dry weight of 51% (Pigna et al., 2012).

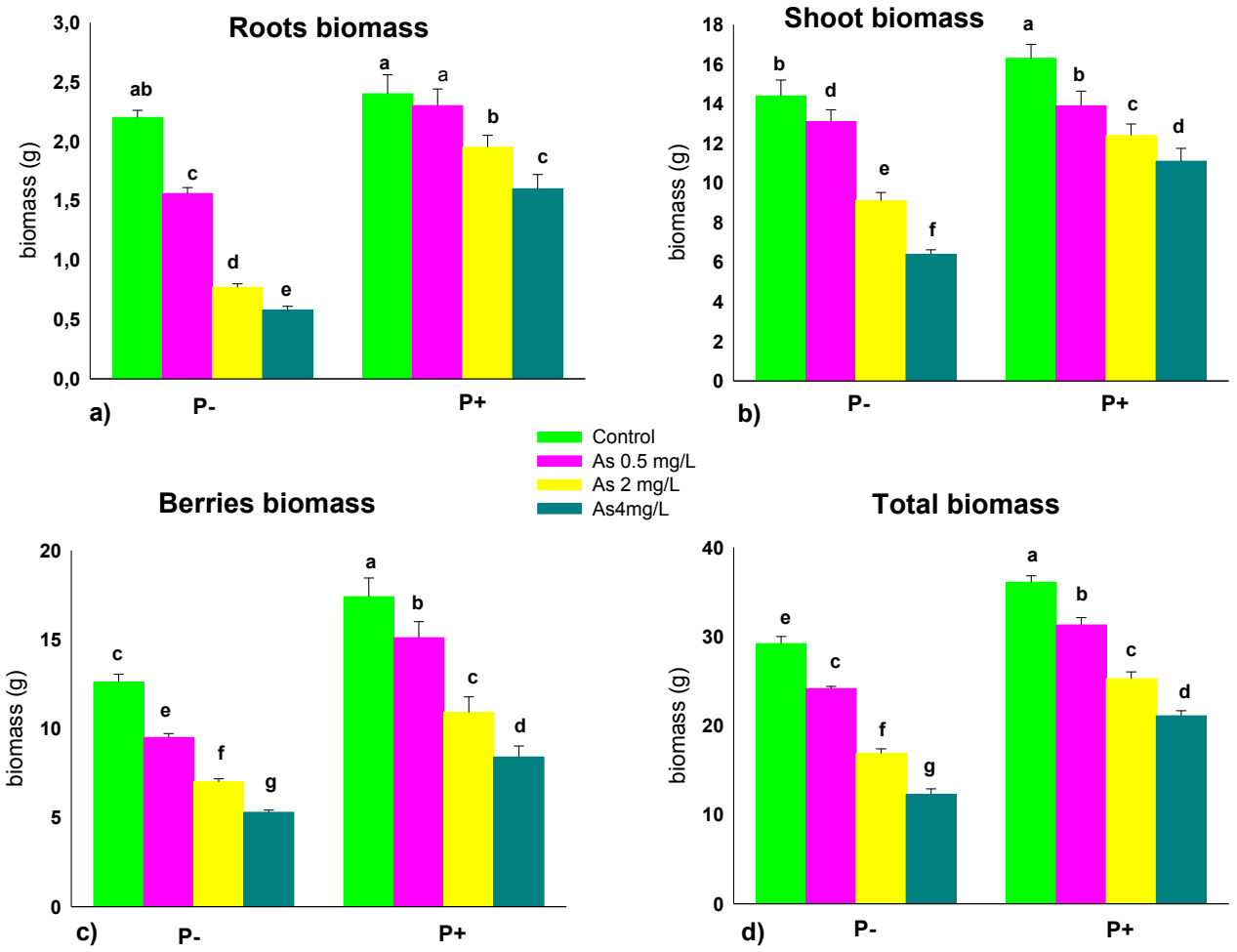

Figure 10. Biomass production (dry weight) in P- and P+ in different vegetal tissue: a) roots; b) shoots; c) berries; d) total. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level. With Anova two ways, As and interaction PO_4 was statistically signicant $p< 0.05$.

The presence of AsO_3 in the irrigation water inhibited tomato plants growth and, consequently, their yield, especially in the absence of P fertilization. The different biomass production of P+ and P- indicated the beneficial role of P in preventing the toxicity of As promoting the growth of plants (Fig. 10). Physiological and electrophysiological studies have shown that arsenate and phosphate share the same transport pathway in higher plants, with the transporters having a higher affinity for phosphate than for arsenate (Asher and Reay, 1979; Ullrich-Eberius et al., 1989; Meharg et al., 1994). The uptake mechanism involves cotransport of phosphate or arsenate and protons, with stoichiometry of at least 2H+ for each H_2PO_4 or H_2AsO_4 (Ullrich-Eberius et al., 1989). Arsenate sensitivity is intimately linked to phosphate nutrition, with increased phosphate status leading to reduced arsenate uptake, through suppression of the high-affinity phosphate/arsenate uptake system (Meharg and Macnair, 1991; 1992).

4.1.2. Arsenic concentration and content in tomato plant

Arsenic concentration in tomato roots, shoots and berries increased with increasing As concentration in irrigation water (Table 4). This trend is particularly evident in the roots of the P+ plants. In fact, by increasing As level from 0.5 to 4.0 mg L^{-1} in irrigation water, the As concentration in the roots increased from 0.68 to 3.85 and from 1.12 to 4.80 mg kg^{-1}, respectively, in P- and P+ plants (Table 4) (Pigna et al., 2012). Similar data were also found by Tao et al. (2006), Pigna et al. (2009), who studied the effect of P addition on the As accumulation in wheat plants. The higher concentration of As in the roots of P+ plants probably occurred because the application of the fertilizer containing P could have inhibited the AsO_4/AsO_3 sorption on the surface of the soil colloids (Violante and Pigna, 2002; Violante et al., 2005) and consequently, promoted the As uptake by plants because of the higher concentration of As in soil solution. Similarly, As content (μg pot^{-1}) also increased with increasing As concentration in irrigation water and P application. Plants irrigated with the highest As concentration in solution showed a higher As content than that of plants non irrigated with As.

Although the P+ plants showed a higher As content than P- plants in all their tissues, the alleviation of As toxicity would be attributed to the greater diluition of As in the higher biomass producted by these plants. This aspect is clearly visible in Figure 11, which shows ratios between the As content in tomato berries and their biomass as a function of As treatment. Regardless As level in the irrigation water, P application has determined a reduction of these ratios, confirming that a greater dilution of the As in the berries biomass occurred in the plants fertilized with P. In addition, a more evident reduction of this ratio between the P- and P+ plants (Table 4) has been determined in the treatments with higher As levels (2.0 and 4.0 mg L^{-1}) versus the lowest one (0.5 mg L^{-1}). The better P nutritional status of P+ plants has also allowed limit the traslocation of As from roots to aboveground plant tissues. Previous studies showed that the effect of phosphate on plant arsenate uptake depends on plant growing conditions (Khattak et al., 1991; Meharg and Macnair, 1991; Pickering et al., 2000). In a hydroponic system, phosphate addiction reduced arsenate uptake in both tolerant and non-tolerant plant genotypes of soft grass (*Holcus lanatus* L.) grown in arsenate solution (Meharg and Macnair, 1991). Alfalfa (*Medicago sativa* L.) shoot arsenate concentrations were also decreased by phosphate (Khattak et al., 1991). Even for Indian mustard (*Brassica juncea* L.), a hyperaccumulator, grown in arsenate hydroponic solution with phosphate additionhave reduced arsenate uptake by 55–72% over the control was reported by Pickering et al. (2000).

As conc. mg L^{-1}	Roots	Shoots	Tomato berries	Roots	Shoots	Tomato berries	As shoot/root
	As concentration (mg kg^{-1})			(As content µg per pot)			
Control P-	0.16 ± 0.04 g	0.13 ± 0.03 e	0.04 ± 0.01 c	0.35 ± 0.03 h	1.87 ± 0.10 f	0.50 ± 0.04 f	0.81
0.5	0.68 ± 0.06 f	0.38 ± 0.04 d	0.18 ± 0.03 ab	1.06 ± 0.06 f	4.98 ± 0.19 d	1.71 ± 0.08 c	0.56
2	2.10 ± 0.14 d	0.55 ± 0.03 c	0.25 ± 0.04a	1.61 ± 0.08 e	5.00 ± 0.22 d	1.75 ± 0.11 c	0.26
4	3.85 ± 0.16 b	0.79 ± 0.07a	0.28 ± 0.04a	2.23 ± 0.13 d	5.05 ± 0.18 d	1.48 ± 0.10 d	0.20
Control P+	0.20 ±0.03 g	0.14 ± 0.02 e	0.04 ± 0.01 c	0.48 ± 0.04g	2.28 ± 0.10 e	0.70 ± 0.04 e	0.70
0.5	1.12 ± 0.07 e	0.42 ± 0.04 d	0.15 ± 0.02 b	2.58 ± 0.12 c	5.84 ± 0.24 c	2.27 ± 0.10 a	0.37
2	2.90 ± 0.12 c	0.64 ± 0.06 b	0.19 ± 0.03 ab	5.65 ± 0.18 b	7.93 ± 0.31 b	2.07 ± 0.11 b	0.22
4	4.80 ± 0.15 a	0.85 ± 0.06 a	0.22 ±0.03 a	7.68 ± 0.26 a	9.43 ± 0.044 a	1.85 ± 0.08 c	0.18

Table 4. Roots, shoots, berries of tomato As concentration and content irrigated with contaminated water (0-0.5-2-4 mg L^{-1}). Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

The higher the As concentration in irrigated water, the higher the As content in tomato berries; however, this content has never reached hazardous values, indicating a little accumulation of As in the tomato berries; in addition, P application has further limited the As accumulation in tomato berries, highlighting the crucial role of P in reducing the translocation of As toward tomato berries (Table 4). The ratios between the As concentration in shoots and roots, decreasing with increasing As concentration in irrigation water and in P+ plants (Fig. 11).

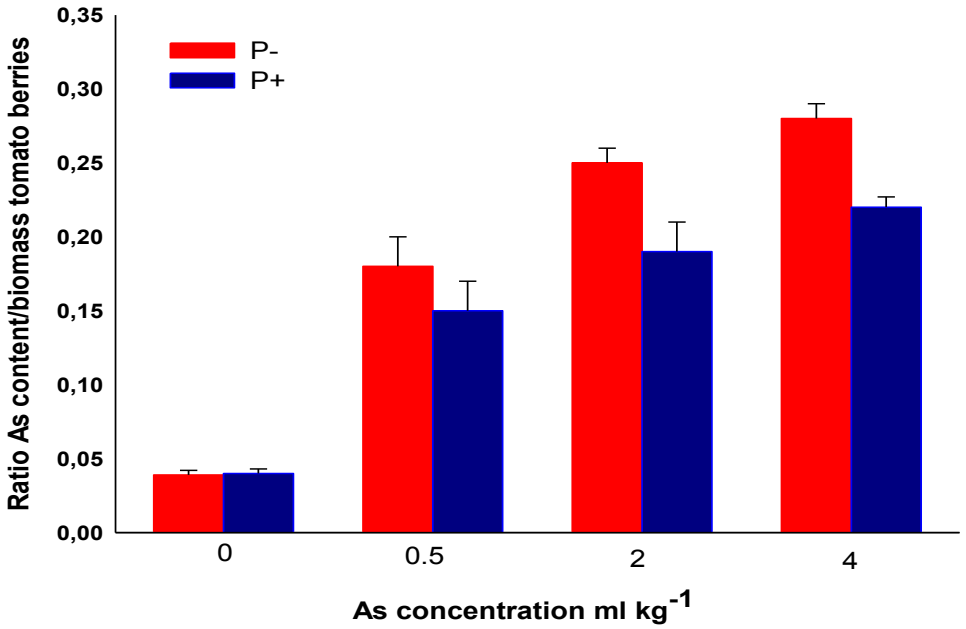

Figure 11. The ratio between As content/biomass in roots, shoot, and berries of tomato.

These results demonstrate that the As concentration in the roots increased more rapidly than that in the shoots; most of the As absorbed by tomato plants, in fact, was accumulated in roots, whereas only a small amount of the toxic element was translocated to the tomato berries; in addition, P application contributed to enhance this positive trend.

In a similar experiment on tomato plants, Carbonell-Barrachina et al. (1997) found that the 83.2 % of all the absorbed As remained in the roots, the 16.8% in the shoots and only 7.3% was accumulated in the leaves. A recent study (Bliek et al., 2008) reported that AsO_4 tolerance in plants is dependent on the P nutritional status of the plant. This behaviour is promoted by the activity of AsO_4 reductase, involved in the reduction of AsO_4 to AsO_3 and subsequent vacuolar sequestration of As (III)-phytochelatin complex. When a toxic metal has been absorbed by plants, then most extended mechanism involved in plant tolerance is limiting the upward transport, resulting in accumulation primarily in the root system (Meharg and Macnair, 1990). The strategy developed by tomato plants to tolerate the different species of As was avoidance, limiting As transport to shoots and increasing As accumulation in the root system. Avoidance, however, does not explain how tomato root tissues tolerate such extremely high As concentrations without exhibiting visual symptoms of toxicity. A possible explanation, not directly deduced from this study, could be that As compartmentalization in tomato roots was so effective that As impact on growth and metabolism

was minimal. Arsenic detoxification and compartmentalization in root cells are topics that will need further research to verify their role in plant tolerance to As (Burlo et al., 1999). There have been a number of reports testing the effect of P on arsenic phytotoxicity (Meharg et al. 1994; Tu and Ma 2003, 2004) and several reports on biochemical responses of plants to arsenic stress (Stoeva et al. 2005; Srivastava et al., 2005). Enhanced uptake of phosphate would have been expected to have given less uptake of arsenate through competition for the phosphate transporter, or at the very least the high internal P status would have been expected to down-regulate the transporter, so preventing further uptake of arsenate (Meharg and Hartley-Whitaker, 2002). Different phosphate transporters may vary in their affinity for arsenate. Use of phosphorus fertilizers to decrease As accumulation in plants has not always been successful, because phosphate competes with arsenate in both root uptake and adsorption on Fe oxides/hydroxides.

4.1.3. Phosphorus concentration and content in tomato plants

Phosphorus concentration in roots of the tomato plants significantly increased with P application and by increasing level of As in irrigation water. The concentration of P increased from 1.16 g kg^{-1} (As control treatment) to 2.30 g kg^{-1} (highest As level) and from 1.65 to 3.40 g kg^{-1}, respectively, in P- and P+ plants The content of P in P- plants roots decreased from 2.55 mg pot^{-1} (As control treatment) to 1.33 mg pot^{-1} (highest As level) because of the lower biomass produced by the plants irrigated with higher As levels; vice versa it increased in the roots of the P+ plants, from 3.96 to 5.44 mg pot^{-1} (Table 5). Similar results were obtained by Meharg (1994) who ascertained that PO_4 is more efficiently taken up and accumulated in plant tissues than AsO_4. These findings may explain how tolerant plants can survive at high levels of AsO_4 in soil solution and, indeed, how plants grown on As contaminated sites are able to obtain enough P to sustain their growth (Meharg, 1994). The P concentration and content in tomato shoots decreased markedly with increasing concentration of As in the irrigation solutions, especially in the plants non-fertilized with P. For instance, the concentration of P in the shoots of P- plants irrigated with solution containing 4 mg As L^{-1} was 64% lower than that of their own As control.

As conc. mg L^{-1}	Roots	Shoots	Tomato berries	Roots	Shoots	Tomato berries	P shoot/root
	P concentration (g kg^{-1})			(P content mg per pot)			
Control P-	1.16 ± 0.03 g	2.10 ± 0.10 d	6.40 ± 0.18 b	2.55 ± 0.12 e	30.24 ± 1.40 e	80.64 ± 3.6 c	1.81
0.5	1.35 ± 0.05 f	1.90 ± 0.06 e	6.30 ± 0.26 b	2.10 ± 0.10 d	24.89 ± 1.30 d	59.85 ± 2.5 e	1.41
2	1.78 ± 0.06 d	1.00 ± 0.04 f	6.12 ± 0.24 b	1.37 ± 0.08 e	9.10 ± 0.40 e	42.84 ± 1.9 g	0.56
4	2.30 ± 0.12 c	0.75 ± 0.02 g	5.90 ± 0.22 bc	1.33 ± 0.06 e	4.80 ± 0.30 f	31.27 ± 0.9 h	0.33
Control P+	1.65 ± 0.04 e	3.20 ± 0.09 a	7.20 ± 0.24 a	3.96 ± 0.15 b	52.16 ± 2.10 a	125.30 ± 5.60 a	1.94
0.5	2.22 ± 0.09 c	2.70 ± 0.07 b	6.90 ± 0.20 a	5.11 ± 0.20 a	37.53 ± 1.70 b	104.20 ± 4.30 b	1.22
2	2.90 ± 0.13 b	2.60 ± 0.08 b	6.65 ± 0.18 ab	5.65 ± 0.25 a	32.24 ± 1.40 c	72.48 ± 3.10 d	0.90
4	3.40 ± 0.16 a	2.45 ± 0.08 c	6.40 ± 0.22 b	5.44 ± 0.28 a	27.20 ± 1.20 d	53.76 ± 2.30 f	0.72

Table 5. P concentration and content in roots, shoot, and berries of tomato plants. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Similarly, the content of the As in these plants decreased from 30.24 to 4.80 mg pot^{-1} (Table 5). The same trend was also found in P+ plants, but it was lesser pronounced. In fact the percentage drop in P concentration of 23% compared to control, with a reduction from 52.16 to 27.20 mg pot^{-1} of P content). The concentration of P (g kg^{-1}) in tomato berries slightly decreased by higher As exposure, both in P and P+ plants, while its content (mg pot^{-1}), being related to the biomass, produced more severe decreases in P- plants.

4.2. Experimental work on total arsenic, inorganic arsenic, and other elements concentrations in Italian rice

4.2.1. Total As concentration

Total As (Ast) concentration was calculated for each 101 samples of Italian commercial rice (Fig. 12). It was found that the highest mean concentration of Ast was for Vialone Nano rice varieties (0.28 mg kg^{-1}, 5 samples), whereas, the lowest total mean concentration was found in Ribe rice samples (0.18 mg kg^{-1}, 21 samples) (Fig. 12, Table 8) (Sommella et al., 2013). According to the region of provenience, it was found that the highest total concentration was in Emilia Romagna region (0.28 mg kg^{-1} 3 samples) and the lowest mean in Calabria (0.11 mg kg^{-1} 5 samples), the range for the entire data set being 0.07-0.47 mg kg^{-1} (Table 9). Meharg et al. (2009) analyzed 38 samples of Italian rice. They found Ast concentration ~ 0.15 mg kg^{-1}; lower than rice from French Camargue production region (0.28 mg kg^{-1}), the highest value found. Also they analyzed US produced rice and they found a mean ~0.25 mg kg^{-1} for Ast concentration. The overall range found for the individual samples was from 0.01 mg kg^{-1} found in Egyptian and Indian rice to 0.82 mg kg^{-1} in Spanish rice (Meharg et. al., 2009). Zavala et al. (2008) found US rice had a mean Ast of 0.20 mg kg^{-1}. D'Ilio et al. (2002) analysed Italian rice varieties and found the following results: 0.20 mg kg^{-1} (Arborio); 0.14 mg kg^{-1}(Carnaroli); 0.28 mg kg^{-1}(Ribe); 0.12 mg kg^{-1} (Ribe parboiled); 0.22 mg kg^{-1} (Vialone Nano); 0.18 mg kg^{-1} (Originario). The study presented here, in comparison, had Ribe at 0.18 mg kg^{-1} and parboiled at 0.20 mg kg^{-1} mean value. Here also, for Ast, concentration means of 8 different varieties varied significantly ($P = 0.03$) when analysed by one way analysis-of-variance (ANOVA). For the region of provenience there was a greater statistical difference in Ast concentration ($P=0.006$) (Sommella et al., 2013).

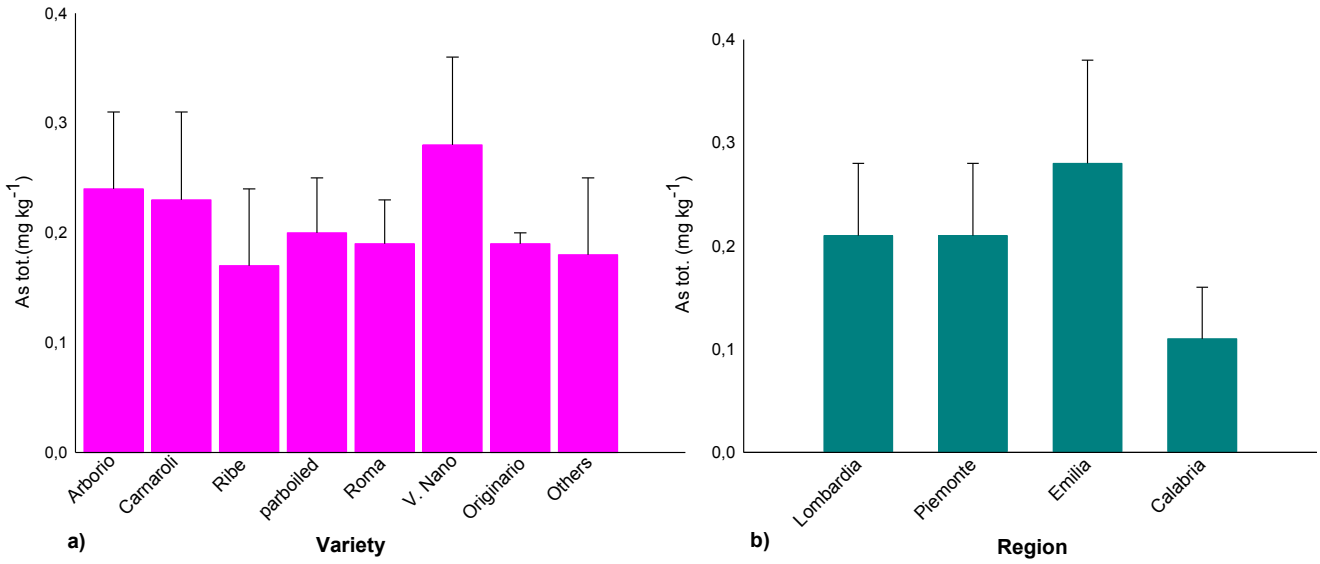

Figure 12. The As total concentration in rice samples, according with a) Variety and b) Region of provenience.

4.2.2. Arsenic speciation

As speciation in rice grain is dominated by inorganic arsenic (Asi) and DMA (Meharg et. al., 2009), although it was found here that MMA was above limits of detection (LOD) in two samples, one of these was Carnaroli type from Lombardia (0.025 mg kg^{-1} MMA) and the other was from a mix of varieties from Piemonte (0.019 mg kg^{-1}MMA). For Asi the highest mean value found in this study by variety was Vialone Nano (0.11 mg kg^{-1}) (Fig. 13, a), while the lowest was for Ribe and Roma type (0.08 mg kg^{-1}). For region of provenience it was found about Asi: Lombardia 0.10 mg kg^{-1}, Calabria 0.06 mg kg^{-1}. As for Ast also the concentration of As_i is strongly influenced by geographical origin (P=0.01) (Fig. 13, b). In this study the percentage of total As represented by Asi varies between 0.4% and 96% (Sommella et al. 2013). In literature this range has been reported as 11% until 91% (Schoof et al., 1998; Heitkemper et al., 2001; D'Amato, 2004; Sanz et al., 2005; Williams et al., 2005).

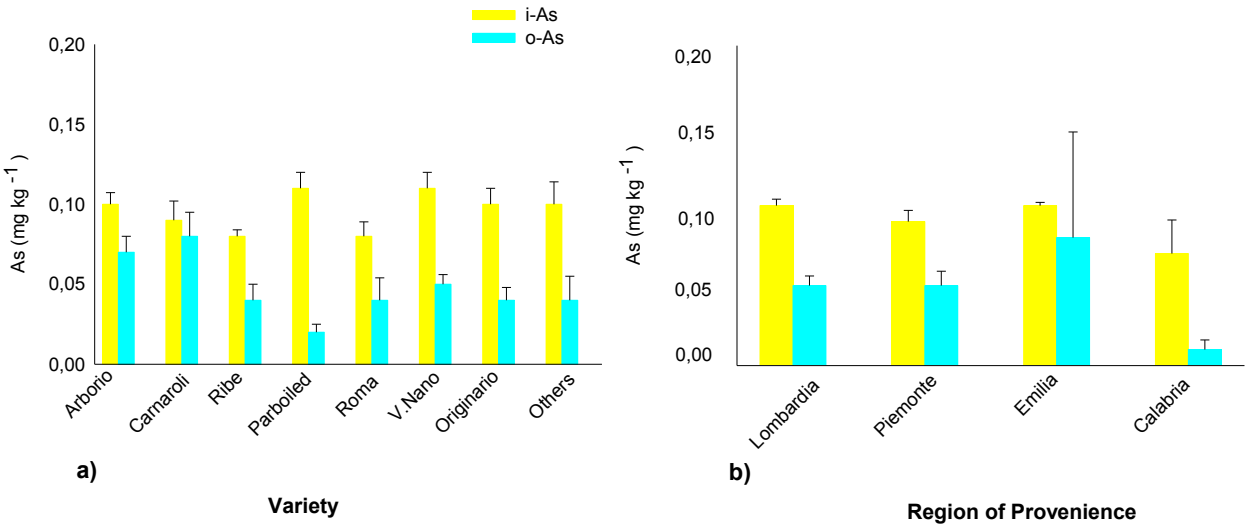

Figure 13. The As species (Asi and Aso) concentration in rice samples, according with a) Variety and b) Region of provenience.

Organic arsenic (Aso) mean varied by variety as follows: 0.08 mg kg^{-1} Carnaroli, 0.08 mg kg^{-1} (Table 6) Emilia, 0.02 mg kg^{-1}parboiled, 0.01 mg kg^{-1} Calabria (Table 7). China had established a limit for the maximum Asi concentration in rice 0.15 mg kg^{-1} (Heikens, 2006). The 10% of total samples analyzed in this study exceeded this value. In 2009 The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain discussed on arsenic human exposure in European countries, concluded that dietary exposure to Asi is high and should be reduced (EFSA, 2009a).

variety	n. samples		tot. As	Σ i-As	Σ o-As	i-As	o-As	Σ As species
			mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	%	mg kg^{-1}
Arborio (japonica, very fine)	29	mean±s.d.	0.23±0.08	0.102±0.04	0.07±0.05	89±13	25±13	0.17±0.08
		min-max	0.09-0.46	0.035-0.20	0-0.30	18-78	0-60	0.03-0.45
Carnaroli (japonica, very fine)	17	mean±s.d.	0.23±0.08	0.090±0.05	0.08±0.06	38±15	30±16	0.17±0.10
		min-max	0.10-0.43	0.001-0.17	0-0.27	0.4-74	0-62	0.04-0.39
Ribe (japonica, fine)	21	mean±s.d.	0.18±0.06	0.081±0.02	0.04±0.04	50±16	22±13	0.12±0.05
		min-max	0.09-0.39	0.05-0.10	0.006-0.22	25-71	3.5-55	0.06-0.32
Ribe/Roma parboiled (japonica)	10	mean±s.d.	0.20±0.05	0.11±0.03	0.02±0.01	54±17	11±8	0.13±0.05
		min-max	0.14-0.28	0.03-0.16	0.006-0.05	15-79	0-24	0.04-0.32
Roma (japonica, very fine)	8	mean±s.d.	0.19±0.03	0.081±0.03	0.05±0.04	48±11	36±47	0.13±0.04
		min-max	0.11-0.22	0.06-0.10	0.006-0.13	34-60	3-36	0.09-0.18
V.Nano (japonica, semi fine)	5	mean±s.d.	0.28±0.08	0.11±0.02	0.05±0.01	41±5	21±6	0.16±0.03
		min-max	0.20-0.40	0.08-0.13	0.04-0.06	32-45	10-25	0.14-0.21
Originario (japonica, common)	3	mean±s.d.	0.19±0.01	0.10±0.02	0.04±0.02	54±9	21±10	0.14±0.04
		min-max	0.18-0.20	0.09-0.12	0.02-0.06	43-61	11-32	0.11-0.18
others (mix of this variety)	8	mean±s.d.	0.18±0.07	0.10±0.04	0.04±0.04	58±20	19±15	0.14±0.08
		min-max	0.07-0.28	0.02-0.16	0-0.13	32-96	0-48	0.02-0.029

Table 6. Summarized speciation data according to variety.

EFSA reported that high consumers of rice in Europe, such as certain ethnic groups, are estimated to have a daily dietary exposure of As_i of about 1 µg kg^{-1} b.w. per day (EFSA, 2009a). According to the information available, As_i values in raw rice varies between 0.01 and 0.51 mg kg^{-1} d.w. (Schoof et al., 1999; Schoof et al., 1998; Heitkemper et al., 2001; Pizarro et al., 2003; D'Amato et al., 2004; Williams et al., 2005; Sanz et al., 2005). Meharg et al. (2009) found in 5 Italian rice samples a mean of 0.11 mg kg^{-1} and a range from 0.07-0.16 mg kg^{-1}similar to US samples (mean 0.10 mg kg^{-1}), Chinese samples (mean 0.16 mg kg^{-1}) and japanese samples (range of mean 0.09-0.23 mg kg^{-1}) (Meharg et al., 2009). Previous studies on Italian rice found a mean value of ~0.1 mg kg^{-1} for Asi (Pizarro et al., 2003; Williams et al., 2005). The FDA (Food and Drugs Administration) analyzed several samples of rice and rice products in US, founding a range of 3.5 to 6.7 µg per serving. The FDA's scientist recommend for reduce arsenic exposure to limit rice consumption: for adult 2 serving/week and for child 1 ¼ serving/week (FDA, 2013).

Region	n. samples		tot. As	Σ i-As	Σ o-As	% i-As	% o-As	Σ As species
			mg kg^{-1}	mg kg^{-1}	mg kg^{-1}			mg kg^{-1}
Lombardia	60	mean±s.d.	0.22±0.07	0.09±0.03	0.05±0.04	46±13	23±13	0.15-0.07
		min-max	0.09-0.46	0.03-0.17	0-0.30	18-79	0-60	0.03-0.45
Piemonte	33	mean±s.d.	0.21±0.07	0.09±0.04	0.05±0.04	47±18	23±13	0.15±0.07
		min-max	0.09-0.43	0.001-0.20	0.006 -0.27	0.40-78	3- 62	0.04-0.39
Emilia	3	mean±s.d.	0.28±0.09	0.09± 0.004	0.08±0.11	37±11	23±28	0.18±0.11
		min-max	0.20-0.39	0.10-0.09	0.006-0.22	25-46	3-55	0.1-0.32
Calabria	5	mean±s.d.	0.11±0.04	0.06±0.05	0.01±0.01	54±25	8±8	0.08±0.06
		min-max	0.07-0.17	0.02-0.13	0 – 0.02	32-96	0-17	0.02-0.16

Table 7. Summarized speciation data according to region of provenience.

4.2.3.Other elements

In this study total concentration of Cr, Cd, Se Mg, K, Zn, Ni was also quantified (Table 8-9).
Cr was detectable in all 101 samples analysed and the total concentration ranged between a mean value from 0.18 (Ribe) to 1 mg kg^{-1} (Vialone Nano), with a maximum single value of 1.51 mg kg^{-1} and a minimum single value of 0.11 mg kg^{-1}, with a significant statistical difference between rice type (P=0.001). On a geographical origin basis, the total Cr ranged between a mean value from 0.15 (Emilia Romagna) to 0.52 mg kg^{-1}(Calabria) (Table 9). D'Ilio et al. (2002) reported a total concentration of Cr ranging between 0.02 (V. Nano) and 0.05 (Arborio) mg kg^{-1} for Italian rice types (Table 8) (Sommella et al., 2013).
Cr is an essential trace element but it may create problems above certain concentration. The recommended dietary intake of chromium for adults is 50–200 mg/day (National Research Council 1989, Ikem, 2002). Currently, there is no formal Recommended Dietary Allowance (RDA) for Cr. The US Food and Nutrition Board (FNB) derived Adequate Intakes (AI) for Cr for different age groups, e.g. 35 μg/day and 25 μg/day for 19 to 50 years old men and women respectively (FNB, 2001). Determination of trace amount of chromium in food samples is important and of great interest due to human exposure and essential role of Cr (III) species for humans (Kabata-Pendias and Pendias, 1999). Cr (III) affects on the mechanism of action of the insulin, the pancreatic hormone, taking part in glucose and fat metabolism (Shils et al., 1994). On the other hand, Cr (VI) is extremely toxic to humans because of its high oxidation potential (Carlosena et al., 1997).

The highest mean value for total Cd concentration was found in Originario rice type (0.08 mg kg^{-1}), followed by Arborio, Ribe and Roma rice type (0.04 mg kg^{-1}), Carnaroli (0.03 mg kg^{-1}), Vialone Nano and parboiled (0.02 mg kg^{-1}). The range found in this study according to region of provenience was from 0.02 mg kg^{-1} (Calabria) to 0.04 mg kg^{-1} (Emilia Romagna and Lombardia). Whereas, D'Ilio et al. (2002) found a total Cd concentration ranging from 0.0015 (V.Nano) to 0.23 (Originario) mg kg^{-1} according to rice types (Table 8-9). No significant statistical difference was found in this study for geographical origin or rice type (Sommella et al. 2013). Meharg et al. 2013, compared commercial rice samples from 12 countries.

Variety	n. samples		Cr	Cd	Ni	Se	Zn	Mg	K
			mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Arborio (japonica, very fine)	29	mean±SE	0.24±0.03	0.04±0.01	0.19±0.02	0.07±0.01	14.36±0.03	461± 12.3	1059±37.3
		min-max	0.11-0.76	0-0.16	0.08-0.80	0-0.30	17.50-10.66	222-587	618-1478
Carnaroli (japonica, very fine)	17	mean±SE	0.37± 0.05	0.03±0.007	0.25±0.02	0.08±0.01	17.51±0.5	485±12.3	1061±45.6
		min-max	0.12-0.89	0.1-0.24	0.08-0.37	0-0.17	13.76-20.82	377-572	792-1218
Ribe	21	mean±SE	0.18±0.01	0.04±0.01	0.19±0.03	0.05±0.006	13.52±0.05	444±8.4	910±27.3
(japonica, fine)		min-max	0.11-0.41	0-0.15	0.08-0.49	0-0.13	9.62-20.16	375-511	808-1236
Ribe/Roma parboiled (japonica)	10	mean±SE	0.41±0.01	0.02±0.006	0.32±0.05	0.06±0.1	6.96±0.3	427±7	2005±42.4
		min-max	0.22-0.56	0.01-0.06	0.13-0.53	0-0.17	4.77-8.01	389-457	1746-2225
roma (japonica, very fine)	8	mean±SE	0.60±0.2	0.04±0.01	0.30±0.05	0.04±0.01	13.90±0.08	444 ±16.8	930±52
		min-max	0.12-1.44	0-0.08	0.15-0.55	0.03-0.08	9.91-16.87	386-493	776-1143
V.Nano (japonica, semi fine)	5	mean±SE	1.06±0.2	0.02±0	0.35±0.04	0.07±0.01	12.59±0.7	483±18.1	1115±82.1
		min-max	1.34-0.76	0-0.04	0.21-0.46	0.04-0.10	10.67-14.80	419-519	1414-915
Originario (japonica, common)	3	mean±SE	0.28±0.2	0.08±0.01	0.15±0.1	0.05±0.01	11.65±1.09	431±33.2	868±118
		min-max	0.15-0.54	0.05-0.1	0.08-0.9	0.02-0.07	9.82-13.55	380-493	665-1074
others (mix of this variety)	8	mean±SE	0.51±0.1	0.03±0.02	0.48±0.05	0.06±0.02	12.61±1.6	569±105	1444±215
		min-max	0.13-1.36	0-0.06	0.08-0.54	0-0.20	6.9-19.21	431-1302	887-2439
LOD (mg Kg^{-1})			0.049	0.023	0.15	0.044	n.a	n.a.	n.a.
spike (%)			100.29	80.34	102.04	70.03	77.75	n.a.	n.a.
CRM (%)			n.a.	74.25	n.a.	66.64	74.19	n.a.	n.a.

Table 8. Trace elements and essential elements concentrations (mg kg^{-1}) in commercial samples of italian rice according to variety.

In this study was included the rice samples analyzed here. It's was found French (0.010 mg kg^{-1}) and Cambodian rice (0.006 mg kg^{-1}) had the lowest grain cadmium concentration, followed by Ghana (0.020 mg kg^{-1}). Italy (0.038 mg kg^{-1}), Japan (0.059 mg kg^{-1}), Nepal (0.050 mg kg^{-1}), Spain (0.024 mg kg^{-1}), India (0.078 mg kg^{-1}), Thailand (0.027 mg kg^{-1}), and the U.S. (0.018 mg kg^{-1} could all be considered as having intermediate grain cadmium. On the other hands the samples come from Bangladesh (0.099 mg kg^{-1}) and Sri Lanka (0.08 mg kg^{-1}), have the highest Cd concentration.

EFSA recently lowered the Cd PTWI (Provisional Tolerable Weekly Intake) from 0.007 to 0.0025 mg kg^{-1} and established that cereals/grains, vegetables/nuts/pulses and animal offal were the main dietary sources of Cd in Europe (EFSA, 2009b).This limits exceeded by ~67% of the samples analyzed here. Cd concentration in rice for Europe was in a range from 0.004 to 0.02 mg kg^{-1}

(FAO/WHO, 2011). Current Cd exposure estimates are close to 2.5 mg kg^{-1} body weight for the average European adult population, whereas some subgroups, such as vegetarians and children, exceed this level, as do many populations worldwide. EFSA therefore concluded that there is no margin of safety between the point of departure for adverse effects of Cd on health and the exposure levels of the general population (Jarup and Akesson, 2009). There is a general consensus about the highly dominant role of food intake for environmental Cd exposure, whereas drinking water and inhalation of Cd in ambient air are minor sources. Cd intake via food is a function of the Cd concentration of the food and the amount consumed (FAO/WHO, 2001).

The mean value of Ni, found here, ranged between 0.15 and 0.48 mg kg^{-1} according to rice type. On the basis for the region of provenience, we found a mean value from 0.12 mg kg^{-1} (Emilia Romagna) to 0.51mg kg^{-1} (Calabria) (Table 8, 9) There was a significant statistical difference for rice type (P= 0.007) and for origin region (P=0.02) (Sommella et al. 2013). Nutritional requirements or recommended dietary allowances for Ni have not been established. The SCF stated explicitly that the data were not sufficiently conclusive to justify setting any recommended intakes (SCF, 1992).

Region	n. samples		Cr	Cd	Ni	Se	Zn	Mg	K
			mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Lombardia	60	mean±s.d.	0.43± 0.41	0.04±0.03	0.25±0.15	0.06±0.05	13.32±3.31	236±24	1175±390
		min-max	0.11-1.46	0.01-0.16	0.08-0.56	0.02-0.17	5.54-20.16	192-299	766-2225
Piemonte	32	mean±s.d.	0.26±0.25	0.03±0.03	0.25±0.17	0.06±0.03	14.10±3.99	229±23,2	1074±357
		min-max	0.11-1.50	0-0.13	0.08-0.80	0.02-0.17	4.77-20.82	191-292	642-2047
Emilia	3	mean±s.d.	0.15±0.04	0.04±0.06	0.12±0.07	0.09±0.01	13.04±1.62	250±17	1056±139
		min-max	0.11-0.17	0.01-0.11	0.08-0.20	0.08-0.10	11.73-14.85	234-268	896-1043
Calabria	3	mean±s.d.	0.15±0.02	0.011±0.004	0.29±0.03	0.13±0.1	14.91±3.89	240±20	964±92
		min-max	0.13-0.17	0-0.1	0.29-0.31	0.02-0.18	11.66-19.21	228-263	904-1069

Table 9: Trace elements and essential elements concentrations (mg kg^{-1}) in commercial samples of Italian rice according to region of provenience.

The total Se concentration was independent of Italian rice type (P>0.0.5), and varied from 0.04 mg kg^{-1} (Roma) and 0.08 (Carnaroli). Whereas on a statistical basis for different geographical origin there was a significant difference (P=0.02), with the highest mean value found in Emilia Romagna

(0.09 mg kg^{-1}) (Table 8, 9) (Sommella et al., 2013). Williams et. al. (2007a) analysed US rice samples found a mean value ranging from 0.21 (Mississippi Delta) to 0.08 mg kg^{-1}(California) and analysed Bangladesh rice samples, finding a mean value ~0.05 mg kg^{-1}(Williams et al., 2007a).
Antagonistic effects or mutual detoxification between As and Se have been reported in humans and other animals (Levander, 1977; Schrauzer, 1992; Zeng, 2001). Interaction of arsenic and selenium promotes the biliary excretion of exogenous selenium and selenite, and also augments the excretion of arsenic into bile. Some authors suggested that arsenic augmented the epatobiliary transport of selenium and facilitated accumulation in red blood cells. Selenium in turn facilitated the biliary excretion of arsenic (Rosen and Liu, 2009). EFSA considers that protection against heavy metals of Se is a beneficial physiological effect. No conclusions can be drawn from the only reference provided for the scientific substantiation of the claimed effect. On the basis of the data presented, EFSA concludes that a cause and effect relationship has not been established between the dietary intake of selenium and protection against heavy metals (EFSA, 2010).

The parboiled rice type contained lower Zn compared to other rice types analysed in this study (Carnaroli 17.5 mg kg^{-1}, parboiled 6.9 mg kg^{-1}). Previous studies found in Italian rice samples a mean value between 8.6 (parboiled) 19.9 mg kg^{-1} (Arborio) (D'Ilio et al., 2002). There was a significant statistical difference ($P<0.001$) on a variety basis for Zn, but not on geographical origin basis, where the total Zn ranged between 13.0 - 14.6 mg kg^{-1} (Table 8, 9).

4.3. Experimental work on *Trichoderma* spp. interacting with lettuce (*Lactuca sativa* L.) roots can alleviate arsenic toxicity

4.3.1. In vitro experiment

The results showed that both strains (*T. harzianum* T22 and *T. atroviride* P1) could tolerate increasing concentration of As into the growing media, with the production of fungal biomass being in some cases promoted by the presence of As (Table 10). For instance, the obtained biomass of either *T. harzianum* T22 or *T. atroviride* P1 was more than doubled at 20 ppm of As of that in the untreated control (Table 10). The content of bioaccumulated and bio-volatilized As was between 0.45 to 1.55% and 0.22 to 0.83% of the total applied toxicant, respectively .Our results are in accordance with those reported for *T. asperellum* strains isolated from As contaminated soil (Su et al., 2010).

Initial As concentration in PDB (µg mL^{-1})	Initial As content in PDB (µg)	Final As content in PDB (µg)		As content in *Trichoderma* (µg)		Trichoderma dry biomass (g)		As concetration in *Trichoderma* biomass (µg g^{-1})		Calculated amount of volatized As (µg)	
		T22	**P1**	**T22**	**P1**	**T22**	**P1**	**T22**	**P1**	**T22**	**P1**
0	0	n.a.	n.a.	n.a	n.a.	0.145±0.010 bc	0.123±0.007 bc	n.a.	n.a.	n.a.	n.a.
3	150	147.51±2.25 e	146.42±2.69 e	1.74±0.09 d	2.33±0.10 c	0.120±0.008 c	0.188±0.010 a	14.52±0.75 d	12.24±0.53 c	0.75±0.06 c	1.25±0.09 b
5	250	246.13±3.25 d	245.70±3.70 d	2.45±0.12 c	3.09±0.12 b	0.163±0.012 b	0.211±0.014 a	15.31±0.75 d	14.69±0.57 c	1.42±0.13 b	1.22±0.10 b
10	500	495.76±4.97 c	495.08±4.60 c	3.41± 0.16 b	3.05±0.14 b	0.169±0.010 b	0.152±0.012 b	20.09±0.94 c	20.34± 0.93 b	1.83±0.15 b	1.87±0.15 a
15	750	743.26±5.58 b	744.46±4.72 b	3.82±0.15 b	3.36±0.18 b	0.121±0.009 c	0.118±0.011 c	31.81±1.25 a	28.02± 1.50 a	2.92±0.20 a	2.18±0.17 a
20	1000	987.45±10.50 a	993.09±5.31 a	9.20±0.31 a	4.72±0.23 a	0.367±0.023 a	0.210±0.015 a	24.87±0.84 b	22.48±1.10 b	3.35±0.29 a	2.19±0.18 a
As concentration		p < 0.0001		p < 0.0001		p < 0.0001		p < 0.0001		p< 0.0001	
***Trichoderma* treatment**		p = 0.5623		p < 0.0001		p < 0.01		p < 0.0001		p< 0.0001	
As and *Trichoderma* interaction		p = 0.81554		p < 0.0001		p < 0.0001		p < 0.05		p< 0.00001	

Table 10: Bioaccumulation and biovolatilization of As by *Trichoderma harzianum* (T22) and *T. atroviride* (P1) in the PGP liquid medium.

4.3.2. Effect of As and Trichoderma on lettuce plants

The lettuce plants inoculated with both species (T22 and P1) of *Trichoderma*, have showed a significant plant growth-promoting effect regardless the level of As in the irrigation water (Fig. 14). On the other hand, the plants didn't inoculate, has been severely limited in growing by the irrigation with the As-contaminated waters (Fig. 14).The effects of As toxicity was mostly visible in the root system production where there was a strong inhibition of the growth in lettuce plants irrigated with As 10 water: with this treatment the plants produced a third of the root biomass's control (Fig. 14). Cozzolino et al. (2010) and Gusman et al. (2013), growing lettuce on As-contaminated soil and nutrient solution, respectively, also observed a stunted growth of the plants, because of the high toxicity of As toward these sensitive leafy vegetables. In fact, reported ranking of relative sensitivity to As are (in order of increasing sensitivity; Wauchope, 1983): asparagus, tomato, potato, carrot, tobacco, grape, raspberry < strawberry, sweet corn, beet, squash < bean, onion, pea, cucumber and alfalfa. Nevertheless, no lettuce plants died during our study, which just showed few symptoms to As toxicity, such as red-brown necrotic spots on the leaves, 12-15 days after the first irrigation with the As-contaminated waters as noticed by Ascher et al (2009). Therefore, it is clearly that these fungal strains (T22, in particular) were able to mitigate, at least in part, the negative effect of As on plant growth. Is to note despite the high level of As in the irrigation water, the beneficial effect of the inoculation with T22 on plants growth. In fact, the lettuce plants increased the biomass production (dry matter) of 30.1, 39.1 and 52.5% in comparison to control, in As 5 and As 10 plants, respectively. The growth-promoting effect of *Trichoderma* spp. has been demonstrated on a wide variety of plants in both laboratory-based and field trials (Adams et al., 2007; Ousley et al., 1994) and is attributed to a number of factors, including biocontrol, increased soil nutrient solubilization (Altomare et al., 1999).

Mechanisms that *Trichoderma* apply in facilitating metal stress tolerance in plants is attributed to enhanced root biomass production, hyperaccumulation of toxicants in plant tissues protection against oxidative damage in plants, and enhanced nutrient availability and efficiency (Harman et al. 2004b; Tu et al. 2004; Arriagada et al. 2009a; Mastouri et al. 2010).

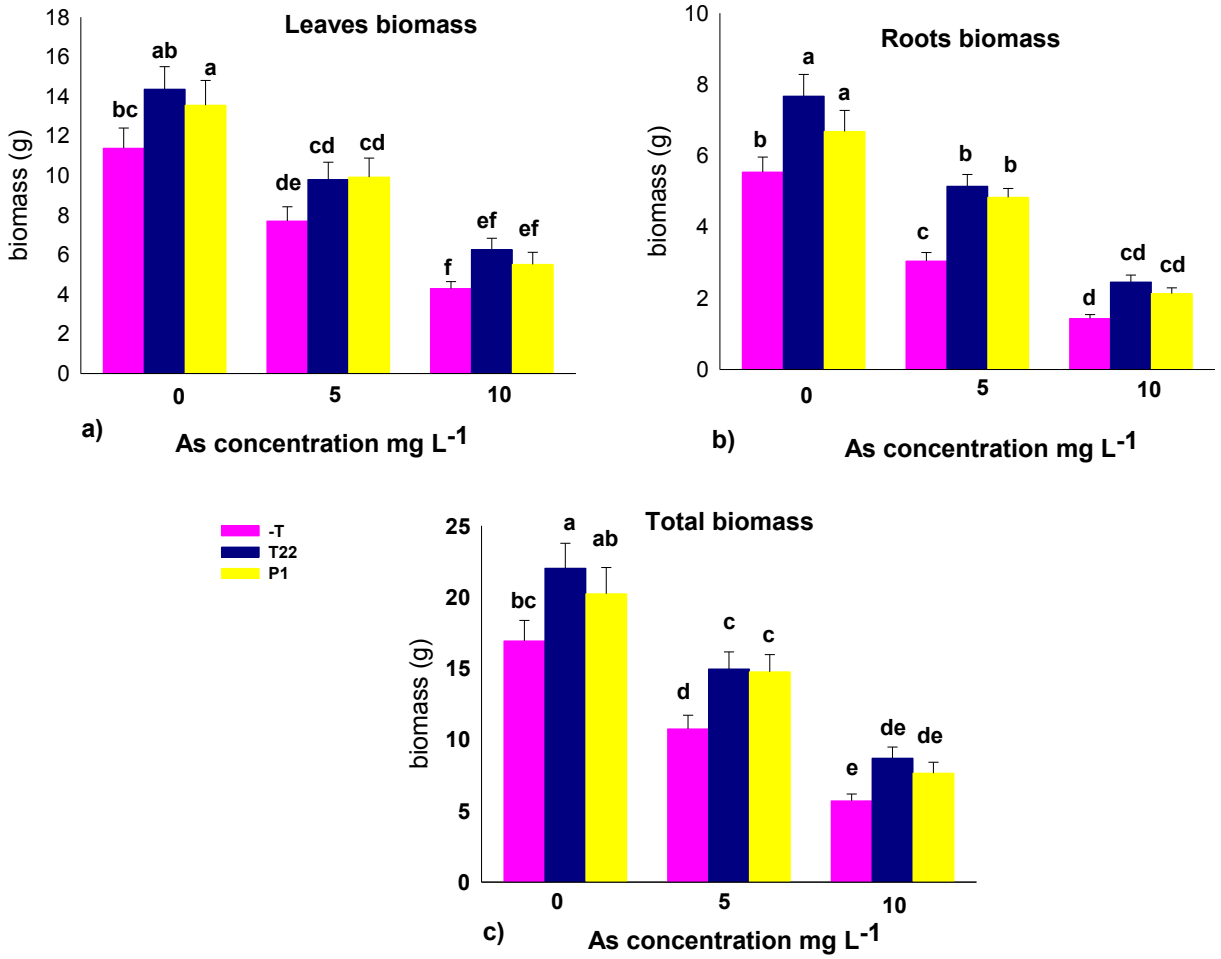

Figure 14. Biomass production (dry weight) in lettuce plants in different vegetal tissue: a) roots; b) leves c) total. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level. Anova two ways, arsenic and *Trichoderma* interaction was statistically significant ($p < 0.05$).

Previuos studies, have dimostrate that Trichoderma promote root growth of arsenic hyperaccumulating fern *Pteris vittata* (Lynch, 2004). Combined application of AM fungi and *T. harzianum* increased the tolerance and accumulation of Eucalyptus globulus to high concentrations of aluminum and arsenic in soil (Arriagada et al. 2007, 2009b). Tripathi et al (2013) have demonstrated that *Trichoderma*-inoculated chickpea plants were healthier (descreases of damage by As, in cellular structure) than the uninoculated control during As exposure. Certain *Trichoderma spp*. have beneficial effects on plant growth and enhance resistance to both biotic and abiotic stresses. Early work revealed that *Trichoderma* promotes growth responses in radish, pepper, cucumber and tomato (Chang et al., 1986). Further studies demonstrated that Trichoderma also increases root development and crop yield, the proliferation of secondary roots, and seedling fresh weight and foliar area (Harman, 2000). Moreover, *T. harzianum* can solubilize several plant nutrients (Altomare et al., 1999), and the colonization of cucumber roots by *T. asperellum* has been shown to enhance the availability of P and Fe to plants, with significant increases in dry weight,

shoot length and leaf area (Yedidia et al., 2001). According to Viterbo et al. (2010), *Trichoderma* can produce1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can contribute to plant growth promotion, such as an increase in root elongation. These isolates did not have a negative impact on plants or soil, and no disease symptoms appeared on the tested plants.

4.3.3. Net photosynthesis rate of the lettuce plants

As long as the As concentration increased in the irrigation water, a continuous reduction in the net photosynthesis rate of the lettuce plants was observed (Fig. 15). The net photosynthesis rate of control plants was, on average, 10.85 μmol CO_2 m^{-2} s^{-1}, while that of the plants irrigated with As was, on average, 8.05 (As5) and 4.51 μmol CO_2 m^{-2} s^{-1} (As10). The lowering of photosynthetic rate is considered to be one of the most damaging effects of this metalloid (Gusman et al., 2013; Stoeva et al. 2005). Arsenic can interfere in photosynthesis system in several ways. The toxic effects on the photosynthetic process can occur in the photochemical or in the biochemical steps or even in both Arsenic accumulation is able to interfere with chlorophyll biosynthesis through the induction of iron (Fe) deficiency or the inhibition of some key steps of the process (Schoefs and Bertrand 2005). In photochemical step of photosynthesis, As can interfere in the electron transporter chain (ETR), resulting in changes in the formation of the reducing power (NADPH) and ATP and/or promote an increase in fluorescence emission or energy liberation as heat. In biochemical step, As can affect stomatal conductance, restricting CO_2 concentration in the plant or inhibiting ribulose-1,5-bisphosphate carboxylase/ oxygenase (RUBISCO) activity (Gusman et al., 2013). In addition, this element is able to affect the photochemical efficiency and heat dissipation, promoting changes in gas exchange and in fluorescence emission (Rahman et al., 2007). These alterations result in reduction of leaves and roots growth, with some symptoms of toxicity as wilt and violet coloration of leaves (Abedin et al. 2002; Rahman and Naidu, 2009).

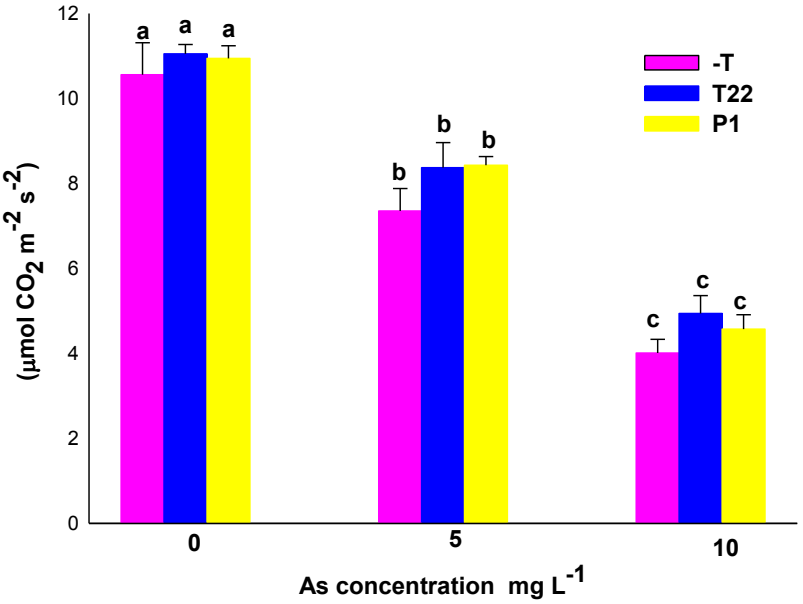

Figure 15. Net photosynthesis rate of the lettuce plants irrigated with arsenic solution (0, 5, 10 mg L^{-1}). Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

As, we have noticed in our studies. However, it is interesting to note that the inoculation of the lettuce plants with two species of *Trichoderma* (T 22, in particular) slightly enhanced the photosynthesis rate of the As 5 (T22 8.43; P1 8.37; control 7.38 µmol CO_2 m^{-2} s^{-1}) and As 10 (T22 4.94; P1 4.57 control 4.01 µmol CO_2 m^{-2} s^{-1}) plants (Fig. 15). Recent literature (Harman et al., 2004b; Tripathi et al., 2013) also demonstrated that the stimulatory effect of *Trichoderma* spp. on plant growth is also due to the rise of photosynthesis rate.

4.3.4. Arsenic accumulation in plant tissues

Significantly higher concentrations of As were found in the root system and leaf biomass, irrigating the plants with water containing increasing levels of As (Table 11). However, lettuce plants, thanks to their high sensitiveness to As toxicity, have accumulated relatively slight amounts of As in their own tissues, essentially in the roots. Regardless treatments, indeed, the concentration of As in the lettuce roots was, on mean value, fourfold higher than that in the above-ground tissue, indicating a limited translocation of the harmful element toward leaf biomass (Table 11). Here has been found that the distribution of As in plant tissue decreased in the following order: roots >> stems > leaves.

In previous works has been reported the same As concentration trend in different species of plant. For instance, Xie and Huang (1998), and Liu et al. (2004) in rice studies, Cobb et al. (2000), and Caporale et al. (2013) in beans, Burlo et al. (1999), lettuce, radish and chard roots (Smith et al., 2009) have observed elevated concentrations of As in plant roots compared to other plant tissues. The lower concentration of As in the lettuce leaves may be also due to the scant ability of the plant to translocate As beyond the roots (Smith et al., 2009). Even the total amount of As accumulated in the leaf biomass resulted to be lower than that stored in the roots. The As content in the lettuce leaves (%) was always lower than 50 %, except As 10 –T.

As concentration in plant tissue (mg kg^{-1})		
Treatment	**roots**	**leves**
Control -T	0.160 ± 0.008 e	0.060 ± 0.004 d
Control T22	0.110 ± 0.006 e	0.031 ± 0.003 d
Control P1	0.120 ± 0.010 e	0.036 ± 0.002 d
As 5 -T	3.84 ± 0.21 c	1.24 ± 0.13 c
As 5 T22	2.95±0.10 d	0.92 ± 0.08 c
As 5 P1	3.14 ± 0.25 cd	0.91 ± 0.07 c
As 10 -T	7.30 ± 0.54 a	2.66 ± 0.25 a
As 10 T22	6.13 ± 0.32 b	2.03 ± 0.22 b
As 10 P1	6.25 ± 0.29 b	2.10 ± 0.18 b
As concentration	P < 0.0001	p < 0.0001
Trichoderma treatment	P < 0.0001	P < 0.0005
As *Trichoderma* interaction	P < 0.01	P < 0.05

Table 11. Arsenic concentration in roots e leaves of lettuce plants. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

Trichoderma-inoculated plants exhibited a significant lower As concentration in their own tissues, essentially because these fungal strains (both T22 and P1) improving plant growth, caused a dilution of As concentration in their roots and leaves. Consequently, T22 plants (grown slightly more than P1) showed a little lower As concentration in their own tissues than that detected in P1 plants. Recently, Harman et al. (2004a) found that mechanisms employed by *Trichoderma* spp. in facilitating metal stress tolerance in plants are essentially attributed to enhanced production of biomass and nutrient use efficiency and availability. This observation suggests that chemical form of As but not its total concentration in tissue decides the toxicity. It is thus presumed that the *Trichoderma* can detoxify As, probably by converting it into a relatively less toxic species mediated by arsenate reductase enzyme. Earlier, Liu et al. (2009) have reported important role of arsenate reductase in the detoxification of As in fern while Yu et al. (2009) have demonstrated the conversion of inorganic As species to organic form in presence of VAM fungi in maize in addition, Mastouri et al. (2010) noted a greater protection against oxidative damage in *Trichoderma*-inoculated plants.

4.3.5. Plants P status

The inoculation of the lettuce plants with both species of *Trichoderma* (T22 and P1) raised significantly their P status, in both roots (Table 12) and leaves (Table 12), regardless the As treatment. Recent literature (Srivastava et al., 2012) also demonstrated that P uptake by plants can be increased by inoculation with soil mineral-phosphate-solubilizing fungi. The largest increases of P concentration in plant tissues (up to 16%), due to the *Trichoderma* inoculation, were found in the lettuce roots, essentially because of the higher availability of the nutrient in the soil. Plants susceptible to As toxicity, such as lettuce, can be made more resistant to As by raising their P status, as the P is taken up more effectively compared to As (Caporale et al., 2013; Lee et al., 2003; Pigna et.al, 2012).

P concentration in plant tissue (g kg^{-1})		
Treatment	**roots**	**leves**
Control -T	1.76± 0.15 ab	1.98 ± 0.20 abc
Control T22	2.38 ± 0.10 a	2.81 ± 0.15 a
Control P1	2.11± 0.16 a	2.37± 0.13 ab
As 5 -T	1.96 ± 0.18 bc	2.14 ± 0.24 cde
As 5 T22	2.76 ± 0.12 ab	2.60 ± 0.19 bcd
As 5 P1	2.37± 0.15 ab	3.13 ± 0.17 bcd
As 10 -T	1.91 ± 0.11 c	2.17 ± 0.17 e
As 10 T22	2.72 ± 0.17 c	3.07 ± 0.21 de
As 10 P1	2.40± 0.10 c	2.58± 0.15 de
As concentration	p < 0.0007	p < 0.01
Trichoderma treatment	p < 0.00001	P < 0.00001
As *Trichoderma* interaction	p = 0.732	P =0. 96

Table 12. Phosphste concentration in roots e leaves of lettuce plants. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

4.3.6. Available As and P in the soil

The presence of two *Trichoderma* strains (T22 and P1) in the soil rhizosphere enhanced significantly the concentration of the biologically available P fraction ($NaHCO_3$-extractable-P) (Table 13). Especially the *Trichoderma* strain T22 increased the available P fraction of 12% (control) 14% (As5), 14.7% (As10) compared with control for each treatment. The P1 strain was not so efficiently, increased the available P fraction of 12% (control) 12% (As5), 13% (As10). Plant growth-promoting microbes, in fact, have the ability to convert nutritionally important elements from their unavailable to available form and so their bioavailability increases uptake and furthers plant growth promotion (Khan et al., 2007; Srivastava et al., 2012). Many soil fungi have been shown to possess the ability to solubilize sparingly soluble phosphates in vitro by secreting inorganic or organic acids. Kim et al. (1990) suggested that the secretion of phosphatases by phosphate solubilizing fungi frequently facilitates the conversion of insoluble forms of phosphate to plant-available forms and enhances plant phosphorus uptake and further growth of the treated

plants. The solubilization of soil nutrients and their increased uptake have also been demonstrated by de Silva et al. (2000) for soil-inhabiting fungal biocontrol agents such as Gliocladium virens and Trichoderma harzianum. Turpeinen et al. (2004) suggested that metal-tolerant microbes can maintain their metabolic activities in contaminated soils. These fungi are able to tollerate, biosorb and detoxify arsenic by several mechanisms, including valence transformation, extra- and intracellular precipitation, active uptake and methylation (Srivastava et al., 2012).

Treatment	P available	As available
	(mg P kg^{-1})	(µg As kg^{-1})
control –T	11.98 ± 0.67 d	21.1 ± 0.9 c
control T22	13.42 ± 0.73 abcd (12.0)*	22.3 ± 1.3 c (5.7)*
control P1	13.31 ± 0.54 abcd (11.1)*	22.4 ± 1.5 c (6.2)*
As 5 –T	12.42 ± 0.49 cd	321.8 ± 20.6 b
As 5 T22	14.20 ± 0.72 abc (14.3)*	330.6 ± 18.6 b (2.7)*
As 5 P1	13.97 ± 0.66 abc (12.5)*	329.0 ± 22.3 b (2.2)*
As 10 –T	12.86 ± 0.68 bcd	518.9 ± 37.5 a
As 10 T22	14.75 ± 0.46 a (14.7)*	536.2 ± 39.4 a (3.3)*
As 10 P1	14.53 ± 0.75 ab (12.9)*	538.0 ± 35.8 a (3.7)*
As level	$p < 0.01$	$p < 0.0001$
Inoculation	$p < 0.0001$	$p < 0.6696$
Interaction	$p = 0.9780$	$p = 0.9726$

Table 13. Concentrations of $NaHCO_3$-extractable-P and $(NH_4)_2SO_4$-extractable-As (biologically available inorganic P and As) in the soils irrigated with As-contaminated waters at plants harvest time. Data are expressed as mean values ± SD (n = 4) and have been analyzed by two-way analysis of variance. Mean values followed by the same letter within columns are not significantly different by Tukey's test at the 5% level.

* The relative change (%) in P and As available was calculated by dividing the T22 or P1 treatment by the –T treatment for each As level (e.g., P available of control T22 treatment (13.42-11.98) x 100 / 11.98) = 12.0 %).

The solubilization of soil nutrients and their increased uptake have also been demonstrated by de Silva et al. (2000) for soil-inhabiting fungal biocontrol agents such as *Gliocladium virens* and *Trichoderma harzianum*. The irrigation with increasing levels of As also slightly raised the available P fraction in the soil, essentially because of the partial replacement of the nutrient by the contaminant on the soil Al- and Fe-(hydr)oxides, since they are chemical analogues (Violante et al., 2013).

Unlike the significant raising of the available P fraction, two fungal strains (T22 and P1) were just able to lightly increase the biologically available As fraction ($(NH_4)_2SO_4$-extractable-As) in the soil (Table 13). This finding is extremely important, because reveals that, even though two elements are chemical analogues, two *Trichoderma* strains (T22 and P1) were able to enhance selectively the phytoavailability of the nutrient vs. that of the contaminant. Turpeinen et al. (2004) suggested that metal-tolerant microorganisms can maintain their metabolic activities in contaminated soils.

CHAPTER V

Conclusions

The accumulation of As in food is quite alarming and is mainly due to irrigation with As contaminated water. The above information asserts that ingestion of arsenic by humans can occur not only through drinking water but also through the food-chain. Crops, receiving arsenic-contaminated irrigation-water take up this toxic element and accumulate it in different degrees depending on the species and varieties.

Human food production systems require rigorous protection against compounds with a potential for bioaccumulation; thus water as the key commodity for agriculture needs the same attention. In addition, the precautionary principle has to be applied in designing potential substitutes for such priority pollutants to make sure that today's solution will not become tomorrow's problem. Global agriculture faces the challenge to increase production yields and at the same time safeguard the environment and protect the food chain against contamination.

The results of experimental work to study the influence on the arsenic uptake by tomato irrigated with contaminated water, draw attention to the important and beneficial role of P fertilization in minimizing the toxic effects of arsenic. Phosphate addition has determined a limited translocation of the toxic element from roots to aboveground plant tissues. Levels of As found in tomato berries were not hazard in edible part of the plant, especially in plant fertilized with P. This point is of great interest for human health and in agricutural industry, where the P fertilization can be very important techniques for safeguard food quality and yield production. These findings could have important implications for human health and agricultural systems, since they suggest that it may limit the ingestion of As through the consumption of crops grown on contaminated soils and reduce yield losses.

Acquisition of knowledge about phosphate transporters and their regulation in plants will undoubtedly lead to a better understanding of the arsenate uptake mechanisms in plants. Specifically, it would be interesting to determine the relative selectivity of different transporters for phosphate and arsenate, and to examine allelic variation in this selectivity. Reduced uptake of arsenate is a well-known mechanism of arsenate resistance employed by many plant species, which

is achieved through a suppression of the high-affinity phosphate/ arsenate uptake system in the resistant plants (Meharg and Hartley-Whitaker, 2002).

Italian rice, analyzed here, present a clear variation in As total concentration in rice samples grown in different areas of Italy. On rice type basis it was observed that total concentration of Zn, Cr, Ni and K are strongly influenced by type. In particular, the total concentration of Zn is influenced by parboiling. This current study draws attention to potential risk to human health from trace elements and their species in rice grain.

The differences in As concentration in rice's grain are dependent from geogenic origin. The natural geochemical background is highly variable in Italy because of the complexity of its geological history and the wide variety of substratum rock types. For this reason, As concentration at regional scale is not homogeneous (Cubadda et al., 2010). The cultivation of rice throughout the Po River Valley is very intensive and so the potential loading of agrochemicals, such as fertilizers, trace elements and pesticides, is high. In the last years pesticides are the main environmental issues so many studies have been conducted to determine the processes of pesticide dissipation in rice paddies. Capri and Cavanna (1999), reported specific pesticides used in rice crops (propanil, molinate, bethiocarb, bensulfuronmethyl) as well as pesticides used in other rotational crops like maize and soya (alachlor, atrazine, metolachlor and bentazone). There is no evidence about use in this zone of As pesticide or As contaminated water. So we can only assert that the contamination cause was about geological origin.

The EFSA calculated total dietary exposure to total arsenic mean in Italy was between 2.1-2.4 μg kg^{-1} b.w. per day (EFSA, 2009a). In the European and South-East Asian diets rice contribute on average 0.8% and 24% of the PTWI, respectively (Jorhem et al., 2007). Those who are reliant on rice as a dietary staple are most at risk from enhanced Asi consumption. Prior study showed that the bioavailability from rice grain is high, particularly for the inorganic arsenic, in an order of 90% (Juhasz et al., 2006).

Inorganic As (arsenate and arsenite) has been classified by the International Agency for Research of Cancer (IARC) as a human carcinogen. Because of this, determination of As speciation in food is very important to realistically assess the risk rapresented by As in the diet. Our results indicated that special precautions should be taken to reduce the concentration of Asi, Cr and Cd in Italian commercial rice.

The results of this study on *Trichoderma*, indicate that the inoculation of food plants, such as lettuce, with *Trichoderma* spp. lay the foundation for future investigations on the potentiality of *Trichoderma* in growth of plants and in alleviating As toxicity. In particular we have obtained

important results from inoculation with T22 strain. The data of the study reveal the plant growth-promoting efficiency of four arsenic-tolerant and -remediating fungi along with their positive effects on soil nutritive properties. The stimulatory efficiency of tested fungal strains on plant growth and soil properties showed their potential to be utilized as bio-culture for better crop cultivation in arsenic-contaminated agricultural soils (Srivastava et al., 2012).

Various studies showed that the *Trichoderma spp*. possess great potential to tolerate and detoxify environmental contaminants from polluted sites; but further researches is necessary for understanding the long-term effects of *Trichoderma* on stabilization and remediation of the contaminated sites. A better understanding of these processes will result in the application of safer and less expensive methods to protect our environment, and increase crop yield in contaminated sites. Recent advances in modern techniques such as genomics, proteomics, and metabolomics may provide novel information about the complex interactions, in particular the ability of *Trichoderma* to sense the environment, the plant, and the microbial community and tolerate various organic and inorganic contaminants at high concentrations (Tripathi et al., 2012). The use of plant growth-promoting rhizofungus (PGPF) as a bioinoculant is a recent area of interest. Metal(loid)-tolerant and metal(loid)-remediating PGPF inoculants can play an important role in maintaining sustainable agricultural production in metal(loid)-contaminated soils. The bioavailability of arsenic in different food materials needs to be further assessed, and a screening of vegetables that contains exceptionally high amount of arsenic needs to be carried out. To solve the problem of As contamination it will require a variety of approaches from different fields of research.

Therefore if becomes of primary importance to perform detailed studies and development of strategies that minimize the water-soil-plant transfer of arsenic or restrict As contamination of edible plant parts, preventing root-to-shoot translocation. These strategies would be sufficient to become another route for increasing food safety.

A better understanding of these processes will result in the application of safer and less expensive methods to protect our environment, and increase crop yield in contaminated sites.

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