Arsenic removal from contaminated waters by Fe-based (hydr)oxides and its phytoavailability in soil-plant system

Ph. D. dissertation by
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To my beloved parents & my wife
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ABSTRACT

History of arsenic (As) in science has been overshadowed by its notoriety as a poison in homicides. Arsenic is considered as being synonymous with toxicity. Alarming As concentrations in natural waters is now a worldwide problem and referred to as the biggest natural calamity 21st century. With greater public concern of As poisoning in human health, there has been a growing interest in developing regulatory guidelines and remediation technologies for mitigating As-contaminated ecosystems. Urgent steps must be taken to reduce these impacts by providing access to safe water as a fundamental human right. Delaying mitigation is increasing death and disease.

Considerable technologies are available to reduce As concentrations in water but most of them are cost-prohibitive for small communities or less developed countries such as Bangladesh, Ghana, which are likely to face expensive and technically imposing challenges to run up against the maximum contaminant level of 10 μg As L\(^{-1}\). Several models show a wide range of costs for the different technologies, which can impede their implementation to As-contaminated sources. For these reason, it is particularly important to investigate and promote new As remediation technologies able to remove As from contaminated areas in an inexpensive and safe way. Hence, it is necessary to conduct researches on novel sorbents at low cost, easily synthesized, or even by-products coming from production processes, available for free or at very low price.

Layered double hydroxides (LDHs) show excellent capacity to sorb and exchange anions. Although As has a high affinity for sorbents containing Fe, only few studies have been carried out on the sorption of arsenite [As(III)] onto a Fe-based LDH. In the present work we evaluated the sorption of As(III) onto a LDH containing Mg and Fe (Fe–Mg-LDH) (easily reproducible anionic
clays at low cost) as affected by: (i) pH; (ii) the presence of increasing concentrations of organic [citrate (CIT) and oxalate (OX)] and inorganic [phosphate (PO$_4$), selenite (SeO$_3$) and sulphate (SO$_4$)] ligands, (iii) the effect of residence time on the desorption of As(III) by these ligands, and; (iv) the kinetics of desorption of As(III) by PO$_4$.

The As(III) sorption isotherms, carried out at different pHs, indicated a good affinity of the harmful oxyanion for the sorption sites of the Mg–Fe-LDH. This material was able to remove efficiently As(III) from contaminated solutions over a wide range of pH, but more in acidic than in alkaline systems. Competing ligands differently prevented the As(III) sorption on Mg–Fe-LDH, according to the following order: SO$_4$ < OX << SeO$_3$ < CIT < PO$_4$. The desorption of As(III) by these anions decreased with increasing As(III) residence time on LDH surfaces. A comparison between the sorption/desorption of As(III) and As(V) on/from Mg–Fe-LDH in the presence or absence of anions highlights that less As(III) than As(V) is sorbed, whereas more As(III) than As(V) is desorbed by all the selected organic and inorganic ligands, but PO$_4$.

In recent decades, Fe-(hydr)oxides (e.g., Ferrihydrite and Goethite) have attracted substantial attention for their potential use as As sorbents. A plethora of scientific works, however, demonstrates their effectiveness in removing arsenate [As(V)] ions from aquatic environments, while few studies have been done for assessing their ability in adsorbing As(III) ions from contaminated water, at varying pHs, in presence of competing anions.

The aim of the work was to study the As(III) removal from contaminated waters by Fe-based minerals, by using Ferrihydrite [non-crystalline Fe-(hydr)oxide)] and Goethite [crystalline Fe-(hydr)oxide] as sorbent media in batch experiments. In particular, it was studied: i) the
Langmuir isotherms of As(III) on Ferrihydrite and Goethite at pH 6.0; ii) arsenite sorption at different pHs; iii) arsenite sorption in presence of selected competing ligands, such as citrate (CIT), molybdate (MoO₄), oxalate (OX), phosphate (PO₄), selenite (SeO₃), selenate (SeO₄), and sulphate (SO₄).

Both Ferrihydrite and Goethite samples show a high affinity for As(III). However, the Ferrihydrite samples, characterized by a higher surface area (104.9 m² g⁻¹), were able to sorb much greater amounts of As(III) than the Goethite, with a lower surface area (42.3 m² g⁻¹). However, Ferrihydrite (Sₘ = 1245.1 mmol kg⁻¹) adsorbed much more As(III) than Goethite (Sₘ = 327.9 mmol kg⁻¹), essentially because of its greater surface area (178.2 vs. 42.3 m² g⁻¹) and, hence, lower degree of crystallinity, as well as to the greater affinity of As(III) for its surfaces. Probably, in our tests, Ferrihydrite adsorbed As(III) on their own external surfaces by forming a greater amounts of inner-sphere complexes vs. those formed by Goethite.

The sorption of As(III) was lightly pH dependent. In fact, in the pH range of 4.0-9.0 the quantities of As(III) adsorbed resulted to be practically constant. At higher pHs we observed a decrease in As(III) sorption by both the sorbents, probably because of the competition with the increasing amounts of hydroxyl ions and the repulsion between the negative charged As(III) ions (pKa = 9.2 and 12.1) and the negative charged surfaces of two Fe-(hydr)oxides.

The organic and inorganic ligands showed different capacities to compete with As(III) for the sorption sites of the Ferrihydrite and Goethite, according to the following increasing sequence: selenate < sulphate < oxalate < citrate < selenite ≈ molybdate < phosphate on Ferrihydrite, and sulphate ≈ selenate < oxalate < citrate < molybdate < selenite ≈ phosphate on Goethite.
Carrot (*Daucus carota* L.) is a very popular root vegetable and widely consumed by humans. However, the study about influence of organic amendment (i.e. humic acid) and inorganic fertilizers (i.e. N, P, K) on As uptake, translocation, and its toxicity in carrot is limited.

The presence of As in soils and/or groundwaters used for agricultural purposes, causes a strong abiotic stress to the cultivated plants, which elucidates itself through the reduction of biomasses and, specially, yields. It is therefore covetable to identify and develop production techniques capable of limiting the mobility and phyto-availability of As in soil, through the stabilization of the metalloid on the more recalcitrant soil fractions. In the present work it was carried out an experiment on an edible plant, carrot (*Daucus carota* L.), irrigated with different solutions containing As(III) (0, 3 and 6 mg L$^{-1}$) and grown in a As-uncontaminated soil amended with increasing amounts (half and full doses) of stabilized humic acid and fertilized (half and full doses) with inorganic fertilizer. The aims of this experiment were to study: i) the influence of the humic acid application and inorganic fertilizer on the mobility and phyto-availability of As in soil; ii) the influence of the humic acids and inorganic fertilizer on the growth of the carrot plants and their uptake of As from contaminated systems. Carrot was selected as a test plant in this study, because this crop is grown in several As-contaminated areas and suffers from As toxicity.

Carrot plants growth (taproots and shoots dry matter production) was significantly affected by As and soil amended with humic acid and fertilization with inorganic fertilizer treatments. Increasing As concentration in the irrigation water decreased markedly the dry biomass of carrot plants, as a consequence of the phytotoxic effect of As.
Carrot plants irrigated with water containing 3 mg As L\(^{-1}\) produced, on average, a biomass 22.1% lower than plants irrigated with As-uncontaminated water. Likewise, the biomass of the plants irrigated with water containing 6 mg As L\(^{-1}\) resulted to be, on average, 39.3% lower, compared to As control plants. Beside As treatment, the growth of carrot plants was also significantly affected by fertilization with humic acids or inorganic fertilizers. It is conceivable as all the fertilized plants grew much more than non-fertilized plants. In comparison with unfertilized As 6 plants, for example, As 6 carrots amended with full dose of humic acids (F.O.D.) produced 90.1% more of biomass, whereas those treated with full dose of inorganic fertilizers (F.I.D.) grew 97.2% more.

As a consequence of negative effects on plants, As causes a reduction of the photosynthesis rate and damages the chloroplasts membranes and their structure. In the present study we also noted that the chlorophyll A+B concentration progressively decreased, as long as increased As levels were supplied to carrot plants by the irrigation water. In comparison to the unfertilized control, significantly higher chlorophyll A+B concentration was found in the leaves of the carrots fertilized either with humic acids or inorganic fertilizers.

Most of the As taken up by our carrot plants was accumulated in their taproots, while a lesser allocation of toxicant occurring in the leaf biomass, in all experimental treatments. Indeed, plants amended with humic acids (H.O.D. and F.O.D.) exhibited a lower concentration of As in their own tissues, while the opposite was occurred in those fertilized with inorganic fertilizer. These findings are strictly related to the higher availability of As in the soil fertilized with inorganic fertilizers vs. that amended with humic acids. A low As allocation in carrot yield is definitely desirable, because a high
content of As in edible part of plant could cause contamination of the human food-chain, being carrots a low-cost carotenoids and dietary fibers, antioxidants source and one of the most consumed root vegetable around the world including Italy.

The concentration of the free-fraction of As in soil samples (non-specifically sorbed As) decreased significantly by increasing level of organic fertilizer (i.e. humic acid) application, whereas the higher the organic dose the higher was the concentration of specifically sorbed As by soil colloidal particles. Moreover, by increasing As concentration in irrigation solutions there was an increase of both concentrations of As non- and specifically sorbed in soil samples.

On the contrary, the fertilization of soil with inorganic fertilizers (H.I.D. and F.I.D.) determined a significant increase of the nonspecifically sorbed As fraction, with a consequent decrease of the specifically sorbed As, compared control.

The use of a classical soil amendant as humic acid in the present work, in addition to improve carrot plants growth and their nutritional status, has allowed to limit the As uptake by biomasses, through the immobilization of the metalloid, derived by irrigation water, on/in their humified organic macromolecules. Furthermore, the soil amendment by humic acids falls within the context of organic farming, eco-friendly production system, which ensures the sustainability of the soil, improving its fertility.
CHAPTER 1 - INTRODUCTION
1.1 Arsenic

Arsenic (As) is one of the most toxic elements found in nature. It ranks 20\textsuperscript{th} in natural abundance, comprising about 0.00005\% of the earth’s crust, 14\textsuperscript{th} in seawater, and 12\textsuperscript{th} in the human body (Mandal and Suzuki, 2002). It is a ubiquitous element, can pollute soil, water, and plants including other compartments of the ecosystem and the environment, and ultimately affects human health and well being. The sources of As in the environment are mainly geogenic, i.e. naturally occurring (Smedley and Kinniburgh, 2002; Nriagu et al., 2007). The king of poisons (i.e. As), has probably influenced human history more than any other element or toxic compound as a class one carcinogen (Zhao et al., 2010; Duan et al., 2012). For the thousands of years that people have worked and lived with this enigmatic element, tens of thousands have died of it and millions have been sickened by it (Nriagu et al., 2007).

In neutral oxygenated waters, As(V) is the thermodynamically favored form, whereas As(III) is stable under reducing conditions (Frankenberger Jr., 2002; Smedley and Kinniburgh, 2002; Mahimairaja et al., 2005). Arsenite is 25-60 times more toxic and mobile than As(V); however, As(III) mainly arises from its state as $\text{H}_3\text{AsO}_3$ at pH $< 9.0$, whereas As(V) is present in the charged species ($\text{H}_2\text{AsO}_4^-$ and $\text{HAsO}_4^{2-}$) which predominate in a wide pH range (Frankenberger Jr., 2002; Smedley and Kinniburgh, 2002).

In general, the inorganic As (such as trivalent arsenite (As(III)) and pentavalent arsenate (As(V)) are more prevalent and toxic than the organic As in terrestrial environments. Arsenite has an affinity for sulfhydryl groups existing in cysteine residues to exert detrimental effects on general protein metabolism with high toxicity (Rai et al., 2011). Arsenite stimulated
The generation of reactive oxygen species (ROS) was proven to damage proteins, lipids, and DNA (Ali et al., 2012). Arsenite is more prevalent in groundwater than previously believed. Indeed, reducing conditions in alluvial aquifers supplying single families may result in significant exposures to naturally occurring As(III). Moreover, the mechanism promoting the mobility of As(III) in groundwater is the onset of reducing conditions in alluvium in which iron oxides have sorbed As. Such conditions may result in concentrations of As in groundwater as high as several hundred micrograms per liter (Korte and Fernando, 1991).

In addition, the degree of toxicity of As compounds is inversely proportional to the rate of excretion from the body and can be shown as follows: arsine > As(III) > As(V) > methylated arsenicals (Puttemans and Massart, 1982).

The acid dissociation constants of the four As species demonstrate why ion exchange chromatography is an effective separation method (Table 1).

**Table 1** Approximate values for the pKa's of the four arsenic species

<table>
<thead>
<tr>
<th>Arsenic species</th>
<th>pK(_1)</th>
<th>pK(_2)</th>
<th>pK(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(III): Arsenious acid</td>
<td>9.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>As(V): Arsenic acid</td>
<td>2.2</td>
<td>6.9</td>
<td>11.5</td>
</tr>
<tr>
<td>(MMA): Monomethylarsonic acid</td>
<td>4.1</td>
<td>8.7</td>
<td>—</td>
</tr>
<tr>
<td>(DMA): Dimethylarsinic acid</td>
<td>1.6</td>
<td>6.3</td>
<td>—</td>
</tr>
</tbody>
</table>

Arsenic contamination of surface- and ground-waters occurs worldwide and has become a socio-political issue in several parts of the globe. For example, millions of people are at risk from drinking As-contaminated water in West Bengal (India) (Nriagu et al., 2007) and Bangladesh (Chakraborti et
al., 2010). Significant number of people from China (Rahman et al., 2009), Vietnam (Smedley and Kinniburgh, 2002), Taiwan (Tseng et al., 2005), Chile (Bundschuh et al., 2012), Argentina (Barringer and Reilly, 2013), and Mexico (Meza et al., 2004) are likely at risk as well.

Hence, there are much attention in developing guidelines and remediation technologies for mitigating As-contaminated ecosystems because of the greater public awareness of As poisoning on human health. A good number of technologies, including chemical immobilization has been applied with varying levels of success either to completely remove As from the system or to reduce its biotoxicity. Layered double hydroxide (LDH), Ferrihydrite and Goethite may offer a low-cost and ecologically viable means for the mitigation of As toxicity in the environment.

1.1.1 Origin and sources of arsenic contamination
Arsenic compounds (both organic and inorganic) are ubiquitous in rocks, soil sediments and various raw materials such as metal ores and concentrates, coal, peat, oil, chemical and industrial products, and its flow in different compartments of the ecosystem (Figure 1). Arsenic contamination occurs by anthropogenic activities (54 countries/territories), coal activities (geogenic coal excluding coal burning) (28 countries/territories), geogenic (68 countries/territories), mining activities (74 countries/territories), petroleum activities (17 countries/territories), volcanogenic activities (35 countries/territories) (Murcott, 2012).
INTRODUCTION

Figure 1 Arsenic distribution from environment to human body and its toxic effect. Arsenic releases into the environment by natural processes and anthropogenic activities such as weathering of rocks and sediments, hydrothermal ore deposits, volcanic eruptions, geothermal activities, wind-blown dust, and mining. Peoples use As-contaminated water for their daily purposes (drinking, cooking, etc). People also use As contaminated water for irrigation and livestock resulting contamination in the food chain. As a result, arsenic easily enters in to the human body through consumption of crops as well as animals products, and may cause of cancer, dermal diseases, cardiovascular diseases, respiratory diseases, neurological diseases, hematological diseases, renal diseases, and hepatic diseases.
1.1.1.1 Geological sources

At varying concentrations, As is widely distributed in all geological materials. An average concentration of 1.5 to 2.0 mg As/kg is expected in the continental crust of the earth. The mean concentrations of As in igneous rocks ranged from 1.5 to 3.0 mg/kg, whereas in sedimentary rocks range from 1.7 to 400 mg/kg (Smedley and Kinniburgh, 2002). Arsenic is a major constituent in more than 245 minerals (Mandal and Suzuki, 2002). Realgar (As$_4$S$_4$) and orpiment (As$_2$S$_3$) are the two common As sulfides where As occurs in reduced form while As occurs in oxidized form in the mineral arsenolite (As$_2$O$_3$), loellingite (FeAs$_2$), safforlile (CoAs), niccolite (NiAs), rammelsbergite (NiAs$_2$), arsenopyrite (FeAsS), cobaltite (CoAsS), enargite (Cu$_3$AsS$_4$), gerdsorfite (NiAsS), glaucodot [(Co,Fe)AsS], and elemental As are other naturally occurring As-bearing minerals (Nriagu et al., 2007). In neutral oxygenated waters, As(V) is the thermodynamically favored form, whereas As(III) is stable under reducing conditions (Mahimairaja et al., 2005).

Geogenic contamination of As in soils and water has been reported in many parts of the world. One typical example is the extensive As contamination of groundwaters in Bangladesh and West Bengal, India. Relatively high concentrations of naturally occurring As can appear in some areas as a result of inputs from geothermal sources or As-rich groundwaters (Sharma et al., 2014). Arsenic mobilization can depend on many other geochemical conditions. A special feature is the large differences in rainfall between seasons and extensive water withdrawal resulting in large groundwater table fluctuations and thus an increased ventilation of deeper sediments for the Bengal Delta. The penetration of oxygen into the sediments in the dry season resulting in oxidation of As bearing pyrites like Pyrite (FeS)
and Arsenopyrite (FeAsS) is leading to As mobilization (Navas-Acien et al., 2008).

In the atmosphere, natural emission of As include windblown dust from weathered continental crust, forest fires, vegetation emissions, volcanoes, sea spray, hot springs, and geysers (Nordstrom, 2002). Volcanoes are also considered as a geogenic source of As to the environment (Centeno et al., 2007). In natural lakes, levels of As range from 0.2 to 56 µg/l (Crecelius, 1975). In deep sea water, its average concentration is reported to be 1.7 mg/l at a salinity of 3.6% (Bruland, 1983). High levels of As are noted in both dissolved and particulate phases in rivers influenced by contamination from anthropogenic sources in Europe and North America (Seyler and Martin, 1991). It has been reported that the concentrations varied between 1 and 8 µg/l in seawater (Mandal and Suzuki, 2002).

Weathering and leaching of geological formations and mine wastes result in elevated concentrations of As in natural waters in several areas. Mobility of As is constrained in the surface water because of the prevalence of oxic conditions. On the other hand, reducing conditions offered by the aquifers lead to the mobilization of As, thereabout increasing the risk of groundwater contamination. 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., 2004; Nguyen and Itoi, 2009).

1.1.1.2 Anthropogenic sources

Arsenic introduces into the environment via various anthropogenic activities. These sources release As compounds that differ greatly in chemical nature
(speciation) and bio-availability. Mining, smelting, and ore beneficiation, pesticides, fertilizers and chemical industries, thermal power plants using coal, wood preservation industries using CCA, and incinerations of preserved wood wastes contribute to significant influx of As to the environment (Bhattacharya et al., 2002). The flue gases and particulate from smelters can contaminate nearby ecosystems downwind from the operation with a range of toxic metal(loid)s, including As (Adriano, 2001). Mining and smelting activities lead to high concentrations of As in sulphide mineral deposits (Thornton, 1996).

Commercial wastes, coal ash, mining industry and the atmospheric fallout from the steel industry are the leading sources of As discharged onto land (Eisler, 2004). Arsenic trioxide (As$_2$O$_3$) is used extensively in the manufacturing of ceramic and glass, electronics, pigments and antifouling agents, cosmetics, fireworks, and Cu based alloys (Winder, 2004). Arsenic is also used for wood preservation in conjunction with copper (Cu) and chromium (Cr), i.e., copper-chromium-arsenate (CCA). In addition, As releases during their mining and smelting processes because As is widely distributed in the sulfide ores of Pb, Zn, Au, and Cu. Elevated concentrations of As, as well as other metals such as Cd, Cu, Fe, Pb, Ni and Zn, are commonly encountered in the acid mine effluents (Bundschuh et al., 2012). In studies of acid mine drainage, adsorption of As on Fe(OH)$_3$ surfaces was found to be the principal sink for As (Webster et al., 1994). Furthermore, agricultural inputs such as fertilizers, pesticides, and desiccants are the major sources of As in soils. There are low levels of As in N and K fertilizers as well as lime. Therefore, the possible As accumulation in soils due to repeated application of As containing fertilizers raise the levels (Jiang and Singh, 1994).
Inorganic arsenicals (e.g. arsenic trioxide, arsenic acid etc.) have been widely used in pigments, pesticides, insecticides, herbicides, and fungicides over hundreds of years (Chisholm, 1972). At present, As is no longer used in agriculture in the developed countries, but persistence of the residues of the inorganic arsenicals in soils is an issue of environmental concern (Smith and Brauning, 1995). The use of pesticides, lead arsenate (PbAsO₄), calcium arsenate (CaAsO₄), magnesium arsenate (MgAsO₄), zinc arsenate (ZnAsO₄), zinc arsenite [Zn(AsO₂)₂], and Paris Green [Cu(CH₃COO)₂ ∙ 3Cu(AsO₂)₂] in orchards has contributed to soil As contamination in several parts of the world (Peryea and Creger, 1994). The use of sodium arsenite (NaAsO₂) 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 (McBride et al., 1998).

Likewise, timber treatment effluent is considered to be the major source of As contamination in aquatic and terrestrial environments in New Zealand (Bolan et al., 2004). Continuous application of fertilizers containing trace levels of As also results in As contamination of soil, thereby reaching the food chain through plant uptake (Zhao et al., 2010). The use of chromated copper arsenate (CCA) and other As-based chemicals in wood preservation industries has caused widespread contamination of soils and aquatic environments (Bhattacharya et al., 1996). CCA had attained wide-scale industrial application as a wood preservative owing to biocidic characteristics of Cu(II) and AsO₄. The preservative chemical used for pressure impregnation comprises a water based mixture of dichromic acid (H₂Cr₂O₇), arsenic acid (H₃AsO₄), and Cu(II) as divalent cation at variable proportions (Jacks and Bhattacharya, 1998). 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 (Ryberg and Haugen, 1990). 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.

Sediments in a drain adjacent to the cemented impregnation platform contained an average 632 mg As/kg. Arsenic concentrations in the reference soils (119 mg/kg) were lower than in the contaminated area, but exceeded the level of As in average glacial till (Bhattacharya et al., 2002). Analyses of water in a stream found As concentration of 238 μg/l (Bhattacharya et al., 1995). Groundwater contamination must be considered as an imminent risk close to wood preservation sites, and especially at older sites where precautions against spills and material handling were not taken adequately.

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). High-As-bearing coals combustion is known to be a principal pathway of As emission in the Guizhou province, China (Zheng et al., 1996). In addition, open coal-burning stoves used for drying chili peppers have been the principal cause of chronic As poisoning in a population of nearly 3,000. Fresh chili peppers have less than 1 mg As/kg, while chili peppers dried over high-As coal fires were reported to contain more than 500 mg As/kg (Zheng et al., 1996). Consumption of other tainted foods, ingestion
of kitchen dust containing as high as 3,000 mg As /kg, and inhalation of indoor air polluted by As from coal combustion are the other causes of chronic As poisoning.

The incidental use of preserved wood in open fires, indoors or outdoors is a possible pathway for exposure through air particulates. CCA impregnated wood burning from a sawmill was found to be a source of As contamination to the environment (Aggett and Aspell, 1980). The content of As in air particulates from open fires was found to exceed the German air quality standards by 100-fold (Bringezu, 1990). The ashes, spread on lawns or vegetable cultivations, pose further risk to human health. 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, 1988). Tannery industries are also route for exposure of As through foods. As concentration in vegetables grown in and around the Hazaribag tannery industrial region, Dhaka, Bangladesh was higher than the safe limit (Islam et al., 2014).

1.1.1.3 Biogenic redistribution

Biological sources contribute small amounts of As into soil and water ecosystems. However, plants and microorganisms affect the redistribution of As through their bioaccumulation (e.g., biosorption), biotransformation (e.g., biomethylation) and transfer (e.g., volatilization) (Azizur Rahman et al., 2012). Arsenic accumulates readily in living tissues because of its strong affinity for proteins, lipids and other cellular components (Ferguson and Gavis, 1972; Ali et al., 2012).

Aquatic organisms are particularly known to accumulate As, resulting in considerably higher concentrations than in the water in which they live (i.e.,
biomagnification). They subsequently become a source of environmental contamination upon their disposal or consumption. Arsenic could be transferred from soil to plants and later to animals and humans, involving terrestrial and aquatic food chains. For example, one of the major sources of As input to soils is poultry manure addition. In United States, 20-50 mg of As is introduced to the environment through the use of As compounds (e.g., Roxarsone, ROX) in poultry feed (Christen, 2001). However, in many situations the soil-plant transfer of As is low (Burlo et al., 1999; Bhatti et al., 2013) and it is important to recognize that metal(loid)s loading through manure application may overestimate their actual net accumulation in soil, as a substantial portion of the metal(loid)s in manure originate in crop uptake and are therefore being recycled within a production system (Bolan et al., 2004).

### 1.1.2 Arsenic contamination in the world

A good number of large aquifers in various parts of the world have been identified with problems from As occurring at concentrations above 50 µg/l (Smedley and Kinniburgh, 2002). Arsenic pollution of drinking water supplies has been reported from over 105 countries, posing a serious health hazard to an estimated 226 million people worldwide (Murcott, 2012). Major As pollution has been reported in the USA, China, Chile, Bangladesh, Vietnam, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, New Zealand, Japan and India (Nickson et al., 2000; Chakraborti et al., 2002; Hossain, 2006; Mondal et al., 2007; Nriagu et al., 2007). In Bangladesh, it has been estimated that 35–77 million people are exposed to As-contaminated well water (Rabbani et al., 2002; Chakraborti et al., 2004). The recent discovery of As enrichment on a large scale in Bangladesh has highlighted the need for a rapid
In Asian countries, the highest number of people consuming and using As-contaminated drinking water and groundwater for crops irrigation purposes suffer from arsenicosis (Chakraborti and Das, 1997). The range of As concentrations found in natural waters is large, ranging from less than 0.5 µg/l to more than 5000 µg/l. The As content in groundwaters as reported (Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002; Liu et al., 2003; Nriagu et al., 2007) is wide in range (West Bengal, India <10–3200 µg/l, Thailand 1–>5000 µg/l, Cambodia 1–1610 µg/l and Bangladesh <0.5–2500 µg/l, Taiwan 34–558 µg/l and Vietnam 3050 µg/l in rural groundwater samples. In 17 districts (out of 64) of Bangladesh, its concentration is as high as 1000 µg/l (Harvey et al., 2002; Hossain, 2006). Surface water is also found to be contaminated with As by the anthropogenic sources to various degrees. In an early experiment, NaCl-dominated brine of Tisakürt, Hungary, contains more than 5800 µg/l of As (White et al., 1963). As-contaminated groundwater is a severe problem in West Bengal, India (Bhattacharya et al., 2011). In Italy, the higher As concentrations were found in groundwaters of some Italian regions including Emilia, Lombardia (Zavatti et al., 1995), Campania (Dall’Aglio, 1996), Po river delta (Di Giuseppe et al., 2014) and Viterbo area (Sappa et al., 2014). In Latin America, many regions are widely (Mexico 50–24000 µg/l, Argentina 10–5000 µg/l, Chile 30-5100 µg/l, Peru 200-1680 µg/l) reported for the occurrence of high As in groundwater and surface water due to a combination of geological processes and/or anthropogenic activities (Bundschuh et al., 2012).

It is well established that As-contaminated groundwater used for irrigation can pose an equally serious health hazard to people eating food from
the crops irrigated (Williams et al., 2006) and that As accumulating in irrigated soils poses a serious threat to sustainable agriculture in affected areas (Heikens, 2006; Brammer and Ravenscroft, 2009). The major cereals such as rice (Rahman and Hasegawa, 2011) wheat (Pigna et al., 2009; Pigna et al., 2010) maize (Rosas-Castor et al., 2014) vegetables (Roychowdhury et al., 2002; Alam et al., 2003) accumulate As more than the hygienic limit. Straw can accumulate high level of As (Abedin et al., 2002). In a later case, it comes into human body through food chain (Rahman et al., 2008).

1.1.3 Bangladesh, a country of huge As contamination

Considering the affected population, As problems in groundwater of Bangladesh represent the most serious occurrences identified globally. A survey looked at the As concentrations of drinking water from deep wells in 64 districts in the country and found that 59 had concentrations >10 µg/l and 43 had concentrations >50 µg/l (Hossain, 2006). The (BGS, 1999) estimated that about 21 million people in Bangladesh are exposed to As concentrations >0.05 mg/l. Numerous scientific papers (Karim, 2000; Nickson et al., 2000; Harvey et al., 2002; Horneman et al., 2004; Zheng et al., 2004; Ahmed et al., 2006; Hossain, 2006) and some organizations (Dhaka Community Hospital (DCH), 1997; British Geological Survey (BGS), 2000; DPHE/BGS/DFID (Department for International Development), 2000) have implemented different As programs and revealed the extent and severity of the problem. To date, different issues such as population exposed to contaminations, assessment and modeling of As transport, As mobility and groundwater extraction, cause of contamination and number of people suffering from arsenicosis have been addressed. Groundwater As contamination is a severe
problem in Bangladesh. About 60% of groundwater sources of this country are affected by As contamination (Joarder et al., 2002).

Bangladesh depends heavily on groundwater for drinking water supply and irrigation purposes. Groundwater development has been actively encouraged in this region over the last 3 decades by government and other agencies as a means of providing an alternative to contaminated surface water and thereby reducing the incidence of water-borne diseases. Almost all the village people use the tubewells.

As a consequence of As-contaminated water and food over prolonged periods resulting chronic As diseases. The As calamity of Bangladesh can be described as the largest known mass poisoning in the history considering the number of population (Rabbani et al., 2002).

In a review article, it was mentioned that (1) is the problem becoming worse and if so, will it continue to worsen? Has the increased use of groundwater for irrigation purposes caused As to be mobilized and enter the groundwater? (3) How acute is the risk that As in irrigation water will enter the food chain (Hossain, 2006)? Later, it was shown in another review that the problem is being worst and it caused As to be mobilized and enter the groundwater by increasing use of groundwater for irrigation purposes (Brammer and Ravenscroft, 2009). In addition, the risk that As in irrigation water is acute in cereals such as rice (Norton et al., 2009) barley (Dago et al., 2014) and other vegetables (Alam et al., 2002; Alam et al., 2003).

There are consolidated evidences on the rice (staple food) and As of Bangladesh have been made such as genotype and environment effects (Ahmed et al., 2011), cooked rice (Bae et al., 2002), temporal variations (the role of iron plaque) and spatial distribution (Dittmar et al., 2007; Garnier et al., 2010), fate of irrigation-water As and bioavailability (Khan et al., 2009; Khan
et al., 2010), wetland condition (Khan et al., 2010), As transport across rice-field soils (Polizzotto et al., 2013), toxicity (photosynthesis, growth and yield) (Azizur Rahman et al., 2007; Panaullah et al., 2009), daily dietary intake (Kile et al., 2007; Rahman et al., 2013). Varietal differences, speciation of As in rice and the levels and impact of As in straw on animal health also need to be considered (Duxbury et al., 2003). Rice consumption may therefore represent a significant risk, mainly when As is present in its inorganic forms, As(III) and As(V) (ATSDR, 2007; Meharg et al., 2009). Rice grain had As levels of 0.03–1.84 mg/kg dry weight (dw) (Meharg and Rahman, 2003) 0.05–2.05 mg/kg dw (Das et al., 2004) collected from groundwater As contaminated area of Bangladesh. In this manner, rice could be considered an important contributor to total As intake in Bangladesh.

No significant research was carried out on the market basket survey to know the As accumulation in food except (Anawar et al., 2012; Saha and Zaman, 2013). They found that all the food items collected from local market accumulate considerable amount of As. Little amount of As accumulation in foods is a matter of concern because almost all the people drink As contaminated water (more than the permissible limit by WHO).

1.1.4 Permissible limit for food and drinking water
In 1903, the first limit on As concentration in water was established at 108 µg/l by the Royal Commission of London (Stöhrer, 1991). International guidelines have been established for As in drinking water since the 1950s and the historical developments in the guideline values for As in drinking water and reflects the increasing awareness to protect the populations (Table 2) (WHO, 2005). The maximum contaminant level of As in drinking water had also been reduced from 50 to 10 µg/l by European Commission (ECD, 1998).
Environmental authorities have taken a more stringent attitude towards the presence of As in water. World Health Organization (WHO) and National Health and Medical Research Committee, Australia, recommended maximum contaminant level of As in drinking water as 10 and 7 µg/l respectively because of the negative impact of As on human health (WHO, 1993; NHMRC, 1996). Japan and Canada reduced the MCL for As in drinking water to 10 and 25 µg/l, respectively. The MCL for As in countries like India, Bangladesh, Taiwan, China, Vietnam, Chile etc. is 50 µg/l where the groundwater As contamination is high (Nordstrom, 2002). The USEPA changed the maximum contaminant level (MCL) for arsenic from 50 µg/l in 1974 to 10 µg/l in 2001 due to its long-term health effect (Melitas et al., 2002). World Health Organization (WHO) As guideline value 50 µg/l to 10 µg/l resulted in a several times increase of the number of population exposed to As. In Honduras (1995), Costa Rica (1997), El Salvador (1997), Colombia (1998), Guatemala (1998), Nicaragua (1999), Panama (1999), Bolivia (2004), Brazil (2005), Argentina (2007), and Chile (2008) made their law following the provisional value of WHO for drinking water. The permissible limit of Uruguay, Peru, and Venezuela are 50 µg/l; Mexico reduced the limit of 25 µg/l in 2005. In Argentina, the national limit is not applied to all provinces but they regulate the permissible As concentration by their own rules and regulations (Bundschuh et al., 2012).

Only a few countries have established national legislative limits for inorganic As including China (CFSA, 2005), Australia and New Zealand (ANZFA, 2011) and India (Awashthi, 1999). The Chinese permissible limits are in rice 0.15 mg/kg, flour 0.10 mg/kg, other cereals 0.20 mg/kg, vegetables 0.05 mg/kg, fruit 0.05 mg/kg, poultry 0.05 mg/kg, egg 0.05 mg/kg, milk powder 0.25 mg/kg, fresh milk 0.05 mg/kg, beans/pulses 0.10 mg/kg, fish 0.10
mg/kg, algae 1.50 mg/kg, shellfish 0.50 mg/kg) (CFSA, 2005) and Australia and New Zealand fixed 1–2 mg/kg for seafood products (ANZFA, 2011). Indian standard for As in foods 1.1 mg/kg (Awashthi, 1999). The Joint FAO/WHO Expert Committee on Food Additives recommends a provisional tolerable weekly intake (PTWI) of inorganic As of 15 μg/kg body weight, equivalent to 130 μg/day for a 60-kg person (WHO, 1989). In China, daily iAs intake by the human population is around 42 μg/day, and rice is the largest contributor of total iAs intake accounting for about 60% (Li et al., 2011). In countries, such as Switzerland, the tolerable limit of As concentration in fodders is 4 mg/kg; shows that this value is not exceeded by the As concentrations found in the leaves and stems (Gulz et al., 2005). The maximum tolerable daily intake of As (III) is 2 μg/kg body mass (WHO, 1993). Food and Agricultural Organization (FAO) permissible limit for irrigation water is 0.10 mg/l (Bhattacharya et al., 2010). Plants application for irrigation of vegetables, fruits, forages, etc. and plants consumption (raw or cooked) are considered to make the provisional limit in some region of the world. Furthermore, the limit of As in water for animal husbandry is regulated individually in some Latin American countries; the national limits vary between 10 μg/l (Chile) and 500 μg/l (Argentina). There is no national regulations for irrigation water and water for animal husbandry in Nicaragua, El Salvador and Guatemala (Bundschuh et al., 2012). Urgent action must be taken to settle up the hygienic level of As for food, drinking water for human and livestock, soil and irrigation water taking into account the As-contamination.
Table 2: Developments in the WHO’s guideline value for maximum As concentration in drinking water (WHO, 2005).

<table>
<thead>
<tr>
<th>Year</th>
<th>Guideline (µg/l)</th>
<th>Reasons for the change in the guideline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>200</td>
<td>Noted possible risk for adverse health effects</td>
</tr>
<tr>
<td>1963</td>
<td>50</td>
<td>No reason was mentioned</td>
</tr>
<tr>
<td>1971</td>
<td>50</td>
<td>It is wise to have a guideline value as low as possible</td>
</tr>
<tr>
<td>1984</td>
<td>50</td>
<td>No health effects were observed at this level yet</td>
</tr>
<tr>
<td>1993</td>
<td>10</td>
<td>Realized risk of excess skin cancer, and practical limit to chemical analysis at that time</td>
</tr>
<tr>
<td>2003</td>
<td>10</td>
<td>Uncertain risk assessment of As carcinogenicity. Prohibitive mitigation costs at lower concentrations</td>
</tr>
</tbody>
</table>

Adopted from (Sharma et al., 2014).

1.1.5 Arsenic toxicity towards plants

It is reported that long term use of As contaminated water for irrigation results elevated As levels in agricultural soils, and thereby in food crops (Williams et al., 2005; Dahal et al., 2008). Additionally, the elevated level of As is reported to inhibit seed germination and seedling establishment (Abedin and Meharg, 2002), and inhibits photosynthesis which eventually affect the growth and often leads to death (Rahman et al., 2007). The bioavailability of As in crop plants depends on several physical and chemical factors including pH, redox potential, organic matter content, texture and clay mineralogy, iron, aluminum and manganese content, presence of inorganic (particularly phosphate) and organic (humic and fulvic acids and roots exudates) ligands, and microbial activity (Violante et al., 2005). Organic matter may form complexes with trace elements and control the mobility and availability of As
in soils. However, in acidic or slightly acidic conditions, organic matter may compete with As for the same adsorption sites and form surface complexes with iron hydroxides, resulting in the release of As to soil solution (Wang and Mulligan, 2006).

Arsenic is non-essential element and generally toxic to plants (Zhao et al., 2009). There is no single level of As in water and soil that can be toxic to plants. Plant species tolerate varying amounts of As in soils and water. Likewise, it does vary in different cultivars (Brammer and Ravenscroft, 2009). Although As is extremely toxic for plant tissues, these can accumulate a small amount of the inorganic forms, such as the As(III) through aquaporin channels and the arsenate (AsV) through the phosphate transporter system (Zhao et al., 2009). Arsenic form has been reported as the authentic factor determining the phytotoxicity in several plants (Marin et al., 1992; Carbonell et al., 1998). Arsenite possesses a high affinity for the sulfydryl groups of amino acids such as cysteine and thereby inactivates a wide range of enzymes in intermediate metabolism (Fendorf et al., 1997). However, the study about influence of organic amendment and inorganic fertilizers on As accumulation, translocation, and its toxicity in plants is limited (Cao and Ma, 2004). Furthermore, only partial attention has been paid to the risks of using contaminated ground water for irrigation.

Various symptoms of As toxicity in different edible plants have been reported. Roots are usually the first tissue to be exposed to As, where the metalloid inhibits root extension and proliferation (Garg and Singla, 2011). The presence of As in irrigation water or in soil at an elevated level could hamper normal growth of plants with toxicity symptoms such as biomass reduction (Carbonell-Barrachina et al., 1997), decrease of plant height (Ahmed et al., 2006), morphological changes (Srivastava et al., 2009), often
leading to death (Jiang and Singh, 1994) affects the photosynthesis (Azizur Rahman et al., 2007), delayed seedling emergence, yellowing and wilting of leaves, brown necrotic spots on older (Huq et al., 2006) and yield losses (Cozzolino et al., 2010).

Increasing As level in the irrigation water decreased markedly the dry matter production, as a consequence of the phytotoxic effect of As. Turnip plant growth (as represented by roots and shoots dry matter production) was significantly affected by As treatments and As chemical form was more important than the As level in solution in determining the phytotoxic effect of As on this turnip cultivar (Burlo et al., 1999). The phytotoxic effect of As determined a strong inhibition of root growth and, to a lesser extent, leaf development. For instance, lettuce plants irrigated with water containing 10 mg/l of As produced less than a third of the root biomass of the control (Caporale et al., 2014).

Straighthead disease reduced grain yield in rice (BRRI 29) irrigated with As, and with the increase in soil As concentration, the severity of straighthead increased significantly (Smith et al., 2010). In sorghum, shoot and root dry matter yield were repressed by higher As levels (Shaibur et al., 2008). In rice, yield decline was regressively from 7–9 to 2–3 t/ha with increasing soil-As concentration (Panaullah et al., 2009). Numerous physiological processes are susceptible to As toxicity. Cellular membranes become damaged in plants exposed to As, causing electrolyte leakage (Singh et al., 2006). Low As burden causes the number of nitrogen-fixing root nodules to be repressed in soybean (Vázquez et al., 2008).

The As tolerance has been identified in a number of plant species (Sharples et al., 2000). The As tolerance in grasses results from suppression of a high-affinity P/As uptake system (Meharg and Macnair, 1992). There are
relatively few species of plants that are naturally As tolerant. Among these are
a group of plants including *Pteris vittata* and other members of the Pteridaceae
that hyperaccumulate As (Ma et al., 2001; Zhao et al., 2009). Even some
medicinal plants such as *Mimosa pudica* L., *Panax notoginseng* (Burkill)
Chen ex Wu & Feng can tolerate high level (5200 mg/kg) of As. Rice plants
can uptake higher As while grown in As-treated soils; plants died at
concentration >1500 mgAs/kg (Nriagu et al., 2007).

Exposure to As(V) causes considerable stress in plants, including
inhibition of growth (Stoeva and Bineva, 2003), physiological disorders
(Stoeva et al., 2005) and finally death. As(III) reacts with sulfhydryl groups (–
SH) of enzymes and tissue proteins, inhibiting cellular function, causing death
(Smith et al., 2010). However, As(V) will not normally have high enough
cytoplasmic concentrations to exert toxicity because As(V) is rapidly reduced
to As(III) in plant tissue (Verbruggen et al., 2009). The majority of the As
remains in roots as an As(III)-tris glutathione. The thiolate donors are
probably either glutathione or phytochelatins. Endogenous plant enzyme
arsenate reductase (ACR) activity converts As(V) to As(III) in roots,
immobilizing As below ground (Verbruggen et al., 2009).

In a significant manner, maize seedlings were reduced in plant fresh
weight with the assayed concentrations of As(III) and/or As(V) (Requejo and
Tena, 2005). The binding of As(III) to the thiol groups of proteins or enzyme
coo-factors may alter or inhibit their activity, also exposing cellular processes
to risk. Oxidative stress brought about by the inevitable production of reactive
oxygen species (ROS) that occurs during As exposure has most recently
gained favor as the main driver of As toxicity in plants. However, which
cellular processes are most sensitive to As toxicity and which As species pose
the greatest threats to plant cell health remain unclear (Finnegan and Chen, 2012).

Arsenite was found to be more toxic than As (V) for rice seed germination (Abedin and Meharg, 2002). Furthermore, it is estimated that the As content of rice is over 10 times greater than that found in other cereals (Duxbury and Panaullah, 2007). The As concentrations in different plant tissues followed the order: root > straw > leaf > husk > grain (Liu et al., 2006). Arsenate (AsO$_4^{3-}$) is an analog of phosphate (P) and competes with the same uptake carriers in the root plasmalemma of plants (Meharg and Macnair, 1992). The negative effects of As on growth and productivity were promoted in the presence of P fertilization (Pigna et al. 2008). Arsenite may decrease all the endpoints more remarkably than arsenate (Liu et al., 2005). In contrast, plants with added P, may a little or moderate decrease in grain yield. In winter wheat, As slightly reduced both root and shoot biomass. P supply slightly reduced root dry weights, but markedly increased shoot dry weights (Geng et al., 2006).

Indeed, the capacity of keeping As(III) away from vital metabolic targets during the translocation and sequestration process in As-hyperaccumulators, the exact nature As toxicity, the combination of mechanisms for toxicity such as inorganic phosphorus replacement, sulfhydryl binding, or ROS production, most vulnerable plants parts metabolism, the most critical molecular targets and protection via breeding or direct engineering, growth stimulation at low concentrations and sustainability, the possibility of As free edible part still remain unknown (Finnegan and Chen, 2012).

1.1.6 Arsenic toxicity to human health
Arsenic is a food chain contaminant and class I carcinogen (IARC, 2012). Foods are the main sources of As intake for humans who are not exposed to elevated As in drinking water. As a result of As-contamination in food and drinking water, it easily enters into the human body. The epidemiological studies showed that the chronic arsenic poisoning can cause serious health effects including cancer, dermal diseases, cardiovascular diseases, respiratory diseases, neurological diseases, hematological diseases, renal diseases, and hepatic diseases (Figure 1) (Jain and Ali, 2000; Liao et al., 2005; Huang et al., 2006; Li et al., 2012). Dietary intake of total As ranges from 10 to 200 μg per person per day in various countries (WHO, 1989; Schoof et al., 1999). Food consumption may represent a significant risk cooked with highly As contaminated water. There is an evidence of As intake through cooked rice based on the As bioaccessibility. It highlights that a few grams of cooked rice (less than 25 g dry weight per day) cooked with highly As contaminated water is equivalent to the amount of As from 2 liter water containing the maximum permissible limit (10 μg As/l) (Signes-Pastor et al., 2012).

Straw is an agricultural by-product, the dry stalks of cereal plants such as rice, wheat, maize, barley, sorghum. Arsenic accumulation in rice straw at very high levels indicates that feeding cattle with such contaminated straw could be a direct threat for their health and also indirectly to human health through presumably contaminated bovine meat, milk and milk products (Abedin et al., 2002).

Many researchers attempted to investigate the various pathways of human exposure to As (Figure 1) (Bae et al., 2002; Roychowdhury et al., 2002; Alam et al., 2003; Duxbury et al., 2003; Meharg and Rahman, 2003; Al Rmalli et al., 2005). In humans, skin is the most sensitive target organ for chronic As exposure. Skin lesions like raindrop pigmentation, palmer and
plantar hyperkeratosis, hypo and hyper pigmentation are regarded as hallmark of As toxicity (Ahsan et al., 2006). Among these skin lesions, palmar and plantar hyperkeratosis are known as premalignant skin lesions, which later develop into skin cancer (Tseng, 1977) like basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and Bowen’s disease (BD) (Banerjee et al., 2011).

People consume As from drinking water, cooked water and from contaminated foodstuffs in the worst As-affected areas of Bengal delta plain, India. The toxicity of As in human system were dependent on the gender, age and exposure routes as well as nutritional status. Assessment on calories intake belongs to poor nutrition in the study areas. Cases of melanosis were higher compared to other dermal diseases (Samal et al., 2013). In another study, brittle nail, patchy alopecia, facial edema as well as nonpitting edema of the feet and rarely conjunctival congestion have been reported as signs of arsenical skin diseases (Stöhrer, 1991). It is well known that high levels of As are associated with increased risk for cardiovascular disease. Vice versa, long-term exposure to low to moderate As levels was also associated with cardiovascular disease incidence and mortality (Moon et al., 2013). The surprising characteristic of As is that it may increase both malignant and nonmalignant respiratory disease. Ingestion of iAs in drinking water results in pulmonary effects manifested by cough, chest sounds in the lungs and shortness of breath (Mazumder et al., 2000). In addition, chronic exposure to iAs is associated with lower intelligence quotient (IQ) and learning disabilities as well as memory impairment. There was a dose-dependent relationship between As levels in water and poor performance scores on intelligence measures. Comparing with different studies, children exposed to >50 μg/l arsenic had poorer intellectual performance than those exposed to < 2 μg/l).
resulted in no measurable effects on IQ, moderate exposures (142 μg/L and 190 μg/l) resulted in decreases of 5 and 10 IQ points, respectively (Tyler and Allan, 2014). Moreover, hematological disorders have been reported in acute and chronic As poisoning. Anemia, leucopenia, and thrombocytopenia were found exposed to As in drinking water in Niigata Prefecture, Japan, half of the patients having arsenical skin lesions. Likewise, West Bengal on 156 people exposed to arsenic-contaminated water (50-14,200 μg/L) showed incidence of anemia in 47.4% of cases (Mazumder and Dasgupta, 2011). Chronic kidney disease is an important global health problem. In a recent review, there was evidence of a positive association between As exposure and kidney disease mortality. Assessment of a small number of studies with three or more categories showed a clear dose-response association between As and prevalent albuminuria and proteinuria, but not with chronic kidney disease outcomes (Zheng et al., 2014). Furthermore, the hepatotoxic effect of As has long been recognized. From hospital-based studies on arsenicosis, hepatomegaly was found in 76.6% in West Bengal, India. Prolong drinking of As-contaminated water is associated with hepatomegaly. Predominant lesion of hepatic fibrosis appears to be caused by As induced oxystress (Mazumder, 2005). Arsenic was shown to adversely impact reproductive and child health, as it was shown to cross the placenta and cause low birth weight, and fetal loss and infant mortality (Hopenhayn et al., 2003; Rahman et al., 2010), adverse pregnancy (Hasnat, 2005). There are increasing reports of chronic arsenicism worldwide, including cutaneous malignancies as also cancer of internal organs, like bladder cancer (Steinmaus et al., 2003) lung cancer, kidney and liver cancer (Chen and Ahsan, 2004; Ahsan et al., 2006; Pimparkar and Bhave, 2010; Banerjee et al., 2011).
INTRODUCTION

More than 700,000 people in the South and East Asian region have been affected by arsenicosis and As-related diseases, especially skin and internal (lung, bladder, kidney) cancers (Rahman et al., 2009). A cancer epidemiology study in Antofagasta found that As exposures in early life were strongly associated with increased mortality from bladder cancer, laryngeal cancer, liver cancer, and chronic renal disease in adults younger than 50 years old (Smith et al., 2012). Similar pattern of cancer risk was demonstrated in people chronically exposed to elevated levels of As in groundwater in northern Argentina (Aballay et al., 2012). A growing concern over the incidence of widespread human exposure of As has been seriously noticed during the past three decades globally (Nordstrom, 2002; Kapaj et al., 2006; Nriagu et al., 2007; Bundschuh et al., 2009). Therefore, it is strongly recommended a total understanding of the adverse effect of this carcinogen on health, potential molecular mechanism and thus paving the strategies to intervene.

1.2 Layered double hydroxide (LDH)

Layered double hydroxides (LDHs), or the so-called anionic clays, consist of layers of $\text{M}^{\text{II}}$ and $\text{M}^{\text{III}}$ cations which are octahedrally co-ordinated by six oxygen anions, as hydroxides (Wells, 1984) and are host-guest materials that, owing to their potential application in these and other areas, have recently gained much attention (Roy et al., 1992). These layers exist with a similar layered structure to that exhibited by natural Mg(OH)$_2$, also known as brucite. The substitution of M cations into the brucite-like hydroxide layer imparts an overall positive charge on the octahedral layer (Reichle, 1986).

In recent decades, a class of anionic clays known as layered double hydroxides (LDHs) or hydrotalcite-like compounds has attracted considerable attention from both industry and academia (Williams and O'Hare, 2006).
Although LDHs exist as naturally occurring minerals (e.g., green rusts), 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. LDH have relatively weak interlayer bonding and as a consequence, exhibit excellent ability to capture and exchange organic and inorganic anions (Cavani et al., 1991; Williams and O'Hare, 2006; Goh et al., 2008; Wang et al., 2009; Chetia et al., 2012). Thus, unlike other anion adsorbents, such as noncrystalline or poorly crystalline Al- and Fe-(hydr)oxides, on which contaminants are adsorbed on their external surfaces, the internal surface of each individual hydroxide sheet in LDHs can provide binding sites for contaminants in addition to those on their external surfaces. Because of these unique characteristics, LDH materials usually show a higher adsorption capacity for the anions in solution.

In the last decade studies have been carried out on the removal of As [mainly As(V)] from contaminated waters using different forms of LDHs as sorbents (You et al., 2001; Yang et al., 2005; Goh et al., 2008; Grover et al., 2009; Violante et al., 2009; Wang et al., 2009; Caporale et al., 2011; Park and Kim, 2011; Chetia et al., 2012). Arsenate was usually removed more easily than As(III) by Mg–Al-LDHs. It was demonstrated that As(III) is sorbed on chloride-LDHs (You et al., 2001) but not on carbonate-LDHs. In fact, As(III) is much more difficult to remove than As(V) both by calcined and uncalcined LDHs (Yang et al., 2005).

However, few studies have been carried out on the removal of As by Mg–Fe-LDH (Carja et al., 2005; Carja et al., 2008; Türk et al., 2009; Türk and Alp, 2010; Caporale et al., 2011; Park and Kim, 2011), but all of them were devoted to As(V) and not to As(III) sorption. Furthermore, the effect of
foreign ligands on the sorption/desorption of As on/from LDHs has received some attention, but the anions taken into consideration were usually chloride, carbonate, nitrate, sulphate and phosphate (You et al., 2001; Yang et al., 2005; Wang et al., 2009), whereas poor attention has been dedicated to the effect of other inorganic ligands (e.g. selenite and selenate) and low molecular mass organic anions, which often are present in As-contaminated waters (Caporale et al., 2011).

Recently, it has been demonstrated that Mg–Fe-LDHs was efficient sorbents for the removal of As(V) from contaminated waters, especially those containing Fe versus Al. The sorption and desorption of As(V) on/from these minerals in the absence or presence of selected organic and inorganic ligands were affected by pH, residence time, nature and concentration of competing anions (Caporale et al., 2011). In contrast, the capacity of LDHs to sorb As(III) in presence of competing ligands has received scant attention (You et al., 2001) and deserves to be studied in detail. Furthermore, to our knowledge till today the sorption/desorption of As(III) on/from a Fe-based LDH was not studied.

In particular, combination of two dimensional layered material and intercalation technique offers new area for developing nanohybrids with desired functionality. Nanohybrids have composites function and most biomolecules that are negatively charged can be incorporated between hydroxide layers as charge compensating anions through ion exchange. Mineral of this family is Hydrotalcite (Mg-Al\textsubscript{3}CO\textsubscript{3}). LDHs have technological importance in catalysis, separation technology, optics, medical science and nanocomposite material engineering (Khan et al., 2001).

Research on LDHs (i.e. Uncalcined carbonate-LDHs, uncalcined chloride-LDHs, uncalcined nitrate-LDHs, uncalcined and calcined Mg–Al
LDHs, calcined LDHs such as Mg–Fe LDHs) were investigated for their adsorption characteristics with oxyanions (e.g. As(III)) (Kovanda et al., 1999; Manju et al., 1999; You et al., 2001; Yang et al., 2005; Gillman, 2006; Shibata and Murayama, 2006).

The charge density and the anion exchange capacity of the LDHs may be controlled by varying the M$^{2+}$/M$^{3+}$ ratio. However, a broad spectrum of charge-balancing anions may be incorporated, namely halides, oxyanions, silicates, polyoxometalate anions, complex anions, and organic anions (Newman and Jones, 1998).

The removal of As(III) from water by hydrotalcite was characterized. The maximum removal to be 78.2% (Manju et al., 1999). In addition, calcined Mg-Al-CO$_3$ LDH is a promising adsorbent for the removal of trace levels of As.

Hydrotalcite- and hydrocalumite-type LDH can be used for removal of As(III) from solutions. Calcined carbonate form was also effective in removal of As(III) from contaminated water. In case of hydrotalcites the mechanism of uptake was topotactic exchange. The kinetics study showed that anion exchange process was very fast and apparently attained a steady-state. Arsenite removal was found to be 87.5% and 83.6% with the nitrate forms of hydrotalcite and hydrocalumite, respectively (Grover et al., 2009).

The LDH samples (LDH-Cl and LDH-CO$_3$) sorbed more PO$_4$ than As(V), but after calcination LDH-CO$_3$ showed a greater capacity to sorb both the oxyanions. Competition in sorption between PO$_4$ and As(V) was affected by pH, reaction time, surface coverage and sequence of addition of the anions. The final As(V) sorbed/phosphate sorbed molar ratio (rf) increased with reaction time or by adding As(V) before phosphate, but decreased by increasing pH and by adding phosphate before As(V) (Violante et al., 2009).
However, LDHs have several shortcomings that may limit their large-scale applications for oxyanion removal such as i) precursor metals could be released in the solution, particularly at lower pH values, ii) regeneration of the spent LDHs can be costly, iii) high basicity would be introduced to the product water if the calcined LDHs are used.

In conclusion, it can be stated that, i) LDHs offer technical as well as economical merits in treating waters and wastewaters containing harmful oxyanions (Gillman, 2006), ii) they consist of hydroxides of common and abundant metals on the earth and can be synthesized economically (Cavani et al., 1991), iii) oxyanion adsorption process using LDHs shows considerably faster kinetics in comparison to most of the biological treatment processes (Yang et al., 2005), iv) The main advantages of LDHs over the conventional anionic exchange resins include their higher anion exchange capacity for certain oxyanions and their good thermal stability (Das et al., 2004), v) LDHs can be fully regenerated in a short time for reuse (Kuzawa et al., 2006).

Furthermore, some other important factors that may influence the efficiency of oxyanion adsorption on LDHs including the presence of competitive anions, LDHs/oxyanions ratio, temperature, nature of LDH precursor metals, crystallinity of LDHs, layer charge and interlayer anion charge of LDHs, LDH particle sizes, and calcination effect.

1.3 Ferrihydrite

Ferrihydrite is one of the most ubiquitous iron (oxyhydr)oxides and identified by poorly crystalline to amorphous phase with stoichiometry near 5Fe$_2$O$_3$·9H$_2$O (Oremland and Stolz, 2003). It is the solid formed upon rapid hydrolysis of ferric iron solutions at 20–30°C (Dzombak, 1990). Despite the ubiquity of ferrihydrite in natural sediments and its importance as an industrial
sorbent, the nanocrystallinity of this iron oxyhydroxide has hampered accurate structure determination by traditional methods that rely on long-range order (Michel et al., 2007).

It is well evaluated that Ferrihydrite has fresher and higher adsorption capacity. Its large surface area, strong adsorptive properties, and low cost make it an attractive material for removal of both cationic and anionic impurities from wastewater and drinking water. Ferrihydrite readily adsorbs arsenic (V) in the form of arsenate anion (AsO$_4^{3-}$), but probably the most effective method of removal of As from aqueous solutions is through coprecipitation of As with ferrihydrite (Richmond et al., 2004).

Moreover, iron oxides, including the poorly crystalline iron oxides, e.g., ferrihydrite, have a strong affinity for both As(III) and As(V). The retention of As(III) and As(V) is predominantly by ligand exchange with surface structural OH$_2$ and/or OH$^-$ at surface adsorption sites. The retention of both As(III) and As(V) is strongly pH dependent, but with opposite trends. At low to moderate As adsorption levels, the adsorption envelopes of As(III) and As(V) usually cross within the pH range of 6-7.5, i.e., ferrihydrite exhibits a relatively greater retention of As(V) at lower pH values, whereas As(III) is more strongly retained at higher pH values. As(III) is less strongly retained than As(V) at pH < 6 (Jain et al., 1999). Viceversa, there is a discrepancy between theoretical considerations and experimental observations. Several studies concluded that As(V) adsorption is pH-dependent whereas As(III) adsorption is less affected by pH (Jeong et al., 2007; Maiti et al., 2008).

Arsenate was better adsorbed than As(III) on ferrihydrite at a low initial concentration, while the opposite occurred at a high initial arsenic concentration (Jain and Loeppert, 2000). It was reported that As(V) adsorption was greater than As(III) adsorption at a lower pH (4) (Raven et al., 1998).
whereas As(III) adsorption was greater at a higher pH (Dhaka Community Hospital (DCH), 1997) or at high As concentrations.

Some important point regarding ferrihydrite: i) It can be effectively used to achieve a low level of As in drinking water supplies for small communities ii) The isotherm plots for iron oxide-coated sand (IOCS) and ferrihydrite (FH) suggested that the As adsorption can be best described by the Freundlich isotherm at the range studied iii) the As adsorption capacities of IOCS and FH were estimated at 18.3 and 285 μg/g, respectively iv) the large difference in surface area could be the reason that the adsorption capacity of FH was higher compared to the adsorption capacity of IOCS v) the results of the speciation studies with natural water containing As were encouraging and showed that the speciation protocol adopted in this study, using nitric acid preservation, can be adopted in the field vi) speciation studies with natural water showed that the particulate and soluble As contributed 11.4 and 88.6%, respectively, of the tAs present in the natural water (Thirunavukkarasu et al., 2003).

Contrary to the theoretical prediction that As(V) should adsorb more strongly than As(III) at pH values below the point of zero charge (pzc) of ferrihydrite of about 7 to 8, As(III) more or less outcompeted As(V) across the pH scale from 4 to 10. At low pH, As(V) impedes the adsorption of As(III) but to a lesser degree than As(III) impedes As(V) adsorption at a pH above 6, which is below the PZC of ferrihydrite. The effect of As(III) on the adsorption of As(V) increased with an increase in pH, and the adsorption of As(V) was almost absent at a pH of 8 to 9 (Qi and Pichler, 2014).

It has been demonstrated that As(III) sorption onto two-line ferrihydrite is considerably fast and the equilibrium is achieved within the reaction time of 3 h. The two-line ferrihydrite is an efficient sorbent for arsenite due to its
peculiar amorphous characteristic, large surface area, and high PZC. The results indicated that As(III) has a strong affinity for two-line ferrihydrite and, in particular. The mechanism for this high retention of As(III) by twoline ferrihydrite was elucidated by spectral evidence and structural data obtained by X-ray absorption spectroscopic measurements (Kim et al., 2014).

The ability of sulfhydryl ligands to compete with ferrihydrite for As(III) was tested in various anoxic mixtures of both adsorbents. It was documented that sulfhydryl ligands are highly competitive As(III) complexing agents that can stabilize As in its reduced oxidation state even under prolonged oxidizing conditions. These findings are particularly relevant for organic S-rich semi-terrestrial environments subject to periodic redox potential changes such as peatlands, marshes, and estuaries (Hoffmann et al., 2014).

At relatively high As concentrations, As(III) reacted faster than As(V) with the ferrihydrite, i.e., equilibrium was achieved sooner, but As(V) adsorption was faster at low As concentrations and low pH. Adsorption maxima of approximately 0.60 (0.58) and 0.25 (0.16) mol\text{As} \text{mol}^{-1}\text{Fe} were achieved for As(III) and As(V), respectively, at pH 4.6 (pH 9.2 in parentheses). The results of this study show that both As(III) and As(V) have strong affinities for ferrihydrite, and arsenite can be retained in much larger amounts than arsenate at high pH (approximately >7.5) or at high As concentrations in solution. Spectral evidence and structural data will be required to obtain molecular explanations for the high retention of arsenite by ferrihydrite (Raven et al., 1998).

Adsorption was observed to increase with increasing pH to a maximum at pH 7–9, after which sorption decreased slightly with further increase in pH (Gao and Mucci, 2001). The sorption capacity of As(III) was greater than that of As(V) in the range of pH 4.0–11.0. The capability of organic and inorganic
ligands in preventing As sorption follows the sequence: SeO$_4$ ≈ SO$_4$ < OX < MAL ≈ TAR < CIT < SeO$_3$ ≪ PO$_4$. The efficiency of most of the competing ligands in preventing As(III) and As(V) sorption increased by decreasing pH, but PO$_4$ whose efficiency increased by increasing pH (Zhu et al., 2011). Complete sorption of As(V) onto ferrihydrite was observed after 8 h, the kinetics of sorption of As(V) was evaluated for the reaction period 0.167 to 8 h for all the oxides for uniformity (Pigna et al., 2006).

1.4 Goethite
Goethite, one of the most thermodynamically stable iron oxides, has been extensively researched especially the structure (including surface structure), the adsorption capacity to anions, organic/organic acid and cations in the natural environment and its potential application in environmental protection. For example, the adsorption of heavy metals by goethite can decrease the concentration of heavy metals in aqueous solution and immobilize; the adsorption to soil organic carbon can decrease the release of carbon and fix carbon (Liu et al., 2014).

As oxyanions, considered as priority pollutants, were removed from dilute aqueous solutions by sorption onto synthetic goethite, a typical inorganic adsorbent (Matis et al., 1997). Goethite can be considered as a useful potential extractant for the removal of priority pollutants from dilute aqueous streams (Matis, 1994).

The combination of bio-oxidation with goethite is a promising approach for As removal from contaminated groundwater, although in a model system at the present stage. The combined use of Aliihoelefa sp. strain 2WW and
goethite is 100-fold higher efficient in adsorbing 200µg/L As(V) than As(III). Resting cells of *Aliihoeflea* sp. strain 2WW oxidize 200 µg/l As(III) in 32 h. 2WW-goethite system remove 95% of the As(III) present, meeting World Health Organization limit adequately (Corsini et al., 2014).

The adsorption of As on goethite in a 0.7 mol/L NaCl solution decreases in competitive experiments with phosphate. The reduction in uptake is proportional to the initial amount of phosphate in solution (Gao and Mucci, 2001).

The surface properties of a well-crystallized synthetic goethite have been studied by acid–base potentiometric titrations, electrophoresis, and phosphate and As(V) adsorption. The charging behavior of the goethite surface is characteristic of a pure sample with no surface carbonate contamination. Phosphate and As(V) adsorption decrease as the pH increases in either 0.1 or 0.01 M electrolyte. However, the effects of pH and electrolyte concentration are more pronounced for phosphate than for As(V). Arsenate adsorption is almost independent on the electrolyte concentration. These differences in the adsorption behavior explain why several reports indicate that As(V) adsorbs more than phosphate on goethite whereas other reports indicate the opposite phenomenon (Antelo et al., 2005).

Most of the articles deal with adsorption on goethite (α-FeOOH), which has become a model solid for adsorption studies because goethite is one of the most common and stable crystalline iron (hydr)oxides in natural environments and is also a solid that can be prepared in a very reproducible way with particles of controlled size and geometry. Whereas in some articles it is reported that As(V) adsorbs more strongly than phosphate, in contrast, the opposite behavior is observed (Gao and Mucci, 2001). It is found that the adsorption decreases as the pH increases in both cases. It is also reported that
although the affinity of both anions for the surface is high, that of As(V) is higher. No data for lower ionic strength were presented (Hongshao and Stanforth, 2001). The maximum sorption capacity is estimated to be 7.0×10⁻⁶ mol m⁻² goethite, under similar experimental conditions (Asta et al., 2009).

Goethite and jarosite are effective As(V) sorbents in highly acidic pH, favouring the natural attenuation of arsenic in acid mine drainage (AMD) environments. These sorption capacities are in the wide range of that of low-cost adsorbents or activated carbons at very low pH. pH has little effect on As(V) removal and ionic strength has no substantial effect on the arsenic sorption on jarosite and goethite. The competitive effect of sulphate, which is the main anion in AMD impacted waters, is greater on the As(V) sorption capacity of jarosite than on that of goethite (Asta et al., 2009).

Different studies have been carried out on the As(III) and/or As(V) sorption on amorphous iron oxides (Wilkie and Hering, 1996; Goldberg and Johnston, 2001) and on the As sorption onto goethite, especially related to the As(V) sorption. In this sense, it was noted that the sorption of As(V) on goethite decreased with the increase of pH in the range 6–11,(Grossl and Sparks, 1995) and was showed that both As(V) and As(III) sorption on goethite decreased at neutral to alkaline and the variation of the sorption with the initial concentration of As(V) followed the Langmuir isotherm (Matis et al., 1997). A decrease of the As(V) sorption on goethite with pH while As(III) had a range of pH of maximum sorption between 5 and 9 (Manning et al., 1998). In another study it was observed that the sorption of As (either As(III) and As(V)) is higher in a natural goethite than in a natural magnetite and higher for As(V) than for As(III) (Bowell, 1994).

Among the various adsorbents, iron bearing minerals especially goethite was observed to be more effective and economically viable. Various synthesis
methods of goethite (Kosmulski et al., 2004) were reviewed and found that the particle size, shape and surface area of goethite depend on the Fe(III):OH ratio, the rate of base titration of iron salt, temperature of neutralization and time of crystallization. Most of the synthesis methods were based on neutralization of ferric nitrate with an alkali and subsequent aging spanning from 20 h to 336 h. The specific surface area of goethite specimens obtained by the above method ranges from 11 m² g⁻¹ to 150 m² g⁻¹ (Kosmulski et al., 2004).

The adsorption amount of goethite to As(V) decreased from 4.7 to 1.1 mg g⁻¹ as the pH increased from 5 to 8 (Lakshmpathiraj et al., 2006). Additionally, competitive adsorption on goethite also has been the subject of much research because the competitive adsorption results at least to some extent, affects the bioavailability of nutrients and immobilization of toxic chemicals, such as the competitive adsorption between phosphate and sulfate/As (Gao and Mucci, 2003).

Both hematite and goethite are able to adsorb more than 80% of As, whatever the initial concentration may be, at natural pH water,. The iron oxides used in this work should be suitable candidates as sorbents for As(V) removal technologies (Mamindy-Pajany et al., 2009). Arsenite adsorbed on goethite under air dry conditions was not stable. After 20 days, more than 20 % of absorbed As(III) was oxidized to As(V). The adsorption-oxidation system composed of goethite may be significant in decreasing As toxicity in terrestrial environments (Sun and Doner, 1998).

The competitive adsorption of phosphate and As on goethite was investigated to better understand the bonding mechanisms for the two ions. The anions were added both simultaneously and sequentially. When added simultaneously, the two ions were adsorbed about equally, with the total
surface coverage being slightly greater than for either ion alone. When added sequentially, the extent of exchange for the first ion depended on the equilibration time before the second ion was introduced the longer the equilibration time the greater the exchange (Hongshao and Stanforth, 2001).

The adsorption of As(V) and As(III) on goethite and ferrihydrite in the presence of several naturally occurring organic ligands was investigated. They found that both As(V) and As(III) adsorption may be decreased which depended on the pH of the system, the type of Fe oxide, and the type of organic ligand (Grafe et al., 2002).

The adsorption of silicic acid at all pH values and concentrations decreased the rate of As adsorption and the total quantity of As adsorbed. Furthermore, the quantity of As adsorbed decreased as the surface concentration of silicic acid increased. No clear pH trend was observed for the inhibition of consist arsenite adsorption in the presence of silicic acid At a silicic acid concentration of 0.10 mM, arsenite adsorption was reduced the greatest at a pH value of 4 followed by a pH value of 8. At a silicic acid concentration of 1.0 mM, arsenite adsorption was reduced the greatest at a pH value of 8 followed by equal quantities at pH values of 4 and 6 (Waltham and Eick, 2002).

Maximum As desorption by goethite was found at pH 8.0 and by clinoptilolite at pH 10.0 and 0.01 M KCl. The experimental results of As adsorption were comparable successfully fitted to Freundlich and Temkin isotherms (Dimirkou et al., 2009).
CHAPTER 2 –
AIMS OF THE WORK
2.1 Aims of the present work

Arsenic contamination in terrestrial and aquatic ecosystems is a very important environmental issue due to its negative impact on livestock and human health. With greater public awareness of As poisoning in animal and human nutrition, there has been a growing interest in developing regulatory guidelines and remediation technologies for mitigating As-contaminated ecosystems. However, numerous treatment technologies are available to reduce As concentrations in contaminated waters (Williams and O'Hare, 2006). The most effective As treatment processes include adsorption, ion exchange, reverse osmosis, and nanofiltration (Mohan and Pittman, 2007). The majority of As removal technologies from contaminated waters treat As(V) much more effectively than As(III), so a pre-oxidation step is recommended for source waters containing As(III) at significant concentrations (Frankenberger Jr., 2002). These As treatment technologies have been successfully applied to remove trace As concentrations from contaminated waters, but most of them are cost-prohibitive for small communities or developing countries, which are likely to face expensive and technically imposing challenges to face the maximum contaminant level of 10 μg As L\(^{-1}\). Several models show a wide range of costs for the different technologies, which can hinder their implementation to As-contaminated sources (Pirnie, 1999). For these reason, it is extremely important to investigate and promote new As remediation technologies able to remove As from contaminated areas in an inexpensive and safe way.

In the present work, the competition between As(III) with organic and inorganic ligands for the sorption sites on LDH, obtained by coprecipitating Mg with or Fe (Fe-Mg-LDH), was studied. LDHs are relatively simple and economical to synthesize by coprecipitation methods under laboratory
AIMS OF THE WORK

conditions (Cavani et al., 1991), thus they can be produced in large quantities. LDHs also form in soil environments (e.g., green rusts) and present peculiar sorption capacities (Violante et al., 2008). In this work we studied the sorption of As(III) onto a LDH containing Mg and Fe (Mg–Fe-LDH), as affected by: (i) pH; (ii) the presence of increasing concentrations of organic [citrate (CIT) and oxalate (OX)] and inorganic [phosphate (PO₄), selenite (SeO₃) and sulphate (SO₄)] ligands, (iii) the effect of residence time on the desorption of As(III) by these ligands, and; (iv) the kinetics of desorption of As(III) by PO₄. Furthermore, competitions in sorption of these anions on LDHs have received scant attention.

Ferrihydrite is known to play an important role in the natural environment. Its large surface area, strong adsorptive properties, high adsorptive capacity and low cost make it an attractive sorbent for removal of As. In addition, Goethite, one of the most thermodynamically stable iron oxides has been extensively researched. The adsorption of As by Goethite can decrease the concentration of As in aqueous solution. The aim of this experiment was to study the As(III) removal from contaminated waters by Fe-based minerals, by using Ferrihydrite [non-crystalline Fe-(hydr)oxide] and Goethite [crystalline Fe-(hydr)oxide] as sorbent media in batch experiments. In particular, it was studied: i) the Langmuir isotherms of As(III) on Ferrihydrite and Goethite at pH 6.0; ii) arsenite sorption at different pHs; iii) arsenite sorption in presence of selected competing ligands, such as citrate (CIT), molybdate (MoO₄), oxalate (OX), phosphate (PO₄), selenite (SeO₃), selenate (SeO₄), and sulphate (SO₄).

To assess the possible health risk to humans consuming crops irrigated with As contaminated water, information is needed regarding the soil-plant transportation of As and to minimize the accumulation of As in plants.
consumed directly by humans, farm animals or wildlife (Zhao et al., 2010). Apart from the health risk, the presence of As in irrigation water and/or in soil at an elevated level could hamper normal growth of plants with toxicity symptoms such as biomass reduction (Caporale et al., 2013) and yield losses (Jiang and Singh, 1994). The spread of contaminants in soil can be obstructed by the soil stabilization technique. The application of stabilizing agents to contaminated soils is a remediation technique that reduces both mobility and bioavailability of trace elements (Kumpiene et al., 2008). Incorporation of low-cost, widely available materials as humic acid into soil for As immobilization offers various potential advantages over other methods such as cost, simple methodology and low environmental impact. In the present work it was carried out an experiment on an edible plant, the carrot (*Daucus carota* L.), irrigated with different solutions containing As(III) and grown in a As-uncontaminated soil amended with increasing amounts of stabilized humic acid and inorganic fertilizer. The aim of this experiment was to study the influence of the organic amendment (i.e. humic acid) and inorganic fertilization on: i) the mobility and phyto-availability of As in soil; ii) the growth of the carrot plants; iii) the As uptake and translocation in plant compounds from contaminated systems. Carrot was selected as the test plant of this study, because this crop is grown in several As contaminated areas throughout the world and suffers from As toxicity.
CHAPTER 3 – MATERIALS AND METHODS
3.1 Study on the LDH

3.1.1 Synthesis and characterization of the Mg–Fe-LDH

The Mg–Fe-LDH was prepared by the coprecipitation method described by Caporale et al., (2011). Briefly, solutions containing MgCl$_2$.6H$_2$O and FeCl$_3$.6H$_2$O (initial Mg/Fe molar ratio equal to 2) were slowly added with stirring at 20°C to a NaOH solution at pH 10.0. The pH was maintained constant for 24 h by adding 2 mol/l L$^{-1}$ NaOH using an automatic titrator (Potentiograph E536 Metrom Herisau) in conjunction with an automatic syringe (burette 655 Dosimat), at 20°C. The suspension was centrifuged at 10,000g for 30 min, rinsed five times with deionized water and then dialyzed for 21 d, freeze dried and lightly ground to pass through a 0.315 mm sieve.

In our previous work Caporale et al., (2011) the nature of this material was studied by X-ray diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy and its surface area (273 m$^2$ g$^{-1}$) was determined by H$_2$O sorption at 20% relative humidity. We showed that this Mg–Fe-LDH sample exhibited to XRD and FT-IR characteristic peaks and bands of hydrotalcite, even though relatively broad and weak. By prolonging the aging of this material for 30 d at 50°C these peaks and bands appeared stronger and sharper (see Caporale et al., (2011), their Figs. 1 and 2B). We considered the material aged at 20°C a mixture of materials of different crystallinity, size and nature, precisely LDH (mainly) and short-range ordered oxides. This material could be similar to that synthesized by (Park and Kim, 2011) at 30°C, which they called “amorphous” Mg–Fe-LDH, in spite of their sample showed distinct XRD peaks.

3.1.2. Arsenite sorption at different pHs
Suitable volumes of 0.1 mol L⁻¹ solution containing sodium arsenite (NaAsO₂) were added to flasks containing 100 mg of Mg–Fe-LDH, in order to have initial As(III) concentrations in the range of 5×10⁻⁴ to 8.75×10⁻³ mol L⁻¹. Each suspension was shaken for 24 h at 20°C on a magnetic stirrer, keeping a constant pH of 6.0 or 9.0 by adding 0.1 or 0.01 mol L⁻¹ HCl or NaOH with the automatic titrator/syringe.

The sorption of As(III) onto the Mg–Fe-LDH was also carried at different pH values (from 4.0 to 10.0) by adding a suitable amount of As(III) (1875 mmol kg⁻¹, a quantity slightly higher than that necessary to reach a maximum sorption at pH 6.0, as determined from the sorption isotherm) to 100 mg of Mg–Fe-LDH. The suspensions were shaken for 24 h at 20°C on a magnetic stirrer and their pHs were kept constant by adding 0.1 or 0.01 mol L⁻¹ HCl or NaOH.

The final volume of the suspensions was adjusted to 20 mL with 0.01 mol L⁻¹ KCl and the LDH/solution ratio was 5 g L⁻¹. Finally, the suspensions were centrifuged at 10,000g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.1.3 Sorption of As(III) in presence of organic and inorganic ligands
To flasks containing 100 mg of Mg–Fe-LDH suitable amounts of solutions containing As(III) as NaAsO₂ (1000 mmol kg⁻¹) and organic (CIT as C₆H₅Na₃O₇.2H₂O or OX as Na₂C₂O₄) or inorganic (PO₄ as KH₂PO₄, SeO₃ as Na₂SeO₃.5H₂O or SO₄ as MgSO₄.7H₂O) ligands were added, in order to have initial ligand/As(III) molar ratios (R) of 1, 2, 3 or 5. The As(III) surface
coverage of the Mg–Fe-LDH was about 75% (based on the sorption isotherm at pH 6.0). The suspensions were shaken for 24 h at 20°C on a magnetic stirrer and their pHs were kept constant at 6.0 as described before. The final volume was adjusted to 20 mL with 0.01 mol L\(^{-1}\) KCl and the LDH/solution ratio was 5 g L\(^{-1}\). Finally, the suspensions were centrifuged at 10,000g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.1.4 Effect of residence time on the desorption of As(III) by organic and inorganic ligands

To 100 mg of Mg–Fe–LDH were added suitable amounts of 0.1 mol L\(^{-1}\) NaAsO\(_2\) solution, so that the As(III) surface coverage of the anionic clay (1000 mmol kg\(^{-1}\)) was about 75% (based on the sorption isotherm at pH 6.0). The suspensions were shaken for 24, 72 or 168 h at 20°C on a magnetic stirrer and their pHs were kept constant at 6.0 as described before. All the As(III) added was completely sorbed within 24 h systems. Organic (CIT or OX) or inorganic (PO\(_4\), SeO\(_3\) or SO\(_4\)) ligands [initial ligand/As(III) molar ratios (R) of 1 and 3] were then added to all these As(III) sorbed LDH systems. The final volume was adjusted to 20 mL with 0.01 mol L\(^{-1}\) KCl and the LDH/solution ratio was 5 g L\(^{-1}\). The suspensions were kept at pH 6.0 with 0.1 or 0.01 mol L\(^{-1}\) HCl or NaOH for further 24 h at 20°C and then centrifuged at 10,000g for 20 min and filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.1.5 Kinetics of desorption of As(III) by PO\(_4\)
The kinetic study was carried out using the 24 h As(III) sorbed Mg–Fe-LDH (1000 mmol kg\(^{-1}\)) system (described in the previous section), to which suitable amounts of PO\(_4\) were added, in order to have initial PO\(_4\)/As(III) molar ratios (R) of 1 and 3. The final volume was 20 mL and the LDH/solution ratio was 5 g L\(^{-1}\). The amount of As(III) desorbed by PO\(_4\) was determined from 0.08 to 50 h at pH 6.0 and 20°C. The suspensions from each sampling period were centrifuged at 10,000 g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

### 3.1.6 Arsenite determination

The concentration of the As(III) in the filtrates was determined by flow-injection hydride generation atomic absorption spectrometer, using a Perkin–Elmer AAanalyst 700 interfaced with the FIAS 100 hydride generator. The reagents used for HG-AAS were 10% (v/v) HCl, 0.2% NaBH\(_4\) in 0.05% NaOH. The samples were reduced prior to analysis with 5% (w/v) KI and 5% (w/v) ascorbic acid to improve sensitivity. The As detection limit provided by this method was 2.7×10\(^{-8}\) mol L\(^{-1}\).

The amount of As(III) sorbed was determined by the difference between the amount initially added and that determined in the filtrates. The ±values in the tables indicate the standard deviation. The intraday repeatability study was carried out by the analysis of the same standard solution five consecutive times (n = 5) in the same day under the same conditions. The interday precision was carried out for three successive days using the same conditions. The relative standard deviation of these measurements ranged from 1.5% to 3.6%.
3.2 Study on the Ferrihydrite and Goethite
3.2.1 Synthesis and characterization of the Ferrihydrite and Goethite

Ferrihydrite was synthesized by precipitating 0.1 mol L\textsuperscript{-1} Fe(NO\textsubscript{3})\textsubscript{3} at pH 5.5 with 0.5 mol L\textsuperscript{-1} NaOH at a rate of 0.5 mL min\textsuperscript{-1} (Cornell and Schwertmann, 1996). The suspension’s pH was maintained constant for 24 hours by adding 2.5 mol L\textsuperscript{-1}NaOH using an automatic potentiometric titrator(Potentiograph E536 MetromHerisau), at room temperature (around 20°C).

Goethite was prepared by precipitating 0.5 mol L\textsuperscript{-1} Fe(NO\textsubscript{3})\textsubscript{3} at pH 12.0 with slow addition of 1.0 mol L\textsuperscript{-1}NaOH (Atkinson et al., 1968). The suspension was aged for 7 days at room temperature (around 20°C) and then for 20 days on a heated plate at 65°C. During all the aging process, suspension’s pH was maintained constant by adding 1.0 mol L\textsuperscript{-1}NaOH using the automatic potentiometric titrator.

Subsequently, both Ferrihydrite and Goethite suspensions were centrifuged at 10,000 rpm for 30 min, rinsed five times with ultrapure water, transferred into porous dialysis membranes for being dialyzed with deionized water for 16 days [molecular weight (MW) cut off of 15000], freeze-dried, and lightly ground to pass through a 0.315 mm sieve.

3.2.2 Arsenite sorption isotherms at pH 6.0

Suitable volumes of 0.1 mol L\textsuperscript{-1} solution containing sodium arsenite (NaAsO\textsubscript{2}) were added to flasks containing 100 mg of either Ferrihydrite or Goethite, in order to have initial As(III) concentrations in the range of 5 \cdot 10\textsuperscript{-4} to 4.0 (Goethite) or 8.0 (Ferrihydrite) \cdot 10\textsuperscript{-3} mol L\textsuperscript{-1}. Each suspension was shaken for
24 h at 20 °C on a magnetic stirrer, keeping a constant pH of 6.0 by adding 0.1 or 0.01 mol L⁻¹ HCl or NaOH with the automatic titrator. The final volume of the suspensions was adjusted to 20 mL with 0.01 mol L⁻¹ KCl and the sorbent/solution ratio was 5 g L⁻¹. Finally, the suspensions were centrifuged at 10,000 x g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.2.3 Arsenite sorption at different pHs
The sorption of As(III) on the Ferrihydrite and Goethite was also carried at different pH values (from 4.0 to 10.0) by adding a suitable amount of As(III) [400 (Goethite) or 1500 (Ferrihydrite) mmol kg⁻¹, a quantity slightly higher than that necessary to reach a maximum sorption at pH 6.0, as determined from the sorption isotherms] to 100 mg of each sorbent. The suspensions were shaken for 24 h at 20 °C on a magnetic stirrer and their pHs were kept constant by adding 0.1 or 0.01 mol L⁻¹ HCl or NaOH. The final volume of the suspensions was adjusted to 20 mL with 0.01 mol L⁻¹ KCl and the sorbent/solution ratio was 5 g L⁻¹. Finally, the suspensions were centrifuged at 10,000 x g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.2.4 Sorption of As(III) in presence of competing ligands
To flasks containing 100 mg of either Ferrihydrite or Goethite, suitable amounts of solution containing As(III) as NaAsO₂ (specifically: 870 mmol kg⁻¹ for Ferrihydrite and 230 mmol kg⁻¹ for Goethite) and organic (CIT as
C₆H₅Na₃O₇ · 2H₂O or OX as Na₂C₂O₄) or inorganic (MoO₄ as Na₂MoO₄·2H₂O, PO₄ as KH₂PO₄, SeO₃ as Na₂SeO₃·5H₂O, SeO₄ as Na₂SeO₄·10H₂O or SO₄ as MgSO₄·7H₂O) ligands were added, in order to have initial ligand/As(III) molar ratios (R) of 1 or 3. The As(III) surface coverage of both sorbents was about 70 % (based on the sorption isotherm at pH 6.0). The suspensions were shaken for 24 h at 20 °C on a magnetic stirrer and their pHs were kept constant at 6.0 as described before. The final volume was adjusted to 20 mL with 0.01 mol L⁻¹ KCl and the sorbent/solution ratio was 5 g L⁻¹. Finally, the suspensions were centrifuged at 10,000 x g for 20 min and then filtered through 0.22-µm membrane filters. The filtrates were stored at 2°C until analysis. The experiments were conducted in triplicate.

3.3 **Study on the carrot plants** (*Daucus carota* L.)

3.3.1 **Soil preparation and characterization**

The soil used in this experiment was collected from the sub-surface layer (10-30 cm) of a grassland in the Portici (Naples) area. The soil samples for carrot cultivation and chemical analysis, after air-drying, were passed through 5 and 2 mm sieves respectively.

Soil fractions (sand, silt and clay) were separated using a pipette and a sieving method, following a pretreatment with H₂O₂ to oxidize organic matter, and dispersion was aided by sodium hexametaphosphate. 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. For determination of CEC, the soil was extracted with 1 M NH₄OAc 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 NaHCO₃ as the extractant. The As background in the soil was determined by extracting the soil with concentrated HNO₃ and HF at 5:1 ratio.

3.3.2 Experimental design
Carrot seeds (*Daucus carota* L.) were seeded into a polystyrene alveolar seedbed consisting of 90 holes (25 mm diameter, 40 mm depth) containing soil. At the moment of transplanting into pots containing 6 kg of soil (one plant per pot, 15 days after plant emerging), plants were placed in an unheated greenhouse. All of them were initially irrigated with As-uncontaminated water (first 5 days after transplanting) and thereafter irrigated for 60 days with water containing sodium arsenite (NaAs³O₂, Sigma-Aldrich, USA) at two different concentrations: 3 (As 3) and 6 (As 6) mg As L⁻¹. The experiment included a control set of plants irrigated with As-uncontaminated water (control) for 60 days. 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 fertilization, by organic or inorganic fertilizers, was also supplied to the carrot plants; in particular, two sets of plants were amended with half (1/2 O) and full (1 O) doses of organic liquid amendant (consisting of commercial humic acids extracted by leonardite: Energizer 70 WDG, Diachem, Italy) and other two sets of plants were fertilized with half (1/2 I) and full (1 I) doses of inorganic liquid fertilizer (consisting of NH₄NO₃, KNO₃ and KH₂PO₄). To these set plants were provided the half (1/2 O and 1/2 I) and full (1 O and 1 I) amounts of NPK taken up by carrot during its entire life cycle (168 mg N, 82 mg P, 220 mg K).
The experiment also included a control set of unfertilized plants (no F). The pots were organized in a completely randomized design with 4 replicates for each treatment, and rearranged every 5 days. The watering of the carrot plants was stopped 4 days before harvesting.

3.3.3 Chlorophyll A and B concentrations in carrot leaves

1 mg leaves of each plant were sampled in order to determine the chlorophyll A and B contents. Extraction of chlorophylls from carrot leaves was carried out using 80% acetone in deionized water (v/v). One gram of fresh leaves was homogenized with 5 ml of acetone and shaken for 1 hour on a reciprocal shaker at 200 rpm. The homogenate was filtered through 0.22 µm filter and the residue was similarly extracted once again and filtered. The two filtrates were combined and the final volume was made up to 10 ml. All extracts were assayed using Bio-red Benchmark Microplate reader. Chlorophyll A and B contents (mg g⁻¹ dry weight) were calculated by absorbance values at 663 nm (D 663) and 645 nm (D 645) using the formula of Arnon, (1949).

3.3.4 Harvest and plant samples collection

The carrot plants were harvested when they reached the typical market size 60 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 carrot plants were weighed, washed with deionized water to remove soil residues and then dried in an oven for 2 days at 70°C. Thereafter, dried plant tissues were weighed and then ground using a PM 200 ball mill (Retsch).
3.3.5 Determination of nonspecifically and specifically sorbed As in the soil

Arsenic fractions in each soil sample were extracted using the first two step sequential extraction procedure (SEP). Briefly, one gram of each soil sample was shaken with 25 mL of a 0.05 M (NH₄)₂SO₄ for 4 h at 20°C, on a reciprocal shaker at 150 rpm. Following the shaking, the suspensions were centrifuged for 15 min at 3500 x g and the supernatants were then filtered through 0.45-μm membrane filters (Step 1). Subsequently, each soil sample was shaken with 25 mL of a 0.05 M (NH₄)H₂PO₄ for 16 h at 20 °C, on a reciprocal shaker at 150 rpm. Following the shaking, the suspensions were centrifuged for 15 min at 3500 x g and the supernatants were filtered through 0.45-μm membrane filters (Step 2). The As fractions extracted by these two sequential steps were assumed to correspond to nonspecifically sorbed As (Step 1) and specifically sorbed As (Step 2). The concentration of the As fractions was determined as discussed below in the section 3.3.6.

3.3.6 Measurement of As and P concentration in dried plant tissues and soil

Dried root and leaf samples were digested in a microwave (Milestone, Digestor/Dring Ethos 900); 0.5 g of dried plant tissues were weighed into PTFE vessels and digested in 5 mL of HNO₃ (65 %), 0.5 mL of HF (50 %) and 2 mL of H₂O₂. All solutions obtained were passed through a 0.22 mm filter and diluted to 50 mL with deionized water. Thereafter, the measurement of As and P concentrations was performed by a flow-injection hydride generation atomic absorption spectrometer (Perkine Elmer AAnalyst 700 interfaced with the FIAS 100 hydride generator), and a inductively coupled
plasma atomic emission spectroscopy (ICP-AES, Varian, Liberty 150), respectively. 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. Each analytical batch contained at least one reagent blank and one internationally certified reference material (CRM) such as oriental tobacco leaves CTA-OTL-1.
CHAPTER 4 - RESULTS AND DISCUSSION
4.1 Study on the LDH

4.1.1 Arsenite sorption isotherms at different pHs

The Langmuir isotherms of As(III) onto the Mg–Fe-LDH at pH and 9.0 are depicted in Figure 2. The shape of the isotherms indicates a good affinity of the harmful oxyanion for the sorption sites of the anionic clay. Greater amounts of As(III) were sorbed by Mg–Fe-LDH at pH 6.0 ($S_m = 1621.7$ mmol kg$^{-1}$) than at pH 9.0 ($S_m = 1406.0$ mmol kg$^{-1}$), probably because of the competition with hydroxyls ions, which have higher affinity for LDHs than As(III) ions (You et al., 2001).

The sorption capacity of the Mg–Fe-LDH resulted to be much lower for As(III) than As(V) ($S_m = 2595$ mmol kg$^{-1}$, (Caporale et al., 2011). You et al., (2001) claimed that As(III) was not sorbed on Mg–Al-LDH because carbonate ions are preferentially sorbed and prevent As(III) exchange. Indeed, in the sample used in this work the presence of carbonate was significant (Caporale et al., 2011).

![Figure 2](image_url)

**Figure 2** Sorption isotherms of As(III) onto Mg–Fe-LDH at 20°C and pH 6.0 or 9.0, after 24 h of reaction time.
In addition, the Langmuir constant $K$ of As(III) (Table 3) was around an order of magnitude smaller compared to that of As(V) (Caporale et al., 2011), indicating a much higher affinity of the Mg–Fe-LDH for As(V) than As(III). This trend was also found by other authors (Yang et al., 2005; Wang et al., 2009; Xing et al., 2009; Wang et al., 2010), who ascertained a higher As(V) removal from aqueous solutions by Mg–Al-LDHs (Yang et al., 2005; Xing et al., 2009) or green rusts (Wang et al., 2010) compared to that of As(III). The difference in the total amounts sorbed of As(III) and As(V) on the LDH adsorbents may be related to the different species found in solution for these two forms of As with different oxidation states $\text{H}_2\text{AsO}_4^-$ and $\text{HAsO}_4^{2–}$ versus $\text{H}_3\text{AsO}_3$).

**Table 3** Langmuir sorption capacity ($Sm$) and the constant $K$ for As(III) as obtained from the sorption isotherms of the Fe-Mg-LDH at pH 6.0 and 9.0.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface area (m$^2$ g$^{-1}$)</th>
<th>pH</th>
<th>$Sm$ (mmol kg$^{-1}$)</th>
<th>$K$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-Mg-LDH</td>
<td>273</td>
<td>6.0</td>
<td>1621.7 (5.94)$^a$</td>
<td>2.02</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.0</td>
<td>1406.6 (5.15)$^a$</td>
<td>1.22</td>
<td>0.97</td>
</tr>
</tbody>
</table>

$^a$ Numbers in parenthesis indicate the amount of As(III) sorbed as mmol m$^{-2}$× 10$^{-3}$.

The sorption of As(III) onto Mg–Fe-LDH in the range of pH from 4.0 to 10.0 is shown in Figure 3 and resulted to be significantly pH dependent. At pH 4.0–5.0 the quantities of As(III) sorbed resulted to be constant, but at pH > 5.0 they decreased. The rate of decline in the amount of As(III) sorbed was relatively constant from pH 5.0 to 9.0 (on average 66.6 mmol As(III)/pH unit),
whereas it was considerably higher between pH 9.0 and 10.0 (184.2 mmol As(III)/pH unit). The decrease in sorption of As(III) with increasing pH could be attributed to the increasing concentrations of hydroxyl ions which have an high affinity for LDH surfaces and at pH > 9.0 also to the repulsion between the negative charged As(III) ions (pKa = 9.2 and 12.1) and the negative charged LDH. According to (Goh et al., 2008; Park and Kim, 2011) the point of zero charge (pzc) of Mg–Fe-LDH was 8.9 and 9.8, respectively. (Caporale et al., 2011) found the same trend with As(V), but the rate of decline of its sorption with increasing pH was more pronounced than that of As(III). Similar results were also found by (Dixit and Hering, 2003; Zhu et al., 2011), who noted a higher rate of decline of the sorption of As(V) with increasing solution pH compared to that of As(III) onto ferrihydrite and Fe-oxide minerals, respectively.

![Graph](image)

**Figure 3** Arsenite sorbed by Mg–Fe-LDH as a function of pH.

### 4.1.2 Effect of organic and inorganic ligands on the sorption of As(III)
The sorption of As(III) in presence of increasing concentrations [initial ligand/As(III) molar ratios (R) of 1, 2, 3 and 5] of organic (CIT or OX) or inorganic (PO$_4$, SeO$_3$ or SO$_4$) ligands on Mg–Fe-LDH, after 24 h of reaction at pH 6.0, is shown in Table 4 and Figure 4. The ligands efficiency (%) in preventing As(III) sorption on Mg–Fe-LDH was calculated by subtracting the amount of As(III) sorbed in presence of ligand from that sorbed in absence of ligand, divided by the amount sorbed in absence of ligand (Figure 4) (Violante and Pigna, 2002).

**Figure 4** The efficiency (%) of the ligands in preventing As(III) sorption on Mg–Fe-LDH as a function of ligand/As(III) molar ratio (R), after 24 h of reaction time at pH 6.0 and 20°C. Initial As(III) concentration was 1000 mmol kg$^{-1}$.

Organic and inorganic ligands showed different capacities to compete with As(III) for the sorption sites of the Mg–Fe-LDH. Sulphate resulted to be the weakest among the competing ligands and its efficiency in preventing As(III) sorption on Mg–Fe-LDH surfaces ranged from 6.3 (R = 1) to 12.1% (R
RESULTS AND DISCUSSION

The amount of As(III) sorbed in presence of OX was quite similar to that in presence of SO$_4$ at $R = 1$, but at higher $R$ values OX showed to have a relatively higher efficiency in inhibiting As(III) (16.6% at $R = 5$) (Figure 4). The efficiency of these anions in preventing As(III) sorption on this sorbent was also similar to that in reducing As(V) sorption (Caporale et al., 2011).

Table 4  Amount of As(III) sorbed on Fe-Mg-LDH in presence of increasing concentrations of competing ligands, after 24 h of reaction time at pH 6.0 and 20 °C. Initial As(III) concentration was 1000 mmol kg$^{-1}$.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$R^a = 1$</th>
<th>$R^a = 2$</th>
<th>$R^a = 3$</th>
<th>$R^a = 5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO$_4$</td>
<td>936.9 ± 66.2</td>
<td>922.5 ± 83.5</td>
<td>893.2 ± 69.4</td>
<td>879.5 ± 57.4</td>
</tr>
<tr>
<td>OX</td>
<td>932.8 ± 52.6</td>
<td>880.1 ± 63.7</td>
<td>859.5 ± 78.0</td>
<td>833.9 ± 61.5</td>
</tr>
<tr>
<td>SeO$_3$</td>
<td>834.1 ± 71.8</td>
<td>714.7 ± 44.5</td>
<td>668.4 ± 37.6</td>
<td>593.1 ± 48.2</td>
</tr>
<tr>
<td>CIT</td>
<td>779.4 ± 47.9</td>
<td>617.1 ± 42.3</td>
<td>551.1 ± 43.8</td>
<td>432.0 ± 30.1</td>
</tr>
<tr>
<td>PO$_4$</td>
<td>763.4 ± 52.0</td>
<td>553.5 ± 33.9</td>
<td>465.3 ± 29.5</td>
<td>325.7 ± 18.8</td>
</tr>
</tbody>
</table>

$^a$ R indicates the initial ligand/As(III) molar ratio. Data are expressed as mean values ± SD (n = 3).

Selenite resulted to be stronger than OX and SO$_4$ in competing with As(III), showing an efficiency ranging from 16.6 (R = 1) to 40.7% (R = 5) (Figure 4). Selenite replaced more As(III) than As(V) (Caporale et al., 2011) from this Mg–Fe-LDH. In fact, at $R = 5$ its efficiency in preventing As(III) sorption was about two times greater than that in preventing As(V) fixation.

The amount of As(III) sorbed in presence of equimolar concentration of CIT (779.4 mmol kg$^{-1}$) was similar to that sorbed in presence of PO$_4$ (763.4 mmol kg$^{-1}$) (Table 4). However, with increasing $R$, the ability of PO$_4$ in inhibiting As(III) sorption resulted to be higher than that of CIT. At $R = 5$, the
efficiency of PO$_4$ in preventing As(III) sorption was 67.4%, versus 56.8% of CIT (Figure 4).

The efficiency of CIT was much higher than that of OX clearly because CIT is easily sorbed on LDH because of its high charge and the capacity to form strong inner-sphere complexes. Zhu et al., (2011) and Violante et al., (2010); Violante, (2013) also found that CIT has an influence much higher than OX in inhibiting As(III) and As(V) sorption onto Al- and Fe-(hydr)oxides. In this work we confirmed that PO$_4$ is the strongest ligand in preventing As sorption also on Mg–Fe-LDH.

In conclusion, many factors seems to affect the sorption of organic and inorganic anions onto Mg–Fe-LDH and, consequently, their ability in competing with As(III) [as well as As(V)], such as: (i) their valency; (ii) their size; (iii) their orientation in the interlayer of LDHs (Meyn et al., 1990) and (iv) their capacity to form outer- or innersphere complexes onto variable charge minerals eventually present as impurities.

4.1.3 Effect of residence time on the desorption of As(III) by organic and inorganic ligands

Figure 5 shows the desorption of As(III) from Mg–Fe-LDH by the competing ligands, at pH 6.0 and R = 1 (Figure 5A) and 3 (Fig. 5B), as a function of As(III) residence time (24 and 168 h) on LDH surfaces. The effect of a residence time of 72 h is not shown for sake of clarity.

The ability of the competing ligands in removing As(III) previously sorbed onto Mg–Fe-LDH surfaces followed the sequence: SO$_4$ < OX << SeO$_3$ < CIT < PO$_4$, regardless the residence time. Obviously, a greater As(III) desorption (in%) occurred when greater amounts of each ligand was added (Figure 5). In
fact, at $R = 3$ all the ligands desorbed nearly twice the amount of As(III) removed at $R = 1$. However, the higher the As(III) residence time the lower the amount of the metalloid desorbed by each competing ligands. Probably, by increasing residence time a better diffusion of As(III) ions occurred into the interlayers of Mg–Fe-LDH, which, consequently, were not easily replaced by other anions. Some authors found that As(III) (You et al., 2001), SeO$_3$ but not selenate (You et al., 2001) and As(V) (Violante et al., 2009) were intercalated with time into the layer spaces of a Mg–Al-LDH.

Recent literature on Fe- and Al-(hydr)oxides (Meyn et al., 1990; Arai and Sparks, 2002; Pigna et al., 2006; Violante et al., 2008; Caporale et al., 2011) also showed that an increase in residence time of As(V) resulted in a decrease in its desorption by competing anions from minerals surfaces, due to the formation of more stable surface complexes. In fact, formation of inner-sphere complexes by As(III) and As(V) onto a Fe(II)–Fe(III)-LDH (green rust) has been demonstrated (Wang et al., 2010).

Figure 5 Effect of the residence time on As(III) desorption from Mg–Fe-LDH by competing ligands, which were added 24 h (black bars) and 168 h (checkered bars) after As(III). Initial ligand/As(III) molar ratios were 1 (A) and 3 (B). The numbers above the bars indicate the As(III) desorbed (%). The initial As(III) concentration was 1000 mmol kg$^{-1}$.  

![Figure 5](image-url)
Greater percentages of As(III) than As(V) (as referred to the amounts of the oxyanion initially sorbed) were replaced by the competing ligands but PO$_4$. For example, at $R = 3$ and after a residence time of 168 h, 7.1%, 9.3% and 23.2% of As(III) versus 0.7%, 1.5% and 1.5% of As(V) (Caporale et al., 2011) were replaced respectively by SO$_4$, OX and SeO$_3$. Vice versa, 40.2% of As(III) versus 54.5% of As(V) were desorbed by PO$_4$. These findings could be due to the fact that the quantities of both PO$_4$ ($S_m = 2470$ mmol kg$^{-1}$) and As(V)($S_m = 2595$ mmol kg$^{-1}$) sorbed on Mg–Fe-LDH (Caporale et al., 2011) were much higher than those of As(III) ($S_m = 1621$ mmol kg$^{-1}$; Table 3). Clearly, when PO$_4$ was added to the LDH on which As(V) was previously sorbed it competed with As(V) for common sites. Vice versa, because As(III) did not occupy all the sites occupied by As(V), PO$_4$ was initially sorbed on empty sites easily accessible and only later this oxyanion competed with As(III) for common sites. However, these findings merit closer attention.

4.1.4 Kinetics of desorption of As(III) by PO$_4$

The kinetics of desorption of As(III) by PO$_4$ (initial $R$ values of 1 and 3) from Mg–Fe-LDH, as a function of time, is shown in Figure 6A. It was well described by the Elovich equation (Figure 6B); the linear form of this equation (Sparks, 2003; Pigna et al., 2006) is given by:

$$q_t = \frac{1}{\beta} \ln(\alpha\beta) + \frac{1}{\beta} \ln(t)$$
where $q_t$ is the amount of As(III) desorbed for a given time ($t$), and $\alpha$ and $\beta$ are the Elovich coefficients, representing the initial desorption rate (mmol kg$^{-1}$ min$^{-1}$) and the desorption coefficient (mmol kg$^{-1}$), respectively.

**Figure 6** Kinetics of desorption of As(III) by PO$_4$ from Mg–Fe-LDH (A), also described by the Elovich equation (B). The initial PO$_4$/As(III) molar ratios were 1 (R = 1) and 3 (R = 3); initial As(III) concentration was 1000 mmol kg$^{-1}$.

The amount of As(III) desorbed from the LDH sites increased with time, being characterized by an initial very fast desorption reaction (0.08–5 h), followed by a much slower desorption reaction until a plateau was reached (Figure 6A). Precisely, after 0.08 h (5 min) of reaction, PO$_4$ removed the 15.8% and 30.4% of the As(III) previously sorbed on Mg–Fe-LDH, at R = 1 and 3, respectively, which represent the fraction of the As(III) less strongly bonds and/or more accessible and then more easily desorbable.

After 48 h of reaction with PO$_4$, the Mg–Fe-LDH released 25.9% and 42.7% of the initially bound As(III), at R = 1 and 3, respectively. Caporale et al., (2011) studied the sorption/desorption of As(V) on/from the same Fe-
based LDH and found that a greater percentage (as referred to the amount initially sorbed) of As(V) was replaced by PO$_4$. A possible explanation of these findings has been given before.

**Table 5** Kinetic paramaters by simply regression analysis of As(III) desorption by PO$_4$ for the reaction period 5 min to 48 h for the Fe-Mg-LDH using the Elovich kinetic equation, at initial PO$_4$/As(III) molar ratios of 1 (R = 1) and 3 (R = 3).

<table>
<thead>
<tr>
<th>PO$_4$/As(III) molar ratio (R)</th>
<th>Elovich Kinetic Equation</th>
<th>$\alpha^b$</th>
<th>$\beta^b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R = 1$</td>
<td>$q_t = 197.50 + 16.34 \ln(t)$</td>
<td>2.901 $\times$ $10^6$</td>
<td>0.0612</td>
<td>0.9834</td>
</tr>
<tr>
<td>$R = 3$</td>
<td>$q_t = 365.38 + 17.42 \ln(t)$</td>
<td>2.244 $\times$ $10^6$</td>
<td>0.0574</td>
<td>0.9146</td>
</tr>
</tbody>
</table>

$^a$ $q_t$ is the amount of As(III) desorbed (mmol kg$^{-1}$) in time $t$ (min).

$^b$ $\alpha$ and $\beta$ are Elovich coefficients obtained from the Elovich kinetic model

$q_t = (1/\beta) \ln(\alpha \beta) + (1/\beta) \ln(t)$.

The initial As desorption rate ($\alpha$) and desorption coefficient ($\beta$) were higher for As(III) (Table 5) than As(V) (Caporale et al., 2011). As reported by (Sparks, 2003) some researchers have used the $\alpha$ and $\beta$ coefficients to estimate reaction rates. An increase in $\alpha$ and/or a decrease in $\beta$ would increase reaction rate, but according to (Sparks, 2003) this interpretation is questionable. Anyway, our data do not permit to give a reasonable explanation on the differences in the kinetics of removal of As(III) and As(V) by PO$_4$ from Mg–Fe-LDH.

### 4.1.5 Conclusions on the work of the LDH
The findings of this study give useful information on the As(III) sorption capacity of a Fe-based LDH and the factors (pH, presence and concentration of organic and inorganic ligands, residence time) which affect As(III) removal by this anionic clay from contaminated solutions. Arsenite sorption isotherms indicated a good affinity of this harmful oxyanion for the sorption sites of the Mg–Fe-LDH, which could be considered a reliable mean to remove efficiently As(III) from contaminated solutions, especially in alkaline environments. Competing ligands strongly prevented the As(III) sorption by Mg–Fe-LDH, following the order: SO₄ < OX << SeO₃ < CIT < PO₄. The desorption of As(III) by these anions decreased with increasing As(III) residence time on LDH surfaces. We found that more As(V) than As(III) was sorbed on this Mg–Fe-LDH and that all the ligands but PO₄ inhibited As(III) sorption more than As(V) and desorbed greater percentages of As(III) than As(V) previously fixed on the sorbent. The kinetics of desorption revealed that the most easily desorbable fraction of As(III) was removed by PO₄ in the first few hours of reaction.

4.2 Study on the the Ferrihydrite and Goethite

4.2.1 Nature of the Ferrihydrite and Goethite

The Fe-(hydr)oxide obtained at pH 5.5 and aged for 24 h at room temperature was identified to be Ferrihydrite. In fact, the X-ray diffraction pattern (XRD) of the sample showed four characteristic broad and weak peaks of the Ferrihydrite, centered at 0.254, 0.225, 0.198, and 0.148 nm (Figure 7), which indicate a poorly crystalline nature of the sorbent, characterized by particles of smaller size with high surface area (178.2 m² g⁻¹, Table 6).
The Fe-based product synthesized at pH 12 and aged at 65°C was identified to be Goethite, characterized by the intense XRD peaks observed at 0.419, 0.338, 0.269, 0.258, 0.244, 0.225, 0.219, 0.172 and 0.168 nm (Figure 7), which indicate a well-crystallized structure of the sorbent (Cornell and Schwertmann, 1996). The surface area of Goethite resulted to be of 42.3 m² g⁻¹ (Table 6).

**Figure 7** Powder X-ray diffraction patterns of Ferrihydrite and Goethite.

### 4.2.2 Arsenite sorption isotherms at pH 6.0

The Langmuir isotherms of As(III) onto Ferrihydrite or Goethite at pH 6.0 are depicted in Figure 8. The sorption data of As(III) sorbed onto two sorbents conformed to the Langmuir equation in the following form: \( S = \frac{S_m K_c}{(1+K_c c)} \) where \( S \) is the amount of the element sorbed per unit mass of sorbent (mmol kg⁻¹), \( S_m \) is the maximum amount of As(III) that may be bound to the sorbent (sorption capacity), \( c \) is the equilibrium solution concentration (mmol L⁻¹), and \( K \) is a constant related to the binding energy.

The sorption isotherms of As(III) onto Ferrihydrite or Goethite were typical H curve (Giles et al., 1974), indicating a very high affinity of As(III)
for the sorption sites of these two sorbents (Figure 8). The high affinity of the harmful anion for the surfaces of Fe-based (hydr)oxides has been widely recognized (Zhu et al., 2011; Caporale et al., 2013; Violante, 2013). However, Ferrihydrite \( (S_m = 1245.1 \text{ mmol kg}^{-1}) \) adsorbed much more As(III) than Goethite \( (S_m = 327.9 \text{ mmol kg}^{-1}) \), essentially because of its greater surface area \( (178.2 \text{ vs. } 42.3 \text{ m}^2 \text{ g}^{-1}) \) and, hence, lower degree of crystallinity, as well as to the greater affinity of As(III) for its surfaces. This latter statement is supported by the K values (which describe the binding energy), obtained from the As(III) sorption isotherms: K value of Ferrihydrite \( (i.e., 38.5) \), in fact, resulted to be almost three-fold higher than that of Goethite \( (i.e., 13.2) \). Goldberg and Johnston, (2001), indeed, stated that Fe-(hydr)oxides usually adsorb As(III) anions on their external surfaces by both inner- and outer-sphere bonds. Probably, in our tests, Ferrihydrite adsorbed As(III) on their own external surfaces by forming a greater amounts of inner-sphere complexes vs. those formed by Goethite. Accordingly, Ona-Nguema et al., (2005), by EXAFS analysis, demonstrated that As(III) forms similar surface complexes on Ferrihydrite and Hematite (stronger) that differed from those formed on Goethite and Lepidocrocite (weaker).
Figure 8 Arsenite sorption isotherms onto Ferrihydrite and Goethite at pH 6.0 and 20°C, after 24 h of reaction time.

4.2.3 Arsenite sorption at different pHs

The sorption of As(III) onto Ferrihydrite and Goethite in the range of pH from 4.0 to 10.0 is shown in Figure 9 and resulted to be lightly pH dependent. In fact, in the pH range of 4.0-9.0 the quantities of As(III) adsorbed resulted to be practically constant, being As(III) practically uncharged (i.e., H$_3$AsO$_3^0$). At higher pHs we observed a decrease in As(III) sorption by two sorbents, probably because of the competition with the increasing amounts of hydroxyl ions and the repulsion between the negative charged As(III) ions (pKa = 9.2 and 12.1) and the negative charged surfaces of two Fe-(hydr)oxides. According to Pigna et al., (2006), the point of zero charge (pzc) of Ferrihydrite and Goethite is expected to be at 8.70 and 7.45, respectively. Similar results were also found in other scientific works (Manning and Goldberg, 1996; Inskeep et al., 2001; Grafe et al., 2002; Dixit and Hering, 2003; Zhu et al., 2011).
RESULTS AND DISCUSSION

Figure 9 Arsenite sorbed by Ferrihydrite and Goethite as a function of pH.

4.2.4 Effect of organic and inorganic ligands on the sorption of As(III)

The efficiency (%) of the ligands in preventing As(III) sorption on Ferrihydrite and Goethite, as a function of ligand/As(III) molar ratio (R) (i.e., 1 and 3), after 24 h of reaction time at pH 6.0, is shown in Figures 10 and 11. The efficiency (%) was calculated by subtracting the amount of As(III) sorbed in presence of ligand from that sorbed in absence of ligand, divided by the amount sorbed in absence of ligand.

The selected organic and inorganic ligands showed different capacities to compete with As(III) for the sorption sites of the Ferrihydrite and Goethite, according to the following increasing sequence: selenate < sulphate < oxalate < citrate < selenite ≈ molybdate < phosphate on Ferrihydrite, and sulphate ≈ selenate < oxalate < citrate < molybdate < selenite ≈ phosphate on Goethite.

Selenate and SO\textsubscript{4} slightly inhibited the sorption of As(III) on Ferrihydrite and Goethite, since these anions usually form outer-sphere
complexes onto the surfaces of variable charge minerals. Peak et al., (2001), Peak and Sparks, (2002), and Zhang and Sparks, (1990) demonstrated, by EXAFS study, that SeO$_4$ forms a mixture of outer- and inner-sphere surface complexes onto Ferrihydrite and Goethite, with a decrease of inner-sphere complexes at increasing pH, whereas SO$_4$ forms both outer-sphere and inner-sphere surface complexes on Goethite at less than pH 6.0, outer-sphere complex at pH $>$ 6.0, and forms predominantly outer-sphere surface complexes on Ferrihydrite.

The efficiency of CIT was much higher than that of OX clearly because CIT is easily sorbed on Fe-based (hydr)oxides, because of its high charge and the capacity to form strong inner-sphere complexes. Zhu et al., (2011) and Violante, (2013) also found that CIT has an influence much higher than OX in inhibiting As(III) and As(V) sorption onto Al- and Fe-(hydr)oxides. Selenite resulted to be one of the strongest anions in competing with As(III) for sorption sites of Ferrihydrite and Goethite, probably because it usually forms inner-sphere complexes on both these Fe-based (hydr)oxides (Peak et al., 2006). By our competitive study, we also noted that MoO$_4$ has a good affinity for Goethite surfaces, and even more those of Ferrihydrite. The efficiency (%) of this anion in preventing As(III) sorption on Ferrihydrite, indeed, resulted to be slightly higher than that of SeO$_3$, and quite similar to that of the strongest competing anion (i.e., PO$_4$).

As expected, PO$_4$ demonstrated to be the strongest among the selected ligands in competing with As(III) for the surfaces of Ferrihydrite and Goethite, essentially because its high affinity for the surfaces of Fe-based (hydr)oxides, which usually bind such anion by strong inner-sphere complexes (Zhu et al., 2011; Caporale et al., 2013).
Table 6 Langmuir sorption capacity (Sm) and the constant K for As(III), as obtained from the sorption isotherms on the Ferrihydrite and Goethite at pH 6.0 and 20°C.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>pH</th>
<th>Surface Area (m² g⁻¹)</th>
<th>Sm (mmol kg⁻¹)</th>
<th>K</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrihydrite</td>
<td>6.0</td>
<td>178.2</td>
<td>1245.1</td>
<td>38.5</td>
<td>0.97</td>
</tr>
<tr>
<td>Goethite</td>
<td>6.0</td>
<td>42.3</td>
<td>327.9</td>
<td>13.2</td>
<td>0.94</td>
</tr>
</tbody>
</table>

4.2.5. Conclusions on the work on Ferrihydrite and Goethite

The results of this study give important information on the As(III) sorption capacity of Fe-based minerals (Ferrihydrite and Goethite) and the factors (pH, presence and concentration of organic and inorganic ligands which affect As(III) removal by this anionic clay from contaminated solutions. Arsenite sorption isotherms indicated high affinity of this harmful oxyanion for the sorption sites of the Ferrihydrite and Goethite, which could be considered to remove efficiently As(III) from contaminated solutions. Competing ligands
strongly prevented the As(III) sorption following the order: selenate < sulphate < oxalate < citrate < selenite ≈ molybdate < phosphate on Ferrihydrite and sulphate ≈ selenate < oxalate < citrate < molybdate < selenite ≈ phosphate on Goethite. Finally, by comparing these data with those obtained with Mg-Fe-LDH it is noteworthy to point out that As(III) adsorption by Fe-based LDH was a little higher if compared with Ferrihydrite, and significant higher if compared with Goethite, since LDH wide interlayer region is easily accessible to a great amount of As(III) ions. However, by studying As(III) adsorption in presence of competing ligands, we observed that As(III) is more strongly adsorbed Fe-(hydr)oxides than LDH, because of the formation of inner-sphere complexes vs. weaker electrostatic bonds.

4. 3. Study on the carrot plants (*Daucus carota* L.)

4.3.1 Soil characterization

The soil used in this experiment was collected from the sub-surface layer (10-30 cm) of a grassland in the Portici (Naples) area. Its principal physical and chemical properties are reported in Table 7. According to the soil classification of Food and Agriculture Organization (FAO) World Reference Base for Soil Resources, we can considerate this soil as a Calcari-Vitric Cambisol. Briefly, this was a sandy-loam neutral soil, with low organic matter content and P availability, and very low As-background.
Table 7 Physical and chemical properties of the soil.

<table>
<thead>
<tr>
<th>Soil Properties</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand (g kg(^{-1}))</td>
<td>473 ± 32</td>
</tr>
<tr>
<td>Silt (g kg(^{-1}))</td>
<td>371 ± 25</td>
</tr>
<tr>
<td>Clay (g kg(^{-1}))</td>
<td>156 ± 11</td>
</tr>
<tr>
<td>pH (in H(_2)O)</td>
<td>7.34 ± 0.18</td>
</tr>
<tr>
<td>Organic carbon (g kg(^{-1}))</td>
<td>10.90 ± 0.45</td>
</tr>
<tr>
<td>CEC (mequiv 100 g(^{-1}))</td>
<td>17.26 ± 0.59</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}))</td>
<td>1.02 ± 0.07</td>
</tr>
<tr>
<td>C/N</td>
<td>10.69</td>
</tr>
<tr>
<td>Available P (mg P(_2)O(_5) kg(^{-1}))</td>
<td>10.35 ± 0.50</td>
</tr>
<tr>
<td>Total As (mg kg(^{-1}))</td>
<td>4.67 ± 0.21</td>
</tr>
</tbody>
</table>

4.3.2. Effect of As and fertilization on the growth of carrot plants

The growth of the carrot plants was significantly (p<0.0001) impaired by the irrigation with As-contaminated waters (Table 8). Increasing As level in the irrigation water, indeed, decreased markedly the dry matter production of carrot plants, as a consequence of the phytotoxic effect of As. For instance, carrot plants irrigated with water containing 3 mg As L\(^{-1}\) produced, on average, a biomass 22.1% lower than plants irrigated with As-uncontaminated water (As 0). Likewise, the biomass of the plants irrigated with water containing 6 mg As L\(^{-1}\) resulted to be, on average, 39.3% lower, if compared to that of As control plants (As 0) (Table 8). Carrot plants stressed by As started to show symptoms of As toxicity (i.e., the appearance of red-brown
necrotic spots on the leaves, in some cases followed by foliar senescence) 12-15 days after the first irrigation with the As 3 and As 6 contaminated waters, but none of them died from As intoxication. The phytotoxic effect of As on edible plants have been shown in different studies. Shri et al., (2009) noted that As is highly toxic during the germination and growth of rice seeds. Sensitive edible plants, such as bean (Caporale et al., 2013), lettuce (Caporale et al., 2014), radish (Bhatti et al., 2013), tomato (Pigna et al., 2012) and wheat (Pigna et al., 2009) suffered considerable stress upon As exposure, with negative outcomes on plant growth, net photosynthesis rate and fruit yield.

Beside As treatment, the growth of carrot plants was also significantly (p<0.0001) affected by fertilization with humic acids or inorganic fertilizers. It is conceivable as all the fertilized plants grew much more than non-fertilized plants (Table 8). Within fertilized plants, carrots treated with inorganic fertilizer grew slightly more than those amended with commercial humic acids, probably because of the faster and higher availability of its nutrients. Accordingly, the P status of fertilized plants resulted to be a little higher than that of the amended plants, significantly higher in comparison to unfertilized plants (data not shown). In comparison with unfertilized As 6 plants, for example, As 6 carrots amended with full dose of humic acids (F.O.D.) produced 90.1% more of biomass, whereas those treated with full dose of inorganic fertilizers (F.I.D.) grew 97.2% more.
Table 8 The dry weight of the roots and shoots biomass at harvest time.

<table>
<thead>
<tr>
<th>Organic, inorganic and As treatments</th>
<th>Taproots (g)</th>
<th>Shoots (g)</th>
<th>Total biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No F As 0</strong></td>
<td>5.71 ± 0.25 efg</td>
<td>3.98 ± 0.31 efg</td>
<td>9.69 ± 0.72 efg</td>
</tr>
<tr>
<td><strong>H.O.D As 0</strong></td>
<td>7.10 ± 0.66 cde</td>
<td>4.90 ± 0.42 cde</td>
<td>12.00 ± 1.08 cde</td>
</tr>
<tr>
<td></td>
<td>(24.34%)</td>
<td>(23.17%)</td>
<td>(23.86%)</td>
</tr>
<tr>
<td><strong>F.O.D As 0</strong></td>
<td>9.48 ± 0.90 ab</td>
<td>6.83 ± 0.60 ab</td>
<td>16.31 ± 1.56 ab</td>
</tr>
<tr>
<td></td>
<td>(66.02%)</td>
<td>(71.56%)</td>
<td>(68.30%)</td>
</tr>
<tr>
<td><strong>H.I.D As 0</strong></td>
<td>7.86 ± 0.65 bc</td>
<td>5.76 ± 0.41 bc</td>
<td>13.62 ± 0.80 bc</td>
</tr>
<tr>
<td></td>
<td>(37.65%)</td>
<td>(44.73%)</td>
<td>(40.56%)</td>
</tr>
<tr>
<td><strong>F.I.D As 0</strong></td>
<td>10.38 ± 0.92 a</td>
<td>7.25 ± 0.61 a</td>
<td>17.63 ± 1.62 a</td>
</tr>
<tr>
<td></td>
<td>(81.79%)</td>
<td>(82.16%)</td>
<td>(81.94%)</td>
</tr>
<tr>
<td><strong>No F As 3</strong></td>
<td>4.30 ± 0.23 gh</td>
<td>3.02 ± 0.19 gh</td>
<td>7.32 ± 0.43 hi</td>
</tr>
<tr>
<td><strong>H.O.D As 3</strong></td>
<td>5.59 ± 0.41efg</td>
<td>3.89 ± 0.32efg</td>
<td>9.48 ± 0.76efghi</td>
</tr>
<tr>
<td></td>
<td>(30.00%)</td>
<td>(28.81%)</td>
<td>(29.51%)</td>
</tr>
<tr>
<td><strong>F.O.D As 3</strong></td>
<td>7.50 ± 0.48 cd</td>
<td>5.38 ± 0.45 cd</td>
<td>12.88 ± 0.89 cd</td>
</tr>
<tr>
<td></td>
<td>(74.42%)</td>
<td>(78.15%)</td>
<td>(75.96%)</td>
</tr>
<tr>
<td><strong>H.I.D As 3</strong></td>
<td>6.05 ± 0.57 def</td>
<td>4.45 ± 0.38 def</td>
<td>10.50 ± 0.91 def</td>
</tr>
<tr>
<td></td>
<td>(40.70%)</td>
<td>(47.35%)</td>
<td>(43.44%)</td>
</tr>
<tr>
<td><strong>F.I.D As 3</strong></td>
<td>8.21 ± 0.64 bc</td>
<td>5.70 ± 0.50 bc</td>
<td>13.91 ± 1.29 bc</td>
</tr>
<tr>
<td></td>
<td>(90.93%)</td>
<td>(88.74%)</td>
<td>(90.03%)</td>
</tr>
<tr>
<td><strong>No F As 6</strong></td>
<td>3.05 ± 0.28 h</td>
<td>2.38 ± 0.21 h</td>
<td>5.43 ± 0.31 i</td>
</tr>
<tr>
<td><strong>H.O.D As 6</strong></td>
<td>4.32 ± 0.40 gh</td>
<td>3.23 ± 0.30 gh</td>
<td>7.55 ± 0.70 ghi</td>
</tr>
<tr>
<td></td>
<td>(41.64%)</td>
<td>(35.71%)</td>
<td>(39.04%)</td>
</tr>
<tr>
<td><strong>F.O.D As 6</strong></td>
<td>5.86 ± 0.50 defg</td>
<td>4.46 ± 0.38 defg</td>
<td>10.32 ± 0.85 defg</td>
</tr>
<tr>
<td></td>
<td>(92.13%)</td>
<td>(87.39%)</td>
<td>(90.06%)</td>
</tr>
<tr>
<td><strong>H.I.D As 6</strong></td>
<td>4.66 ± 0.36 fgh</td>
<td>3.59 ± 0.25 fgh</td>
<td>8.25 ± 0.68 fgh</td>
</tr>
<tr>
<td></td>
<td>(52.79%)</td>
<td>(50.84%)</td>
<td>(51.93%)</td>
</tr>
<tr>
<td><strong>F.I.D As 6</strong></td>
<td>6.04 ± 0.43 def</td>
<td>4.67 ± 0.29 cdef</td>
<td>10.71 ± 0.72 def</td>
</tr>
<tr>
<td></td>
<td>(98.03%)</td>
<td>(96.22%)</td>
<td>(97.24%)</td>
</tr>
</tbody>
</table>

As treatment: p < 0.0001
Fertilization: p < 0.0001
Interaction: 0.3432
4.3.3 Chlorophyll A+B concentration in the carrot leaves

By this study we also noted that the chlorophyll A+B concentration progressively decreased (p<0.0001), as long as increased As levels were supplied to carrot plants by the irrigation water (Figure 12). The photosynthetic pigments are some of the most important cellular components of plants, hence, they are targets of the toxic As effect (Miteva, 2002). In fact, the presence of As in the plant leaves has been shown to damage the chloroplast’s membrane structure, drastically reducing the rate of photosynthesis carried out by plants (Marques and Anderson, 1986; Miteva, 2002; Miteva and Merakchiyska, 2002). In a previous study, As was reported to reduce the chlorophyll biosynthesis in maize (Jain and Gadre, 1997). Later, it was observed that both chlorophyll A and B concentrations in rice leaf decreased significantly with the increase of As concentrations (Rahman et al., 2007).

In comparison to the unfertilized control (no F As 0, no F As 3 or no F As 6), significantly (p<0.0001) higher chlorophyll A+B concentration was found in the leaves of the carrots fertilized either with humic acids or inorganic fertilizers (Figure 12). A positive effect on chlorophyll A and B concentrations, due to organic fertilization, was also noted by Caporale et al., (2013), who treated As-stressed bean plants with commercial stabilized compost.
4.3.4 Arsenic concentration in carrot taproots and shoots

Significantly (p<0.0001) higher concentrations of As were found in carrot tissues when plants were irrigated with water containing increasing levels of As (Table 9). 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 including carrot (Alam et al., 2003; Roychowdhury et al., 2003; Williams et al., 2006), with As present only in inorganic forms (Williams et al., 2006).

Most of the As taken up by our carrot plants was accumulated in their taproots, while a lesser allocation of toxicant occurring in the leaf biomass. A greater accumulation of As in carrot taproots vs. leaves was also observed in other studies (Bohari et al., 2002) and (Codling et al., 2014) with As-treated carrot plants.

Within each set of As treated plants (As 0, As 3 and As 6), the concentration of As in carrot taproots and leaf biomass significantly
(p<0.0001) changed according to the kind of fertilizer supplied (Table 9). In comparison to unfertilized controls (no F As 0, no F As 3 or no F As 6), indeed, plants amended with humic acids exhibited a lower concentration of As in their own tissues (up to -18.8%), while the opposite was observed in those fertilized with inorganic fertilizer (up to +12.4%) (Table 9). These findings are strictly related to the higher availability of As in the soil fertilized with inorganic fertilizers vs. that amended with humic acids (as discussed below in the 4.3.5 section, and shown in Table 10).
Table 9 Arsenic concentration (mg As kg\(^{-1}\) dry weight) in the taproots and shoots of carrot determined at harvest time

<table>
<thead>
<tr>
<th>Organic, inorganic and As treatments</th>
<th>Taproots (mg/kg)</th>
<th>Shoots (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No F As 0</td>
<td>0.120±0.008 e</td>
<td>0.073±0.005 e</td>
</tr>
<tr>
<td>H.O.D As 0</td>
<td>0.115±0.007 e</td>
<td>0.070±0.004 e</td>
</tr>
<tr>
<td>F.O.D As 0</td>
<td>0.102±0.005 e</td>
<td>0.061±0.002 e</td>
</tr>
<tr>
<td>H.I.D As 0</td>
<td>0.124±0.010 e</td>
<td>0.075±0.006 e</td>
</tr>
<tr>
<td>F.I.D As 0</td>
<td>0.132±0.011 e</td>
<td>0.080±0.005 e</td>
</tr>
<tr>
<td>No F As 3</td>
<td>2.15±0.14 d</td>
<td>0.97±0.06 d</td>
</tr>
<tr>
<td>H.O.D As 3</td>
<td>2.04±0.17 d</td>
<td>0.91±0.05 d</td>
</tr>
<tr>
<td>F.O.D As 3</td>
<td>1.79±0.13 d</td>
<td>0.80±0.04 d</td>
</tr>
<tr>
<td>H.I.D As 3</td>
<td>2.23±0.16 d</td>
<td>1.01±0.06 d</td>
</tr>
<tr>
<td>F.I.D As 3</td>
<td>2.40±0.15 d</td>
<td>1.07±0.07 d</td>
</tr>
<tr>
<td>No F As 6</td>
<td>4.83±0.31 ab</td>
<td>2.13±0.15 ab</td>
</tr>
<tr>
<td>H.O.D As 6</td>
<td>4.51±0.27 bc</td>
<td>1.98±0.15 bc</td>
</tr>
<tr>
<td>F.O.D As 6</td>
<td>3.92±0.28 c</td>
<td>1.75±0.13 c</td>
</tr>
<tr>
<td>H.I.D As 6</td>
<td>5.06±0.35 ab</td>
<td>2.25±0.16 ab</td>
</tr>
<tr>
<td>F.I.D As 6</td>
<td>5.43±0.38 a</td>
<td>2.38±0.17 a</td>
</tr>
</tbody>
</table>

As treatment p < 0.0001  Fertilization p < 0.0001  Interaction p < 0.005
4.3.5 Non- and specifically-sorbed As concentrations in soil samples

The irrigation of the soil with As-contaminated waters significantly (p<0.0001) increased both the nonspecifically and specifically sorbed As fractions in the soil, following the order: As 6 > As 3 > As 0, which indicated an increased availability of As in the soil, and the contemporary occupation of the soil sorption sites with increasing As concentration (Table 10).

Within two set of plants irrigated with As-contaminated waters (As 3 and As 6), the supply of commercial humic acids (H.O.D. and F.O.D.) to the soil, led to a significant (p<0.0001) decrease of the nonspecifically sorbed As (up to -21.7%), with the contemporary increase of the specifically sorbed fraction (up to +21.4%), in comparison to the unfertilized control (no F) (Table 10); this trend was probably due to partial immobilization of the As by the organic functional groups on the humic acids, either directly or through cation bridging. Anions are usually not sorbed by soil organic matter, but As(III) and As(V) have been found to be bound to humic acids (Thanabalasingam and Pickering, 1986). Indeed, many binding mechanisms have been proposed for the sorption of As anions to organic matter, which include the formation of: i) outer-sphere complexes with protonated amino groups, ii) covalent bonds with carboxylate or phenolate groups and iii) the formation of ternary complexes with polyvalent cations (Fe, Al) forming a bridge between As anions and organic matter (Mikutta and Kretzschmar, 2011). Arsenic may form complexes with humic acid with a higher affinity for As(V) than for As(III) (Fakour and Lin, 2014). Humic acids has a great potential for influencing sorption behavior and redox transformation of As species through interacting with Fe oxide surfaces and/or with As itself, and thus may play a significant
role in the fate, transfer and release of As from terrestrial environments into the groundwater (Fakour and Lin, 2014).

On the contrary, the fertilization of soil with inorganic fertilizers (H.I.D. and F.I.D.) determined a significant (p<0.0001) increase of the nonspecifically sorbed As fraction (up to +13.0%), with a consequent decrease of the specifically sorbed As (up to -12.8%), if compared to data found in the soil of unfertilized control (no F) (Table 10); this behavior was probably due to the competition between the contaminant and the phosphorus, supplied by fertilization and ready available, for the sorption sites of the soil Al- and Fe-(hydr)oxides (Violante and Pigna, 2002; Violante, 2013). A slight enhancement of As mobility in soil (i.e., increase of nonspecifically sorbed As fraction), due to P application by inorganic fertilizers, was also observed by Cozzolino et al., (2010), in a study with lettuce plants grown in a natural As-contaminated soil (containing 250 mg As kg⁻¹).
Table 10: Non- and specifically-sorbed As concentrations in soil samples

<table>
<thead>
<tr>
<th>Organic, inorganic and As treatments</th>
<th>Nonspecifically sorbed As in soil (µg/kg)</th>
<th>Specifically sorbed As in soil (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No F As 0</td>
<td>18.65±1.55 g (-4.66%)</td>
<td>0.183±0.015 e (4.69%)</td>
</tr>
<tr>
<td>H.O.D As 0</td>
<td>17.78±1.23 g (-16.09%)</td>
<td>0.192±0.016 e (16.03%)</td>
</tr>
<tr>
<td>F.O.D As 0</td>
<td>15.65±1.18 g (-10.63%)</td>
<td>0.213±0.016 e (16.03%)</td>
</tr>
<tr>
<td>H.I.D As 0</td>
<td>19.42±1.75 g (-4.03%)</td>
<td>0.176±0.014 e (-4.03%)</td>
</tr>
<tr>
<td>F.I.D As 0</td>
<td>20.71±1.79 g (11.05%)</td>
<td>0.164±0.012 e (-10.63%)</td>
</tr>
<tr>
<td>No F As 3</td>
<td>213.4±17.6 ef (-5.67%)</td>
<td>0.778±0.058 d (5.66%)</td>
</tr>
<tr>
<td>H.O.D As 3</td>
<td>201.3±13.5 ef (-10.63%)</td>
<td>0.822±0.063 d (5.66%)</td>
</tr>
<tr>
<td>F.O.D As 3</td>
<td>173.6±12.3 f (-18.65%)</td>
<td>0.925±0.065 d (18.89%)</td>
</tr>
<tr>
<td>H.I.D As 3</td>
<td>224.0±17.8 e (-4.88%)</td>
<td>0.740±0.050 d (-4.88%)</td>
</tr>
<tr>
<td>F.I.D As 3</td>
<td>239.2±19.9 de (-11.70%)</td>
<td>0.687±0.037 d (-11.70%)</td>
</tr>
<tr>
<td>No F As 6</td>
<td>357.1±18.5 bc (-7.22%)</td>
<td>1.380±0.134 bc (7.61%)</td>
</tr>
<tr>
<td>H.O.D As 6</td>
<td>331.3±18.4 c (-21.38%)</td>
<td>1.485±0.137 ab (21.38%)</td>
</tr>
<tr>
<td>F.O.D As 6</td>
<td>279.8±13.9 d (-5.22%)</td>
<td>1.675±0.150 a (-5.22%)</td>
</tr>
<tr>
<td>H.I.D As 6</td>
<td>375.0±18.9 ab (-12.83%)</td>
<td>1.308±0.110 bc (-12.83%)</td>
</tr>
<tr>
<td>F.I.D As 6</td>
<td>403.6±20.3 a (-12.83%)</td>
<td>1.203±0.084 c (-12.83%)</td>
</tr>
</tbody>
</table>

4.3.6 Conclusions on the study on the carrot plants

The irrigation of carrot plants with As-contaminated waters caused a severe phytotoxic effect, as indicated by the drastic reduction of biomass and
chlorophyll A and B concentrations in the leaves. The supply of either humic acids or inorganic fertilizers alleviated, at least in part, the toxicity of the As, essentially by stimulating plant growth and promoting nutrient uptake (e.g., P), with a consequent dilution of As inside plant tissues. The mobility of As in the soil was slightly reduced by application of humic acids, because of the partial immobilization of the As on their organic functional groups, whereas was slightly enhanced by the treatment with inorganic fertilizers, probably because of the competition between the contaminant and the P, supplied by fertilization, for the sorption sites of the soil Al- and Fe-(hydr)oxides.
5.1 Overall conclusions

At present, As-contaminated soil and aquatic environment management is an important issue and a global concern because it is the biggest mass poisoning in history. It is necessary to improve sustainable environmental health and also to minimize the adverse impact on humans to be successful.

Numerous technological developments occurred in the last decade are leading to a gradual economic growth of many countries, but, at the same time, they cause serious environmental risks and damages, including As contamination. Concentrations of As more than the hygienic level appear frequently in drinking-water, posing an important environmental issue, particularly in densely populated area such as Bangladesh, India where the demand for water is very high. In fact, 226 million people in 105 countries/territories across the world are exposed to levels of As in their drinking-water that exceed the WHO recommended permissible limit of 10 μg L \(^{-1}\) and 100 million of these are exposed to level of over 50 μg L \(^{-1}\) (Murcott, 2012). The epidemiological studies showed that the chronic arsenic poisoning can cause serious health effects including cancer, dermal diseases, cardiovascular diseases, respiratory diseases, neurological diseases, hematological diseases, renal diseases, and hepatic diseases. Therefore, necessary steps must be taken to reduce these impacts by providing access to safe water as a basic human right. Delaying mitigation will increase death and disease.

The immobilization of As on the surfaces of sorbents is considered by the scientific community, a effective and safe removal method of As from contaminated water. However, the high cost of production of most sorbents capable to remove As(III) and As(V) ions from contaminated solutions, strongly limits their spread and use, on a large scale. It is therefore necessary
to research on novel sorbents at low cost, easily synthesized, available for free or at very low price. Surveys conducted in the present work have followed exactly this line of thought, through the study of the removal capacity of As(III) from contaminated solutions by LDHs (easily reproducible anionic clays at low cost) and Ferrihydrite and Goethite. The implementation of decontamination systems of As-contaminated waters by providing for LDHs, Ferrihydrite and Goethite use would be able to unify the lower costs of remediation and effective removal of the metalloid from them. In fact, the As(III) sorption capacity exhibited by LDHs, Ferrihydrite and Goethite is absolutely comparable to that of the more expensive sorbents (Kapadia, 2000).

It is well established that As can enter the food chain, affecting food safety. This poses a potential dietary risk to human health in addition to the risk from drinking contaminated water. Food chain contamination by As, in fact, has become a burning issue in recent decade because of their potential uptake and translocation in the edible part of plants by contaminated soil and/or groundwater (Bundschuh et al., 2012). Therefore monitoring programs for As contribute to improving food safety, alert of actual and potential food scares, and facilitate evaluation of possible health hazards by providing continuous information on levels of environmental pollution.

Less well-known but potentially more serious is the risk of As to crop production. Continuous build up of As in the soil from As-contaminated irrigation water reduces crop yields in the long term.

The presence of As in soils and/or groundwaters used for agricultural purposes, causes a strong abiotic stress to the cultivated plants, which manifests itself through the reduction of biomasses and, specially, yields, mostly non-tradable, both for the size that the high As concentration. It is therefore desirable to identify and develop production techniques capable of
limiting the mobility and phyto-availability of As in soil, through the stabilization of the metalloid on the more recalcitrant soil fractions.

The use of a classical soil amendant as humic acids in the present work, in addition to improve carrot plants growth and their nutritional status, has allowed to limit the As uptake by biomasses, through the immobilization of the metalloid, derived by irrigation water, on/in their humified organic macromolecules. Furthermore, soil amendment by humic acids falls within the context of organic farming, eco-friendly production system, which ensures the sustainability of the soil, improving its fertility.
CHAPTER 6 – REFERENCES
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Shah Md. Golam gousul Azam

March 31, 2015
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>As: arsenic</td>
<td>PO₄: phosphate</td>
</tr>
<tr>
<td>As(III): arsenite</td>
<td>SO₄: sulphate</td>
</tr>
<tr>
<td>µg/l: microgram/liter</td>
<td>MoO₄: molybdate</td>
</tr>
<tr>
<td>As(V): arsenate</td>
<td>SeO₃: selenite</td>
</tr>
<tr>
<td>H.O.D.: half organic dose</td>
<td>SeO₄: selenate</td>
</tr>
<tr>
<td>F.O.D.: half organic dose</td>
<td>OX: oxalate</td>
</tr>
<tr>
<td>H.I.D.: half organic dose</td>
<td>CIT: citrate</td>
</tr>
<tr>
<td>F.I.D.: half organic dose</td>
<td>MMA: monomethylarsonic acid</td>
</tr>
<tr>
<td>As₃S₄: realgar</td>
<td>DMA: dimethylarsinic acid</td>
</tr>
<tr>
<td>NiAs: niccolite</td>
<td>NiAs₂: rammelsbergite</td>
</tr>
<tr>
<td>CoAsS: cobaltite</td>
<td>Cu₃AsS₄: enargite</td>
</tr>
<tr>
<td>AsS (Co,Fe):glaucodot</td>
<td>FeS: Pyrite and</td>
</tr>
<tr>
<td>CCA: copper-chromium-arsenate</td>
<td>Cd: cadmium</td>
</tr>
<tr>
<td>Fe: iron</td>
<td>Pb: lead</td>
</tr>
<tr>
<td>Zn: zink</td>
<td>PbAsO₄: lead arsenate</td>
</tr>
<tr>
<td>MgAsO₄: magnesium arsenate</td>
<td>ZnAsO₄: zinc arsenate</td>
</tr>
<tr>
<td>Paris Green: Cu(CH₃COO)₂ · 3Cu(AsO₂)₂</td>
<td>H₂Cr₂O₇: dichromic acid</td>
</tr>
<tr>
<td>ROX: Roxarsone</td>
<td>DCH: Dhaka Community Hospital</td>
</tr>
<tr>
<td>SCC: squamous cell carcinoma</td>
<td>BD: Bowen’s disease</td>
</tr>
<tr>
<td>KCl: potassium chloride</td>
<td>h: hour</td>
</tr>
<tr>
<td>KCl: potassium chloride</td>
<td>K: potassium</td>
</tr>
<tr>
<td>µg/l: microgram/liter</td>
<td>mg: milligram</td>
</tr>
<tr>
<td>As₂S₃: orpiment</td>
<td>As₂O₅: arsenolite</td>
</tr>
<tr>
<td>FeAsS₂: loellingite</td>
<td>CoAs: safforlite</td>
</tr>
<tr>
<td>NiAsS₂: gersdorffite</td>
<td>FeAs₅: arsenopyrite</td>
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<tr>
<td>NiAs: niccolite</td>
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<td>CoAs: safforlite</td>
<td>FeAs₅: Arsenopyrite</td>
</tr>
<tr>
<td>Cu: copper</td>
<td>Ni: Nickel</td>
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<tr>
<td>CaAsO₄: calcium arsenate</td>
<td>Zn(AsO₂)₂: zinc arsenite</td>
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<tr>
<td>H₃AsO₄: arsenic acid</td>
<td>BCC: basal cell carcinoma</td>
</tr>
<tr>
<td>LDHs: layered double hydroxides</td>
<td>WHO: World Health Organization</td>
</tr>
<tr>
<td>FAO: Food and Agricultural Organization</td>
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